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Microbial reduction of alpha-substituted-alpha-acetyl-gamma-butyrolactones

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Abstract

The microbial reduction of alpha-methyl, allyl and benzyl derivatives of alpha-acetyl-gamma-butyrolactones was carried out. It has been shown that different microorganisms, *Kluyveromyces marxianus*, *Hansenula* sp., *Geotrichum candidum* and *Aspergillus niger*, are capable to carry out the enantiospecific/selective reduction of these compounds. In the examples studied *K. marxianus* afforded the best results in terms of diastereo and enantioselectivity. It was found that depending on the particular microorganism used, a particular enantiomer can be obtained. Since these microorganisms are wild type, that is, are not engineered, they are robust and can be used to scale up these processes, which is going to be the next step of our study. It has been definitively proved that this microbial reduction occurs via the keto form, so clarifying the mechanism of these reactions firstly carried out with unsubstituted compounds.

Keywords: Yeasts; Mold; Reduction; Chiral

1. Introduction

In the past years our group has been involved in enzymatic [1–4] and microbial processes [5–9] suitable to be applied in organic synthesis. Recently, 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 [8,9]. In addition, it has been shown that microbial reduction of unsubstituted alpha-acetyl-gamma-butyrolactone proceeded well giving rise to the corresponding alcohols (Scheme 1). *K. marxianus*, in particular, is capable to reduce enantioselectively and specifically alpha-acetyl-butyrolactones in very high enantiomeric (ee) and diastereomeric excesses (de) [7]. Mechanistically, it was suggested that reduction does occur via the keto form. To further evidence this pathway and also to provide new compounds with potential medicinal interest [10] it was proposed to study the reduction of the title compounds, alpha-methyl, allyl and benzyl derivatives of alpha-acetyl-gamma-butyrolactones.

2. Experimental

Microorganisms, media, growth conditions and biotransformation with free cells: *Hansenula* sp., *Geotrichum candidum*, *K. marxianus*, *Dekera* sp. were collected from different fruits, and belong to the collection of the 'Departamento de Engenharia Bioquímica, Escola de Química, UFRJ' and are freely available upon request. Cells were allowed to grow for 48 h, under 150 rpm and 30 °C in a medium containing 1% glucose, 0.5% yeast extract, 0.5% peptone, 0.1%

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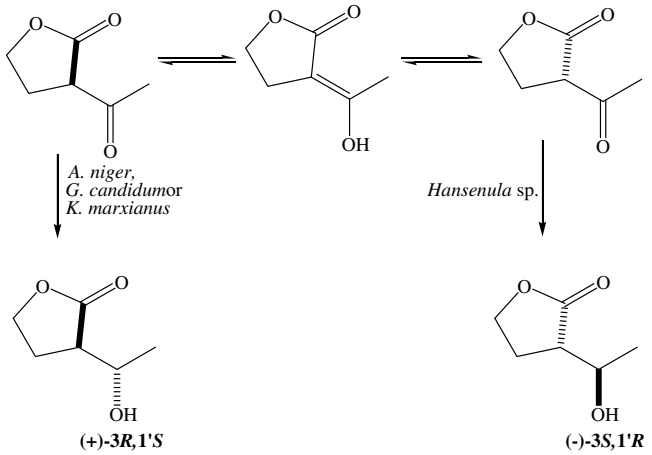


Scheme 1.

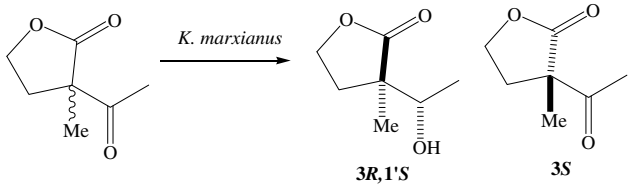
$(NH_4)_2SO_4$, and 0.1% $MgSO_4 \cdot 7H_2O$. After that period, they were harvested by centrifugation, re-suspended in water and used for the reaction. After centrifugation the cells (3.8 g/l, dried weight) were added to the reduction's medium containing: glucose (5%), $MgCl_2$ (0.1%) in a final volume of 100 ml. After 30 min of addition of the microorganisms, the substrate (0.5%) in 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 again to separate the cells and the liquid phase was extracted with ethyl acetate. The organic phase was dried (anhydrous Na_2SO_4), filtered, and concentrated under vacuum.

3. Results and discussion

Production of alpha-methyl, allyl and benzyl derivatives of alpha-acetyl-gamma-butyrolactones was carried out via known procedures [11].

