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# Dynamic kinetic resolution in the microbial reduction of α-monosubstituted β-oxoesters[1]: the reduction of 2-carbethoxy-cycloheptanone and 2-carbethoxy-cyclooctanone

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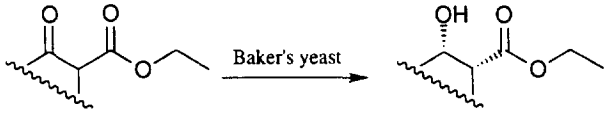
***Abstract:*** The microbial reduction of the title compounds by various yeasts or filamentous fungi strains affords the corresponding (1*S*,2*R*)- and/or (1*S*,2*S*)-hydroxyesters in good yield and ee. The determination of their absolute configuration was achieved by transformation into known 2-methylcycloalkanone stereoisomers. © 1997 Elsevier Science Ltd

The microbial reduction of racemic α-monosubstituted β-oxoesters, and among them cyclic β-oxoesters, is known to afford some of the corresponding hydroxyesters with high diastereo- and enantiomeric excesses[2]. This selectivity results from the fast equilibrium existing between both enantiomers of the oxoester in the incubation conditions, and the occurrence in the microorganism of either a single active dehydrogenase with a high stereospecificity (enantiomeric specificity and stereogenic specificity) or several active enzymes, all of them having the same stereospecificity. This methodology, used for the first time in the reduction of 2-carbethoxy -cyclopentanone and -cyclohexanone[3] and which has been then extended to purely chemical resolution processes, has been designated by the term of "dynamic kinetic resolution"[4–6].

Baker's yeast, being easily available as a grown biomass, is by far the most commonly used microorganism. It catalyses the reduction of 5- or 6-membered ring cyclic β-oxoesters to give mostly or exclusively *cis*-(1*S*,2*R*)-hydroxyesters[3,7–13] (Scheme 1). This stereospecificity is also observed for the reduction of heterocyclic oxoesters having an oxygen atom[14], a sulfur atom[15–20] or a nitrogen atom[9,21–24] in the ring, and for the reduction of various bicyclic oxoesters[9,25–31]. We have recently proposed a model to explain this stereospecificity[31].




**Scheme 1.**

Other microorganisms have been shown by us[8] and others[27,32,33] to be able to reduce some of these oxoesters with different stereospecificities (enantiospecificity and/or stereogenic specificity) and they have been effectively used to prepare the corresponding hydroxyesters in good yield and to derive from them useful asymmetric synthons[30,34]. As a continuation of our investigations on the microbial reduction of cyclic β-oxoesters, we report now our results concerning the reduction of 7- and 8-membered ring cyclic substrates **1** and **2**, and the determination of the absolute configuration of the hydroxyesters **3–6** respectively obtained.

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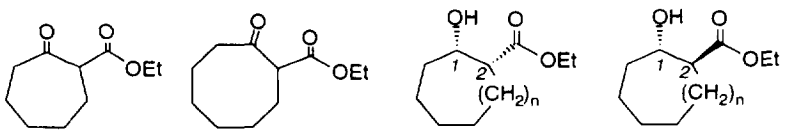
**Table 1.** Reduction of oxoesters **1** and **2** by yeasts and fungal microorganisms[a]

| | 1 | | | | | 2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Microorganims | Time (h)[b] | side-products (%)[c,d] | 3/5 ratio[d] | 3 (% ee) | 5 (% ee) | Time (h)[b] | side-products (%)[c,d] | 4/6 ratio[d] | 4 (% ee) | 6 (% ee) |
| Baker's yeast[e] | 72[f] | 15 | 68/32 | 99 | 99 | *g* | - | - | - | - |
| *Saccharomyces montanus* CBS 6772 | 72 | 12 | 54/46 | 99 | 93 | 96[f] | 15 | 90/10 | 99 | 94 |
| *Rhodotorula mucilaginosa* | 24 | 23 | 74/26 | 97 | 95 | 42 | 96 | 60/40 | 97 | 92 |
| *Rhodotorula glutinis* NRRL Y-1091 | 24 | 6 | 87/13 | 97 | 94 | 24 | 30 | 70/30 | 98 | 94 |
| *Kloekera magna* NRRL Y-1611 | 96 | 4 | 100/0 | 99 | - | *g* | - | - | - | - |
| *Cunninghamella echinulata* NRRL 3655 | 50 | 3 | 97/3 | 99 | - | *g* | - | - | - | - |
| *Beauveria bassiana* ATCC 7159 | 24 | 50 | 94/6 | 97 | 96 | 24 | 72 | 100/0 | 98 | - |
| *Mucor racemosus* | 24 | 0 | 45/55 | 89 | 92 | 24 | 4 | 11/89 | 99 | 96 |
| *Mucor griseocyanus* ATCC 1207a | 30 | 1 | 12/88 | 74 | 95 | 30 | 15 | 0/100 | - | 88 |
| *Rhizopus arrhizus* ATCC 11145 | 48 | 8 | 31/69 | 89 | 97 | 48 | 19 | 44/56 | 99 | 93 |
| *Mortierella isabellina* NRRL 1757 | 24 | 2 | 67/33 | 96 | 96 | 72 | 60 | 50/50 | 96 | 91 |

