Bioreduction of α-haloacetophenones by *Rhodotorula glutinis* and *Geotrichum candidum*

Lucídio C. Fardelone, J. Augusto R. Rodrigues, and Paulo J. S. Moran*

Universidade Estadual de Campinas, Instituto de Química, CP 6154, 13084-971, Campinas-SP, Brazil
E-mail: moran@iqm.unicamp.br

Dedicated to Prof. E. A. Ruveda on his 70th birthday

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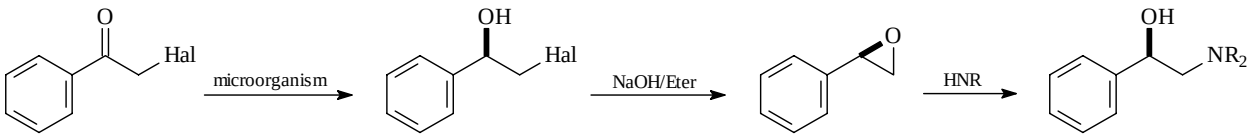
Abstract

Enantioselective reductions with enantiocomplementarity of α-haloacetophenones by *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205 afforded the corresponding (*R*)- and (*S*)-halohydrins (halo = Cl, Br and I), respectively, in high chemical yields (89-99%) and enantiomeric excesses (92-99%). These halohydrins are potential chiral building blocks for the stereoselective syntheses of valuable compounds.

Keywords: Bioreduction, α-haloacetophenones, *Rhodotorula glutinis*, *Geotrichum candidum*

Introduction

The reduction of α-haloketones mediated by whole cell of microorganisms has been frequently used to obtain chiral halohydrins,[1] that are potential chiral building blocks for use in stereoselective synthesis of valuable compounds such as enantiomerically pure 1,2-aminoalcohols *via* epoxides (Scheme 1).[1-2]



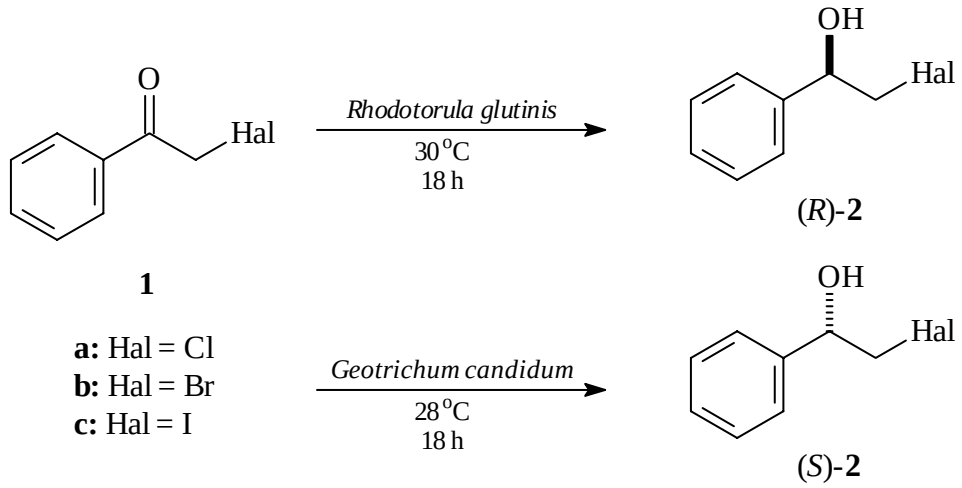
1 (*R*)-**2** (*R*)-**3** (*R*)-**4**

Scheme 1

The microorganism frequently used for reduction of α-haloacetophenones is baker´s yeast (*Saccharomyces cerevisiae*). However, the results are only fair with fluoro- and chloroacetophenone and inferior with α-bromoacetophenone giving poor yields of the corresponding bromohydrin. Also, a dehalogenation product, *i.e.* acetophenone, was obtained when α-iodoacetophenone was used as substrate.[1a,3] Recently, other microorganisms have been used with success for reduction of a large number of 4-substituted acetophenones.[1f,4] A previous report on the microbial reduction of α-haloacetophenones gave α-bromo- and α-iodo-1-phenylethanols in poor yields and low enantioselectivites.[1e] In this work, we present results of the asymmetric bioreduction of α-haloacetophenones mediated by *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205 which gave halohydrins with opposite enantioselectivity and without any dehalogenated by-products.

