Biocatalysis and Biotransformation, March–April 2010; 28(2): 137–143

ORIGINAL ARTICLE

Rhodotorula minuta-mediated bioreduction of 1,2-diketones

LEANDRO N. MONSALVE[1], PATRICIA CERRUTTI[2], MIGUEL A. GALVAGNO[2,3], & ALICIA BALDESSARI[1]

[1]*Departamento de Química Orgánica y UMYMFOR, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina,* [2]*Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad de Buenos Aires, Buenos Aires, Argentina, and* [3]*IIB–Intech–CONICET–UNSAM, Buenos Aires, Argentina*

Abstract

The reduction of cyclic and acyclic 1,2-diketones was investigated by employing whole cells of the yeast *Rhodotorula minuta* as biocatalyst. The reactions showed a variable degree of regio- and enantioselectivity depending on the nature of the substrate. In the case of cyclic diketones, the reduction afforded a mixture of diastereomeric diols only. The reduction of acyclic diketones allowed production of both the hydroxy ketone and the diol, in a two-step reaction. The first step was highly regio- and stereoselective, affording the hydroxy ketone of (*S*)-configuration with high enantiomeric excess. After longer reaction times the corresponding (*S*,*S*)-diols were obtained in high yield and diastereomeric excess.

Keywords: *1,2-Diketones, bioreduction, Rhodotorula minuta*

Introduction

Regioselective and stereoselective reactions have been frequently used in the synthesis of biologically active compounds or natural product precursors (Hashimoto & Maruoka 2007; Mohr et al. 2008; Seki 2008). Among them, considerable effort has been made to obtain hydroxy ketones and diols from prochiral dicarbonyl derivatives or olefins (Bode et al. 2006; Biradar et al. 2008; Neisius & Plietker 2008). It is well known that enantiomerically pure α-hydroxy ketones are useful building blocks in the synthesis of various pharmaceuticals and fine chemicals, such as inhibitors of β-amyloid protein production (Wallace et al. 2003), antidepressant drugs (Fang et al. 2000) or antitumor agents (Anisimov 2006).

However, their preparation is not always efficient and generally requires use of a toxic transition metal catalyst such as Rh, Ru, etc. (Ikariya & Blacker 2007). An approach to this class of compounds involves the reduction of 1,2-diketones (Nakamura et al. 1996) but it is not easy to control the regioselectivity. In order to circumvent these problems, several methods have been developed. For example, the use of an electrochemical approach (Boutoute et al. 1998), Ti, V and Zn halides (Clerici & Porta 1985; Hayakawa et al. 2000) and activated alkyl phosphines (Zhang & Shi 2006) allowed production of α-hydroxy ketones in good yield but no data about the stereoselectivity of the reactions has been reported.

Reduction of 1,2-diketones may be conducted using biocatalysts (Nakamura et al. 2003; García-Urdiales et al. 2005). Enantiopure diols and hydroxy ketones have been produced biocatalytically by employing alcohol dehydrogenases from various sources (Stampfer et al. 2003; Kosjek et al. 2004). Whole cells rather than isolated enzymes are used preferentially to avoid enzyme purification and cofactor addition or the requirement for an associate system for cofactor regeneration, since such reactions often require stoichiometric amounts of nicotinamide cofactors (Moore et al. 2007).

The reduction of different 1,2-diaryl-ethanediones with whole cells from various microorganisms such as *Saccharomyces cerevisiae* (Besse et al. 1993), *Rhizopus oryzae* (Demir et al. 2004), and more recently *Aspergillus oryzae*, *Fusarium roseum* (Demir et al. 2008) and *Pichia glucozyma* (Hoyos et al. 2008) has been studied. Moreover, whole cells of the yeast

Correspondence: A. Baldessari, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2 – Piso 3 – Ciudad Universitaria, C1428EGA Buenos Aires, Argentina. Tel/Fax: +54-11-4576-3385. E-mail: alib@qo.fcen.uba.ar

ISSN 1024-2422 print/ISSN 1029-2446 online © 2010 Informa UK Ltd. (Informa Healthcare, Taylor & Francis AS)
DOI: 10.3109/10242420903515445

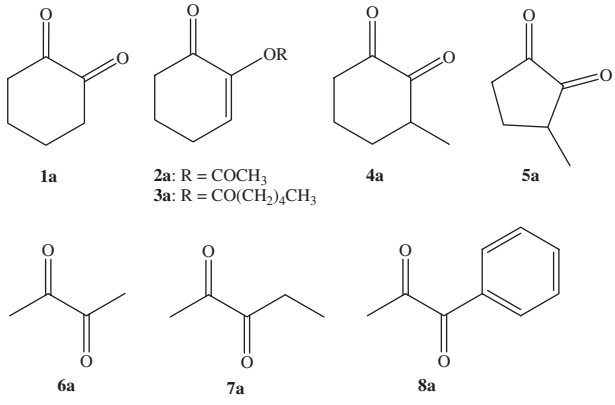
Rhodotorula spp. have been used in biotransformations performed in aqueous media (Yang et al. 2006) and macroemulsions (Cinelli et al. 2006). In our laboratory we have recently reported the application of *Rhodotorula minuta* whole cells in the regio- and stereoselective reduction of dialkyl esters of 2-oxoglutaric acid (Rustoy et al. 2008).

