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Biocatalytic reduction of a racemic selenocyclohexanone by Brazilian basidiomycetes

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Abstract—An efficient synthesis of the chiral cyclic secondary alcohols, *trans*-2-(phenylseleno)cyclohexanol **1a** and *cis*-2-(phenylseleno)cyclohexanol **1a**, was obtained by enzymatic reduction of 2-(phenylseleno)cyclohexanone **1** using whole fungal cells. Five strains of white-rot basidiomycetes were examined; *Irpex lacteus* CCB 196, *Pycnoporus sanguineus* CCB 196, *Trametes rigida* CCB 285, *Trametes versicolor* CCB 202 and *Trichaptum byssogenum* CCB 203. Cells of *T. rigida* CCB 285 gave alcohols *cis*-(*RS*)-**1a** and *trans*-(*SS*)-**1a** in high enantiomeric excesses (ca. 99%).

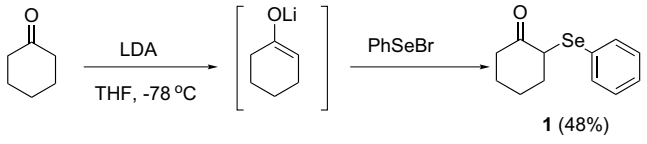
1. Introduction

Chiral alcohols are important intermediates in organic syntheses in the food, agrochemical and, most importantly, pharmaceutical industries.[1–7] Reactions that are catalysed by micro-organisms and isolated enzymes are amongst the best methods currently available for the preparation of enantiomerically pure compounds.[1,2] In this context, the microbial reduction of carbonyl compounds represents a particularly convenient route for the preparation of chiral alcohols. In nature, oxidoreductase enzymes, such as alcohol dehydrogenases, facilitate the interconversion between alcohols and aldehydes or ketones. Such dehydrogenases catalyse the removal of hydrogen from a substrate and the transfer of the hydrogen to an acceptor, either $NAD^+/NADP^+$ or a flavin enzyme, in an oxidation–reduction reaction.[3]

We have recently demonstrated that enzymes from fungal strains[8] and carrot root[9] can be employed as excellent biocatalysts in the syntheses of chiral organoselenium and organosulfur compounds. In order to extend this biocatalytic protocol to the preparation of enantiomerically pure selenoalcohols, the bioreduction of a selenoketone by some Brazilian basidiomycetes has been investigated. Previously we have employed white-rot basidiomycetes as biocatalysts in the oxidation of sulfides into sulfoxides,[10] and recently the bioreduction of ketones by basidiomycetes has been reported.[11] As a result, we herein report the preparation of enantiomerically pure cyclic secondary alcohols **1a** via the biocatalytic reduction of the racemic selenocyclohexanone **1**.

2. Results and discussion

(*R*,*S*)-2-(Phenylseleno)cyclohexanone **1** was prepared by the treatment of cyclohexanone with lithium diisopropylamide (LDA), followed by the addition of a solution of PhSeBr in THF (Scheme 1).[12] Selenoketone **1** was obtained in 48% yield following purification by column chromatography (CC) over silica gel eluted with *n*-hexane/EtOAc (10:1).



Scheme 1. Synthesis of 2-(phenylseleno)cyclohexanone **1**.

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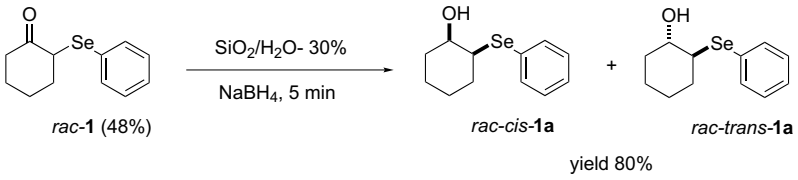


Scheme 2. Synthesis of racemic *cis*-**1a** and *trans*-**1a** alcohols.

In order to prepare the racemic diastereoisomers of β-hydroxyselenides **1a**, selenoketone **1** was reduced using a mixture of $NaBH_4$ and SiO_2/H_2O (30%) (Scheme 2).[13] A mixture of *cis*-**1a** and *trans*-**1a** isomers was purified by CC eluted with *n*-hexane/EtOAc (5:1) to afford the alcohols in 80% yield (*cis*-**1a** 68%; *trans*-**1a** 12%) for use as standards in subsequent GC and HPLC analyses. The alcohols were identified by their NMR, IR and HRMS data, which were in agreement with those reported in the literature (see Section 4.4).[14–16]

