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Journal of Molecular Catalysis B: Enzymatic 31 (2004) 159–163
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
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Chemo- and stereo-selective biocatalytic reduction of α,β-unsaturated ketones employing a chemo-tolerant ADH from *Rhodococcus ruber* DSM 44541

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Received 2 August 2004; received in revised form 15 September 2004; accepted 17 September 2004

Abstract

Biocatalytic reduction of the keto-moiety of α,β-unsaturated ketones (enones) was achieved with absolute chemo- and stereo-selectivity employing whole lyophilized cells of *Rhodococcus ruber* DSM 44541 to furnish the corresponding allylic alcohols in e.e. up to >99%. It was shown that a stereocenter in γ-position of the ketone moiety to be reduced is too distant from the reaction center to induce any significant diastereoselectivity, thus no kinetic resolution of an racemic ketone occurred.
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Keywords: Chemoselective; Stereoselective; Biocatalytic reduction; *Rhodococcus ruber*

1. Introduction

The chemo-selective hydrogenation of α,β-unsaturated ketones (enones) to the corresponding allylic alcohols is a very intriguing challenge in metal catalysis [1]. Several metal-catalyzed chemo- and stereo-selective reduction protocols for the carbonyl moiety have been proposed [2–5], which avoid undesired cross-reactions with the conjugated C=C double bond via 1,4-addition. The bioreduction of enones has been shown to occur via two different pathways involving two distinct classes of oxidoreductases. (i) On the one hand, alcohol dehydrogenases (carbonyl reductases) exclusively reduce the ketone functionality by leaving the C=C double bond untouched. On the other hand, (ii) enoate reductases show absolute chemo-selectivity (and often good stereoselectivity) for the C=C double bond by leaving the carbonyl group untouched. Due to its easy availability and its facile handling, baker's yeast, which possesses a large number of alcohol dehydrogenases and enoate reductases, has been widely employed [6]. As a rule of thumb, baker's yeast reduces the C=C bond of enones first by the action of enoate reductases to yield saturated ketones, which are further reduced to saturated alcohols (in a slower process) by alcohol dehydrogenases [7]. Depending on the substrate structure, this order of reduction steps is reversed and the ketone is reduced first in rare cases [8]. A competition in reduction steps is avoided, when isolated enzymes (e.g. ADH from *Lactobacillus brevis* [9,10], horse liver and *Thermoanaerobium brockii* [10]) are used, allowing the reduction of the C=O moiety in the presence of a conjugated carbon–carbon double or triple bond with excellent chemo-selectivity. An ADH from a *Pseudomonas* sp. showed only very low activity but good stereoselectivity for α,β-unsaturated ketones [11]. The opposite case of chemo-selectivity, i.e. C=C reduction in favor of the carbonyl group by using isolated enoate reductases, is impeded by the non-availability of these enzymes due to their sensitivity in (partially) purified form.

We recently presented an NADH-dependent alcohol dehydrogenase from *Rhodococcus ruber* DSM 44541 employed as a whole cell preparation as an efficient redox catalyst for

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doi:10.1016/j.molcatb.2004.09.004

the reduction of aliphatic, aromatic [12,13] and heteroaromatic ketones [14]. The strength of this biocatalyst results from its ability to recycle the cofactor NADH itself by accepting and tolerating high substrate concentrations (up to 2 M, depending on the substrate) [15] and 2-propanol (up to 80%, v/v) [16] employed as co-solvent and co-substrate. In order to extend the applicability of this simple and efficient bioreduction system, its chemo- and stereo-selectivity was investigated for ketones bearing conjugated carbon–carbon double and triple bonds.