Now we have found that *R. minuta* cells also carry out the reduction of 1,2-diketones. In the present work we report the ability of these cells to produce chiral α-hydroxy ketones and diols depending on the structure of dicarbonyl substrate. Scheme 1 shows the 1,2-diketones used as substrates in the biotransformation.

Materials and methods

Substrates and analysis

All solvents and reagents were of reagent grade and used without purification. Diketones **1a** and **4a**–**8a** were available commercially from Sigma Aldrich (Argentina SA). Sabouraud broth was purchased from Merck KGaA (Darmstadt, Germany). For column chromatography, Merck silica gel 60 (70–230 mesh) was used. Microbial reductions were carried out with a Sontec incubator shaker (Scientifica, Buenos Aires, Argentina) at 33°C and 200 rpm. The courses of reactions were followed by TLC on Merck silica gel 60F-254 aluminum sheets (0.2 mm thickness). GLC analysis for determination of percentage of conversion was obtained on a Finnigan Focus GC instrument (Thermo Electron Co., Boston, MA, USA), the capillary column being HP-ULTRA-2 (Hewlett-Packard, Newtown, PA, USA), 25 m×0.2 mm, film thickness 0.2 μm (6 min at 80°C, 5°C/min, 150°C). GLC analyses for determination of enantiomeric excess were performed on the same apparatus, the capillary column being CHIRALDEX G-TA (Sigma-Aldrich Argentina, Buenos Aires, Argentina) 40 m×0.32 mm, film thickness 0.2 μm (80°C). Retention times in chiral separations (min): **1a**, 7.92; **1b**, 11.30 (*R*,*R*), 11.42 (*S*,*S*); **1c**, 10.94 (*meso*); **6b**, 7.64 (*R*), 7.38 (*S*); **6c**, 9.90 (*S*,*S*), 9.82 (*R*,*R*), 9.40 (*meso*); **7b**, 9.84 (*R*), 9.91 (*S*); **7c**, 13.73 (*R*,*R*), 13.81 (*S*,*S*), 14.03 (*erythro* diol); **8b**, 17.43 (*S*), 17.52 (*R*); **8c**, 18.22 (*S*,*S*), 18.36 (*R*,*R*), 18.54 and 18.70 (*erythro* diol). The absolute configuration of products **1b**, **6b**–**8b** and **6c**–**8c** was determined by comparison with the sign of the specific rotation reported in literature (Hummel et al. 1989; Besse et al. 1993; Boutoute et al. 1998). ^{1}H NMR and ^{13}C NMR spectra were recorded at 200 MHz with a Bruker AC 200 NMR spectrometer (Bruker Biospin, Billerica, MA, USA). Chemical shifts are reported in units relative to tetramethylsilane set at δ=0 ppm, and coupling constants are given in Hz. EI-MS were obtained at 70 eV with TRIO-2VG Masslab Shimadzu QP-5000 (Shimadzu America Inc., Columbia, MD, USA) and Finnigan TSQ70 mass spectrometers (Thermo Electron Co., Boston, MA, USA), in *m*/*z* (%). Elemental analysis was carried out with a Perkin-Elmer 240 apparatus. Optical rotation measurements of products were performed with Perkin-Elmer 343 instrument (Perkin Elmer, Waltham, MA, USA). Solvents are indicated throughout.



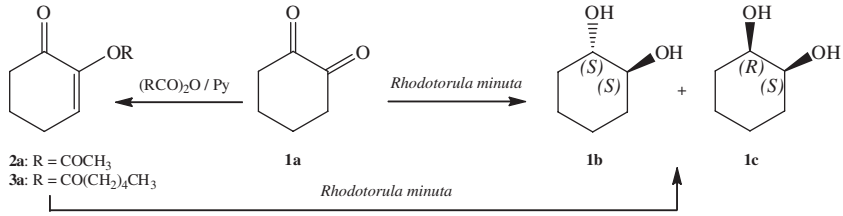
Scheme 1. 1,2-Diketones employed as substrates for microbial reduction.

