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Whole Cells Mediated Biocatalytic Reduction of Alpha-Keto Esters: Preparation of Optically Enriched Alkyl 2-hydroxypropanoates

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Abstract

Biocatalytic reduction of alkyl 2-oxopropanoates were carried out by utilizing the whole cells of *Candida parapsilosis* ATCC 7330 to form the optically enriched alkyl 2-hydroxypropanoates with good enantiomeric excess (ee) (≤91%) and isolated yields (≤68%). Enantiomerically enriched (*S*)-ethyl 3-bromo-2-hydroxypropanoate thus synthesized by biocatalytic reduction of ethyl 3-bromo-2-oxopropanoate is presented in this study for the first time in water under ambient reaction conditions in a reaction time of 4 h which is considerably less than earlier reported procedures.

Keywords: Asymmetric reduction, alkyl 2-oxopropanoates, alpha-keto esters, *Candida parapsilosis*, ethyl 3-bromo-2-oxopropanoate

Introduction

In recent times, microbial flora has been explored well to produce various industrially important products like xylitol (1), clinically useful enzymes such as L-asparaginase (2) etc. In addition to these, they were also explored to produce pharmaceutically important chiral compounds (3). Industrially, optically enriched α-hydroxy esters find great value due to their applications in the synthesis of liquid crystals (4, 5) and as 'green' solvents (6). Food and Drug Administration approved ethyl lactate, an important α-hydroxy ester which is used as an additive in both food and pharmaceutical industries. It is utilized as an efficient solvent for the extraction of vitamin E and important phytoconstituents. In addition, it is also considered an environmental friendly solvent due to its non-corrosive and biodegradable properties (7). Optically enriched α-hydroxy esters are used in pesticides as an additive and also used in insect repellents which are intended for human applications (8). Optically pure (*S*)-ethyl lactate finds application in the treatment of acne (9). In combination with a biopolymer,

optically pure (*S*)-ethyl lactate finds application as an embolising medium to treat vascular injuries (10). It also acts as a chiral precursor for carbapenem antibiotics (11, 12), as an important pheromone scaffold present in common wasp (13), in the synthesis of (*R*)-(+)-phenoxypropionic acid (14) among others.

Enantiomerically enriched aliphatic alpha hydroxy esters are reportedly synthesized by means of chemo-catalytic or biocatalytic reductions. Brown and coworkers, (15) reported the catalytic hydrogenation of different carbonyl functionalities with the help of chiral trialkylborane B-(3-pinanyl)-9-borabicyclo [3.3.2]-nonane. In this study, different alkyl 2-oxopropanoates including ethyl pyruvate 1a were converted to the respective (*S*)-enantiomers in appreciable enantiomeric excess (72-79%). Likewise, the substrate ethyl 3-bromo-2-oxopropanoate 1b was reduced to form the (*S*)- product 2b' (enantiomeric excess:7.6%) (15). Likewise, several chemical catalysts like alkaloid modified platinum nanowires, (16) cinchonidine containing layered double hydroxides (LDH) were utilized for the catalytic reduction of ethyl pyruvate (1a) to produce optically enriched ethyl lactate (2a') (17). In another study, the reduction of ethyl 2-oxopropanoate was performed with ruthenium nanoparticles and surprisingly, no enantioselectivity was displayed (18). It is therefore very important to design efficient "green" methodologies for this reduction and biocatalytic methods offer a good possibility (19, 20). Immobilized *Candida antarctica* lipase B was used as a biocatalyst for the resolution of ethyl 2-hydroxypropanoate 2a (racemic mixture) to selectively produce the (*S*)-product 2a' (enantiomeric excess: 43->99%) (21). The reactions were carried out using different solvents, where the (*R*)-enantiomer reacted selectively with different vinyl esters to produce the respective acylated (*R*)-products with excellent enantiomeric excess (>99%) (21). Kinetic resolution is useful, only when both optical counterparts are utilized. Otherwise, it is of limited use due to its less theoretical yield (50%). In this perspective, asymmetric reduction is a preferred choice as it can produce the product in good theoretical yield.

Ishihara and coworkers reported the biocatalytic reduction of different alpha keto esters involving *Chlorella sorokiniana* as biocatalyst. The green algae were grown under different growth conditions for about 72 h. Due to varied growth conditions, the products thus formed differed in their conversion as well as in enantiomeric excess (up to >99%) (22). The study had shown that the biocatalyst expresses different reductases and thus controls the stereoselectivity of the product, when cultivated under different growth conditions (22). Some marine microalgae and actinomycetes including few strains of *Salinispora* and *Streptomyces*, were employed for

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the biocatalyst mediated reduction of various alpha-keto esters to form their respective (*S*)-alcohols with up to 99% conversion and enantiomeric excess in 20-48 h (23-25). In addition, there are a number of reports that has been published for the biocatalytic reduction of alkyl-2-oxopropanoates using several purified enzymes and over-expressed cells (26-31).

