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Kinetic resolution of racemic secondary alcohols via oxidation with *Yarrowia lipolytica* strains

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Abstract

Cyclic and alicyclic racemic secondary alcohols are kinetically resolved via oxidation with *Yarrowia lipolytica* strains. The comparison of the oxidation reactions with the reductions of the corresponding ketones supports the hypothesis of the presence of two alcohol dehydrogenases with opposite enantioselectivity. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introducton

Enantiomerically pure secondary alcohols are important as intermediates and chiral auxiliaries in organic synthesis[1,2] and common as pheromones,[3] aroma and flavor enhancing compounds. Among the current methodologies to obtain these chiral compounds there are the microbial or enzymatic reductions of the corresponding ketones and the kinetic resolution of the racemic alcohols via esterification or hydrolysis of the appropriate esters with lipases. Only recently has the kinetic resolution of racemic secondary alcohols been carried out via oxidation with baker's yeast (BY)[4] and *Bacillus stearothermophilus*,[5,6] and the combination of this methodology with the complementary microbial reduction afforded excellent results.[7,8]

In this paper we describe the kinetic resolution of various cyclic and alicyclic alcohols via oxidation with *Yarrowia lipolytica* strains (Scheme 1). These microorganisms are yeasts, distributed over a wide range of food systems, that use oils and fats as the sole carbon source, and in previous work have been efficiently employed in the anti-Prelog microbial reduction of various prochiral carbonyl compounds.[9]

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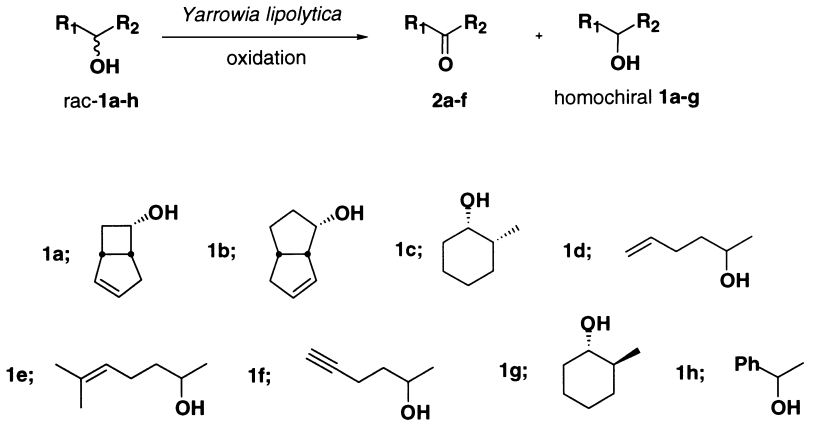


Scheme 1.

2. Results and discussion

Initially ten *Y. lipolytica* strains were screened on an analytical scale in the oxidation of the racemic alcohols **1a–h** varying the culture medium, and subsequently the reaction was repeated on a preparative scale using *Y. lipolytica* YL2 that showed good activity towards most of the substrates. The most significant results of the microbial oxidations are summarised in Table 1.

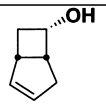
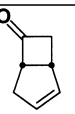
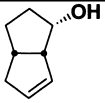
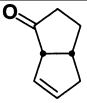
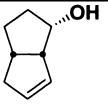
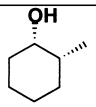
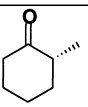
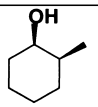
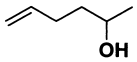
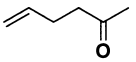
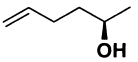
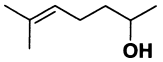
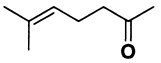
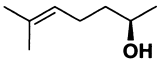
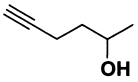
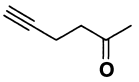
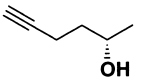
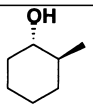
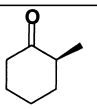
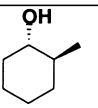
All *Y. lipolytica* strains oxidize the racemic *endo*-bicycloheptenol **1a** to (1*R*,5*S*)-ketone **2a** leaving the unreacted (6*S*)-alcohol with variable yields and different enantiomeric excesses. The best results are obtained with the YL2 strain that produces the pure ketone **2a** (40%, ee 100%) and resolves the (6*S*)-alcohol (60% yield, ee 78%) after 32 h incubation (Table 1). Surprisingly, the oxidation of the racemic mixture of **1a** leaves the same enantiomer obtained by reduction of the corresponding ketone with YL2 (57% yield, ee 34% of the (*S*)-enantiomer).