Table 1
Asymmetric bioreduction of enones and ynones

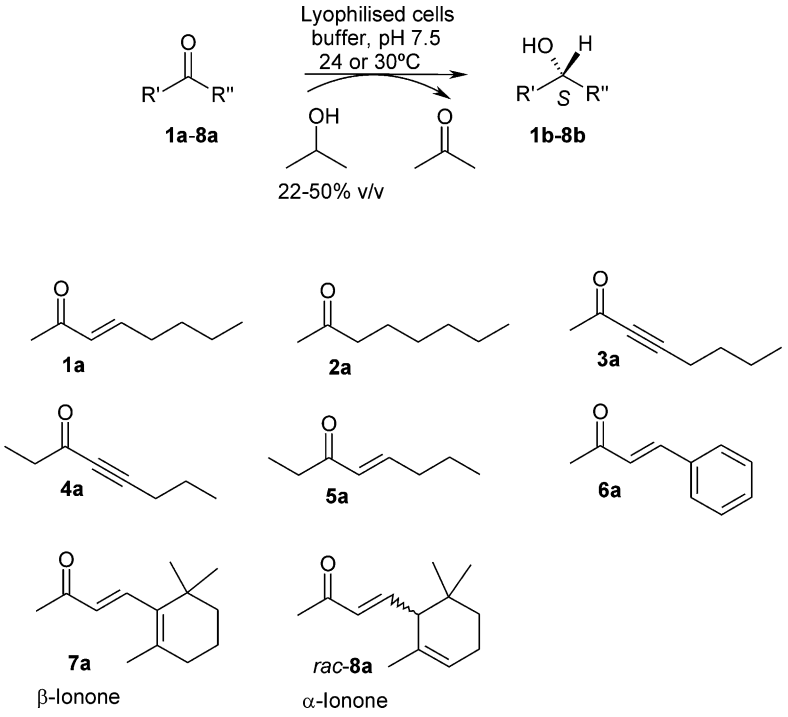
Substrate	Time (h)	2-Propanol (% v/v)	Temperature (°C)	Conv. (%)	e.e. (%)
1a	22	50	30	67	>99 (*S*)
1a	20	50	24	26	>99 (*S*)
2a	22	22	30	92	>99 (*S*)
3a	20	50	24	92	75 (*S*)
4a	20	50	24	50	>99 (*S*)
5a	20	50	24	54	>99 (*S*)
6a	22	22	30	52	>99 (*S*)
7a	22	22	30	57	>99 (*S*)

2. Results and discussion

The reduction of α,β-unsaturated (aryl)alkenyl- and alkynyl-ketones was performed employing whole lyophilized cells of *R. ruber* DSM 44541 (therefore enoate reductases are present as well) as catalyst in buffer at the expense of 2-propanol as hydrogen donor (Scheme 1). The substrate concentration was 7 g/L. For all ketones (**1a**–**8a**) investigated, (*S*)-alcohols were obtained.

E-Configured α,β-unsaturated ketone oct-3-en-2-one (**1a**) was transformed to enantiopure (*S*)-alcohol (*S*)-**1b** [12] with the same excellent selectivity as the corresponding saturated analogue 2-octanone (**2a**) [13] (Table 1). In contrast, the acetylenic derivative oct-3-yn-2-one (**3a**) was reduced with low stereopreference to give the corresponding (*S*)-alcohol (*S*)-**3b** in only 75% e.e. When the carbonyl moiety bearing the triple bond was moved from the (ω − 1)- to the (ω − 2)-position (substrate **4a**), the stereoselectivity became absolute again, which raised the e.e. again to >99% going in hand with a slightly reduced activity, ascribable to decreased steric accessibility of the (ω − 2)-position. The analogue substrate bearing a C=C double bond in this position (**5a**) was converted to alcohol **5b** in similar good selectivity (e.e. >99%). Remarkably, a sterically rather demanding group adjacent to the C=C double bond (substrates **6a** [13] and **7a**) did not exert any negative influence neither on enantioselectivity nor reaction rate and, as a consequence, both ketones were reduced to enantiopure alcohols (*S*)-**6b**, (*S*)-**7b** with excellent e.e. (>99%). In comparison, the enantiomeric excess of β-ionol (**7b**) obtained by asymmetric hydrogenation of **7a** using chemo-catalysts varied from low (∼20% e.e.: chiral Zn [17] or iridium catalyst [18]) to good (94% e.e. for a XylBINAP/DAIPEN-ruthenium catalyst [19]).

It is worth to emphasize that for all α,β-unsaturated substrates investigated, no corresponding enoate reductase activity could be detected as no trace of the corresponding



Scheme 1. Chemo-selective asymmetric bioreduction of enones and ynones.

saturated ketones nor saturated alcohols nor any other byproduct was identified. Therefore, in contrast to baker's yeast, whole lyophilized cells of *R. ruber* do not exert an interfering enoate reductase activity under the conditions employed.

The reduction of a α,β-unsaturated ketone already possessing a stereocenter in γ-position allowed to investigate any chiral recognition (i.e. diastereoselectivity) of the alcohol dehydrogenase. As a model compound, racemic α-ionone *rac*-**8a** was chosen. On the one hand, the reduction of the ketone moiety proceeded with excellent e.e. >99% (Scheme 2), however, the diasteromeric excess of alcohols **8b** was not significant (<1%), which leads to the conclusion that a stereocenter in γ-position is too distant from the reaction center to influence the stereochemical outcome of the bioreduction.