It is noteworthy that the chemical reduction of **1** led to the preferential formation of the *cis*-**1a** isomer (*cis*:*trans* ratio 85:15), since this result complements the methodology for the preparation of β-hydroxyselenides involving the ring opening of epoxides by nucleophilic selenium species to yield alcohols with a *trans*-configuration.[14]

The results obtained following the biotransformation of selenoketone **1** promoted by whole cells of the fungal species *Irpex lacteus* CCB 196, *Pycnoporus sanguineus* CCB 501, *Trametes rigida* CCB 285, *Trametes versicolor* CCB 202 and *Trichaptum byssogenum* CCB 203 are summarised in Table 1. In each experiment, the enantiomeric purities of alcohols **1a** produced were determined by HPLC analyses using a chiral column (Fig. 1).

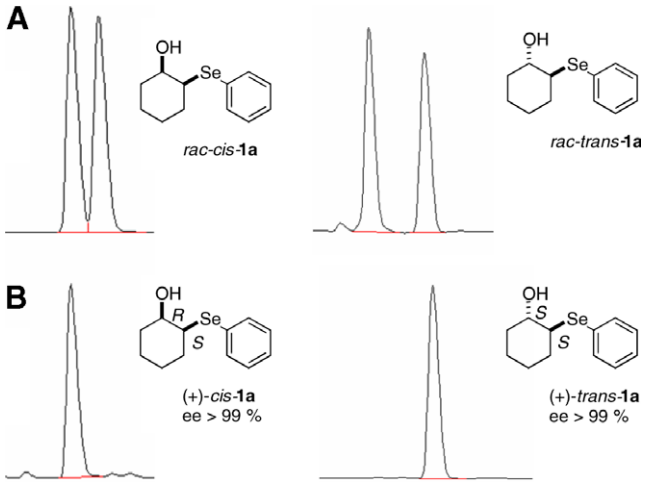
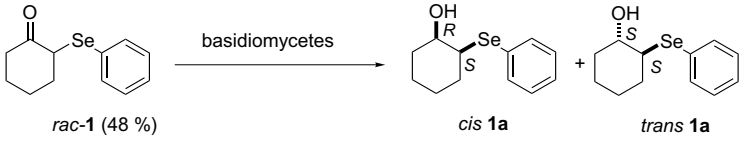


Figure 1. HPLC chromatograms of: (A) (±)-*cis*-**1a** and (±)-*trans*-**1a**; and (B) the bioreduction of (±)-ketone **1** by *Trametes rigida* CCB 285 (96 h).

As can be seen from Table 1, the bioreduction reactions promoted by different basidiomycetes varied significantly, especially with respect to the stereoselectivity. The fungus *I. lacteus* CCB 196 biotransformed **1** to alcohols *cis*-**1a** (ee 73%) and *trans*-**1a** (ee 68%) with moderate enantiomeric

Table 1. Reduction of 2-(phenylseleno)cyclohexanone **1** by whole cells of Brazilian basidiomycetes



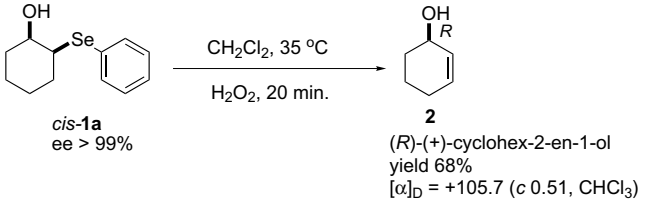
Entry	Microorganism	Time (h)	**1** c^a (%)	*cis*-**1a**		*trans*-**1a**		$(PhSe)_2$ c^a (%)
				c^a (%)	ee^b (%)	c^a (%)	ee^b (%)	
1	*Irpex lacteus*	48	10	30	—	55	—	10
2	CCB 196	96	10	22	—	45	—	23
3		144	7	18	73	42	68	33
4	*Trametes rigida*	48	20	30	—	50	—	—
5	CCB 285	96	15	34	—	51	—	—
6		144	12	34	99	46	99	8
7	*Trichaptum byssogenum*	48	43	20	—	37	—	—
8	CCB 203	96	41	21	—	38	—	—
9		144	30	26	77	44	62	—
10	*Trametes versicolor*	48	100	—	—	—	—	—
11	CCB 202	96	88	8	—	5	—	—
12		144	83	8	—	5	—	—
13	*Pycnoporus sanguineus*	48	31	29	—	40	—	—
14	CCB 501	96	19	29	—	40	—	—
15		144	19	30	68	37	79	14

[a] Concentration (%): determined by GC analysis using an achiral column.
[b] Enantiomeric excess (%): determined by HPLC analysis using a chiral column.

excess (entry 3). This was accompanied, however, by the formation of a small amount of diphenyldiselenide after a 48 h incubation (entry 1), the concentration of which increased appreciably after 96 and 144 h incubation (entries 2 and 3). The formation of $(PhSe)_2$ may occur via complex-enzymatic oxidoreduction reactions of the selenocompound **1**.[17] An investigation of these mechanistic sequences is currently underway in our laboratory.

