Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Tetrahedron: *Asymmetry* 15 (2004) 3397–3400
Tetrahedron: *Asymmetry*

Rhizopus arrhizus mediated asymmetric reduction of alkyl 3-oxobutanoates

Neeta A. Salvi and Subrata Chattopadhyay*

Bio-Organic Division BARC, Mumbai 400 085, India

Received 14 July 2004; accepted 24 September 2004

Abstract—Alkyl 3-oxobutanoates (alkyl: methyl, ethyl, allyl, isobutyl, *t*-butyl) were reduced enantioselectively to the corresponding (*S*)-alcohols by the fungus *Rhizopus arrhizus* and other *Rhizopus* sp. The best result obtained was with *t*-butyl 3-oxobutanoate, which was reduced by *R. arrhizus* with 99% enantiomeric excess and ~68% isolated yield.
© 2004 Published by Elsevier Ltd.

1. Introduction

The synthesis of chiral compounds with the aid of microorganisms is a useful method in organic synthesis with the reduction of ketones to chiral alcohols being a typical example. In this connection, the reduction of β-keto esters to β-hydroxy esters is extremely useful as the products are important building blocks for the synthesis of a large number of bioactive compounds, intermediates and chiral auxillaries.[1a–f] Chiral β-hydroxy esters can be obtained in enantiomeric forms by chemical asymmetric reducing agents.[2a,2b] In comparison, the enzymatic protocol offers the advantage of better enantioselectivity, but their widespread use for asymmetric reduction is restricted due to the prohibitive costs of commercial enzymes and the required co-factors. In this regard, the use of a whole cell system is emerging as an economical and eco-friendly alternative. 3-Hydroxybutanoic acid and its esters are prominent members in 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. In particular its ethyl ester has been exploited extensively for the synthesis of compounds with diverse structural features viz. macrolides, pheromones, antibiotics, etc.[3a–f] Amongst the enzymatic methods, baker's yeast is extensively used for the asymmetric reduction of ethyl 3-oxobutanoate,[4] while other microbial methods have also been found inadequate.[5] Although the baker's yeast-mediated method often proceeds with a poor chemical yield and varying enantioselectivity (70–97%),[4b] better results have been reported using non-fermenting yeast in an organic solvent.[6] More recently, we have developed an efficient method for the enantiomeric synthesis of both the enantiomers of 3-hydroxybutanoates via a lipase-catalyzed esterification protocol.[7]

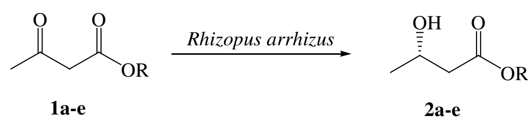
During our work on microbial transformations,[8a–d] we found that the fungus *Rhizopus arrhizus* can efficiently carry out the asymmetric reduction of a wide variety of ketones. Herein, we report the potential of the fungus for the enantiomeric synthesis of the 3-hydroxybutanoates.

2. Results and discussion

One of the prime requirements of a microbial transformation is the optimization of the reaction parameters such as substrate structure, reaction conditions etc. Hence, for the present study, we chose a series of alkyl 3-oxoalkanoates **1a**–**e** differing in the alkyl chain length (R) of the alcohol moiety and studied their bio-reduction with *R. arrhizus* (Scheme 1). The reactions with all the substrates were carried out for 4 and 8 days using almost the same substrate concentrations. After the usual work-up, product alcohols **2a**–**e** were isolated from the respective substrates, purified by preparative TLC and characterized by IR and ^{1}H NMR spectra. The enantiomeric excesses (ees) of the products were determined from the ^{1}H NMR spectra of the corresponding MTPA esters,[9] while their configurations were

* Corresponding author. Fax: +91 22 25505151; e-mail: schatt@apsara.barc.ernet.in

0957-4166/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.tetasy.2004.09.026



a: R = CH_3; **b**: R = Et; **c**: R = Allyl; **d**: R = *iso*-Bu; **e**: R = *t*-Bu

Scheme 1.

assessed by comparison of their specific rotation values with those reported.[10d,g,6] The results are summarized in Table 1.