Chemical reduction

Substrates **1a** and **4a**–**8a** (1 mmol) were dissolved in methanol (10 mL). Sodium borohydride (74 mg, 2 mmol) was added to the solution and the reaction was stirred for 30 min. The solvent was evaporated off; 10 mL methanol was added and then evaporated. The mixture of products was separated by column chromatography and used as standards for chromatographic identification.

Enol carboxylate synthesis

2-Acetoxy-2-cyclohexenone (**2a**): 1,2-cyclohexanedione (180 mg, 1.6 mmol) was dissolved in a 1:1 (v/v) acetic anhydride–pyridine mixture (3 mL). The resulting solution was shaken overnight at 33°C and 200 rpm and poured into cold water (10 ml). Hydrochloric acid (20% v/v) was added dropwise and, once acid, the solution was extracted with ethyl acetate (2×15 mL). The combined organic phases were washed with water (3×10 mL), then dried and evaporated. **2a** was obtained as colorless crystals (226 mg, 92% yield). ^{1}H NMR ($CDCl_3$), δ (ppm): 2.0–2.2 (m, 2H); 2.2 (s, 3H); 2.4–2.6 (m, 4H); 6.55 (t, 1H). ^{13}C NMR ($CDCl_3$), δ (ppm): 20.0, 22.2, 24.4, 37.6, 136.1, 144.9, 168.4, 191.5. EI-MS, *m*/*z* (relative intensity): 154 [M^+] (14); 95 (100). Calc. for $C_8H_{10}O_3$: C, 62.33%; H, 6.47%. Found: C, 61.01%; H, 6.47%.



2-Hexanoyloxy-2-cyclohexenone (**3a**): As described for **2a** but using 1:1 (v/v) caproic anhydride–pyridine mixture (3 mL). **3a** was obtained as colorless oil (255 mg, 76% yield). ^{1}H NMR ($CDCl_3$), δ (ppm): 0.85 (t, 3H); 1.2–1.4 (m, 4H); 1.64 (m, 2H); 1.9–2.1 (m, 4H); 2.3–2.6 (m, 4H); 6.55 (t, 1H). ^{13}C NMR ($CDCl_3$), δ (ppm): 13.9, 20.0, 22.7, 24.4, 25.2, 30.2, 33.7, 37.6, 136.0, 144.9, 171.4, 191.5. EI-MS, *m/z* (relative intensity): 210 [M^+] (12); 181 (7), 153 (20), 95 (100). Calc. for $C_{12}H_{18}O_3$: C, 68.54%; H, 8.63%. Found: C, 67.10%; H, 8.89%.

Microorganism

R. minuta strain was provided by Dr N. Refojo from Laboratorio de Micología of the Instituto de Microbiologia Dr. C. Malbran (Buenos Aires, Argentina). The strain is available to the public from the same institute under the culture number 062693.

Yeast cells previously grown in Sabouraud broth plus 0.3% w/v yeast extract at 28°C and 160 rpm were inoculated in 8.33× volume Erlenmeyer flasks containing the same medium to initial *OD* of 0.025 at 630 nm. Cultures thus prepared were incubated at 28°C for 48 h in a rotary shaker at 160 rpm. Then, cells were harvested by centrifugation (10 000***g*** for 10 min), washed twice with sterile deionized water and centrifuged again. The pellets obtained were used for the biotransformation experiments described below.

General procedure for redution by R. minuta

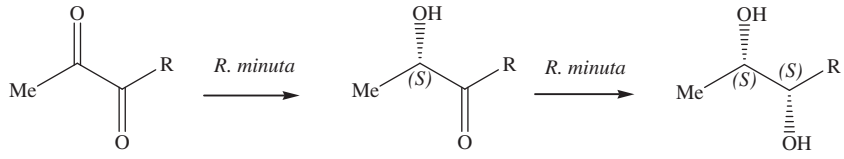
Substrate (1 mmol) and glucose (50 mg) were added to a suspension of yeast (0.5 to 8 g wet weight) in the appropriate solvent (10 mL) and the resulting mixture was incubated at 33°C and shaken at 200 rpm in 25-mL sterile Erlenmeyer flasks stoppered and sealed. Water incubations were performed using sterile water alone. The course of reactions was followed by TLC and GC. Aliquots (1 mL) were centrifuged at 10 000***g*** and the supernatants were extracted with ethyl acetate (3×1 mL). The organic phases were combined, dried over anhydrous Na_2SO_4, evaporated and analyzed by GLC.

The conversions were determined by GLC by comparing the retention time of the product obtained with that of the diketone (**1a**–**8a**) and standard compounds: hydroxy ketone (**6b**–**8b**) or diols (**1b**, **1c**, **6c**, **7c** and **8c**). After the indicated time, ethyl acetate was added (10 mL) and the contents of the flask were centrifuged. The supernatants were removed and,

Table I. Bioreduction of 1,2-cyclohexanedione **1a** and 1,2-cyclohexanedione enol esters **2a** and **3a** by *Rhodotorula minuta* whole cells.

