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The enantioselectivity of reduction of ethyl 4-halo-3-oxobutanoate catalyzed by *Geotrichum candidum* depends on the cofactor

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Abstract

Enantioselective reductions of ethyl 3-oxobutanoates with fermenting cells or acetone treated cells of *Geotrichum candidum* gave 3-hydroxyesters with different ee and different predominant configurations depending on reaction conditions. Ethyl 4-bromo-3-oxobutanoate was reduced with APG4 and NADH to give predominantly ethyl (*R*)-4-bromo-3 hydroxybutanoate while the (*S*)-configuration was predominant when NADPH was the cofactor. Moreover, when the catalyst was heated before the reaction, the ee was increased indicating that the enzyme giving the (*S*)-alcohol is more thermolabile than the other.

Keywords: *Geotrichum candidum*; NADPH; Ethyl 3-oxobutanoates; Enantioselective reduction

1. Introduction

The importance of enantiopure 3-hydroxybutanoates is well known. They are valuable synthons for a wide range of important bioactive molecules, such as carnitine [1,2], 4-amino-3-hydroxybutanoic acid (GABOB), pyrrolidin-3-ol and 5-(chloromethyl)-1,3-oxazolidin-2-one [3]. The methods for their production include lipase-catalyzed resolutions [4] and ammonolysis [3], and asymmetric reduction of the corresponding 3-oxo-butanoates catalysed by BINAP [5,6], binaphtylbiphosphine ruthenium complexes [7] or microorganisms [8,9].

Methyl ketones have been reduced by *Geotrichum candidum*, and the enantiomeric excesses are normally very high [10].

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2. Results and discussion

2.1. Synthesis of starting compound

Ethyl 4-chloro-3-oxobutanoate (**2**) and ethyl 4-bromo-3-oxobutanoate (**3**) were obtained via the diketene route [4] using either chlorine or bromine (Scheme 1). Only after carefully monitoring of the reaction temperature, a reasonable yield of **3** was obtained. Attempts to make **3** starting with either ethyl 3-butenoate or 2,2-dimethyl-1,3-dioxane-4,6-dione were unsuccessful.

2.2. Microbial enantioselective reduction

The 3-oxobutanoates **1**–**3** were reduced with fermenting cells of *Geotrichum candidum* (IFO 4597); or cells that had been treated with ice-cold acetone (acetone powder, APG4). The reducing agents in the latter case were either NADH or NADPH which were regenerated *in situ* using cyclopentanol (Scheme 2). Hydride attack from the *re*-face of **1**, gave predominance

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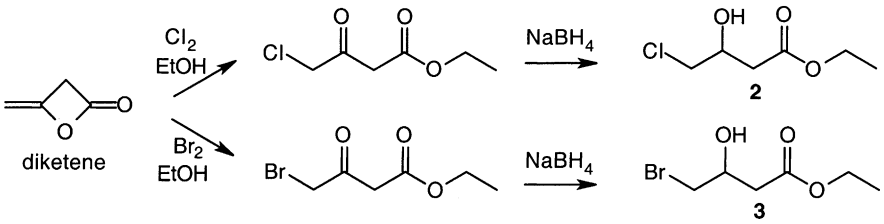
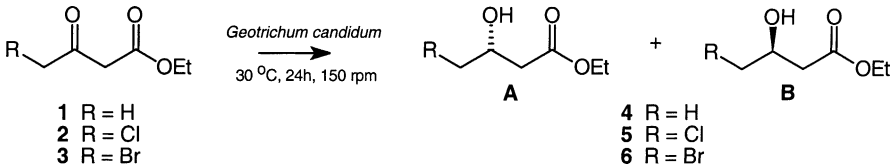


Scheme 1.



Scheme 2.

of the **A**-isomer, i.e. (*S*)-**4**. Hydride attack from the same face, with regards to the size of the substituents, also led to the **A**-isomer of **5**. However, this is now actually the *si*-face and the product is predominantly (*R*)-**5**. The stereoselectivity of the reduction of **3** depended on the catalyst and cofactor. Whole cell-reduction or APG4 with NADPH gave, by attack from the opposite side, predominance of **B** and (*S*)-**6**, while APG4 and NADH gave mostly **A** and (*R*)-**6**.