Table 1. *Rhizopus arrhizus* mediated reduction of alkyl 3-oxobutyrates **1a**–**e**[a]

Entry	Substrate	Incubation time (d)	Yield[b] (%)	% Ee[c]
1	**1a**	4	15	70
2	**1a**	8	22	71
3	**1b**	4	37	74
4	**1b**	8	39	89
5	**1c**	4	17	80
6	**1c**	8	26	89
7	**1d**	4	51	89
8	**1d**	8	59	90
9	**1e**	4	71	94
10	**1e**	8	65	94

[a] The reactions were carried out 27 ± 2 °C by adding the substrates (~0.1 g) in EtOH (1.5 mL) to the microbial culture (150 mL) and shaking (90–95 rpm) the mixture.
[b] Isolated yield.
[c] From ^{1}H NMR spectra of the respective MTPA esters.

Irrespective of the alkyl chain length (R) of the alcohol moieties, all esters **1a**–**e**, were reduced without any noticeable decarboxylation furnishing the (*S*)-hydroxy esters **2a**–**e** with good ees. Elongation of the alkyl chain (methyl to *t*-butyl) in the alkoxy group led to a gradual increase in the yields and enantioselectivities of the reduced products. For the lower homologous **1a**–**c** containing the linear chain alcohols, the yields and % ees of the products were poor. However, increasing the chain length of R-group improved the yields and even the % ees of the products. The apparent lesser yield of **2c** was because of the recovery of significant amounts of the unreacted substrates, which were not accounted in the data shown in Table 1. With **1a** and **1b** also, the amounts of the recovered substrates could partly explain the poor yields. In these cases, the substrates and the resultant products could not be completely extracted from the reaction mixture, in view of their lower hydrophobicity. The reactivities of these lower homologues **1a**–**c** were poor and even after 8 days of incubation, the products were only obtained in modest yields. This, however, could be improved significantly by using the substrates **1d** and **1e**, which possess sterically bulky branched R-groups.

Better enantioselectivity with these substrates was anticipated, as the enzyme could easily differentiate between the small and large groups flanking the carbonyl function. The higher yields of **2d** and **2e**, however, may be partly due to the their higher hydrophobicity, which assisted better recovery during isolation. In these cases, the incubation period did not matter much, both in terms of yields and % ees. The *t*-butyl ester **1e** was the best substrate furnishing (*S*)-**2e** with 94% ee and 65–70% yield.

Table 2. Asymmetric reduction of *t*-butyl acetoacetate **1e** with different *Rhizopus* sp.[a]

Entry	Organism	Yield[b] (%)	% Ee[c]
1	*Rhizopus arrhizus*	68	99
2	*Rhizopus arrhizus* (NCIM 877)	67	91
3	*Rhizopus arrhizus* (NCIM 878)	67	91
4	*Rhizopus arrhizus* (NCIM 879)	69	91
5	*Rhizopus arrhizus* (NCIM 997)	62	91
6	*Rhizopus oryzae* (NCIM 1009)	49	93
7	*Rhizopus nivius* (NCIM 958)	75	96
8	*Rhizopus nivius* (NCIM 959)	86	93

[a] The reactions were carried out at 27 ± 2 °C by adding the substrates (~0.1 g) in EtOH (1.5 mL) to different *Rhizopus* cultures (150 mL) and shaking (90–95 rpm) the mixture for 2 days.
[b] Isolated yield.
[c] From ^{1}H NMR spectrum of the MTPA ester of **2e** obtained from different experiments.

