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Microbial reduction of α-acetyl-γ-butyrolactone

Joyce Benzaquem Ribeiro,[a] Livia Maria Andrade de Sousa,[a] Mariana da Volta Soares,[a] Maria da Conceição Klaus V. Ramos,[a] Francisco Radler de Aquino Neto,[a] Carlos Alberto Mansour Fraga,[b] Selma G. Ferreira Leite,[c] Yraima Cordeiro[d] and Octavio A. C. Antunes[a,*]

[a]*Instituto de Química, Universidade Federal do Rio de Janeiro, CT Bloco A, Cidade Universitária, Rio de Janeiro RJ 21949-900, Brazil*
[b]*Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio), Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, CCS Bloco B, Cidade Universitária, Rio de Janeiro RJ 21949-900, Brazil*
[c]*Escola de Química, Universidade Federal do Rio de Janeiro, CT Bloco E, Cidade Universitária, Rio de Janeiro RJ 21949-990, Brazil*
[d]*Instituto de Bioquimica Médica, CCS Bloco E Sala 42, Cidade Universitária, Rio de Janeiro RJ 21949-900, Brazil*

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Abstract—Isomers of α-1′-hydroxyethyl-γ-butyrolactone can be considered as potential GHB receptor ligands designed by the molecular hybridization of GLB **2** and GHV **4**. Using *Aspergillus niger*, *Geotrichum candidum*, and *Kluyveromyces marxianus*, it was possible to obtain (+)-(3*R*,1′*S*)-α-1′-hydroxyethyl-γ-butyrolactone in good to excellent conversions, diastereoisomeric and enantiomeric excesses. The corresponding enantiomer, (−)-(3*S*,1′*S*)-α-1′-hydroxyethyl-γ-butyrolactone was also produced in good conversion, and diastereoisomeric and enantiomeric excesses using *Hansenula* sp.
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1. Introduction

Gamma-hydroxybutyric acid **1** (GHB) is a putative neurotransmitter discovered about 30 years ago, which exerts several inhibitory actions over the CNS through the interaction with specific neuronal high-affinity receptors.[1,2] GHB and its chemical precursors, γ-butyrolactone **2** (GLB) and 1,4-butanediol,[3] **3**, have been implicated in many cases of suspected surreptitious administration, potentially for the purpose of increasing victim vulnerability to sexual assault.[4] Additionally, it was recently described that γ-hydroxyvaleric acid[5] **4** (GHV), a 4-methyl substituted analogue of GHB **1**, is also able to bind to the GHB receptor with a twofold lower affinity than the natural ligand, promoting a change in behavioral profile only in higher doses.[6] This distinct biological profile is a good indication that the affinity and intrinsic activity of new functionalized GHB or GBL analogues could be modulated by the introduction of the adequate functional groups (Fig. 1).

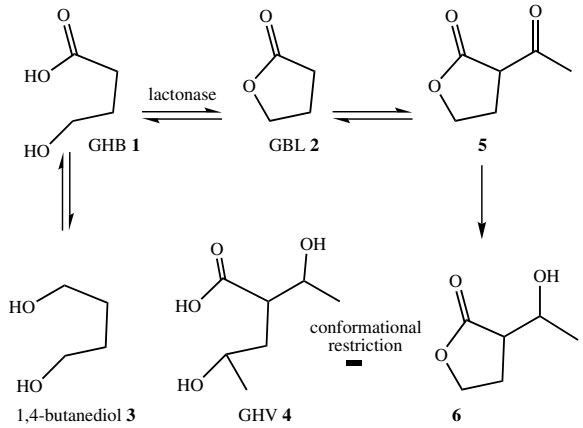

Figure 1. α-1′-Hydroxyethyl-γ-butyrolactone **5** as molecular hybridization of GLB **2** and GHV **4**.

In this context, our group have been interested in the preparation of new functionalized butyrolactone derivatives, obtained from commercial (±)-α-acetyl-γ-butyrolactone **5**, and its corresponding (±)-*anti* and (±)-*syn* hydroxy analogues **6a**–**d** (Figs. 1 and 2) as potential CNS ligands or as attractive intermediates to new bioactive compounds.[7]

* Corresponding author. Tel.: +55 21 25627818; fax: +55 21 25627559; e-mail: octavio@iq.ufrj.br

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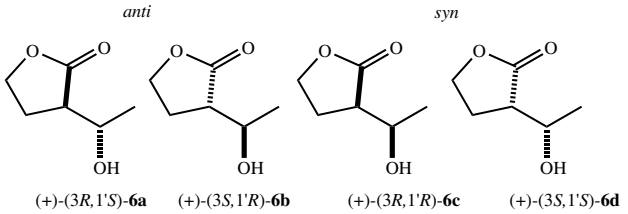


Figure 2. Cyclic chiral α-1′-hydroxyethyl-γ-butyrolactones.