				Products		
				1b		**1c**
Entry	Substrate	Solvent	Time (h)	% yield[a]	% ee	% yield[a]
1	**1a**	water	24	19	58	74
2	**1a**	glycerol	24	4	61	14
3	**1a**	hexane	24	8	54	50
4	**1a**	toluene	24	9	56	23
5	**1a**	water–hexane[b]	24	17	55	67
6	**1a**	water–isooctane[b]	24	13	57	50
7	**1a**	water–toluene[b]	24	7	57	24
8	**1a**	water–ethyl acetate[b]	24	7	53	25
9	**2a**	water	2	40	57	57
10	**2a**	water	24	42	56	57
11	**3a**	water	2	2	–	5
12	**3a**	water	24	32	57	64

Reaction conditions are described in the Materials and methods section; biomass (g)/substrate (mmol) ratio=4:1.
[a]Isolated yield.
Ratio=1:1 (v/v).



6a: R = Me
7a: R = Et
8a: R = Ph

6b: R = Me
7b: R = Et
8b: R = Ph

6c: R = Me
7c: R = Et
8c: R = Ph

when applied, water phases were extracted with ethyl acetate (3×10 mL). The organic phase was separated, dried over anhydrous Na_2SO_4, filtered and concentrated. The products were purified by flash chromatography (eluant: hexane–ethyl acetate, 2:1 to 1:1 v/v). All experiments were performed in duplicate. The identity of isolated compounds was determined by ^{1}H NMR, ^{13}C NMR and MS, and the percentage enantiomeric excess (% ee) was determined by chiral GLC. Yields of isolated compounds **1b**, **1c** and **6b**, **6c**, **7b**, **7c** and **8b**, **8c** obtained by reduction using *R. minuta* cells are reported in Tables I and II.

Results and discussion

A series of cyclic and acyclic 1,2-diketones were submitted to reduction by fresh cells from *R. minuta* as the source of reductase activity. The results are shown in Tables I and II.

We began studying the behavior of cyclic ketones and used 1,2-cyclohexanedione **1a** as model substrate. Table I (entries 1–8) shows the results obtained in the yeast reduction of **1a** in various solvent systems, such as water, glycerol, hexane, toluene and 1:1 (v/v) biphasic mixtures of water–hexane, water–toluene, water–isooctane and water–ethyl acetate.

In every case, 1,2-cyclohexanediol was obtained as a mixture of *cis* and *trans* isomers. Most of the *trans* isomer **1b** had the configuration (*S*,*S*); therefore **1b** is (*S*,*S*)-1,2-cyclohexanediol and **1c** is (*S*,*R*)-1,2-cyclohexanediol (see scheme in Table I).

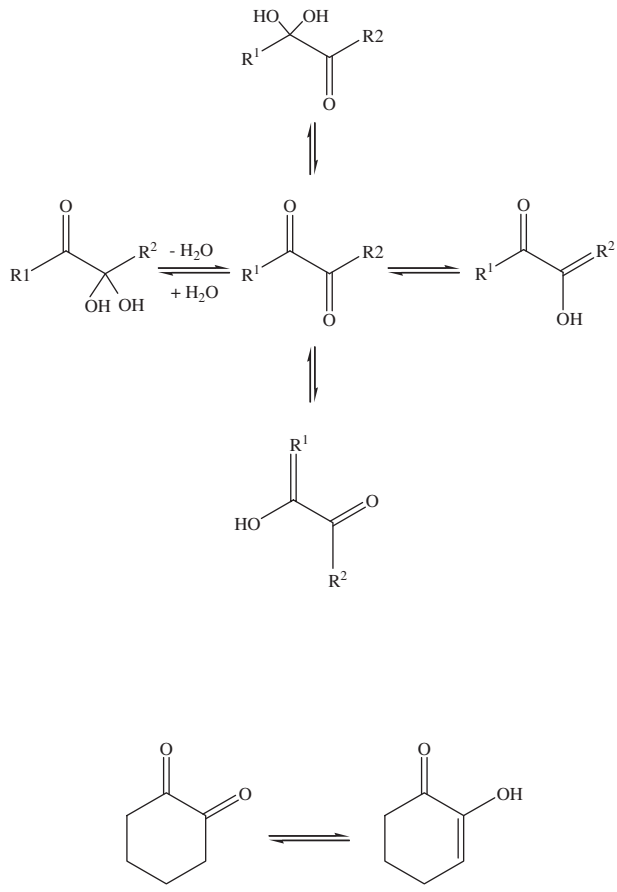
Yeast cells were active in water and, to a lesser extent, in organic or biphasic systems. As experiments using water as solvent gave the best conversions, we optimized the biomass/substrate ratio by following reactions with diverse biomass loading at different times (Figure 1).

