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On the microbial reduction of ethyl α-methylacetoacetate

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ABSTRACT

In the present work several microorganisms were screened in the reduction of methyl and ethyl acetoacetates. Good to excellent ees were obtained with up to quantitative substrate conversions. These microorganisms were also tested in the reduction of ethyl α-methylacetoacetate with good des and excellent ees. It was noticed that *Kluyveromyces marxianus* and *Trichoderma harzianum* were found to lead to products of opposite configuration with the use of methyl or ethyl acetoacetates, which is a very unusual finding.

1. Introduction

Enantiomerically pure hydroxy esters are very versatile building blocks for enantioselective synthesis of fine chemicals, including pharmaceuticals, flavours and fragrances.[1–3] Chemical and enzymatic asymmetric reduction of ketoesters have been the most extensively investigated routes for producing these chiral building blocks. Chemical approaches involve the use of chiral hydride reagents,[4] chiral transition metal catalysts such as BINAP-Ru complexes,[5] Lewis acid mediated borohydride reductions[6] and also oxygenation of chiral imide enolates.[7]

In recent years biocatalytic reduction of prochiral ketoesters has gained increasing importance in view of their application in asymmetric synthesis.[3,5,8] As now stated biocatalytic reductions are now 'First Choice'.[8c] These reactions can be performed in two different ways: either by using isolated, purified enzymes[9] or by using whole cells containing the enzymes of interest. There are some advantages to using whole cells as biocatalysts rather than purified enzymes.[10] One of them is that enzymatic reductions require the presence of expensive cofactors that, of course, can (and must) be recycled, while whole cells are an economic source of cofactors that can be easily regenerated during the reaction.[11,12]

3-Hydroxybutanoic acid and its esters are prominent members of this category and have been used as synthetic building blocks and intermediates for the syntheses of several classes of natural products and many therapeutic agents. This has been exploited extensively for the synthesis of compounds with diverse structural features, for example, macrolides, pheromones and antibiotics, among others.[13] Ethyl 2-methyl-3-hydroxybutanoate (2Me-3OHBu) is a useful starting material for the synthesis of various biologically active substances and liquid crystal compounds.[14] When ethyl 2-methyl 3-oxobutanoate (2Me-3OXOBu) is microbially reduced, four isomers of 2Me-3OHBu are expected. Since it is difficult to separate these isomers, several attempts[15] have been made to explore the diastereo- and enantioselective reduction of 2Me-3OXOBu in hope of finding optimized conditions for the predominant formation of a certain isomer.[16]

Nakamura et al.[15g] tested plant cell cultures in the bio-reduction of ethyl 2-methylacetoacetate and observed that conversion, enantio- and diastereoselectivity were dependent on the species used. Different diastereoisomers were obtained with high conversions: the enantiomeric excess of (2*S*,3*S*)-isomer was over 99% for the reduction with *Marchantia polymorpha*, while the *syn*-isomer (2*R*,3*S*) was obtained with *Glycine max* with 97% ee, 88% and 100% conversions, respectively. Although plant cell cultures provided good results, their maintenance and growth are in generally more difficult and more expensive than microbial cultures.[17]

Over the past several years, we have been investigating the reduction of various carbonyl compounds by enzymes recycling the cofactors with excellent results including some cascade systems which we investigated in kinetic terms.[9a–c] More recently we have been using microorganisms to obtain detailed knowledge of their reducing abilities.[3,8a,8b] In the present work we wish to describe the bio-reduction of ethyl 2-methylacetoacetate using different yeasts and fungi.

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2. Results and discussion

To carry out the present study we started using unsubstituted β-ketoesters, ethylacetoacetate and methylacetoacetate (Fig. 1) as model compounds to search for the best reaction conditions with 12 microorganisms. Then we moved to our substrate ethyl 2-methylacetoacetate (Fig. 2).

Bio-reductions were carried out using free cells after growing. Using ethyl acetoacetate as a substrate it was possible to verify that all microorganisms showed excellent conversions, over 90%, but the enantiomeric excesses were not so high. *Kluyveromyces marxianus*, *Aspergillus niger* and *Trichoderma harzianum* furnished the (*R*)-enantiomer, according to chiral GC data compared to a standard sample, while the other microorganisms provided the (*S*)-enantiomer, the one predicted by Prelog's rule (Table 1).