Previous papers from our laboratory described the enantio-[8,9] and diastereoselective[7,10–12] preparation of cyclic β-hydroxy ester derivatives, exploring chemoselective reduction of the ketone carbonyl group of 2-alkyl-2-carboalkoxycyclopentanone, 2-alkyl-2-carboalkoxyhexanone, and α-acetyl-α-alkyl-γ-butyrolactone derivatives.

As previously stated,[14] α-acetyl-γ-butyrolactone **5** represents a useful intermediate in the synthesis of (chiral) triols by enantioselective reduction and subsequent reductive cleavage of the lactone ring, or by initial chemical reduction and subsequent resolution of the racemic alcohols with a lipase.

Fantin et al.[13] studied some microorganisms, which were able to carry out this reduction. In their study, almost all of the microorganisms preferentially gave (3*R*,1′*R*)-α-1′-hydroxyethyl-γ-butyrolactone. In this paper, it is claimed that this compound is obtained in quantitative yield, with excellent enantiomeric excess (100%), by reduction with *Saccharomyces cerevisiae* ML77 and *Yarrowia lipolytica* PFL9CE. Among the yeasts, only *S. cerevisiae* RM1 and RM74 afforded the (3*S*,1′*S*)-enantiomer although with poor ee (32–44%). On the other hand, the same *S. cerevisiae* RM1 produced the pure (3*R*,1′*S*)-enantiomer (39%, ee 100%). It is worth mentioning that only this microorganism gave the same distribution of products obtained with Baker's yeast by Takeshita et al.[14] It should also be noted that the mould strains were less diasteroselective than yeasts giving (3*R*,1′*R*) as well as (3*R*,1′*S*) in some cases, with excellent enantiomeric excesses (100%; *Mucor spirescens* and *Ceratocysts moniliformis*). Only *Trichoderma* sp. afforded the (3*S*,1′*R*)-enantiomer (29% yield, ee 50%).

Table 1. Conversion, enantiomeric excesses (ees) and diastereoisomeric excesses (des) for the enantiomeric bio-reduction of α-acetyl-γ-butyrolactone using free cells after growing (24 h reaction time)

Microorganism	Conversion (%)	ee (%)	de (%)
Hansenula sp.	100	100	90
Saccharomyces cerevisiae	0	—	—
Dekera sp.	4	17	100
Kluyveromyces marxianus	100	100	100
Candida utilis	9	27	58
Aspergillus niger	100	93	98
Geotrichum candidum	80	100	94