Although the above results, especially with **1e**, were encouraging, further improvement was warranted for the application of the protocol as a synthetic route. A reduced reaction time with concurrent increases in yields and ees would be an ideal combination towards this objective. In principle, this can be accomplished by a subtle change in the substrate or screening an alternate microorganism. Very recently, we were able to improve the enantioselectivity of a *Rhizopus* mediated hydrolysis protocol by proper choice of a microbial strain.[8d] Hence, a similar strategy was extended for the present work. For this, the microbial reduction of **1e** was carried out using various *Rhizopus* species for 2 days. All the microorganisms transformed **1e** to (*S*)-**2e**, albeit in different yields and enantiomeric excesses (Table 2). With the exception of *Rhizopus oryzae* NCIM 1009 (Table 2, entry 6), the reactions with other microorganisms proceeded with better enantioselectivities (90–99% ee) furnishing the product in 62–85% isolated yields. The two-day incubation protocol with *R. arrhizus* was found to be the best furnishing (*S*)-**2e** in appreciable yield (~68%) and excellent enantiomeric purity (99%). The fungi *Rhizopus nivius* NCIM 958 and 959 were also very efficient providing (*S*)-**2e** in excellent yields (75% and 86%, respectively), and enantiomeric excesses (96% and 93%, respectively).

3. Conclusion

In view of its simplicity, the baker's yeast-mediated reduction of alkyl 3-oxobutanoates is the most preferred method for the synthesis of the corresponding (*S*)-3-hydroxybutanoates. However, the ees of the products are known to be strongly dependent on the reduction conditions. In view of this, several modifications in the experimental conditions have been suggested to improve the results. Different protocols, such as stirring condi-

tions,[10a] and pretreatment of yeast with 5% ethanol,[10b] have been employed, especially with ethyl 3-oxobutanoate. For a large scale preparation, a continuous feed of the substrate and nutrient was prescribed, as the % ee of the product was found to be dependent on the substrate concentration.[5a] A related paper reported better results (94–96% ee) by carrying out the reaction in tap water and terminating it after 4 h.[10c,d]

The problem of enantioselectivity in the baker's yeast reduction is ascribed to the presence of multiple reductases that show complementary enantiopreferences. Innovative approaches involving selective suppression of some of these enzymes with allyl alcohol and by immobilization on polyurethane (for anticipated change in configuration) proved counter productive, as both these led to the destruction of the *pro*-(*S*) reductases.[10e,f]

In contrast, the fungus *Rhizopus arrhizus* has been found to be an excellent bioreduction system for the enantiomeric synthesis of 3-hydroxybutanoates. By judicious choice of substrate, microorganism and incubation period, it is possible to obtain enantiomerically pure 3-hydroxybutanoates in very high yields and ees with high reproducibility. It is worth noting that the baker's yeast reduction in aqueous medium often furnishes low yields of the products due to the difficulty in their extraction. The results obtained herein compare very well, with one recent report,[6] even surpassing the reported results in some cases.

4. Experimental

4.1. General

Substrates **1a**–**e** (Aldrich, Fluka and Lancaster) were used as received. (*R*)-(+)-α-Methoxy-α-trifluoromethylphenylacetic acid (MTPA) was supplied by Aldrich. The *Rhizopus* species were received from the National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India. Fungus, from potato/dextrose/agar slants were grown[8d] on sterilized modified Czepek Dox medium (150 mL) in 500 mL Erlenmeyer flasks at 27 ± 2 °C with shaking (150 rpm). The IR spectra were recorded as films on a Nicolet FT-IR model Impact 410 spectrometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ with a Bruker AC-200 (200 MHz) spectrometer. The optical rotations were recorded with a Jasco 360 DIP digital polarimeter.

4.2. General procedure for microbial reductions

Substrates (0.1 g) in EtOH (1.5 mL) were added to the 72 h grown culture in cotton plugged flasks and incubated on a rotary shaker (90–95 rpm) at room temperature for the period specified in Tables 1 and 2. At the end of the reaction, the mycelial mass was removed, washed thoroughly with water and squeezed. The aqueous washings were mixed with the aqueous filtrate and extracted with $CHCl_3$ (3 × 50 mL). The organic extract was washed with H_2O, dried and concentrated in vacuo to obtain a residue, which was subjected to preparative TLC (silica gel, 10% EtOAc/hexane) to furnish the product alcohols.