Good results (70–90% conversion and 90–98% ee) were also obtained in previous studies by other groups[18] in the reduction of ethyl acetoacetate to the corresponding (*S*)-hydroxy ester with baker's yeast (*Saccharomyces cerevisiae*), the most used microorganism in bio-reductions. However, larger amounts of the biocatalyst were frequently used.

Using methyl acetoacetate as a substrate it was possible to notice some differences compared with the reaction using ethyl acetoacetate. First, in general, conversions were lower and it was found that *K. marxianus* and *T. harzianum* lead to the product of opposite configuration than the one with the ethyl ester (Table 2). So, with this substrate, only *A. niger* was able to furnish the anti Prelog product, the (*R*)-enantiomer, based on the retention times of (*S*)- and (*R*)-ethyl hydroxy butyrate in chiral GC using the same column.

In the literature some examples exist in which chain length affects the enantioselectivity of the reaction, probably due to changes in the active site substrate–enzyme fitting and, consequently, influencing on the enzyme selectivity. In the reduction of 4-chloroacetoacetic esters by baker's yeast, for example, the octyl ester was reduced predominantly to the (*R*)-hydroxy ester, while ethyl ester was reduced predominantly to the (*S*)-isomer.[19] Otherwise only the (*S*)-hydroxy ester was obtained when ethyl and *t*-butyl acetoacetate were reduced in the presence of bryophyte cell cultures.[20] However, this subtly difference in chain length was not supposed to result in different enantiomer productions and this result must be further investigated with other substrates.

Having shown that the above microorganisms were able to carry out the reduction of the unsubstituted substrate, they were tested for the ability to reduce ethyl 2-methylacetoacetate (Table 3). Traditionally, *S. cerevisiae* catalyzes the reduction of ethyl 2-methylacetoacetate to the predominant production of the corresponding *syn* (2*R*,3*S*)-hydroxy ester.[15c] Baker's yeast selectively also reduces the racemic ester to *syn*-(2*R*,3*S*) although some *anti*-(2*S*,3*S*)-isomer is obtained.[15e] This information was used by us to propose the stereochemistry of the products. *K. marxianus*, *Candida* sp., *Hansenula* sp., *T. harzianum*, *M. ramannianus* and *A. niger* led to

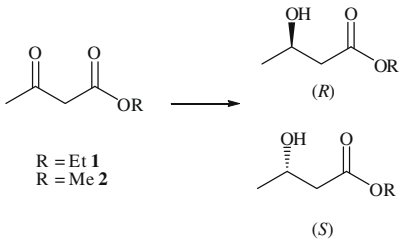


Figure 1. Microbial reduction of β-ketoesters **1** and **2**.

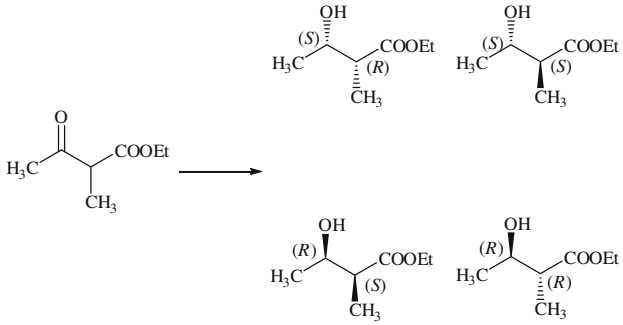


Figure 2. Microbial reduction of ethyl 2-methylacetoacetate.