Candida parapsilosis ATCC 7330 has displayed its versatility as a very efficient and robust biocatalyst (32) for the synthesis of enantiomerically improved chiral alcohols by asymmetric hydrogenation (33, 34) or deracemisation of the racemic alcohols (35-38) with most of them being aryl substituted compounds. Although, multiple reports on the biocatalytic reduction of alkyl keto esters are available, nevertheless a more efficient methodology is however required for a scaled-up process. It is to be highlighted that no biocatalytic reduction was reported for ethyl 3-bromo-2-oxopropanoate so far. Therefore, in the present study, the biocatalytic reduction of alkyl 2-oxopropanoates employing *C. parapsilosis* ATCC 7330 is discussed.

Materials and Methods

The substrates ethyl pyruvate and ethyl bromo pyruvate, for the study, were purchased from M/s Sigma-Aldrich, India. The racemic α-hydroxy esters were synthesised using the reported method from our lab (39). Proton (500 MHz) and ^{13}C (125 MHz) Nuclear Magnetic Resonance spectroscopy were carried out using Bruker AVANCE III 500 MHz spectrometer using deuterated chloroform as solvent. Tetra methyl silane was utilized as internal reference and chemical shifts were mentioned in parts per million. IR spectra of the products were studied using JASCO FT/IR - 4200 instrument. Optical purity of the products were analysed by fitting a chiral stationary column (0.25µm x 25mm x 25m) in a gas chromatograph (Perkin Elmer CLARUS 600) with helium as the carrier gas. Rudolph, Autopol IV digital polarimeter was used to determining the optical rotations.

Candida parapsilosis ATCC 7330 was procured from the repository of ATCC, USA and maintained as per the prescribed conditions. The biocatalyst was cultured using the optimized growth conditions and harvested as per the earlier report (40). The harvested cells were washed thoroughly with water and the same cells were subjected for the biotransformation.

Biocatalytic reduction of ethyl 2-oxopropanoate 1a

The earlier optimised reaction protocol (41) was used for the bioreduction of ethyl 2-oxopropanoate (0.9 mmol) in water. The progress of the reaction was observed using TLC and the time was optimised as 4 h. The product was extracted from the reaction medium using ethyl acetate; the combined extract was treated with

sodium sulphate (anhydrous) to remove any moisture that was present. Using a rotary evaporator, the excess solvent was removed. Optically enriched (*S*)-ethyl 2-hydroxypropanoate was obtained as a faint yellow compound. The crude compound was purified using column chromatography with a mixture of hexane (95%) and ethyl acetate (5%) as eluting solvents. Optical purity of the resultant product and yield was calculated to be 72% and 61% respectively. The spectral details of the compound were in consensus with the earlier report (42).

The asymmetric reduction of ethyl bromo pyruvate 1b was also carried out using the same reaction conditions up to 4 h and spectral data were compared with the reported values. The configuration was assigned as (*S*) as compared with the specific rotation value reported in the literature (15).

The experiments were carried out thrice to ensure reproducibility of the results. In addition, control experiments (using heat-killed cells) were also carried out under same experimental conditions.

Spectroscopic characterization of products

(S)-Ethyl 3-bromo-2- hydroxypropanoate 2b' (15)

Light yellow oil; IR (cm^{-1}): 3435, 2979, 2922, 2853, 1730, 1089; ^{1}H NMR ($CDCl_3$; 500 MHz; δ in ppm): 1.26 (t, 3H, *J* = 7 Hz), 1.38 (d, 3H, *J* = 6.5 Hz), 2.99 (br s, 1H-OH), 4.18-4.25 (m, 3H); ^{13}C NMR ($CDCl_3$; 125 MHz; δ in ppm): 14.09, 20.78, 61.56, 66.72, 175.68; Specific rotation: $[\alpha]_D^{24}$: -7.2 (c 1, $CHCl_3$) {$[\alpha]_D^{23}$: -8.51 (c 1, EtOH); enantiomeric excess: 91%}. GC condition: injector and detector temperature: 250 °C, Oven: 40 °C for 3 min @ 5 °C to 90 °C, hold for 2 min; flow rate: 2.0 mL/min; R_t: 11.90 min (*R*, minor), 12.39 min (*S*, major).