The microbial reduction of selenoketone **1** by whole cells of *T. rigida* CCB 285 led to the formation of alcohols *cis*-**1a** (ee >99%) and *trans*-**1a** (ee >99%) with high stereoselectivity and in yields, following CC purification (see Section 4.3), of 15% and 27%, respectively, (Fig. 1). In this case, only a small amount of diphenyldiselenide was obtained after 144 h of incubation (entry 6). The low isolated yields of selenoalcohols **1a** were caused by their degradation over time. The absolute configuration of *trans*-**1a** alcohol was assigned by comparison of the sign of the measured specific rotation with those reported in the literature.[14–16]

Selenoalcohol *cis*-**1a** was transformed into the corresponding allylic alcohol **2** by selenoxide *syn*-elimination using hydrogen peroxide as the oxidant[12] (Scheme 3). The absolute stereochemistry of **2** was assigned as (*R*) by comparison of its specific rotation with published data[18] As a consequence of the *cis* relation-ship of the substituents in **1a**, the absolute configuration of the selenium containing stereogenic centre was assigned as *S*.



Scheme 3. Selenoxide *syn* elimination of *cis*-**1a** by reaction with H_2O_2.

When the fungus *Trichaptum byssogenum* CCB 203 was used to promote the biotransformation of **1**, *trans*-**1a** was obtained as the main product. HPLC analysis showed that *cis*-**1a** and *trans*-**1a** were produced in enantiomeric excesses of 77 and 62%, respectively (Table 1, entry 9). Similar results were obtained following the biotransformation of **1** with *P. sanguineus* CCB 501, but the bioreduction was not as selective and the alcohols showed only modest enantiomeric excesses (*cis*-**1a**, ee 68% ee; *trans*-**1a**, ee 79%; Table 1, entry 15). The reaction with *T. versicolor* CCB 202 was attempted but with little success since *cis*-**1a** and *trans*-**1a** were obtained only in very small amounts (Table 1, entries 11 and 12).

3. Conclusion

Whole cells of some Brazilian basidiomycetes efficiently catalyse the reduction of racemic selenoketone **1** to the corresponding alcohols **1a**. *T. rigida* CCB 285 afforded alcohols *cis*-**1a** and *trans*-**1a** with two stereogenic centres in high stereoselectivity (ee >99%). It can be concluded that the stereospecific reduction of **1**, which contains a selenium atom and a ketone group in some structure, can be accomplished using whole fungal cells.

4. Experimental

4.1. General methods

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 (500 MHz, ^1H; 125 MHz, ^{13}C) spectrometer. All spectra were measured in $CDCl_3$ and the chemical shifts δ are quoted with respect to the internal standard tetramethylsilane (TMS). IR spectra of the product oils were determined on a Bomem MB 100 spectrometer. HRMS analyses were performed using a Bruker Daltonik Esquire 3000 Plus instrument equipped with an ion trap detector. Optical rotations were measured in a Jasco DIP-378 polarimeter using a 1 dm cuvette and are referenced to the Na–D line value.

Substrates, reagents and solvents were purchased from commercial sources and purified and/or dried, where necessary, by methods described in the literature.[19] Stock cultures of basidiomycetes were stored on solid culture medium (agar 20 g/L, peptone 5 g/L, yeast extract 2 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, KH_2PO_4 1 g/L, NaCl 0.06 g/L) in Petri dishes maintained at 4 °C. All manipulations involving micro-organisms were carried out under sterile conditions in a Veco laminar flow cabinet. Technal TE-421 or Superohm G-25 orbital shakers were employed in the biocatalysed transformation experiments. Purification of the products of the reduction reactions was carried out by CC over silica gel (230–400 mesh) eluted with mixtures of *n*-hexane and EtOAc. The column effluent was monitored by TLC using pre-coated silica gel 60 F_{254} layers (aluminium-backed: Merck) eluted with *n*-hexane/EtOAc and visualised by spraying with *p*-anisaldehyde/sulfuric acid reagent followed by heating at ca. 120 °C. Reaction products were analysed using a Shimadzu model GC-17A (FID) gas chromatograph equipped with a J&W Scientific HP5 column (30 m × 0.25 mm i.d.; 0.25 μm). The chromatographic conditions were: oven temperature initially at 50 °C and increased at 10 °C/min; run time 20 min; injector temperature 230 °C; detector temperature 250 °C; injector split ratio 1:20; hydrogen carrier gas at a pressure of 100 kPa. The enantiomeric excesses of the alcohols were determined by HPLC analyses performed using a Shimadzu model SPD-10Av instrument with UV–vis detector and equipped with a Chiralcel OD-H column (25 × 0.46 cm) eluted with *n*-hexane/2-propanol (99:1). For (±)-*trans*-2-(phenylseleneo)-1-cyclohexanol **1a** the retention times of the (1*S*,2*S*)- and (1*R*,2*R*)-isomers were 8.266 and 10.170 min, respectively. In the case of (±)-*cis*-2-(phenylseleneo)-1-cyclohexanol **1a** the retention times of the stereoisomers were 7.318 and 8.06 min.