Table 1
Microbial reduction of ethylacetoacetate

Microorganisms	Conversion (%)	ee (%)	Configuration
Kluyveromyces marxianus	97	67	(*R*)
Hansenula sp.	92	85	(*S*)
Candida sp.	100	20	(*S*)
Saccharomyces cerevisiae 80	90	81	(*S*)
Saccharomyces cerevisiae 60	92	84	(*S*)
Saccharomyces cerevisiae 40	93	85	(*S*)
Rhodotorula rubra	99	73	(*S*)
Rhodotorula minuta	96	67	(*S*)
Pichia sp.	99	66	(*S*)
Aspergillus niger	85	51	(*R*)
Mucor ramannianus	100	39	(*S*)
Trichoderma harzianum	100	19	(*R*)

Table 2
Microbial reduction of methylacetoacetate

Microorganisms	Conversion (%)	ee (%)	Configuration
Kluyveromyces marxianus	84	5	(*S*)
Hansenula sp.	79	88	(*S*)
Candida sp.	98	76	(*S*)
Saccharomyces cerevisiae 80	91	84	(*S*)
Saccharomyces cerevisiae 60	47	82	(*S*)
Saccharomyces cerevisiae 40	63	79	(*S*)
Rhodotorula rubra	87	37	(*S*)
Rhodotorula minuta	90	35	(*S*)
Pichia sp.	96	80	(*S*)
Aspergillus niger	90	45	(*R*)
Mucor ramannianus	100	64	(*S*)
Trichoderma harzianum	100	24	(*S*)

an excess of the *anti*-(2*S*,3*S*)-isomer, while the other microorganisms provided the *syn*-(2*R*,3*S*)-hydroxy ester. The configuration of products obtained in presence of *T. harzianum*, *M. ramannianus* and *A. niger* was compatible with that observed by Iwamoto et al.,[16] who obtained predominantly the *anti*-(2*S*,3*S*)-isomer when filamentous fungi were used as biocatalysts.

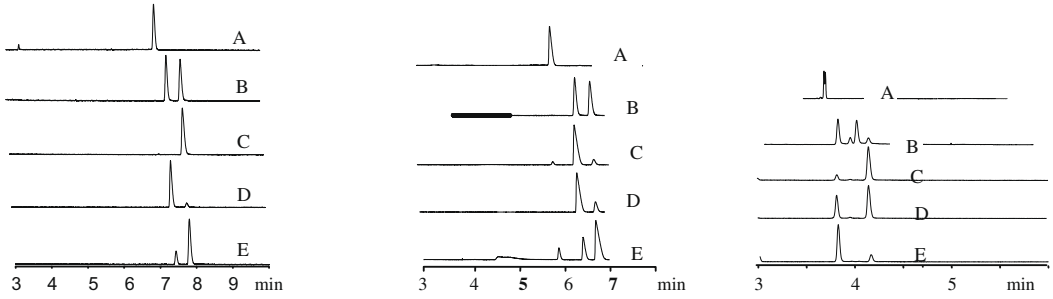
There have been a few reports on fungi and yeasts other than baker's yeast that provided *anti* ethyl (2*S*,3*S*)-2-methyl 3-hydroxybutanoate. Bingfeng et al.[15d] reported a 75/25 ratio of this isomer using *Geotrichum* sp. *Penicillium purpurogenum* also lead to a high excess of *anti*-(2*S*,3*S*)-hydroxy ester, that is, 97/3.[16] In our study, the maximum *anti-syn* ratio with the above configurations was 7.6/1 obtained with *A. niger*. *Candida* sp. also lead to a good excess of *anti*-(2*S*,3*S*)-hydroxy ester (7.2/1) as shown.

Of course the results described in the present work in terms of ees and des are not so good compared to those previously published by us in α-substituted-β-keto derivatives. However, we previously investigated the reduction of a system (lactone)[21] which has an inherent rigidity. In the present work it was demonstrated

Table 3
Microbial reduction of ethyl 2-methylacetoacetate

Microorganisms	Conversion	ee (2*R*,3*S*) (*syn*)	ee (2*S*,3*S*) (*anti*)	(2*R*,3*S*) (*syn*)/(2*S*,3*S*) (*anti*) ratio	de[a]
Kluyveromyces marxianus	99	14	100	1/4.9	66
Candida sp.	93	59	100	1/7.2	76
Hansenula sp.	40	78	100	1/1.5	20
Pichia sp.	73	78	100	1.7/1	27
Saccharomyces cerevisiae 60	10	83	100	2.8/1	47
Saccharomyces cerevisiae 40	24	73	100	2.2/1	37
Saccharomyces cerevisiae 80	15	80	100	2.1/1	68
Rhodotorula minuta	89	79	100	4/1	61
Rhodotorula rubra	93	79	100	3.9/1	60
Trichoderma harzianum	100	76	100	1/1.6	24
Mucor ramannianus	100	77	100	1/1.8	29
Aspergillus níger	87	50	100	1/7.6	77

[a] Comparison between the 2 *syn* isomers and the 2 *anti* isomers. de = *syn* − *anti*/*syn* + *anti*.