Similar results are described for the racemic *endo*-bicyclo octenol **1b** in 48 h. The oxidation with YL2 gives the (1*R*,5*R*)-ketone **2b** (49%, ee 67%) and the (5*S*)-alcohol (51%) with good enantiomeric excess (ee 74%). Also in this case the reduction of the corresponding ketone with *Y. lipolytica* YL2 produces, as observed in previous work with *Y. lipolytica* Y9,[10] the (5*S*)-alcohol (48% yield) with good enantiomeric excess (ee 68%).

Only *Y. lipolytica* YL13 and YL2, however, oxidize the racemic *cis*-2-methylcyclohexanol **1c**. In both cases the (*R*)-2-methylcyclohexanone **2c** is produced (50 and 31% yield, respectively) with satisfying ee (55% with YL13 and 92% with YL2). In these reactions the (1*R*,2*S*)-alcohol **1c** was kinetically resolved: with YL13 50% yield, ee 76%; with YL2 69% yield, ee 66%. Worst results have been detected in the oxidation screening of *trans*-2-methylcyclohexanol **1g**. Only YL2 strain oxidizes the racemic alcohol **1g** to give the (*S*)-ketone **2c** (11% yield, ee $>95\%$) leaving the unreacted (1*S*,2*S*)-*trans*-2-methylcyclohexanol **1g** (80%, ee 20%). The absolute configuration of the alcohols **1c** and **1g** are confirmed by comparison with the reduction products of 2-methylcyclohexanone with baker's yeast (Scheme 2).

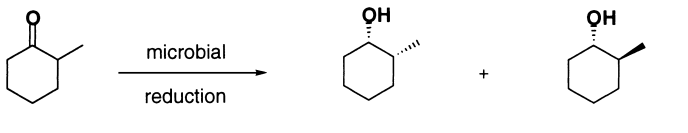
Various 2-alkyl cyclohexanones are reduced with BY to give a mixture of *cis*- and *trans*-2-alkyl (*S*)-alcohol derivatives.[11] As in the previous examples BY-mediated reduction of 2-methylcyclohexanone affords about a 1:1 mixture of *cis*- and *trans*-2-methylcyclohexanol **1c** and **1g** (30 and 27% yield, respectively). While the *cis*-(1*S*,2*R*)-isomer is produced with good enantiomeric excess

Table 1
Kinetic resolution of alcohols **1a**–**g** with *Yarrowia lipolytica* strains

Alcohol	*Yarrowia lipolytica*[a]	Time (h)	Ketone	Resolved alcohol
 **rac-1a**	YL2 (**A**)[c] YL6 (**A**)	32 32	 **(1*R*,5*S*)-2a** 40 %, ee 100 % 43 %, ee 82 %	 **(1*S*,5*R*,6*S*)-1a** 60 %, ee 78 % 57 %, ee 72 %
 **rac-1b**	YL2 (**B**)[c]	48	 **(1*R*,5*R*)-2b** 49%, ee 67%	 **(1*S*,2*S*,5*S*)-1b** 51%, ee 74%
 **rac-1c**	YL13 (**A**) YL2 (**B**)[c]	48 48	 **(*R*)-2c** 50 %, ee 55 % 31 %, ee 92 %	 **(1*R*, 2*S*)-1c** 50 %, ee 76 % 69 %, ee 66 %
 **rac-1d**	YL2 (**A**)[c] YL17 (**A**)	96 120	 **2d** --[b] --[b]	 **(*R*)-1d** 40 %, ee 82 % 37 %, ee 90 %
 **rac-1e**	YL2 (**A**)[c] YL6 (**A**) YL10 (**A**)	32 32 72	 **2e** --[b] --[b] 42 %	 **(*R*)-1e** 30 %, ee 100 % 34 %, ee 100 % 58 %, ee 91 %
 **rac-1f**	YL2 (**B**)[c]	120	 **2f** --[b]	 **(*S*)-1f** 30%, ee 97%
 **rac–1g**	YL2 (**B**)	48	 **(S)-2c** 11%, ee> 95%	 **(1S,2S)-1g** 80%, ee 20%

[a] In parenthesis culture media. [b] The yields of ketones (determined by GLC with internal standard) are very low (6-10%). [c] On preparative scale.