4.3. Typical procedure for the synthesis of (*S*)-*t*-butyl-3-hydroxybutanoate 2e via microbial reduction

In five cotton plugged Erlenmeyer flasks each containing the 72 h grown *R. arrhizus* culture (150 mL) was added **1e** (0.570 g, 3.6 mmol) in EtOH (7.5 mL) in equal amounts. The mixtures were incubated on a rotary shaker (90–95 rpm) at room temperature for 2 days. The mycelial mass was removed, washed thoroughly with water and squeezed. The aqueous washings were mixed with the aqueous filtrate and extracted with $CHCl_3$ (3 × 50 mL). The organic extract was washed with H_2O, dried and concentrated in vacuo to obtain a residue, which was subjected to preparative TLC (silica gel, 10% EtOAc/hexane, visualization by staining with I_2 vapour) to furnish **2e** (0.390 g, 68%).

4.4. Methyl 3-hydroxybutanoate 2a

$[\alpha]_D^{22} = +24.2$ (*c* 0.82, $CHCl_3$) (71% ee), lit.[10g] $[\alpha]_D = +33.3$ (*c* 1.2, $CHCl_3$); IR: 3423, 2977, 2933, 1728 cm^{-1}; ^{1}H NMR: δ 1.18 (d, *J* = 6.2 Hz, 3H), 2.32–2.41 (m, 2H), 3.1 (br s, 1H), 3.67 (s, 3H), 4.07–4.20 (m, 1H).

4.5. Ethyl 3-hydroxybutanoate 2b

$[\alpha]_D^{22} = +30.0$ (c 0.77, $CHCl_3$) (89% ee), lit.[10d] $[\alpha]_D^{23.5} = +43.9$ (*c* 1.35, $CHCl_3$); IR: 3432, 2977, 2933, 1728 cm^{-1}; ^{1}H NMR: δ 1.17 (d, *J* = 7.0 Hz, 3H), 1.23 (t, *J* = 6.2 Hz, 3H), 2.22–2.48 (m, 2H), 3.2 (br s, 1H), 4.06–4.22 (m, 3H).

4.6. 2′-Propenyl 3-hydroxybutanoate 2c

$[\alpha]_D^{22} = +28.2$ (*c* 0.97, $CHCl_3$) (89% ee); IR: 3433, 2986, 2933, 1728 cm^{-1}; ^{1}H NMR: δ 1.16 (d, *J* = 7.0 Hz, 3H), 2.32–2.49 (m, 2H), 3.2 (br s, 1H), 4.08–4.16 (m, 1H), 4.54 (dd, *J* = 4.5, 6.0 Hz, 2H), 5.18–5.26 (m, 2H), 5.78–5.92 (m, 1H). Anal. Calcd for $C_7H_{12}O_3$: C, 58.31; H, 8.39. Found: C, 58.16; H, 8.58.

4.7. Isobutyl 3-hydroxybutanoate 2d

$[\alpha]_D^{22} = +27.0$ (*c* 1.16, $CHCl_3$) (90% ee); IR: 3432, 2968, 2881, 1745 cm^{-1}; ^{1}H NMR: δ 0.88 (d, *J* = 6.7 Hz, 6H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.82–1.98 (m, 1H), 2.31–2.52 (m, 2H), 3.2 (br s, 1H), 3.84 (d, *J* = 6.7 Hz, 2H), 4.06–4.19 (m, 1H). Anal. Calcd for $C_8H_{16}O_3$: C, 59.97; H, 10.07. Found: C, 59.77; H, 10.25.

4.8. *t*-Butyl 3-hydroxybutanoate 2e

$[\alpha]_D^{22} = +32.3$ (*c* 1.03, $CHCl_3$) (99% ee), lit.[6] $[\alpha]_D = +34.05$ (*c* 1.0, $CHCl_3$); IR: 3423, 2977, 2933, 1727 cm^{-1}; ^{1}H NMR: δ 1.09 (d, *J* = 7.0 Hz, 3H), 1.35 (s, 9H), 2.24–2.27 (m, 2H), 3.25 (br s, 1H), 4.02–4.06 (m, 1H).