The best results were achieved using a biomass ratio of 4 g of fresh cells over 1 mmol of substrate. Under these reaction conditions a conversion of 100% was observed, yielding *meso*-1,2-cyclohexanediol (**1c**) (74%) and (*S*,*S*)-1,2-cyclohexanediol (**1b**) (19%, 58% ee) (Table I, entry 1). According to previous work on microbial reduction of 1,2-cyclohexanedione,

Table II. Bioreduction of acyclic α-diketones 6a, 7a and 8a with Rhodotorula minuta whole cells.

			Products			
			2-Hydroxy ketone		1,2-Diol	
Entry	Substrate	Time (h)	% yield[a]	% ee	% yield[a]	% ee
1	**6a**	2	**6b** (39)	99	**6c** (58)	33
2	**6a**	24	–	–	**6c** (91)	85
3	**7a**	2	**7b** (53)	97	**7c** (44)	40
4	**7a**	24	–	–	**7c** (88)	77
5	**8a**	2	**8b** (82)	99	**8c** (15)	95
6	**8a**	24	**8b** (5)	97	**8c** (82)	95
7	**8a**	48	**8b** (5)	93	**8c** (81)	95

Reaction conditions are described in the Materials and methods section; biomass (g)/substrate (mmol) ratio=4:1.
[a]Isolated yield.



Scheme 2. 1,2-Diketones tautomeric forms in aqueous medium

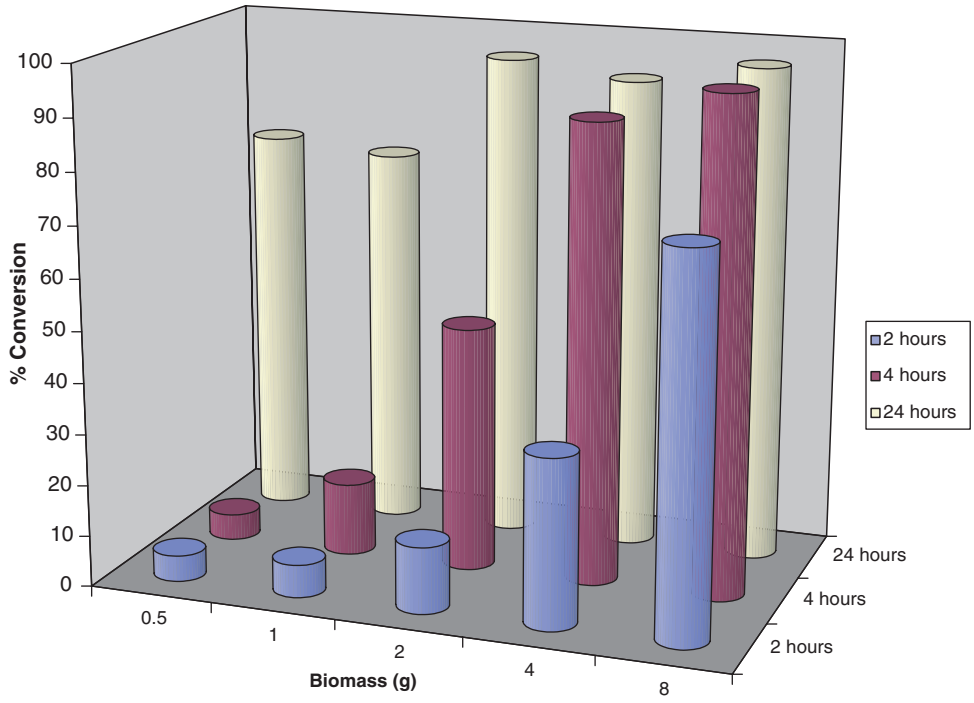


Figure 1. Time/biomass/conversion plot for the reduction products of 1,2-cyclohexanedione **1a** in water; reaction performed under standard conditions.

the reaction was not chemoselective and the formation of the 1,2-hydroxy ketone intermediate was not observed. The enzymatic pathway for 1,2-diketone reduction has been investigated using Baker's yeast and it seems that the 1,2-hydroxy ketone is reduced much faster than the diketone, therefore it cannot be detected (Besse et al. 1993).

