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Short communication

Microbial enantioselective reduction of ethyl-2-oxo-4-phenyl-butanoate

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Abstract

Different microorganisms (MOs) were used to carry out the enantioselective reduction of ethyl-2-oxo-4-phenylbutanoate to (*S*)-(+)-2-hydroxy-4-phenylbutanoate or (*R*)-(+)-2-hydroxy-4-phenylbutanoate. Commercially available *Saccharomyces cerevisiae* and *Dekera* sp. led to over 92% ee of (*S*)-(+)-2-hydroxy-4-phenylbutanoate. *Kluyveromyces marxianus* gave the opposite isomer with 32% ee (*R*). All reactions, except those with *Hansenula* sp., proceeded to greater than 90% conversion. This the first report on the use of *Dekera* sp., *Hansenula* sp. and *K. marxianus* in the reduction of α-ketoesters.

Keywords: *Saccharomyces cerevisiae*; *Dekera* sp.; *Hansenula* sp.; *Kluyveromyces marxianus*; Lisinopril; ACE inhibitors

1. Introduction

Asymmetric reduction of prochiral ketones is one of the most investigated methods to produce chiral compounds [1–6]. Other approaches that have been so far published included catalytic use of chiral oxazaborolidine for hydride transfer [7–9], hydrogenation using homogeneous chiral metal complexes [10–12] and heterogeneous hydrogenation on chiral modified surfaces [13–24].

The search for alternative microorganism to *Saccharomyces cerevisiae* to effect enantioselective reduction with control on the chiral center has been the objective of several groups [25–30], included our own [31].

Chadha et al. reported that the enantioselective reduction of ethyl-2-oxo-4-phenylbutanoate to (*R*)-(+)-2-hydroxy-4-phenylbutanoate was not achieved by using *S. cerevisiae*, but was made possible by using cell free aqueous extracts of the callus of *Daucus carota* (wild carrot) with excellent chemical yields (90%) and ee >99% (*R*) [32]. However, since the use of cell cultures is not very useful to be scaled up and this procedure was time consuming (10 days), the use of microorganisms deserves further investigation. In that way, Oda et al. reported the production of (*R*)-(+)-2-hydroxy-4-phenylbutanoate in up to 95% ee, although in low yields (58%), by using *Rhodotorula minuta* and *Candida holmii* in interface bioreactors [33] and Dao et al. reported that *S. cerevisiae*, preincubated in the presence of phenacyl chloride, can be used in the production of (*R*)-(+)-2-hydroxy-4-phenylbutanoate [34].

Since our group has been using microorganisms to produce useful active pharmaceutical ingredients (APIs), for instance to fluoxetine [35], and that *Hansenula* sp., *Dekera* sp. led a higher ee than *S. cervisiae* and *Aspergillus niger* for enantioselective reduction of β-ketoesters [31,35], the purpose of the present paper is to briefly describe our study with different microorganisms in the reduction of ethyl-2-oxo-4-phenylbutanoate.

Our interest in this system has risen on the analysis of the production routes of lisinopril [36] and other angiotensin converting enzyme (ACE) inhibitors. In general, aminative reduction is the process of choice. The distereoisomeric mixture is separated and, after deprotection, lisinopril is obtained.

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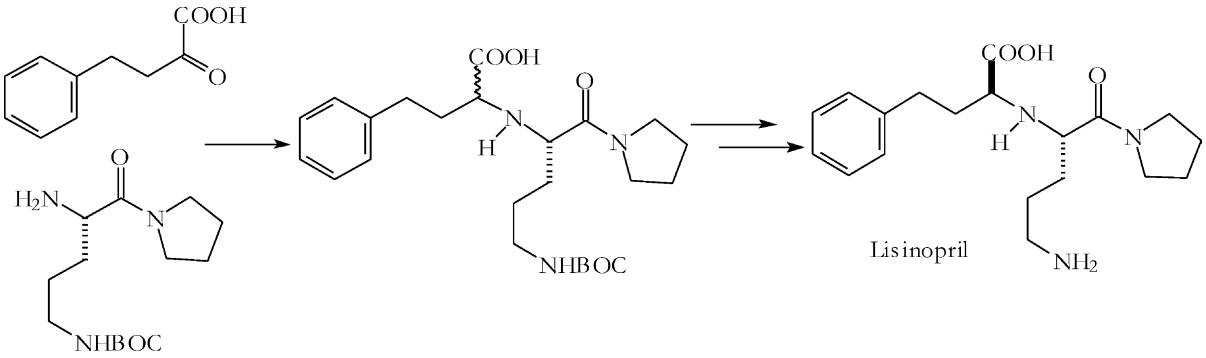
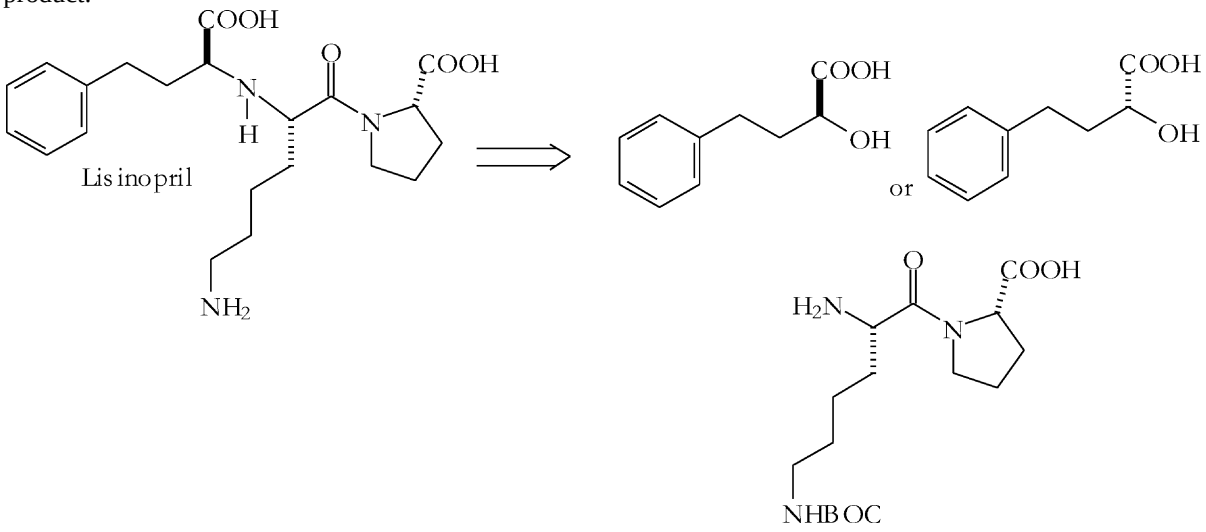