3. Experimental

Lyophilized cells of *R. ruber* DSM 44541 were prepared as previously described [20,21].

NMR spectra were recorded using a Bruker AMX 360 at 360 MHz (^{1}H) and 90 MHz (^{13}C). All chemical shifts are reported relative to TMS with $CDCl_3$ as internal standard. TLC and column chromatography was performed with silica gel Merck 60 (F_{254}). The compounds on TLC were visualized by spraying with vanillin (5 g/L in H_2SO_4) or by dipping in $KMnO_4$ solution in H_2O.

3.1. Biotransformations

3.1.1. Analytical scale

Lyophilized cells (40 mg) of *R. ruber* DSM 44541 were rehydrated in phosphate buffer (500 μL, 50 mM, pH 7.5) in Eppendorf vials (2.5 mL) for half an hour. After addition of substrate (7 μL) and *i*-propanol (143 or 500 μL, respectively) the vials were shaken for the given reaction time and temperature on a rotary shaker (140 rpm) in horizontal position. The reactions were stopped by addition of ethyl acetate (400 μL), centrifugation (2 min, 13,000 rpm), and repeated extraction with ethyl acetate (400 μL). The combined organic phases were dried (Na_2SO_4) and analyzed by GC.

3.1.2. Preparative scale

Same as above, but 30-fold scale in 50 mL round-bottomed flasks.

3.2. Substrates

2-Octanone (**2a**), 3-octyn-2-one (**3a**), 4-phenyl-but-3-en-2-one (**6a**), β-ionone (**7a**) and *rac*-α-ionone *rac*-**8a** were commercially available. 4-Octyn-3-one (**4a**) and (*E*)-4-octen-3-one (**5a**) were synthesized as previously described [22].

3.3. (*E*)-3-Octen-2-one (**1a**)

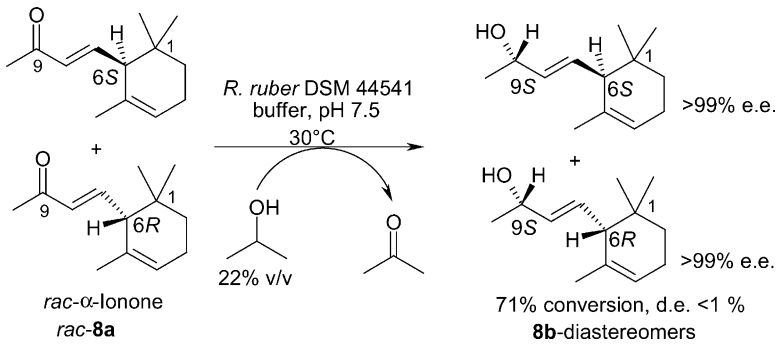
Lyophilized cells of *R. ruber* DSM 44541 (1.0 g) were rehydrated in phosphate buffer (50 mM, pH 8.0, 12.5 mL) for 30 min at room temperature by shaking (140 rpm) in a round-bottomed flask (50 mL). *rac*-(*E*)-3-Octen-2-ol *rac*-**1b** (390 mg, 3 mmol) and acetone (3.1 mL) were added to the suspension, which was shaken for 38 h at room temperature and 140 rpm. The reaction was stopped by addition of ethyl acetate (15 mL) and centrifugation (15 min, 5000 × *g*). The aqueous phase and the cells were extracted once again with ethyl acetate (15 mL). The combined organic phases were dried (Na_2SO_4), the solvent was evaporated and the crude product was purified by silica gel chromatography (*n*-hexane/ethyl acetate = 9:1) to give pure (*E*)-3-octen-2-one (**1a**) in 34% yield (130 mg). The product was identified by NMR comparison with literature data [23].

3.4. Reference material

Alcohols *rac*-(*E*)-3-octen-2-ol *rac*-**1b**, *rac*-2-octanol *rac*-**2b**, *rac*-3-octyn-2-ol *rac*-**3b** and the mixture of all isomers of α-ionol (**8b**) were commercially available. Alcohols *rac*-4-octyn-3-ol *rac*-**4b**, *rac*-(*E*)-4-octen-3-ol *rac*-**5b** and *rac*-4-phenyl-but-3-en-2-ol *rac*-**6b** were synthesized as previously reported [13,22].

3.5. *rac*-β-*Ionol rac*-**7b**

β-Ionone **7a** (1.10 g, 5.15 mmol, ∼90% purity) was reduced with $NaBH_4$ (0.25 g, 96%) in EtOH abs. (15 mL) at



Scheme 2. Simultaneous reduction of the enantiomers of α-ionone.