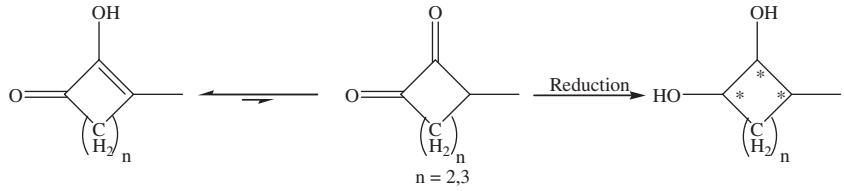
In aqueous medium 1,2-diketones may exist under three forms in equilibrium, as shown in Scheme 2. The enolic form is predominant in the case of cyclic α-diones such as 1,2-cyclohexanedione (Segretario et al. 1986) and it has been reported that the presence of nucleophiles in the substrate can lead to deactivation of reductase activity of the enzyme via complexation of the essential Zn^{2+} ion in the active site (Fries et al. 1979). In order to avoid the possible deactivation of the reductase and to obtain 2-hydroxycyclohexanone, we decided to try the reduction of an enol ester of 1,2-cyclohexanedione.

In order to evaluate the influence of the length in the acid moiety of the ester group in the *R. minuta* reduction, we prepared two enol esters: 1,2-cyclohexanedione enol acetate **2a** and 1,2-cyclohexanedione enol hexanoate **3a**. Compounds **2a** and **3a** are not commercially available, so we synthesized them by treatment of the diketone **1a** with the corresponding acid anhydride and pyridine. Both products were obtained in very good yield and were identified by spectroscopic methods.

Subsequently, the reduction of **2a** and **3a** was performed using whole cells of *R. minuta*. The results at 2 h and 24 h of reaction are depicted in Table I (entries 9–12). Unfortunately we could not obtain the desired hydroxycyclohexanone either with the enol acetate **2a** or enol hexanoate **3a**. The microbial reduction again afforded a mixture of the diasteromeric diols: *meso*-1,2-cyclohexanediol **1c** and (*S*,*S*)-1,2-cyclohexanediol **1b** in a ratio 1:1 from **2a** and 2:1 from **3a**, respectively. It is evident that the reaction did not show any regioselectivity. The fact that **2a** and **1a** gave the same results might suggest that the hydrolysis of the enol ester occurred prior to reduction of the carbonyl group, as previously observed with microbial reactions on cyclic ketone enol esters (Kume & Ohta 1992). Moreover, although the reaction with enol hexanoate **3a** afforded the same products, the reaction was slower than with **1a**, perhaps due to the steric hindrance caused by the hexanoyl group.

In order to study the effect of substitution in the microbial reduction we decided to test two other cyclic 1,2-diketones: 3-methyl-1,2-cyclohexanedione **4a** and 3-methyl-1,2-cyclopentanedione **5a**. Bioreduction of these substrates is a challenging job because the complete reduction of the dione system to the diols would lead to the generation of up to three new stereocenters (Scheme 3).

As with 1,2-cyclohexandione **1a**, these cyclic diketones remain predominantly in their enolic form. We performed the reduction employing *R. minuta*



Scheme 3. Generation of asymmetric centers in the reduction of 4a and 5a.

cells under the same experimental conditions for the bioreduction of 1,2-cyclohexanedione. In this case the cells were not active and no reduction products were obtained from the substituted diketones **4a** and **5a**. In general, yeast alcohol dehydrogenase has very narrow substrate specificity and accepts only small carbonyl compounds. As a consequence, it seems reasonable that acyclic ketones bearing bulky groups or substituted cyclic ketones are excluded as substrates. Our results support the suggestion that the catalytic pocket of yeast alcohol dehydrogenase is not big enough to achieve the reduction of enol esters or cyclic 1,2-diketones substituted in the position 3 (Ramaswamy et al. 1994; Trivic´ & Leskovac 2000).

Finally, we tried *R. minuta* in the reduction of acyclic 1,2-diketones: 2,3-butanedione **6a**, 2,3-pentanedione **7a** and 3-phenyl-2,3-propanedione **8a**. The biotransformation was carried out following the same procedure described for the reduction of cyclic diketones. It can be observed in Table II that these diketones were readily bioreduced. After 2 h of incubation a mixture of the corresponding 2-hydroxy ketone and diol was detected for the diketones **6a** (ratio 2:3) and **7a** (ratio about 1:1) (Table II, entries 1 and 3). A higher proportion of the hydroxy ketone was observed using 3-phenyl-2,3-propanedione **8a** as substrate where the ratio of hydroxy ketone to diol increased to 5:1 (Table II, entry 5). In all cases, the reduction was highly regio- and stereoselective and the hydroxy ketone corresponding to the reduction of the less hindered carbonyl group was obtained as the only product. The microbial reduction of **6a** with *R. minuta* is particularly interesting compared with the results obtained with other microorganisms. Some of them show less regioselectivity (Boutoute et al. 1998); in others it is not possible to stop the reaction at the hydroxy ketone and the diol is produced (Edegger et al. 2006).