Results and Discussion

The bioreductions of α-haloacetophenones **1a-c** mediated by the yeast *Rhodotorula glutinis* CCT 2182 afforded the corresponding halohydrins **2a-c** with the *R* configuration, while the halohydrins **2a-c** with the *S* configuration were obtained when *Geotrichum candidum* CCT 1205 mediated the bioreductions (Scheme 2). Excellent yields and ee were obtained in both cases as shown in Table 1. These results are superior to those reported elsewhere[1a,1e] and therefore, the microorganisms used in this work should be chosen to mediate these biotransformations to obtain halohydrins **2a-c** with desired configuration *R* or *S* in excellent yields and ee. To our knowledge, this is the first time that (*S*)-(+)- and (*R*)-(-)-2-bromo-1-phenylethanol **2b** and (*S*)-(+)- and (*R*)-(-)-2-iodo-1-phenylethanol **2c** are obtained in high yields and enantioselectivity.



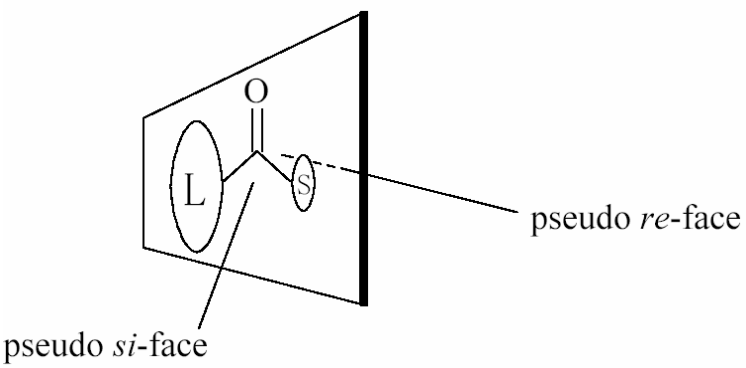
Scheme 2

Table 1. Bioreduction of aromatic ketones **1a-c** by *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205[a]

Ketone	Yeast	T (^oC)	Alcohol	Yield (%)	$[\alpha]_D^{25}$	E.e.(%)[d]
1a	*Rhodotorula glutinis*	30	(*R*)-**2a**	98	- 48.0[b]	92
1a	*Geotrichum candidum*	28	(*S*)-**2a**	89	+ 52.0[b]	> 99
1b	*Rhodotorula glutinis*	30	(*R*)-**2b**	97	- 44.4[c]	> 99
1b	*Geotrichum candidum*	28	(*S*)-**2b**	99	+ 40.0[c]	90
1c	*Rhodotorula glutinis*	30	(*R*)-**2c**	98	- 35.0[c]	94
1c	*Geotrichum candidum*	28	(*S*)-**2c**	96	+ 37.1[c]	> 99

[a] Reaction time: 18 h; 2 mmol of ketone/1.5 mL of EtOH was added to 15 g of *R. glutinis* CCT 2182 or *G. candidum* CCT 1205 (wet weight) / 400 mL of YM (yeast-malt extract) or ME (malt-extract) nutrient broth respectively; [b] *c* 1, cyclohexane; [c] *c* 1, $CHCl_3$; [d] determined by GC-MS analysis (chiral capillary column CHIRASIL-DEX).

Both *Rhodotorula glutinis* CCT 2182 and *Saccharomyces cerevisiae* give products following the Prelog rule,[5] which predicts that in general, hydrogen transfer to the prochiral ketone occurs from the pseudo *re*-face (Scheme 3) were L represent a large and S a small substituent group.[6] On the contrary, the *Geotrichum candidum* CCT 1205 gives anti-Prelog products. This ability of *Geotrichum candidum* to furnish anti-Prelog reduction products has been observed elsewhere.[4a,7]



Scheme 3

The α-haloacetophenones have been used as mechanistic probe in the reduction reactions of NADH-dependent horse liver alcohol dehydrogenase,[8] for identification of reductants in sediments[9] and even in the whole cells.[3] This probe enables differentiation between reduction processes which proceed via hydride transfer (H^-) or by a multistep electron transfer (e^-, $H^\bullet$ or e^-,

H^+, e^- as has been suggested).[10] Acetophenone is the reduction product obtained by electron transfer while optically active halohydrin is obtained when an enzyme mediates a hydride transfer process.