[a] Microorganisms were grown in 100 mL cultures for 60 h then substrate (100 mg) in ethanol solution (1 mL) was added [b] time necessary for complete reduction. [c] mainly the decarboxylated cycloalkanone, and small amounts of the corresponding cycloalkanol. [d] determined by GC on OV-1701 [37]. [e] Lyophilised baker's yeast (Sigma type II, 5 g) and glucose (2.5 g) in water (100 mL); substrate added as in *a*. [f] partial reduction (~70%). [g] no reduction.




**1** **2** **3**: n = 1 **4**: n = 2 **5**: n = 1 **6**: n = 2

Substrates **1** and **2** were obtained by carboxylation of the corresponding cycloalkanones[35]. Analytical samples of hydroxyesters were obtained by $NaBH_4$ reduction, affording mixtures of the racemic *cis*- and *trans*-diastereomers[36]. Microbial reductions were performed as previously described[8]. The diastereomer ratio in the hydroxyesters produced was measured by GC analysis[37]. Enantiomeric excesses were determined after derivatization with (*S*)-O-acetyllactyl chloride followed by GC analysis[38]. Some representative results of a screening of yeasts and fungal microorganisms are summarised in Table 1: entries 1–5 refer to yeasts, whereas entries 6–12 refer to filamentous fungi.

The reduction times are much longer than those observed for the reduction of 5- or 6-membered oxoesters: 2-carbethoxycyclohexanone was completely reduced in the same conditions in 4 hours. Baker's yeast reduced slowly **1** and was unable to reduce **2**, just as several microorganisms (yeasts or fungi). Long reaction times often resulted in the formation of cycloheptanone or cyclooctanone as side-products, as a consequence of oxoester hydrolysis and decarboxylation. The ketones thus produced were very slowly reduced.

A mixture of both diastereomeric hydroxyesters was generally obtained. The biotransformation of **1** and **2** by *B. bassiana* afforded as unique reduction products the *cis*-hydroxyesters **3** and **4**, in a relatively short reaction time. Unfortunately, the simultaneous formation of large amounts of the corresponding cycloalkanones made this strain unsuitable for the production of **3** or **4**. *K. magna* and *C. echinulata* reduced **1** with a high stereospecificity, affording **3** in 80 and 49% isolated yields, respectively. *trans*-Hydroxyesters **5** and **6** were obtained with the best diastereoselectivity using *M. griseocyanus*, but the enantiomeric excesses obtained were lower than those observed for the reduction of **1** and **2** by *R. arrhizus* and *M. racemosus* respectively. It is interesting to note that the enantiospecificity of the reduction by *M. racemosus*, for example, is different for the cyclohexanone-derived oxoester[8] and **1** or **2**: a gradual change of the *cis*:*trans* ratio (from 100:0 to 45:55 and 11:89, respectively), paralleling the ring size increase, is observed.

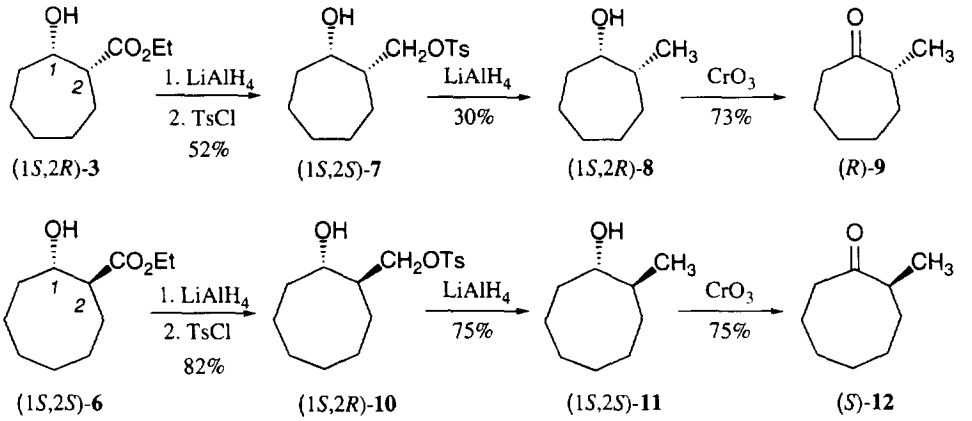
We have reduced oxoesters **1** and **2** in a 1 g-scale with selected strains without special optimisation

**Table 2.** Preparative microbial reductions[a] of **1** and **2**

| Microorganisms | Product | yield (%) | ee (%) | $[\alpha]_D^{20}$ (c 1, $CHCl_3$) |
|---|---|---|---|---|
| *K.magna* | **3** | 80 | 94 | + 37 |
| *R. arrhizus* | **5** | 40 | 93 | +15 |
| *R. glutinis* | **4** | 24 | 96 | +36 |
| *M. racemosus* | **6** | 50 | 93 | +22 |

a) Reductions were performed in 1 L cultures, as described in the screening conditions.