The enantiomeric excess was very good in the three products (**6b**, **7b** and **8b**) with values between 97 and 99%. In the case of the bioreduction of **8a** it was observed that longer reaction times did not change the stereoselective behavior of the biocatalyst (Table II, entries 6 and 7). The configuration of the new chiral center in the hydroxy ketones **6b**, **7b** and **8b** is (*S*), following Prelog's rule.

At longer reaction times the second reduction step occurred, showing a moderate to high stereoselectivity, since the (*S*)-enantiomer of each hydroxy ketone (**6b**, **7b** and **8b**) was reduced further to yield the corresponding (*S*,*S*)-1,2-diol (**6c**, **7c** and **8c**, respectively) (Table II entries 2, 4 and 6).

According to early reports on microbial reduction of **7c**, depending on the microbial source, the configuration of **7c** can be preferentially (*S*,*S*) as with our results (Boutoute et al. 1998) or the diastereomer (*S*,*R*) as with *S. cerevisiae* and *Rhodococcus ruber* (Edegger et al. 2006), where further reduction of the remaining carbonyl group yields the corresponding diol predominantly in the anti-configuration.

It is interesting to observe that the diastereomeric excess for (*S*,*S*)-diols increased with reaction time, suggesting the participation of multiple enzymatic pathways with different rates in the reduction of the diketones.

Conclusions

We have described the bioreduction of various diketones as a convenient methodology to obtain hydroxy ketones and diols. The reactions showed a variable degree of regio- and enantioselectivity depending on the substrate. In the case of cyclic diketones, the reduction afforded a mixture of diastereomeric diols only, and the hydroxy ketone was not obtained as an intermediate from the reduction of only one carbonyl group in the diketone. Similar results were obtained using the enol esters of 1,2-cyclohexanedione as substrates.

The reduction of acyclic diketones allowed both hydroxy ketone and diol to be obtained, in a two-step reaction. The first step was highly regio- and stereoselective, affording the expected hydroxy ketone of (*S*)-configuration with high enantiomeric excess. After longer reaction times the corresponding (*S*,*S*)-diols were obtained in high yield and diastereomeric excess.

Acknowledgements

We thank UBA (X089 (2004-2007) and X010 (2008-2010), ANPCyT (PICT 2005 06-32735) for partial financial support. A.B. and M.A.G. are Research Members of CONICET. The authors

gratefully acknowledge M. M. Rivero for his assistance in GLC analysis.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Anisimov VN. 2006. Premature ageing prevention: limitations and perspectives of pharmacological interventions. Curr Drug Targets 7:1485–1503.

Besse P, Bolte J, Fauve A, Veschambre H. 1993. Baker's yeast reduction of α- diketones: investigation and control of the enzymatic pathway. Bioorg Chem 21:342–353.

Biradar AV, Sathe BR, Umbarkar SB, Dongare MK. 2008. Selective *cis*-dihydroxylation of olefins using recyclable homogeneous molybdenum acetylide catalyst. J Mol Catal A: Chem 285:111–119.

Bode SE, Wolberg M, Muller M. 2006. Stereoselective synthesis of 1,3-diols. Synthesis 557–588.

Boutoute P, Mousset G, Veschambre H. 1998. Regioselective or enantiogenic electrochemical and microbial reductions of 1,2-diketones. New J Chem 247–251.

Cinelli G, Cuomo F, Hochkoeppler A, Ceglie A, Lopez F. 2006. Use of *Rhodotorula minuta* live cells hosted in water-in-oil macroemulsion for biotransformation reaction. Biotechnol Prog 22:689–695.

Clerici A, Porta O. 1985. Reduction of aromatic carbonyl compounds promoted by titanium trichloride in basic media. Stereochemistry studies. J Org Chem 50:76–81.

Demir AS, Hamamci H, Ayhan P, Duygu AN, Igdir AC, Capanoglu D. 2004. Fungi mediated conversion of benzil to benzoin and hydrobenzoin. Tetrahedron Asymm 15:2579–2582.

Demir AS, Ayhan P, Demirtas U, Erkilic U. 2008. *Fusarium roseum* and *Aspergillus oryzae*-mediated enantioselective reduction of benzyls to benzoins. J Mol Catal B: Enzym 55:164–168.

Edegger K, Stampfer W, Seisser B, Faber K, Mayer SF, Oehrlein R, Hafner A, Kroutil W. 2006. Regio- and stereoselective reduction of diketones and oxidation of diols by biocatalytic hydrogen transfer. Eur J Org Chem 1904–1909.

Fang QK, Han Z, Grover P, Kessler D, Senanayade CH, Wald SA. 2000. Rapid access to enantiopure bupropion and its major metabolite by stereospecific nucleophilic substitution on an α-ketotriflate. Tetrahedron Asymm 11:3659–3663.

Fries RW, Bohlken DP, Plapp BV. 1979. 3-Substituted pyrazole derivatives as inhibitors and inactivators of alcohol dehydrogenase. J Med Chem 22:356–359.