(S)-Ethyl 2-hydroxypropanoate 2a' (42, 43)

Light yellow oil; IR (cm^{-1}): 3454, 2980, 2923, 2854, 1730, 1090, 640.64; ^{1}H NMR ($CDCl_3$; 500 MHz; δ in ppm): 1.33 (t, 3H, *J* = 7 Hz), 3.23 (d, 1H, *J* = 6 Hz), 3.68 (dd, 1H, *J* = 10.5, 3.5 Hz), 3.72 (dd, 1H, *J* = 10.5, 3.5 Hz), 4.25-4.37 (m, 2H), 4.49-4.51 (m, 1H); ^{13}C NMR ($CDCl_3$; 125 MHz; δ in ppm): 14.16, 35.07, 62.54, 69.73, 171.48. Specific rotation: $[\alpha]_D^{24}$: -31.2 (c 1, $CHCl_3$) {$[\alpha]_D^{23}$: -6.13 (c 2.4, CCl_4); enantiomeric excess: 7.6%}. GC condition: injector & detector temperature: 220 ºC; Oven :40 ºC for 3 min @ 5ºC to 90 ºC hold for 2 min; R_t: 12.04 min (*R*, minor), 12.50 min (*S*, major).

Results and Discussion

C. parapsilosis ATCC 7330 mediated bioreduction of ethyl pyruvate (1a)

Applying the earlier reported experimental conditions, asymmetric biotransformation of ethyl pyruvate 1a was carried out (40). The wet cells of the biocatalyst were suspended

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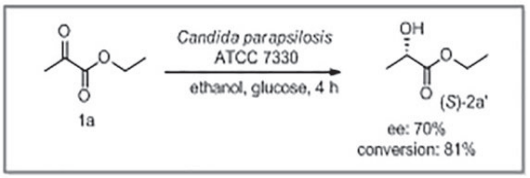
in an aqueous medium with glucose as a cosubstrate. Ethyl pyruvate was dissolved in ethanol and the reaction was carried out till completion (4 h) (Scheme 1).



Scheme 1 Asymmetric reduction of ethyl 2-oxopropanoate 1a using *C. parapsilosis* ATCC 7330

Bioreduction of ethyl pyruvate: role of cosolvents

Earlier reports have suggested the importance of solvents in various biocatalytic reactions (44-46). The reports from our lab also have shown that by choosing suitable cosolvents, the solubility of the reactant in the reaction medium is enhanced which further showed improved conversion and optical purity of the product alcohols (47-49). Therefore, in an attempt to enhance the optical purity and conversion, multiple cosolvents were tested for the biocatalytic reduction of ethyl pyruvate 1a (Figure 1). The products of biotransformation using different cosolvents were analyzed for their enantiomeric excess and conversion (in parentheses). The reactions carried out in acetonitrile (53%; 87%), hexane (62%; 87%), chloroform (65%; 84%) and tetrahydrofuran (63%; 83%) showed better conversion to product with respect to ethanol, whereas solvents like dimethyl sulphoxide (56%; 64%) and 1,4-dioxane (66%; 79%) displayed less conversion. It was observed that the optical purity of the product formed in reactions using different solvents was found to be less as compared with ethanol. Hence, it was considered ideal to use only ethanol as the cosolvent for the bioreduction. In addition, the reaction time of the biotransformation was further extended to improve the conversion of the product, but unexpectedly there observed a notable decrease in both enantiomeric excess (67%) and conversion (77%), which could be attributed to the degradation of the product alcohols in water and hence accounts for the low yield of product 2a' (61%) in this work.

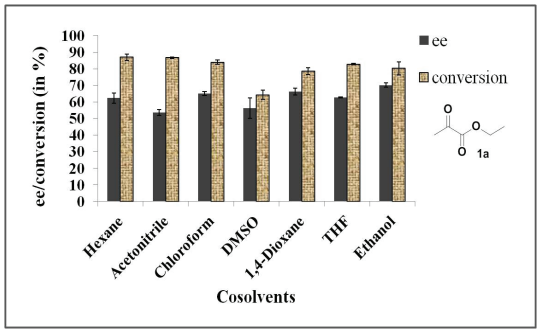


Figure 1 Screening of different cosolvents in the asymmetric reduction of ethyl 2-oxopropanoate 1a

C. parapsilosis ATCC 7330 mediated bioreduction of ethyl bromo pyruvate

Notably, different halogen containing chiral precursors have displayed a marked enhancement in