Ethyl acetoacetate. **A**. substrate; **B**. racemate; **C.** R-enantiomer; **D.** *R.rubra*; **E.** *K.marxianus*. BGB 176 ; isoterma75°C (10min).

Methyl acetoacetate. **A**. substrate; **B**. racemate; **C**. *Pichia* sp.; **D.** *M. ramannianus*; **E**. *A.niger*. BGB 176 isoterma 70ºC

Ethyl 2-methylacetoacetate: **A**. substrate; **B**. racemate; **C**. *Candida* sp.; **D**.*T.harzianun*; **E**.*R.rubra*. BGB-176 100°C (7min)

Figure 3. Typical chromatograms showing the selectivities achieved.

that good results can be obtained even with flexible substrates, so confirming Scilimati et al. previous findings.[22]

Examples of chromatograms obtained are shown in Figure 3.

3. Conclusions

Twelve different microorganisms were used in the reduction of ethyl 2-methylacetoacetate. Diastereoisomer ratios were good and 100% *anti* enantioselectivity was achieved. In addition, with the model compounds, ethyl and methyl acetoacetates very high ees were obtained and an unexpected stereochemical reversion was noticed changing the ester from methyl to ethyl using *K. marxianus* and *T. harzianum*.

References

1. Mori, K. *Tetrahedron* **1989**, *45*, 3233–3298.
2. Sheldon, R. A. *Chirotechnology*; Dekker: New York, 1993.
3. Ribeiro, J. B.; Ramos, M. C. K. V.; Aquino Neto, F. R.; Leite, S. G. F.; Antunes, O. A. C. *J. Mol. Catal. B* **2003**, 24–25. 121–124.
4. Soai, K.; Takashi, Y.; Hitoshi, H.; Oyamada, H. *J. Chem. Soc., Chem. Commun.* **1985**, 138–139.
5. Noyori, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 2008–2022.
6. Alessandrini, S.; Bartoli, G.; Bellucci, M. C.; Dalpozzo, R.; Malavolta, M.; Marcantoni, E.; Sambri, L. *J. Org. Chem.* **1999**, *64*, 1986.
7. (a) Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346–4348; (b) Ribeiro, L. P.; Antunes, O. A. C.; Bergter, L.; Costa, P. R. R. *Tetrahedron: Asymmetry* **1994**, *5*, 1873–1874.
8. (a) Lacerda, P. S. B.; Ribeiro, J. B.; Leite, S. G. F.; Coelho, R. B.; Lima, E. L. S.; Antunes, O. A. C. *Biochem. Eng. J.* **2006**, *28*, 3, 299–302; (b) Ribeiro, J. B.; Ramos, M. C. K. V.; Aquino Neto, F. R.; Leite, S. G. F.; Antunes, O. A. C. *Catal. Commun.* **2005**, *6*, 131–133; (c) De Wildeman, S. M. A.; Sonke, T.; Shoemaker, H. E.; May, O. *Acc. Chem. Res.* **2007**, *40*, 1260.
9. (a) Gonçalves, L. P. B.; Antunes, O. A. C.; Oestreicher, E. G. *Org. Process Res. Dev.* **2006**, *10*, 673–677; (b) Gonçalves, L. P. B.; Antunes, O. A. C.; Pinto, G. F.; Oestreicher, E. G. *J. Fluorine Chem.* **2003**, *124*, 219; (c) Gonçalves, L. P. B.; Pinto, G. F.; Antunes, O. A. C.; Oestreicher, E. G. *Tetrahedron: Asymmetry* **2000**, *11*, 1465; (d) Gonçalves, L. P. B.; Pinto, G. F.; Antunes, O. A. C.; Oestreicher, E. G. *J. Mol. Catal. B* **1998**, *4*, 67; (e) Gonçalves, L. P. B.; Pinto, G. F.; Antunes, O. A. C.; Oestreicher, E. G. *Tetrahedron: Asymmetry* **1996**, *7*, 1237; (f) Adam, W.; Lazarus, M.; Boss, B.; Saha-Möller, CR.; Humpf, H. U.; Schreier, P. *J. Org. Chem.* **1997**, *62*, 7841–7843; (g) Sordedrager, M. J.; van Ranjwijk, F.; Huisman, G. W.; Sheldon, R. A. *Adv. Synth. Catal.* **2008**, *350*, 2322; (f) Perrone, M. G.; Santandrea, E.; Scilimati, A.; Syldatk, C. *Adv. Synth. Catal.* **2007**, *349*, 1111.