We initially studied microbial reduction of the alpha-methyl derivative, scheme 2, using *K. marxianus*, *G. candidum* and *Hansenula* sp., due to the fact that it was previously observed that *K. marxianus* and *Hansenula* sp. afforded distinct enantiomers.

Observation of the chromatograms, Fig. 1, indicated that chemical reduction of the parent lactone (racemate, Fig. 1A, peaks a and b) resulted exclusively in the corresponding alcohols in very high diastereoisomeric excess (Fig. 1B). Microbial reduction with *K. marxianus* is very effective affording practically only one reaction products at 52% conversion (Table 1, Fig. 1C), that is, reaction is almost absolutely specific, and only one enantiomer of the parent lactone is consumed. In addition, the enantiose-



Scheme 2.

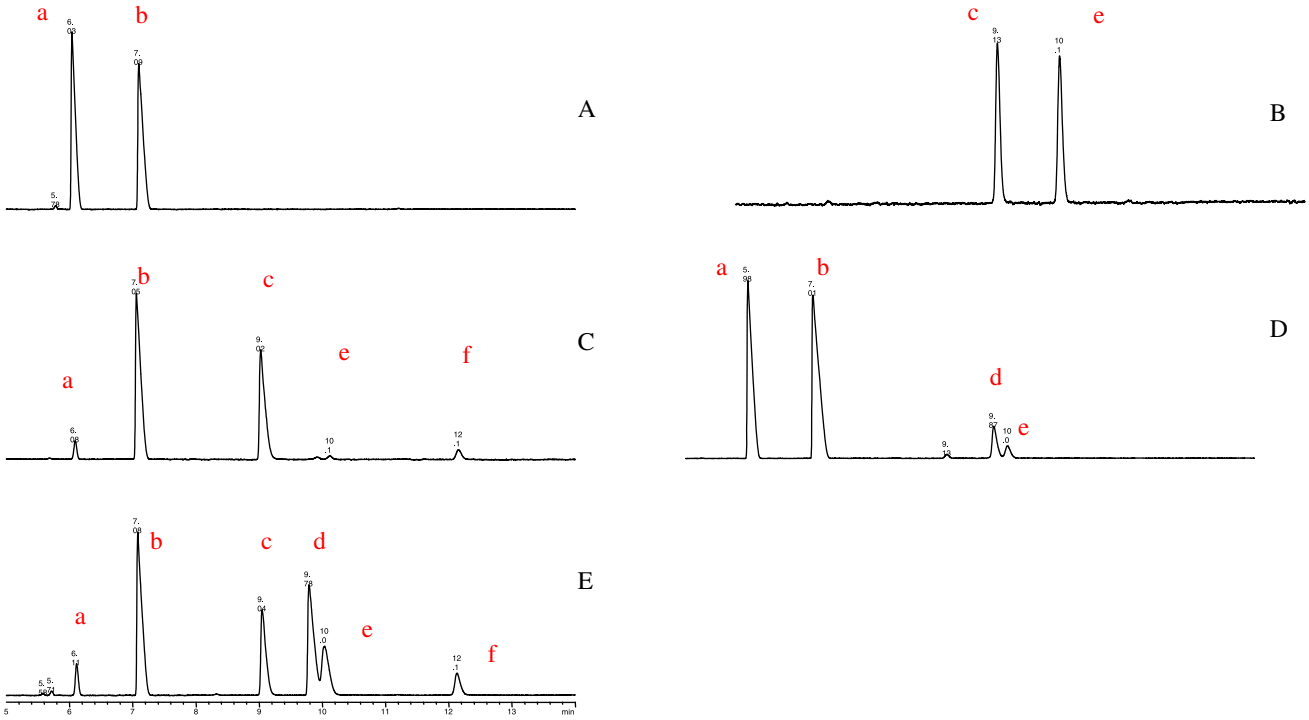


Fig. 1. A – methyl lactone; B – corresponding alcohols via NaBH4 reduction; C – reaction product *K. marxianus*; D – reaction product *Hansenula* sp.; E – reaction product *G. Candidum*. Column (25 m × 0.25 mm × 0.25 μm) BGB 175, 50% 2,3-diacetyl-6-*tert*-butyldimethylsilylated-γ-cyclodextrin dissolved in BGB-1701, 14% cyanopropylphenyl-, 86% methylpolysiloxane. 170 °C (3 min) to 180 °C (2 min) at 0.5 °C/min.