In this work, the yields of optically active halohydrins were high and no dehalogenation product was obtained when iodoacetophenone was used as substrate; therefore, the hydride transfer is the unique mechanism observed in the reduction of α-haloacetophenones mediated by both *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205.

Conclusions

In conclusion, *Rhodotorula glutinis* CCT 2182 and *Geotrichum candidum* CCT 1205 should be used to mediate reduction of α-haloacetophenone to obtain halohydrins **2a-c** with the desired configuration *R* or *S* in excellent yields and ee's. Achieving these asymmetric bioreductions with enantiocomplementarity is remarkable and highlights the potential of such an approach.

Experimental Section

General Procedures. IR spectra ware recorded on a Bomem MB Series spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer in $CDCl_3$. The gas chromatographic analyses were performed using a Shimadzu GC/MS Class 5000 and with helium as carrier gas, with a chiral GC-column CHIRASIL-DEX (30 m x 0.25 mm x 0.25 μm). Optical rotation was measured with a J-720, VRDM306 JASCO, 589.3 nm spectropolarimeter.
Ketones **1a-b** were acquired from Aldrich Co. Ketone **1c** was obtained by reacting **1a** with NaI in acetone at rt. The racemic **2a-c** were obtained by reacting the corresponding **1a-c** with $NaBH_4$ in water/methanol at rt. All other reagents and solvents were reagent grade.

Growth conditions for yeast culture

The yeasts *Rhodotorula glutinis* CCT 2182 and Geotrichum candidum CCT 1205 were stored at Fundação André Tosello Pesquisa e Tecnologia.[11] *Rhodotorula glutinis* was cultivated in YM (yeast-malt extract) nutrient broth (400 mL) at 30°C and *Geotricum candidum* was cultivated in ME (malt extract) nutrient broth (400 mL) at 28°C. Both yeasts were incubation for 2 days on an orbital shaker (200 rpm) before use. Sterile material was used to perform the experiments and the yeasts were manipulated in a laminar flow cabinet.

General procedure for bioreduction of α-haloacetophenones

The compound **1** (2 mmol), dissolved in 1.5 mL of ethanol, was added to slurry of growing yeast (400 mL). The resulting suspension was stirred in an orbital shaker (200 rpm) at 28°C for *Geotricum candidum* and at 30°C for *Rhodotorula glutinis* until full conversion of **1** (18 h). The

product was extracted with CH_2Cl_2 and was purified by column chromatography using hexane/ethyl acetate (7:3).

(*R*)-2-Chloro-1-phenyl-1-ethanol (2a). When 0.31 g (2 mmol) of **1a** was subjected to the general procedure for bioreduction using *Rodothorula glutinis* CCT 2182, the product isolated was **2a** (0.30 g, 98%) as an oil; $[\alpha]_D^{25}$ -48.0^o (*c* 1, cyclohexane) ($[\alpha]_D^{25}$ lit. -43.3^o (*c* 1.8, cyclohexane), (*R*))[1a], giving an optical purity of 92%; IR (film) 3401; 3087; 3064; 3031; 2956; 28 95; 1494; 1453; 1426; 1248; 1200; 1085; 1064; 1012; 1064; 768; 724; 698 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.70 (sl, 1H); 3.61-3.68 (dd, 1H, *J* = 8.8 Hz, *J* = 11.3 Hz); 3.72-3.77 (dd, 1H, *J* = 3.3 Hz, *J* = 11.3 Hz); 4.87-4.92 (dd, 1H, *J* = 3.3 Hz, *J* = 8.8 Hz); 7.25-7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.84; 73.94; 125.81; 128.24; 128.43; 139.64. MS m/z 156-158 (M^+).