10. Wong, C. H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Elsevier Science Ltd: Oxford, 1994. pp 131–194.
11. Kitayama, T. *Tetrahedron: Asymmetry* **1997**, *22*, 3765–3774.
12. Panke, S.; Wubbolts, M. G.; Schmid, A.; Withold, B. *Biotechnol. Bioeng.* **2000**, *69*, 91–100.
13. Salvi, N. A.; Chattopadhyay, S. *Tetrahedron: Asymmetry* **2004**, *15*, 3397–3400.
14. Matsumura, K.; Kawada, M.; Sugihara, Y. *Japan Kokai Tokkyo Koho* **1990**, 2–138235.
15. (a) Miya, K.; Kawada, M.; Sugiyama, Y. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 95–98; (b) Isihara, K.; Iwai, K.; Yamaguchi, H.; Kakajima, N.; Nakamura, K.; Ohshima, T. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 1896–1898; (c) Nakamura, K.; Myiai, T.; Nozaki, K.; Ushio, K.; Oka, S.; Ohno, A. *Tetrahedron Lett.* **1986**, *27*, 3155–3156; (d) Bingfeng, P.; Jianxing, G.; Zuyi, L.; Ward, O. P. *Enzym. Microb. Technol.* **1995**, *17*, 853–855; (e) Kuramoto, T.; Iwamoto, K.; Izumi, M.; Kirihata, M.; Yoshizako, F. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 598–601; (f) Buisson, D.; Sanner, C.; Larcheveque, M.; Azerad, R. *Tetrahedron Lett.* **1987**, *28*, 3939–3940; (g) Nakamura, K.; Myioshi, H.; Sugiyama, Y.; Hamada, H. *Phytochemistry* **1995**, *40*, 1419–1420.
16. Iwamoto, K.; Kuramoto, T.; Izumi, M.; Kirihata, M.; Dohmaru, T.; Yoshizako, F. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 194–197.
17. (a) Assuncao, J. C. C.; Machado, L. L.; Lemos, T. L. G.; Cordell, G. A.; Monte, F. J. Q. *J. Mol. Catal. B* **2008**, *52–53*, 194–198; (b) Villa, R.; Molinari, J. *Nat. Prod.* **2008**, *71*, 693–696.
18. (a) Pereira, R. S. *Quim. Nova* **1995**, *18*, 452–459; (b) Molinari, F.; Gandolfi, R.; Villa, R.; Occhiato, E. G. *Tetrahedron: Asymmetry* **1999**, *10*, 3515–3520; (c) Nakamura, K.; Higaki, M.; Ushio, K.; Oka, S.; Ohno, A. *Tetrahedron Lett.* **1985**, *26*, 4213–4216.
19. Shieh, W.; Gopalan, A. S.; Sih, C. J. *J. Am. Chem. Soc.* **1985**, *107*, 2993–2994.
20. Speichner, A.; Roeser, H.; Heisel, R. *J. Mol. Catal. B* **2003**, *22*, 71–77.
21. Ribeiro, J. B.; Sousa, L. M. A.; Soares, M. V.; Ramos, M. C. K. V.; de Aquino Neto, F. R.; Fraga, C. A. M.; Leite, S. G. F.; Cordeiro, Y.; Antunes, O. A. C. *Tetrahedron: Asymmetry* **2006**, *17*, 984.
22. Perrone, M. G.; Santandrea, E.; Scilimati, A.; Tortorella, V.; Capitelli, F.; Bertolasi, V. *Tetrahedron: Asymmetry* **2004**, *15*, 3501.