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Microbial reduction of ethyl 2-oxo-4-phenylbutyrate. Searching for *R*-enantioselectivity. New access to the enalapril like ACE inhibitors

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Abstract—Herein, different microorganisms were tested in the enantioselective reduction of ethyl 2-oxo-4-phenylbutyrate in aqueous medium for the preparation of ethyl (*R*)-2-hydroxy-4-phenylbutyrate, a key intermediate in the production of angiotensin converting enzyme (ACE) inhibitors. The use of *Pichia angusta* led to the (*R*)-enantiomer in 81% ee.

1. Introduction

In our previously published results,[1] we described the use of *Saccharomyces cerevisiae*; *Dekera* sp.; *Hansenula* sp. and *Kluyveromyces marxianus* in the reduction of ethyl 2-oxo-4-phenylbutyrate, based on our expertise with these microorganisms.[2] However, although very high conversions were obtained with *S. cerevisiae*, *Dekera* sp. and *K. marxianus* (96%, 92% and 91%, respectively) high enantioselectivity was only observed for the (*S*)-enantiomer (95% ee *S. cerevisiae* and 92% *Dekera* sp.) and was poor for the (*R*)-enantiomer (32% ee *K. marxianus*). We envisaged that the production of ACE inhibitors of lisinopril/enalapril type (Fig. 1) would involve the (*R*)-enantiomer (via, e.g., the corresponding triflate), and as a result decided to investigate the use of other microorganisms.

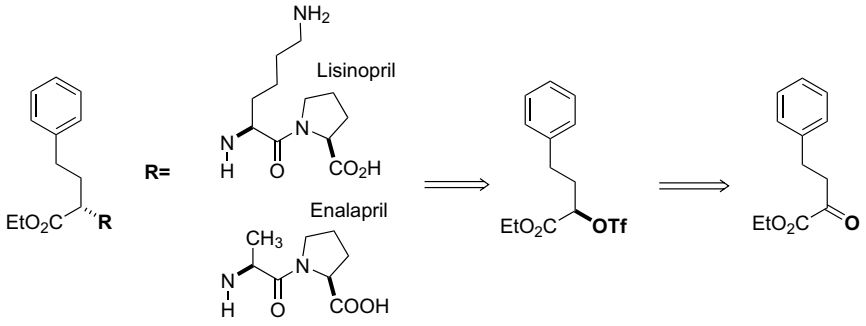

Figure 1. Process for the production of ACE inhibitors of lisinopril/enalapril type.

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Several collections have become available in Brazil in recent years and as *Candida guilliermondii*, *Aureobasidium pullulans*,[3] *Pichia pastoris*, *Pichia anomala*[4] and *Pichia angusta*[5] have not been so used in microbial reductions, these were chosen as potential tools for the desired transformation. It is worth noting, however, that this empirical search is based upon our previous experience that the microorganism contains both pro-*S* and pro-*R* enzymes and under particular circumstances, a particular activity is preponderant.[6] In addition, there are many literature examples of the use of several *Pichia* sp.[7] mediated enantioselective reductions.

2. Results and discussion

The results obtained: yield (*Y*) enantiomeric excess (ee) and the relative main configuration (*R* or *S*), obtained in the reduction of the ketoester by means of each strain, are summarized in Table 1. *Candida guilliermondii* and *Aureobasidium pullulans* showed an (*S*)-preference with high ee. For comparison purposes, other microorganisms including *K. marxianus* were used. *K. marxianus*, *P. pastoris* and *P. anomala* showed *R* preference with low ee. However, using *Pichia angusta* 100% conversion and 81% ee of ethyl (*R*)-2-hydroxy-4-phenylbutyrate were obtained (Scheme 1).

Table 1. Microbial reduction of 2-oxo-4-phenylbutyrate

Microorganism	Conversion (%)	% ee (configuration)
S. cerevisiae	99[a]	100 (*S*)
Dekera sp.	89	100 (*S*)
C. guilliermondii	20[a]	100 (*S*)
A. pullulans	92	60 (*S*)
K. marxianus	82	35 (*R*)
P. pastoris	95	35 (*R*)
P. anomala	94	35 (*R*)
P. angusta	100	81 (*R*)

[a] 80% Decomposition occurred.