(ee 72%), the *trans*-(1*S*,2*S*)-alcohol **1g** is obtained with poor ee (10%). The reduction was also carried out with YL2 strain obtaining the same couple of the *cis*- and *trans*-enantiomers but with different yields and much better enantiomeric excesses. We can point out that the reduction with YL2 of 2-methylcyclohexanone afforded the same enantiomer obtained from the oxidation of the *trans*-alcohol **1g** but the opposite one from the oxidation of the *cis*-alcohol **1c**.



	cis -(*1S,2R*)-**1c**	trans-(*1S,2S*)-**1g**
BY reduction	30%, ee 72%	27%, ee 10%
YL2 reduction	44%, ee 100%	14%, ee 100%

Scheme 2.

The oxidation screening has been also carried out with a series of unsaturated alicyclic and aromatic secondary alcohols **1d–f** and **1h**. The racemic mixture of the alcohol **1d** and **1e** are kinetically resolved by *Y. lipolytica* YL2. In both cases the pure (*R*)-5-hexen-2-ol (40% yield, ee 82%) and (*R*)-6-methyl-5-hepten-2-ol (30% yield, ee 97%) are recovered but only traces of the corresponding ketones are detected (6–10%) probably because they are partially metabolized by the microorganism. On the other hand the oxidation of the racemic 5-hexyn-2-ol **1f** with *Y. lipolytica* YL2 leaves the pure (*S*)-alcohol (30% yield, ee 97%) while no oxidation products are detected with phenyl methyl carbinol **1h**. As in the previous cases the resolved alcohols **1d–f** have the same configuration of the corresponding reduction products.[9,10]

In conclusion, if we compare the results obtained in the *Y. lipolytica* oxidations of the racemic alcohols **1a–g** with those of the reductions of the corresponding ketones, we can point out that they are quite different to those observed in the baker's yeast oxido-reduction of homochiral secondary alcohols[4] and in *Bacillus stearothermophilus* alcohol dehydrogenase (BSDH) redox[12] of bicyclic octen- and heptenols. In these cases the oxidation and reduction are complementary reactions: (*S*)-enantiomer is produced by reduction, (*R*)-enantiomer is left by oxidation. On the contrary, in all oxidation experiments *Y. lipolytica* produces the same enantiomer obtained in the reduction of the corresponding ketone. The only exception is the oxidation of *cis*-2-methylcyclohexanol. This feature agrees with the hypothesis that *Y. lipolytica* is gifted with two distinct alcohol dehydrogenases (probably one (*S*)-stereospecific and the other (*R*)-stereospecific) susceptible to the nature of the substrate both in reduction and oxidation reactions. Studies are in progress to activate and deactivate these enzymes varying the reactions conditions.

3. Experimental

cis-2-Methylcyclohexanol, *trans*-2-methylcyclohexanol, 5-hexen-2-ol, 5-hexyn-2-ol and 2-methylcyclohexanone are commercially available. *endo*-Bicyclo[3.2.0]hept-2-en-6-ol and 6-methyl-5-hepten-2-ol are prepared from the corresponding available ketones (Merck and Fluka) by reduction with $NaBH_4$. The *endo*-bicyclo[3.3.0]oct-7-en-2-ol is obtained according to the literature procedure.[13]

3.1. Enantiomer separation

Gas chromatographic analyses were performed on a Carlo Erba GC 6000 Vega series 2. Enantiomer separation on Megadex 5 column (25 m×0.25 mm) containing *n*-pentyl dimethyl