(*S*)-2-Chloro-1-phenyl-1-ethanol (2ª). When 0.31 g (2 mmol) of 1a was subjected to the general procedure for bioreduction using *Geotrichum candidum* CCT 1205, the product isolated was 2a (0.27 g, 89%) as an oil; $[\alpha]_D^{25}$ +52.0^o (*c* 1, cyclohexane) ($[\alpha]_D^{25}$ lit. -43.3^o (*c* 1.8, cyclohexane), (*R*))[1a], giving an optical purity of > 99%; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

(*R*)-2-Bromo-1-phenyl-1-ethanol (2b).When 0.40 g (2 mmol) of **1b** was subjected to the general procedure for bioreduction using *Rodothorula glutinis* CCT 2182, the product isolated was **2b** (0.39 g, 97%) as an oil; $[\alpha]_D^{25}$ -44.4^o (*c* 1, $CHCl_3$) ($[\alpha]_D^{25}$ lit. -38.2^o (*c* 6.3, $CHCl_3$), (*R*))[1a], giving an optical purity of > 99%; IR (film) 3348; 2975; 2928; 2882; 1593; 1489; 1402; 1371; 1256; 1202; 1086; 1010; 899; 824; 770; 717 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.71 (sl, 1H); 3.46-3.53 (dd, 1H, *J* = 8.8 Hz, *J* = 10.6 Hz); 3.58-3.63 (dd, 1H, *J* = 3.3 Hz, *J* = 10.6 Hz); 4.87-4.92 (dd, 1H, *J* = 3.3 Hz, *J* = 8.8 Hz); 7.25-7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 39.85; 72.95; 127.12; 128.63; 133.98; 138.48. MS m/z 200-202 (M^+).

(*S*)-2-Bromo-1-phenyl-1-ethanol (2b). When 0.40 g (2 mmol) of **1b** was subjected to the general procedure for bioreduction using *Geotrichum candidum* CCT 1205, the product isolated was **2b** (0.40 g, 99%) as an oil; $[\alpha]_D^{25}$ +40.0^o (*c* 1, $CHCl_3$) ($[\alpha]_D^{25}$ lit. -38.2^o (*c* 6.3, $CHCl_3$), (*R*))[1a], giving an optical purity of 90%; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

(*R*)-2-Iodo-1-phenyl-1-ethanol (2c). When 0.49 g (2 mmol) of **1c** was subjected to the general procedure for bioreduction using *Rodothorula glutinis* CCT 2182, the product isolated was **2c** (0.49 g, 98%) as an oil; $[\alpha]_D^{25}$ -35.0^o (*c* 1, $CHCl_3$) ($[\alpha]_D^{25}$ lit. +36.3^o (*c* 5.29, $CHCl_3$), (*S*))[3], giving an optical purity of 94%; IR (film) 3392; 3085; 3061; 3029; 2956; 2919; 1600; 1494; 1452; 1269; 1176; 1056; 1002; 964; 763; 745; 699 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.70 (sl, 1H); 3.61-3.68 (dd, 1H, *J* = 8.8 Hz, *J* = 11.3 Hz); 3.72-3.77 (dd, 1H, *J* = 3.3 Hz, *J* = 11.3 Hz); 4.87-4.92 (dd, 1H, *J* = 3.3 Hz, *J* = 8.8 Hz); 7.25-7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.28; 77.28; 125.45; 128.07; 128.39; 140.82.

(*S*)-2-Iodo-1-phenyl-1-ethanol (2c). When 0.49 g (2 mmol) of **1c** was subjected to the general procedure for bioreduction using *Geotrichum candidum* CCT 1205, the product isolated was **2c** (0.48 g, 96%) as an oil; $[\alpha]_D^{25}$ +37.1^o (*c* 1, $CHCl_3$) ($[\alpha]_D^{25}$ lit. +36.3^o (*c* 5.29, $CHCl_3$), (*S*))[3], giving an optical purity of > 99%; NMR and IV spectra were identical to those observed with its (*R*) enantiomer.

Acknowledgments

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