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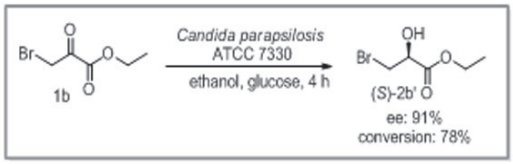
their pharmacological activity due to their electronegative and lipophilic properties in comparison with the unsubstituted compounds (50). Hernandes et al. studied that in drug design, halogenated drug candidates, especially the chloro and bromo derivatives are preferred due to their bulky nature to occupy the binding pockets and their ability to form halogen bonds in ligand-target complexes, which is now being recognised as a favourable interaction to contribute to the stability of such complexes (51, 52).

Size-wise, iodine is bulkier than bromine and chlorine. In drug design, iodine was less preferred due to its high molecular weight and increased polarizability (52). On the other hand, other members of the halogen family contribute to a greater extent in the MDL drug data report (52). Among them, chlorine and fluorine-containing drugs have established a greater contribution in pharmaceutical preparations. Fluorine, which is considered as a hydrogen-mimic forms an important scaffold in several top-selling pharmaceuticals like atorvastatin, fluticasone etc. (50). Interestingly, the biocatalytic synthesis of both optical antipodes of ethyl 4-chloro-3-hydroxybutanoate, a chiral precursor was reported using this biocatalyst with excellent enantiomeric excess (>99%) and yield (up to 96%). This chlorine-containing chiral alcohol is an important precursor for the preparation of L-carnitine and the side chain of atorvastatin (34). In addition, the same biocatalyst has established its versatility in the preparation of fluorinated chiral alcohol with a good enantiomeric excess (84%) and yield (68%) (41). With this perspective, we wished to explore the substrate repertoire of the biocatalyst towards brominated compound and hence ethyl 3-bromo-2-oxopropanoate is the model substrate in this study.

The bioreduction of ethyl bromo pyruvate 1b was performed under optimized reaction conditions (Scheme 2). The product alcohol formed was determined as (*S*)-hydroxy compound 2b' with high enantiomeric excess (91%) and conversion (78%).



Scheme 2 Asymmetric reduction of ethyl 3-bromo-2-oxopropanoate 1b using *C. parapsilosis* ATCC 7330

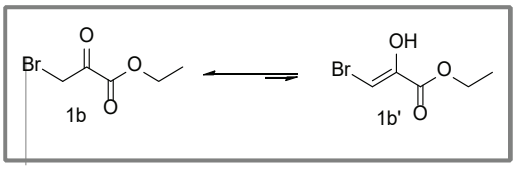


Figure 2 Keto-enol tautomerism of ethyl 3-bromo-2-oxopropanoate 1b

The preparative scale bioreduction was carried out and the isolated yield thus obtained was found to be 68%. Upon extending the reaction time (until 5 h) showed no marked

difference in the conversion as well as optical purity. It is interesting to note that keto esters with active -CH_2- functionality exhibit tautomerism (keto-enol type). Ethyl bromo pyruvate also exhibits keto (1b) as well as in enolic state (1b') (Figure 2). Notably, the enolic compound 1b' is found to be less reactive due to its vinylic bromide structure, which makes the substrate remain stable in water and further do not allow it to take part in any nucleophilic reactions. *C. parapsilosis* ATCC 7330 preferentially reduced the bromo compound (1b) to its (*S*)-alcohol 2b' with good enantiomeric excess (91%), which is accountable due to the electron-withdrawing nature of bromo substituent in the keto ester as opposed to the unsubstituted substrate (1a). Further, the presence of bromo substituent in the substrate is expected to initiate and promote the attack of hydride on carbonyl functionality. In addition, the presence of the halogen in the substrate could have significantly attributed to better substrate-binding confirmation with the reducing enzyme (53) and hence accounts for better enantiomeric excess and yield of the brominated product.

Conclusion

An efficient biocatalytic reduction method was developed for alkyl 2-oxopropanoates like ethyl pyruvate and ethyl bromo pyruvate. The reaction conditions were optimized and the resultant products were found to be the (*S*)-alcohols with good enantiomeric purities (≤ 91%) and isolated yields (≤ 68%) in a very short duration (4 h). For the first time, the biotransformation of ethyl bromo pyruvate to produce the corresponding enantiomerically enriched (*S*)-ethyl 3-bromo-2-hydroxy-propanoate is reported in the present study with a good enantiomeric excess (91%) and isolated yield (68%).

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