García-Urdiales E, Alfonso I, Gotor V. 2005. Enantioselective enzymatic desymmetrizations in organic synthesis. Chem Rev 105:313–354.

Hashimoto T, Maruoka K. 2007. Recent development and application of chiral phase-transfer catalysts. Chem Rev 107:5656–5682.

Hayakawa R, Sahara T, Shimizu M. 2000. Reduction of 1,2-diketones with titanium tetraiodide: a simple approach to α-hydroxy ketones. Tetrahedron Lett 41:7939–7942.

Hoyos P, Sansottera G, Fernandez M, Molinari F, Sinisterra JV, Alcantara AR. 2008. Enantioselective monoreduction of different 1,2-diaryl-1,2-diketones catalysed by lyophilised whole cells from *Pichia glucozyma*. Tetrahedron 64:7929–7936.

Hummel W, Boermann F, Kula MR. 1989. Purification and characterization of an acetoin dehydrogenase from *Lactobacillus kefir* suitable for the production of (+)-acetoin. Biocatal Biotransform 2:293–308.

Ikariya T, Blacker J. 2007. Asymmetric transfer hydrogenation of ketones with bifunctional transition metal-based molecular catalysts. Acc Chem Res 40:1300–1308.

Kosjek B, Satmpfer W, Pogorevc W, Goessler W, Faber K, Kroutil W. 2004. Purification and characterization of a chemotolerant alcohol dehydrogenase applicable to coupled redox reactions. Biotechnol Bioeng 86:55–62.

Kume Y, Ohta H. 1992. Enzyme-mediated enantioface-differentiating hydrolysis of α-substituted sulfur-containing cyclic ketone enol esters. Tetrahedron Lett 33:6367–6370.

Mohr JT, Krout MR, Stoltz BM. 2008. Natural products as inspiration for the development of asymmetric catalysis. Nature 455:323–332.

Moore JC, Pollard DK, Kosjek B, Devine PN. 2007. Advances in enzymatic reduction of ketones. Acc Chem Res 40:1412–1419.

Nakamura K, Kondo S, Kawai Y, Hida K, Kitano K, Ohno A. 1996. Enantio- and regioselective reduction of α-diketones by baker's yeast. Tetrahedron Asymm 7:409–412.

Nakamura K, Yamanaka R, Matsuda T, Harada T. 2003. Recent developments in asymmetric reduction of ketones with biocatalysts. Tetrahedron Asymm 14:2659–2681.

Neisius NM, Plietker B. 2008. Diastereoselective Ru-catalyzed cross-metathesis-dihydroxylation sequence. An efficient approach toward enantiomerically enriched syn-diols. J Org Chem 73:3218–3227.

Ramaswamy S, Kratzer DA, Hershey AD, Rogers PH, Arnone A, Eklund H, Plapp BV. 1994. Crystallization and preliminary crystallographic studies of *Saccharomyces cerevisiae* alcohol dehydrogenase I. J Mol Biol 235:777–779.

Rustoy E, Cerrutti P, Galvagno M, Baldessari A. 2008. An efficient biotransformation of dialkyl esters of 2-oxoglutaric acid by *Rhodotorula minuta* whole cells. Biocatal Biotransform 26:204–209.

Segretario JP, Slezynski N, Zuman P. 1986. Polarographic reduction of aldehydes and ketones: Part XXVIII. 1,2-cyclohexanedione. J Electronanal Chem 214:259–273.

Seki, M. 2008. A practical synthesis of a key chiral drug intermediate via asymmetric organocatalysis. Synlett 164–176.

Stampfer W, Kosjek B, Faber K, Kroutil W. 2003. Biocatalytic asymmetric hydrogen transfer employing *Rhodococcus ruber* DSM 44541. J Org Chem 68:402–406.

Trivić S, Leskovac V. 2000. Structure and function of yeast alcohol dehydrogenase. J Serb Chem Soc 65:207–227.

Wallace OB, Smith DW, Deshpande MS, Polson C, Felsenstein KM. 2003. Inhibitors of Aβ production: solid phase synthesis and SAR of α-hydroxycarbonyl derivatives. Bioorg Med Chem Lett 13:1203–1206.

Yang W, Xu JH, Xie Y, Xu Y, Zhao G, Lin GQ. 2006. Asymmetric reduction of ketones by employing *Rhodotorula* sp. AS2-2241 and synthesis of the β-blocker (*R*)-nifelalol. Tetrahedron Asymm 17:1769–1774.

Zhang W, Shi M. 2006. Reduction of activated carbonyl groups by alkyl phosphines: formation of α-hydroxyesters and ketones. Chem Commun 1218–1220.

This paper was first published online on Early Online on 26 January 2010.