References

1. (a) Poppe, I.; Novak, I. *Selective Biocatalyst A Synthetic Approach*; VCH, Verlagsgsellschaff mbH: Weinhein, 1992; (b) Hirama, M.; Uei, M. *J. Am. Chem. Soc.* **1982**, *104*, 4251; (c) Hashiguchi, S.; Kawada, A.; Natsugari, H. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2435; (d) Hayashi, Y.; Rode, J. J.; Corey, E. J. *J. Am. Chem. Soc.* **1996**, *118*, 5502; (e) Senanayake, C. H.; Fang, K.; Grover, P.; Bakale, R. P.; Vandenbossche, C. P.; Wald, S. A. *Tetrahedron Lett.* **1999**, *40*, 819; (f) Genov, M.; Dimitrov, V.; Ivanova, V. *Tetrahedron: Asymmetry* **1997**, *8*, 3703.
2. (a) Dhar, R. K. *Aldrichim. Acta* **1994**, *27*, 43; (b) Noyori, R.; Ohkume, T.; Kitamura, M.; Takaya, H.; Oayo, N.; Kumobayashi, H.; Akutagawa, S. *J. Am. Chem. Soc.* **1987**, *109*, 5856.
3. (a) Tsachen, D. M.; Fuentes, L. M.; Lynch, J. E.; Laswell, W. L.; Volante, R. P.; Shinkai, I. *Tetrahedron Lett.* **1988**, *29*, 2779; (b) Mori, K. *Tetrahedron* **1989**, *45*, 3233; (c) Chiba, T.; Nagatsuma, M.; Nakai, T. *Chem. Lett.* **1985**, 1343; (d) Seebach, D.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 264; (e) Adams, G.; Zibuck, R.; Seebach, D. *J. Am. Chem. Soc.* **1987**, *109*, 6176; (f) Kahn, M.; Fujita, K. *Tetrahedron* **1991**, *47*, 1137.
4. (a) Faber, K.; Riva, S. *Synthesis* **1992**, 895; (b) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071, and references cited therein.
5. (a) Wipf, B.; Kupfer, E.; Bertari, R.; Leuenberger, H. G. W. *Helv. Chim. Acta* **1983**, *66*, 485; (b) Hochuli, E.; Taylor, K. E.; Dulter, H. *Eur. J. Biochem.* **1977**, *75*, 433; (c) Aragozzini, F.; Maconi, E.; Craveri, R. *Appl. Microbiol. Biotechnol.* **1985**, *24*, 175.
6. Medson, C.; Smallridge, A. J.; Trewhella, M. A. *Tetrahedron: Asymmetry* **1997**, *8*, 1049.
7. Sharma, A.; Chattopadhyay, S. *J. Mol. Catal. B. Enzym.* **2000**, *10*, 531.
8. (a) Salvi, N. A.; Patil, P. N.; Udupa, S. R.; Banerji, A. *Tetrahedron: Asymmetry* **1995**, *6*, 2287; (b) Salvi, N. A.; Udupa, S. R.; Banerji, A. *Biotechnol. Lett.* **1998**, *20*, 201; (c) Salvi, N. A.; Chattopadhyay, S. *Tetrahedron* **2002**, *57*, 2833; (d) Salvi, N. A.; Badheka, L. P.; Chattopadhyay, S. *Biotechnol. Lett.* **2003**, *25*, 1081.
9. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.
10. (a) Seebach, D.; Roggo, S.; Maetzke, T.; Braunschweiger, H.; Cercus, J.; Krieger, M. *Helv. Chim. Acta* **1987**, *70*, 1605; (b) Ehrler, J.; Giovannini, F.; Lamatsch, B.; Seebach, D. *Chimia* **1986**, *40*, 172; (c) Mori, K. *Tetrahedron* **1981**, *37*, 1341; (d) Mori, K.; Watanabe, H. *Tetrahedron* **1984**, *40*, 299; (e) Nakamura, K.; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. *Chem. Lett.* **1987**, 679; (f) Nakamura, K.; Higaki, M.; Ushio, K.; Oka, S.; Ohno, A. *Tetrahedron Lett.* **1985**, *26*, 4213; (g) Lemieux, R. U.; Giguere, J. *Can. J. Chem.* **1951**, *29*, 678.