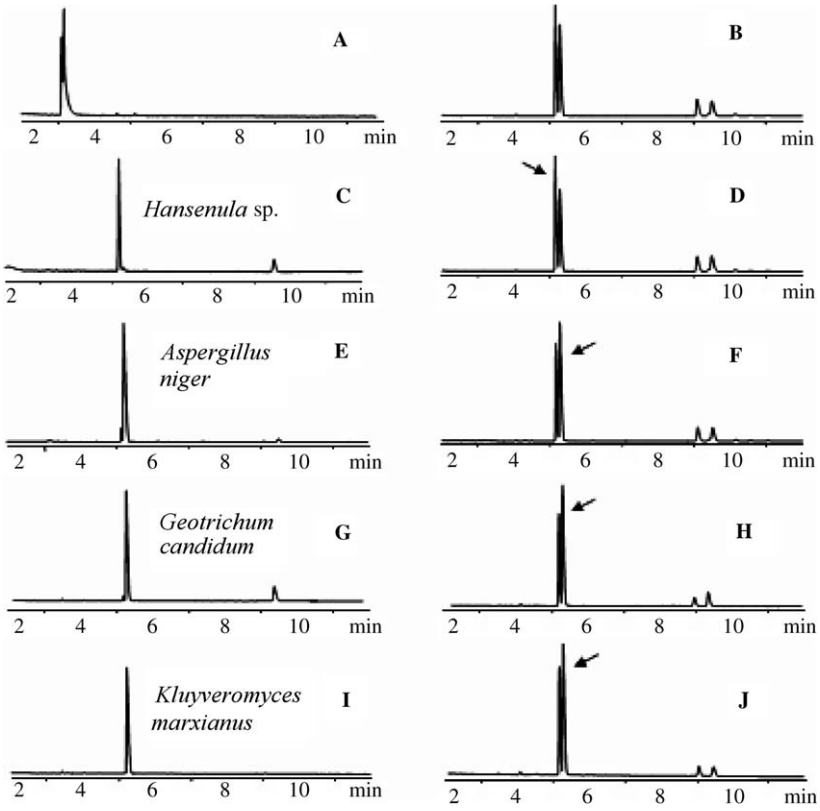


Figure 3. Chiral GC analysis of the microbiological reduction of 2-acetyl-γ-butyrolactone, on BGB-176 (2,3-di-methyl-6-*tert*-butyldimethylsilyl-β-cyclodextrin/polymethylsiloxane) capillary column (25 m × 0.25 μm × 0.25 μm), at 125 °C. (A) Typical chromatogram of racemic α-acetyl-γ-butyrolactones; (B) typical chromatogram of racemic *anti* and *syn* α-1′-hydroxyethyl-γ-butyrolactones; (C) *Hansenula* sp. reduction (LIV 12); (D) co-elution of (B) and (C); (E) *Aspergillus niger* reduction (LIV 10); (F) co-elution of (B) and (E); (G) *Geotrichum candidum* reduction (LIV 11); (H) co-elution of (B) and (G); (I) *Kluyveromyces marxianus* reduction (LIV 30); (J) co-elution of (B) and (I).

In our previously published experiments, it was observed that several microorganisms showed excellent performance in the reduction of α- and β-keto esters.[15–17] Considering the outstanding importance of the target compounds, we decided to reinvestigate the biocatalytic behavior of (±)-α-acetyl-γ-butyrolactone **5**.

Herein, we report the performance of six different yeasts, that is, *S. cerevisiae*, *Hansenula* sp., *Dekera* sp., *Kluyveromyces marxianus*, *Geotrichum candidum* and *Candida utilis*, and a mould strain, *Aspergillus niger*, in the preparation of the target chiral butyrolactone derivative **6**.

2. Results and discussion

Bio-reductions were carried out using free cells after growing. It is possible to verify that four microorganisms, out of the seven tested, *Hansenula* sp., *K. marxianus*, *A. niger* and *G. candidum*, showed excellent conversions and enantiomeric excesses over 90% for one of the four possible alcohols (Table 1).

It is noteworthy that with *K. marxianus* 100% conversion can be achieved within 7–9 h reaction time, while *Hansenula* sp. requires 20–22 h and *A. niger* 24 h. Within a 24 h reaction time, reasonable conversion was attained with *G. candidum*, although it was very low with *S. cerevisiae*, *Dekera* sp., and *C. utilis*. In view of the literature,[13,14] it was very surprising that no conversion was observed with the *S. cerevisiae* strain used in the present work, but since the other microorganisms gave good results, we decided not to investigate further with this or any other *S. cerevisiae* strains.

Using *K. marxianus*, *A. niger*, and *G. candidum*, chiral GC analysis (Fig. 3) indicated that the same alcohol was obtained, while the use of *Hansenula* sp. led to the corresponding enantiomer (see below). These results were confirmed by circular dichroism,[18] (Fig. 4) where the CD curves of the former three alcohols [*A. niger* (LIV 10), *G. candidum* **11** (LIV), and *K. marxianus* **30** (LIV)] showed an opposite signal to the latter [*Hansenula* sp. **12** (LIV)].

Co-elution experiments (Fig. 3) between the microbial reduction products with the racemic, *anti* and *syn*, alcohols, α-1′-hydroxyethyl-γ-butyrolactones, obtained by $NaBH_4$ reduction, indicated that with *A. niger* **10** (LIV) *G. candidum* **11** (LIV) and *K. marxianus* **30** (LIV) major products correspond to the second peak of the first eluted enantiomeric pair, while with *Hansenula* sp. **12** (LIV) the major product corresponds to the first peak. Therefore, chiral GC analysis supported the circular dichroism results and indicated that the microbial reduction with *A. niger*,