The enantiomeric excesses of the produced halogenated alcohols **5** and **6** were not satisfactory (Table 1). Low stereoselectivity in biocatalytic

Table 1
Enantiomeric excess and configurations of predominant product (**4**–**6**) obtained in reduction of ethyl 3-oxobutanoates after 24 h and 30 °C

Substrate	Product	Fermenting cells	APG4 (NADH)	APG4 (NADPH)
1	**4**	63% (*S*) **A**	99.5% (*S*) **A**	91% (*S*) **A**
2	**5**	30% (*R*) **A**	48% (*R*) **A**	25% (*R*) **A**
3	**6**	84% (*S*) **B**	84% (*R*) **A**	54% (*S*) **B**

Formula **A** represents the (*S*)-configuration for **4** while **B** is the (*S*)-configuration for **5** and **6**.

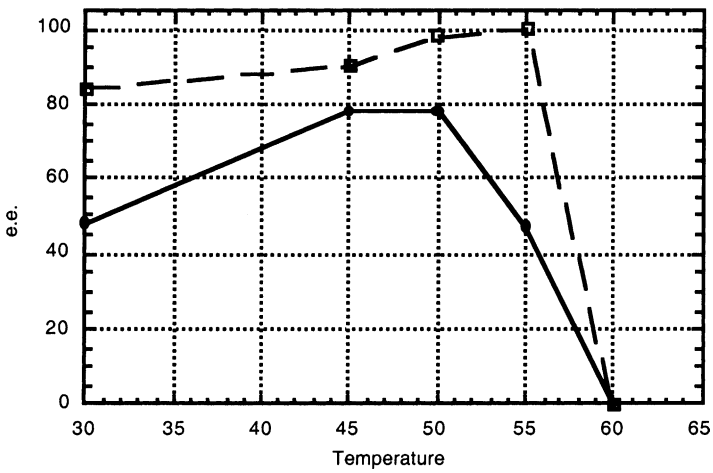


Fig. 1. Enantiomeric excesses in the reduction of **2** (full lines) and **3** (stippled lines) using APG4 with NADH as cofactor. The catalyst was pre-heated at different temperatures for 20 min.

reductions is often caused by the presence of two or more enzymes with different stereoselectivity. Ways to overcome this problem have been reported, such as the addition of a selective inhibitor [11–13] and heat treatment of the biomass prior to reactions [14]. We therefore heated the acetone-dried cells at different temperatures for 20 min prior to the addition of substrates. The ee's were increased to an optimum of 78% for ethyl 4-chloro-3-oxobutanoate (**2**) and 99% for ethyl 4-bromo-3-oxobutanoate (**3**), before complete inactivation of the enzymes occurred (Fig. 1).

3. Conclusion

It is known that *Geotrichum* sp. contains several oxidoreductases with opposite stereochemical preference [15–17]. Our results indicate that one enzyme is more thermolabile than the other. Moreover, in the reduction of **3**, the predominant reduction product changed from (*R*)-configuration with NADH as cofactor to (*S*)-configuration when NADPH was used, indicating that the "(*R*)-enzyme" is NADH-dependent while the "(*S*)-enzyme" depends on NADPH. Further investigations with pure enzymes are needed in order to elucidate the true origin of the observed effects.

4. Experimental

4.1. General

Geotrichum candidum was cultivated under standard conditions, cells were filtered off and washed five times with ice-cold acetone to furnish a white powder (APG4) [10]. Both living cells and APG4 were used for enzymatic reductions [18]. The synthesized substrates had spectroscopic properties in agreement with structures **2** and **3**. Ethyl 3-oxobutanoate (**1**) was obtained from Fluka.

4.2. Analyses

Chiral analyses were performed using Varian 3300 and 3400 gas chromatographs equipped with CP-Chirasil-dex CB columns from Chrompack (25 m, 0.25 mm, 0.25 or 0.35 μm film density) or Chiraldex G-TA (10 m, 0.25 mm, 0.125 μm film density) from Astec (Whipany, NJ, USA).

4.2.1. Chromatographic properties of alcohols ***4–6***

R_{tS} and R_{tR} retention time of (*S*) and (*R*)-enantiomer respectively, R_s resolution.

- *Ethyl 3-hydroxybutanoate*(***4***): analyzed on CP-Chirasildex column as the acetate. Temp. prog. 83 (10 min)-180 °C 15°/min R_{tS} 11.24 min, R_{tR} 13.21 min R_s 2.52.
- *Ethyl 4-chloro-3-hydroxybutanoate*(***5***): analyzed on CP-Chirasildex column as the acetate. Temp. prog. 83 (10 min)-180 °C, 15°/min R_{tR} 11.24 min, R_{tS} 13.21 min R_s 3.36.
- *Ethyl 4-bromo-3-hydroxybutanoate*(***6***): analyzed on Chiraldex G-TA column as the TFA-ester. Temp. prog. 90 (5 min)-150 °C, 15°/min R_{tS} 2.66 min, R_{tR} 2.96 min R_s 2.94.

Acknowledgements

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