Chadha et al.[8] reported that the enantioselective reduction of **1** to (*R*)-**2** could not be 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 ees (99%). However, in this process, a large amount of cells was needed, cell: substrate = 100:1, while a reaction time of 10 days was required.

S. cerevisiae preincubated in the presence of phenacyl chloride was used in the production of (*R*)-**2** to give excellent conversion (96%) and good ee (81%).[9] The disadvantages of this process are the presence of ether in the medium (ethyl ether–water 20:1), the large amount of cells required for the process (1 g cell/12 mg substrate) and the toxicity of the phenacyl chloride.

Since it was observed herein that by using *P. angusta*, 100% conversion and 81% ee of ethyl (*R*)-2-hydroxy-4-phenylbutyrate could be obtained and based on the hypothesis that two different enzymes should be present, we have decided to investigate the conversion *x* ee, that is, the kinetics of this transformation. Other experiments, including immobilization and other growing effects, are also currently under investigation.

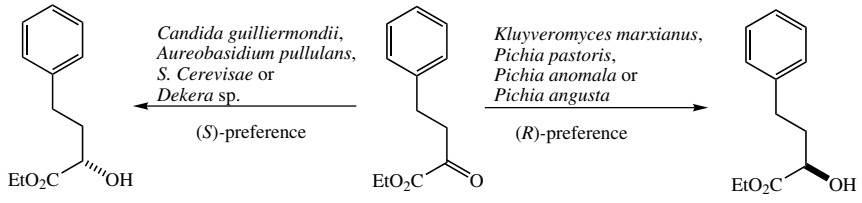
3. Conclusion

In conclusion, we have reported for the first time the preparation of ethyl (*R*)-2-hydroxy-4-phenylbutyrate via microbial reduction of ethyl 2-oxo-4-phenylbutyrate in aqueous medium using an attractive process in terms of potential industrial application and green chemistry.

4. Experimental

All microorganisms used were collected from different fruits (*Hansenula* sp., *Dekera* sp., and *K. marxianus*), and belong to the collection of the 'Departamento de Engenharia Bioquímica, Escola de Química, UFRJ', purchased (*S. cerevisiae*), belong to the collection of the 'Departamento de Bioquímica, Instituto de Química, UFRJ (*P. pastoris*)', or belong to the collection of 'Instituto Nacional de Controle de Qualidade em Saúde (INCQS), FIOCRUZ', and are freely available upon request.

Cells were allowed to grow for 48 h, under 150 rpm at 30 °C 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, they were harvested by centrifugation, re-suspended in water and used in the reaction. The cells (3.8 g/l, dried weight), which were centrifuged, were added to the reaction medium which contained 5% glucose, and 0.1% $MgCl_2$ in a final volume of 100 ml. After 30 min of the addition of the microorganisms, ethyl 2-oxo-4-phenylbutyrate (0.14% in volume) in 1% aqueous ethanol was added to the medium. The reaction was carried out for 24 h at 30 °C and 150 rpm. After 24 h, the medium was centrifuged to separate the cells and the liquid phase then extracted with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The ee and the absolute configuration of the



Scheme 1.

reaction were determined by chiral high-resolution chromatography, performed on 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-phenylbutyrate and *rac*-ethyl 2-oxo-4-phenylbutyrate were used as chromatographic standards. The elution order was *R* (50.3 min) followed by *S* (50.9 min). The reaction products were isolated and characterized by ^{1}H nuclear magnetic resonance (NMR) and infrared spectroscopy (IR). ^{1}H NMR (200 MHz/$CDCl_3$): δ 7.20–7.30 (m, 5H, Ar–*H*); 4.16–4.27 (m, 3H, *CH*(OH)CO and CH_2(OCO)CH_3); 2.73–2.82 (m, 2H, PhCH_2CH_2); 2.3 (s, OH); 1.93–2.09 (m, 2H, CH_2CH_2-CHOH); 1.30 (t, 3H, $COCH_2CH_3$) and IR (film) ν cm^{-1}: 3467 (ν O–H), 1732 (ν C=O) 1214 and 1100 (ν C–O), 701 (ν C=C).

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