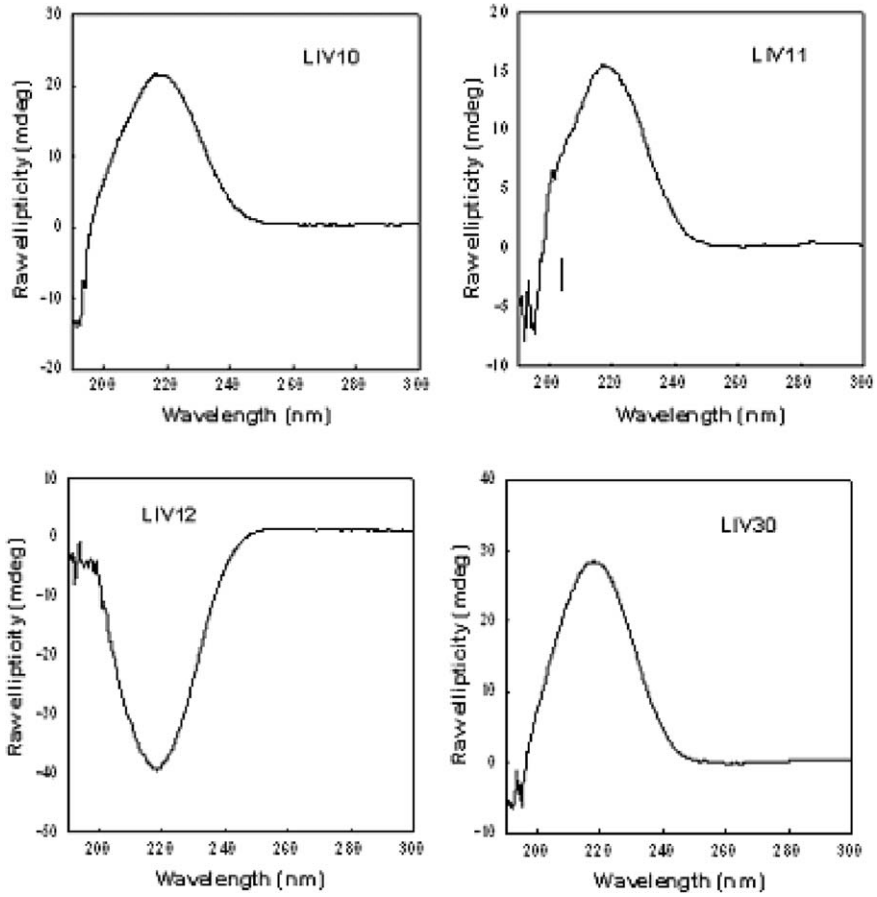


Figure 4. Circular dichroism curves corresponding to reaction products (MeOH solutions) using *A. niger* (LIV 10), *G. candidum* (LIV 11), *Hansenula* sp. (LIV 12), and *K. marxianus* (LIV 30).

G. candidum and *K. marxianus* resulted in the same enantiomeric alcohol, while the *Hansenula* sp. reduction resulted in the corresponding enantiomer.

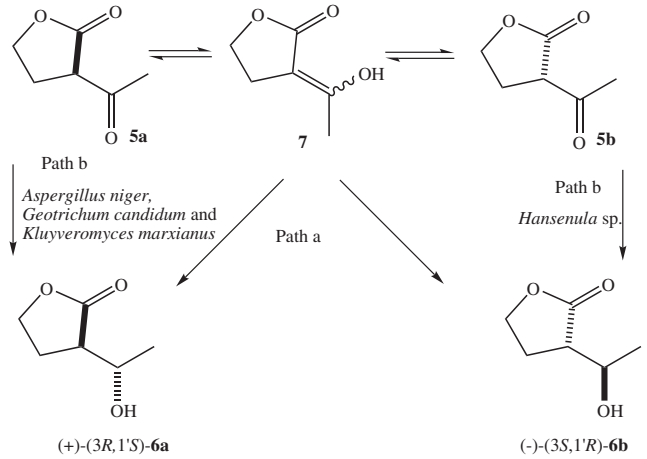
Herein it can be stated that the *anti*-diastereoisomer was produced using any of the four microorganisms in good conversion and enantioselectivity (and enantiospecificity), since, as reported by Teixeira et al., chemical reduction ($NaBH_4$) of α-acetyl-γ-butyrolactone yields the *anti*-diastereoisomer, which under our chiral GC conditions eluted as the first enantiomeric pair, as described by Ramos et al.[19]

Based on the work of Fantin et al.,[13] Takeshita et al.[14] and Trincone et al.,[20] who have isolated and analyzed the four isomers from this reduction, the enantioenriched mixtures are dextrorotatory [*A. niger* **10** (LIV), *G. candidum* **11** (LIV), *K. marxianus* (LIV 30)]; the (+)-(3*R*,1′*S*)-**6a** configuration can be attributed to the product of these bio-reductions, while the levorotatory product [*Hansenula* sp. **12** (LIV)] should have the (−)-(3*S*,1′*R*)-**6b** configuration.

Therefore, it was possible to develop microbiological accesses to either enantiopure *anti*-(+)-(3*R*,1′*S*)-**6a** or enantioenriched *anti*-(3*S*,1′*R*)-**6b**, which complements the results of Fantin et al.[13] who reported the production of the *syn* enantiomeric compounds.