(Table 2) and have determined the configuration of the hydroxyesters **3**–**6** produced. Their relative configurations were established by examination of $^1$H-NMR data and determination of coupling constants[39]. The assignment of their absolute configurations[40] was carried out by transformation into the corresponding known 2-methylcycloalkanones as shown in Scheme 2. The carboxyester group of **3** and **6** was reduced to a hydroxymethyl group which was selectively tosylated to give **7** and **10** respectively. The tosylates were then reduced with $LiAlH_4$ to 2-methylcycloheptanol **8** ($[\alpha]_D^{20}$ +22; c 1.6, acetone) or 2-methylcyclooctanol **11** ($[\alpha]_D^{20}$ +19; c 1, acetone). Jones' oxidation of these alcohols afforded the known ketones **9** ($[\alpha]_D^{20}$ −81; c 1, $CHCl_3$. Lit. for *S*-enantiomer[41]: +84) and **12** ($[\alpha]_D^{20}$ +39; c 1, $CHCl_3$. Lit. for *S*-enantiomer[41]: +40).




**Scheme 2.**

As such transformations take place without any modification of the initial stereochemistry, the hydroxyesters **3** and **6** were identified as the (1*S*,2*R*) and (1*S*,2*S*) stereoisomers, respectively. The (1*S*,2*R*) configuration of **4** and the (1*S*,2*S*) configuration of **5** were ascertained by C-2 epimerisation. Treatment of the *trans* isomers **5** and **6** (obtained from microbial reductions) with DBU in acetonitrile at 80°C during 48 h afforded an equilibrated mixture of diastereomeric hydroxyesters containing the *cis* isomers **3** and **4**, respectively, as demonstrated by GC analysis of their (*S*)-O-acetyllactate derivatives[38].

In conclusion, our results show some of the limitations of the baker's yeast reduction of cyclic β-oxoesters, and afford new indications to be reported in the proposed model[31]. However, other microorganisms are able to reduce **1** and **2** in respectable yields, often with a slightly lower stereospecificity when compared to 2-carbethoxy-cyclohexanone. The asymmetric hydroxyesters we have obtained are being currently used for the synthesis of various natural products.

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36. *Cis/trans* ratios (by GC analysis, see ref. 37) for the reduction of **1** and **2**: 46/54 and 78/22 respectively.
37. Flexibond$^{TM}$ OV-1701 capillary column (Pierce Chem. Co, 15 m × 0.25 mm), 135°C, ret. times: **3**, 7.08 min; **5**, 8.41 min; **4**, 12.4 min; **6**, 13.7 min.
38. OV1701 capillary column, 135°C (10 min) then 135–170°C (2°C/min), ret. times: **3**, 32.6 min; *ent*-**3**, 32.9 min; *ent*-**5**, 34.0 min; **5**, 34.4 min. BP20 capillary column (SGE, 25 m × 0.25 mm), 160°C (20 min) then 160–190°C (4°C/min): *ent*-**4**, 51.2 min; **4**, 51.8 min; *ent*-**6**, 54.4 min; **6**, 55.2 min.
39. Values of coupling constants ($CDCl_3$) for H-1 (C*H*OH, ddd, 3.9–4.1 ppm) and H-2 (C*H*$CO_2$Et, ddd, 2.4–2.6 ppm) in hydroxyesters: **3**: $J_{H1H2}$=2.8 Hz; **5**: $J_{H1H2}$=9.2 Hz; **4**: $J_{H1H2}$=2.4 Hz; **6**: $J_{H1H2}$=7 Hz.
40. Enantiomers of *cis*- and *trans*-hydroxyesters **3** and **5** have been occasionally reported in the literature (Xie, Z.-F.; Nakamura, I.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1988**, 966, cited in Kitamura, M.; Ohkuma, T.; Tokunaga, M.; Noyori, R. *Tetrahedron: Asymmetry* **1990**, *1*, 1), but without any optical rotation measurement, and without clear and justified stereochemical

assignments. However, the levorotatory optical rotations attributed to the 1*R*-isomers in the referred paper are in agreement with our results.

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