β-cyclodextrin in OV 1701 from Mega s.n.c.: carrier gas: helium 82 kPa. For the oxidation of **1a**: temperature 90–200°C (1°C/min), retention time (min): (1*S*,5*R*)-**2a**, 6.73; (1*R*,5*S*)-**2a**, 7.22; (1*S*,5*R*,6*S*)-**1a**, 12.65; (1*R*,5*S*,6*R*)-**1a**, 12.83. For the oxidation of **1b**: temperature 100–200°C (1.5°C/min), retention time (min): (1*S*,5*S*)-**2b**, 11.56; (1*R*,5*R*)-**2b**, 11.81; (1*S*,2*S*,5*S*)-**1b** (as acetyl derivative), 16.96; (1*R*,2*R*,5*R*)-**1b** (as acetyl derivative), 16.82. For the oxidation of **1c**: temperature 70–200°C (1.5°C/min), retention time (min): (*S*)-**2c**, 11.71, (*R*)-**2c**, 11.88, (1*R*,2*S*)-**1c** (as acetyl derivative), 17.43; (1*S*-2*R*)-**1c** (as acetyl derivative), 18.00. For the oxidation of **1d**: temperature 80–150°C (0.5°C/min), retention time (min): **2d**, 4.41; (*S*)-**1d**, 6.95, (*R*)-**1d**, 7.08. For the oxidation of **1e**: temperature 80–200°C (1.5°C/min), retention time (min): **2e**, 9.45, (*S*)-**1e**, 13.27, (*R*)-**1e**, 13.85. For the oxidation of **1f**: temperature 80–200°C (0.5°C/min), retention time (min): **2f**, 6.34; (*R*)-**1f**, 10.51; (*S*)-**1f**, 11.15. For the oxidation of **1g**: temperature 70–200°C (1.5°C/min), retention time (min): (*S*)-**2c**, 11.27; (*R*)-**2c**, 11.47; (1*R*,2*R*)-**1g** (as acetyl derivative), 16.41; (1*S*-2*S*)-**1g** (as acetyl derivative), 17.45. For reduction with baker's yeast (or YL2): temperature: 85°C, retention time (min): (*S*)-**2c**, 12.31; (*R*)-**2c**, 12.49; (1*R*,2*R*)-**1g** (as acetyl derivative) 22.17; (1*R*,2*S*)-**1c** (as acetyl derivative), 23.58; (1*S*,2*S*)-**1g** (as acetyl derivative) 24.07; (1*S*-2*R*)-**1c** (as acetyl derivative), 24.58.

The absolute configurations of the compounds were determined comparing the sign of their specific rotation with those of the literature: for (1*R*,5*S*,6*R*)-**1a**[6] $[\alpha]_D = -68$ (*c* 1.1, $CHCl_3$); for (1*S*,5*R*)-**2a**[6] $[\alpha]_D = -63$ (*c* 1.2, $CHCl_3$); for (1*R*,2*R*,5*R*)-**1b**[6] $[\alpha]_D = 151$ (*c* 1.5, $CHCl_3$); for (1*S*,5*S*)-**2b**[6] $[\alpha]_D = -502$ (*c* 1.3, $CHCl_3$); for (*R*)-**2c**[14] $[\alpha]_D = 14$ (*c* 0.23, MeOH); for (1*S*,2*R*)-**1c**[14] $[\alpha]_D = 18$ (*c* 1.0, MeOH); for (*R*)-**1d**[7] $[\alpha]_D = -12.1$ (*c* 4.6, $CHCl_3$); for (*R*)-**1e**[7] $[\alpha]_D = -14.5$ (*c* 1.3, EtOH); for (*R*)-**1f**[7] $[\alpha]_D = -25.9$ (*c* 3.5, $CHCl_3$); for (1*R*,2*R*)-**1g**[15] $[\alpha]_D = -38.2$ (*c* 9.6, EtOH).

3.2. Culture media

Culture medium A (Saboraud): glucose (40 g/L) and peptone (10 g/L). Culture medium B: yeast extract (4 g/L), malt extract (10 g/L) and glucose (4 g/L).