Scilimati et al.[21–23] have also succeeded in carrying out microbial reductions of α-substituted-β-keto esters with excellent ees and des. In particular, corroborating the present results, *K. marxianus* showed an excellent performance in these reductions.[22,23]

To better understand this methodology, which allows the diastereo- and enantio-reduction of β-keto esters, and can be carried out under very high pressures such as under Noyori conditions, two possible pathways can be envisaged (Scheme 1).



Scheme 1.

In one pathway, the enol form would be attacked by the enzyme (path a) and hydride transfer, followed by rapid proton transfer. In the other route (path b), hydride transfer (from a NADH coenzyme)[24,25] should occur in any of the two keto forms. By considering the very high epimerization rate, via enol form **7**, and the slower microbial reduction rate, a very high enantio- and diastereoselective process would result.[26] In this case, the enzyme must exhibit a much greater enantiomeric ratio[27,28] for one enantiomer over the other (enantiospecificity) and than must show a very high degree of facial selectivity (enantioselectivity), which was the case. In this way, Scilimati et al.[21] and our group[29] showed that α,α-disubstituted-β-keto esters can be reduced with these microorganisms in very high enantiomeric and diastereoisomeric excesses, which practically exclude path a.

3. Conclusions

By using *A. niger*, *G. candidum*, and *K. marxianus*, it was possible to obtain (+)-(3*R*,1′*S*)-α-1′-hydroxyethyl-γ-butyrolactone **6a** in good to excellent yield, diastereoisomeric and enantiomeric excesses, while using *Hansenula* sp. (−)-(3*S*,1′*R*)-α-1′-hydroxyethyl-γ-butyrolactone **6b** was produced also in good conversion, diastereoisomeric, and enantiomeric excesses. These results, to the best of our knowledge, represent the first report on the chiral production of *anti* (+)-(3*R*,1′*S*)- and (−)-(3*S*,1′*R*)-α-1′-hydroxyethyl-γ-butyrolactone, and reinforce the microbial chiral alternative to the asymmetric reduction of α-substituted-β-keto esters.[26]

4. Experimental

CD analyses were performed using a Jasco J-715 spectropolarimeter (1.00 optical path) using MeOH solutions.

^1H and ^{13}C NMR spectra were determined in deuterochloroform containing ca. 1% tetramethylsilane as an internal standard with Brucker AC 200 and Varian V × R 300 spectrometers. Splitting patterns were as follows: s, singlet; d, doublet; t, triplet; q, quartet; qt, quintet; dd, double doublet; ddt, double double triplet; m, multiplet. The mass spectra (MS) were obtained on a GC–MS VG Micromass 12 at 70 eV.

Gas chromatography (GC) was performed in a Hewlett–Packard (Palo Alto, CA, USA) 5890 model series II, and chromatographic data were processed by a Chemstation Plus Family (Agilent Technologies, Palo Alto, CA, USA). GC separations were carried out in a BGB-176 (BGB Analytik AG, Zürich) (2,3-di-methyl-6-*tert*-butyldimethylsilyl-β-cyclodextrin/polymethylsiloxane) capillary column (25 m × 0.25 μm × 1.0 μl, 0.25 μm), at 125 °C. Carrier gas, hydrogen (1.7 ml/min); volume samples 1.0 μl; split injection 1/100; 260 °C; FID (280 °C).

^{13}C NMR data: major δ 20.7, 25.6, 45.7, 66.9, 68.1, and 179.6; minor δ 20.9, 22.0, 46.6, 65.4, 67.1, 178.5. ^1H NMR data: δ 1.28 (3H, d, $J = 6.0$ Hz), 2.06 (1H, dt, $J = 6.0$, 18.0 Hz), 2.36 (1H m), 2.80 (1H, dt, $J = 8.0$, 12.0 Hz), 3.55 (1H, br s), 3.95 (1H, dq, $J = 6.0$, 9.0 Hz), 4.25 (1H, ddd, $J = 7.0$, 9.0, 11.0 Hz), 4.40 (1H, dt,

$J = 2.0$, 9 Hz). GC data: 3.10 and 3.20 min (*anti*), 9.10 and 9.50 min (*syn*). Mass spectra (relative intensity): *anti* 129 (1), 115 (7), 86 (100), 85 (30), 71 (13), 68 (10), 58 (10), and 55 (13); *syn* 129 (1), 115 (7), 102 (4), 86 (100), 85 (28), 71 (20), 68 (8), and 45 (34).

Microorganisms, media, growth conditions and biotransformation with free cells: *Hansenula* sp., *G. 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% $(NH_4)_2SO_4$, 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. The cells (3.8 g/l, dried weight) that were centrifuged 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.

Acknowledgements

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