5 °C. The reaction was stopped after 5 h by acidification with 5% aq. HCl (15 mL) and extraction with EtOAc (2 × 20 mL). The organic phase was washed with H_2O, 10% aq. $NaHCO_3$ and again with H_2O. The organic phase was dried and evaporated. Flash chromatography afforded pure β-ionol (0.79 g, 4.07 mmol, 79.0%).

^{1}H (360 MHz, $CDCl_3$): δ = 6.06 (1H, d, J = 15.9 Hz), 5.48 (1H, dd, J = 15.9, 6.7 Hz), 4.36 (1H, p, J = 6.5 Hz), 1.97 (2H, t, J = 6.2 Hz), 1.74 (bs, OH), 1.66 (3H, s), 1.56–1.61 (2H, m), 1.44 (2H, dd, J = 6.1, 2.6 Hz), 1.31 (3H, d, J = 6.3 Hz), 0.98 (6H, s) ppm. ^{13}C (90 MHz, $CDCl_3$): δ = 137.7, 136.7 (q), 128.8 (q), 127.5, 69.5, 39.4, 33.9 (q), 32.7, 28.7 (2C), 23.6, 21.4, 19.3 ppm.

3.6. GC analyses

3.6.1. Non-chiral methods

Column J&W Scientific Agilent Technologies HP 1701 (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2) was used with the following temperature program: 75 °C/5 min–10 °C/min–125 °C/0 min–30 °C/min–250 °C/2 min. Retention times: **1a**, 9.2 min; **1b**, 8.8 min; **3a**, 9.6 min; **3b**, 9.1 min.

Column J&W Scientific Agilent Technologies HP-1 (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2) was used with the following temperature program: 120 °C/0 min–20 °C/min–250 °C/5 min. Retention times: **7a**, 5.3 min; **7b**, 4.8 min; **8a**, 4.9 min; **8b**, 4.6 min.

3.6.2. Chiral methods

Column Chrompack Chirasil-DEX CB (25 m × 0.32 mm × 0.25 μm, 1.0 bar H_2) was used with the following temperature program: 50 °C/8 min–10 °C/min–150 °C/2 min. Retention times of acetylated derivatives: **1b**, 8.9 min (*S*), 9.3 min (*R*); **3b**, 10.4 min (*S*), 10.7 (*R*). The second temperature program used was: 80 °C/6.5 min–10 °C/min–160 °C/7.5 min. Retention times of acetylated derivatives: **7b**, 12.6 min (*S*), 12.8 min (*R*); **8b**, 12.2 min (6*S*,9*S*), 12.4 min (6*S*,9*R*), 12.5 min (6*R*,9*S*), 12.6 min (6*R*,9*R*).

All other compounds were analyzed by GC as previously described: **2a**, **2b**, **6a** and **6b** [13]; **4a**, **4b**, **5a** and **5b** [22].

3.7. Derivatization of alcohols for e.e. determination by chiral GC

Acetic anhydride (0.2 mL) and DMAP (5 mg) were added to the dried sample of alcohol in ethyl acetate. After 30 min at room temperature, water (1.0 mL) and pentane (0.5 mL) were added, the vial was shaken, the aqueous phase removed and the organic phase was washed with water (3 × 1 mL), dried (Na_2SO_4) and analyzed by GC.

3.8. Determination of absolute configuration

The absolute configuration of (*S*)-2-octanol (*S*)-**2b** was proven by co-injection on GC with a commercially available non-racemic reference sample. The assignment of the absolute configuration of (*S*)-**1b**, (*S*)-**3b**, (*S*)-**4b**, (*S*)-**5b** was performed via catalytic reduction of the corresponding unsaturated acetate (H_2, 1 atm, 5% Pd/C, MeOH, room temperature) and co-injection with acetate esters of racemic and non-racemic 2-octanol **2b** and 3-octanol [22], respectively. The proof of the absolute configuration for (*S*)-**6b** was published before [13]. The assignment for (*S*)-**7b** and the isomers of **8b** was performed by comparison of the elution order on chiral GC [24].

Acknowledgements

This study was performed within the Spezialforschungsbereich 'Biokatalyse' and the Centre of Competence Applied Biocatalysis. Financial support by TIG, SFG, Province of Styria, the City of Graz, and the Fonds zur Förderung der Wissenschaftlichen Forschung (Vienna, Project No. F115) is gratefully acknowledged.

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