3.3. Microorganisms

Yarrowia lipolytica strains[9] were isolated from various habitat and belong to DPVA (Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Italy). Ten strains[9] were tested in the oxidation of racemic alcohols: YL2 (Y9), YL6 (Y5), YL8 (1A), YL12 (RO13), YL13 (RO18), YL14 (RO21), YL15 (16B), YL17 (PO6), YL18 (PO17), YL19 (PO23).

3.4. Screening of oxidation with Y. lipolytica strains of the alcohols *1a–h* on analytical scale. General procedure

The sterilized (120°C for 20 min) culture medium A (or B) (10 mL) was pre-inoculated with a spore suspension of the selected *Yarrowia lipolytica* and grown for 24 h at 28°C. The grown culture (0.2 mL) was added to 10 mL of culture medium A (or B) and grown for a further 48 h at 28°C. To the culture was added the selected substrate solution (100 μL, 10 mg) (the solution was prepared dissolving 0.1 g of the selected substrate in 1 mL of DMF). Aliquots were withdrawing periodically, extracted with diethyl ether, dried over anhydrous Na_2SO_4 and monitored by GLC on chiral column using 2-hexanol as internal standard. The most significant results are summarized in Table 1.

3.5. Oxidation of alcohols **1a–f** with Yarrowia lipolytica *YL2* on preparative scale. General procedure

The reaction was carried out as above starting from 150 mL of the selected culture medium and 0.2 g of the substrate in 2 mL of DMF (see Table 1). After the appropriate time (monitored by GLC) the reaction mixture was extracted with diethyl ether (200 mL) by a continuous liquid extractor, dried over anhydrous Na_2SO_4. The crude reaction products and the enantiomeric excesses were analysed by GLC. Chromatography of the crude reaction mixture (silica gel, cyclohexane:diethyl ether, 80:20) gave the purified alcohols (see Table 1).

3.6. Reduction of ketones **2a–c** with Yarrowia lipolytica *YL2* on preparative scale. General procedure

The sterilized (120°C for 20 min) culture medium B (10 mL) was pre-inoculated with a spore suspension of the *Yarrowia lipolytica* YL2 and grown for 24 h at 28°C. The grown culture (1 mL) was added to 150 mL of medium B and grown for a further 48 h at 28°C. To the culture was added the selected substrate solution (0.2 g of ketone in 2 mL of DMF). After 48 h incubation, the reaction mixture was extracted with diethyl ether (200 mL) by a continuous liquid extractor, dried over anhydrous Na_2SO_4. The crude reaction products and the enantiomeric excesses were analysed by GLC. Chromatography of the crude reaction mixture (silica gel, cyclohexane:diethyl ether, 80:20) gave the purified alcohols: *endo*-(1*S*,5*R*,6S)-bicyclo[3.2.0]hept-2-en-6-ol **1a** (57% yield, ee 34%) from the reduction of **2a**, *endo*-(1*S*,2*S*,5*S*)-bicyclo[3.3.0]oct-7-en-2-ol **1b** (48% yield, ee 68%) from the reduction of **2b**, and a mixture of (1*S*,2*R*)-*cis*-2-methylcyclohexanol **1c** (44% yield, ee 100%) and (1*S*,2*S*)-*trans*-2-methylcyclohexanol **1g** (14% yield, ee 100%) from the reduction of **2c**.

3.7. Baker's yeast reduction of 2-methyl cyclohexanone

To a suspension of baker's yeast (40 g) and glucose (6.2 g) in tap water (200 mL) 2-methylcyclohexanone (0.2 g, 1.78 mmol) in DMF (2 mL) was added. The suspension (monitored by GLC) was incubated for six days at 28–30°C. The reaction mixture was extracted with diethyl ether (200 mL) by a continuous liquid extractor, dried over anhydrous Na_2SO_4 and column chromatography (silica gel, cyclohexane:diethyl ether, 80:20) afforded unreacted 2-methylcyclohexanone (0.07 g, 35%), (1*S*,2*S*)-*trans*-2-methylcyclohexanol **1g** (27%, ee 10%) and (1*S*,2*R*)-*cis*-2-methylcyclohexanol **1c** (30%, ee 72%). The enantiomeric excesses are determined by GLC.

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