The obvious choice to avoid the troubleshooting diastereoisomeric separation is to make use of a proper intermediate, the (*S*)-(+)-2-hydroxy-4-phenylbutanoate or the (*R*)-(+)-2-hydroxy-4-phenylbutanoate. In the first case, ethyl-(*S*)-(+)-2-hydroxy-4-phenylbutanoate would produce, via chloride formation or via a Mitsunobu modified displacement [37,38], upon two inversions, the desirable final product. In the latter case, using a (*R*)-(+)-2-hydroxy-4-phenylbutanoate derivative, a much better protocol was envisaged; a simple S_N2 reaction, of the corresponding triflate, would provide the desirable product.



The main points in favor of the biotechnological process are its green and "natural" appeal. The plethora of MOs available in nature [39–41] and our previous experience encouraged us into the search of a suitable microbiological process to produce (*S*)-(+)-2-hydroxy-4-phenylbutanoate or (*R*)-(+)-2-hydroxy-4-phenylbutanoate.

2. Materials and methods

The ee and the absolute configuration of the reaction were determined by chiral high-resolution chromatography, performed in a commercial available Cyclodex B capillary column (30 m × 0.25 mm, i.d.); initial temperature, 393 K (30 min); rate, 2 °/min; final temperature, 473 K. Ethyl-(*S*)-(+)-2-hydroxy-4-phenylbutanoate and *rac*-ethyl 2-hydroxy-4-phenylbutanoate were used as chromatographic standards. The elution order was *R* (50.3 min) followed by *S* (50.9 min).

To obtain an analytical sample, the reaction products were isolated and characterized by ^{1}H RMN (200 MHz/$CDCl_3$) δ: 7.20–7.30 (m, 5H, Ar–**H**); 4.16–4.27 (m, 3H, **CH**(OH)CO e $\mathbf{CH_2}(OCO)CH_3$); 2.73–2.82 (m, 2H, $Ph\mathbf{CH_2}CH_2$); 2.3 (s, OH); 1.93–2.09 (m, 2H, $CH_2\mathbf{CH_2}CHOH$); 1.30 (t, 3H, $COCH_2\mathbf{CH_3}$) and IR (film) ν cm^{-1}: 3467 (ν O–H), 1732 (ν C=O) 1214 e 1100 (ν C–O), 701 (ν C=C).

The microorganisms used in the present work were isolated from different fruits (*Hansenula* sp., *Dekera* sp., and *Kluyveromyces marxianus*) or purchased (*S. cerevisiae*). The microorganisms *Hansenula* sp., *Dekera* sp., and *K. marxianus* belong to the collection of the "Departamento de Engenharia Bioquímica, Escola de Química, UFRJ" and are freely available upon request.

The cells were allowed to grow during 48 h in a medium containing 1% glucose, 0.5% of yeast extract, 0.5% peptone, 0.1% $(NH_4)_2SO_4$ and 0.1% $MgSO_4$. After that period, the cells (3.8 g/l, dried weight) were harvested by centrifugation, resuspended in water and added to the reaction medium which contained 5% glucose, 0.1% $MgCl_2$, 1% ethanol and 0.5% of the substrate. Reactions were carried out under stirring (150 rpm) at 303 K during 24 h. Centrifugation, decantation and extraction with EtOAc were then done. The organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated under vacuum and analyzed [31,35].