4.2. Synthesis of standard racemic alcohols[7]

Selenocyclohexanone **1** (1 mmol, 254 mg) and $NaBH_4$ (1.1 mmol, 42 mg) were added to a stirred mixture of silica

(100 mg) and H_2O (30 mg), contained in a 5 mL two-necked round-bottomed flask equipped with a magnetic stirrer, and the whole mixture stirred for 5 min at room temperature. Following work-up with CH_2Cl_2, the solvent was removed under vacuum and the residue was purified by CC over silica gel eluting with *n*-hexane/EtOAc (9:1) to afford the racemic alcohols **1a**. The spectral data of these compounds were in agreement with those previously reported (see Section 4.4).[14–16]

4.3. Biotransformation of 1 by basidiomycetes

Small slices of solid medium (0.5 × 0.5 cm) bearing a mycelia of the basidiomycete were cut from the stock culture and inoculated into liquid culture medium (1 L) contained in Erlenmeyer flasks (2 L). The fungal cells were incubated at 32 °C for 8 days on a rotatory shaker (160 rpm), harvested by filtration and suspended in a buffer solution contained in Erlenmeyer flasks (250 mL). Initially, reactions were carried out with 3.0 g (wet weight) of cells and 20 μL of selenoketone **1** in a phosphate buffer (Na_2HPO_4/KH_2PO_4, 40 mL, pH 7), and the mixture was incubated for 6 days on an orbital shaker at 32 °C. The progress of the reaction was assessed every 2 days by GC using an achiral column (Table 1). In further experiments, cells of the most efficient biocatalyst (*T. rigida* CCB 285) were transferred, together with 20 μL of selenoketone **1**, to 10 Erlenmeyer flasks (250 mL) and incubated under the above conditions for 96 h. After this time, the reaction mixtures were filtered and the combined aqueous phases extracted with EtOAc (5 × 30 mL). The yellow organic phase was dried over $MgSO_4$. The solvent removed under vacuum and the residue purified by CC over silica gel to yield the *cis* and *trans* alcohols **1a**.

4.4. Characterisation of alcohols

4.4.1. *trans*-(1*S*,2*S*)-2-(Phenylseleno)-1-cyclohexanol 1a. Yellow oil; isolated yield 27%; $[\alpha]_D^{25} = +44.8$ (*c* 0.53, $CHCl_3$); ee 99%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56–7.65 (m, 2H), 7.26–7.36 (m, 3H), 3.30–3.39 (1H), 2.87–2.95 (m, 1H), 2.11–2.21 (m, 2H), 1.71–1.75 (m, 1H), 1.61–1.65 (m, 1H), 1.36–1.45 (m, 1H), 1.19–1.34 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.1, 129.0, 128.1, 126.6, 72.3, 53.6, 33.8, 33.4, 26.9, 24.5. IR (film) ν/cm^{-1} 3044, 2921, 2851, 1732, 1577, 1427, 1440, 739, 692. HRMS (ESI) $[M+Na]^+$ calcd for $C_{12}H_{16}ONaSe$: 279,0265, C, 56.47; H, 6.32. Found: $C_{12}H_{16}ONaSe$ 279.0260.

4.4.2. *cis*-(1*R*,2*S*)-2-(Phenylseleno)-1-cyclohexanol 1a. Yellow oil; isolated yield 15%; $[\alpha]_D^{25} = +10.1$ (*c* 0.42, $CHCl_3$); ee 99%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51–7.63 (m, 2H), 7.18–7.37 (m, 3H), 3.70–3.73 (1H), 3.50–3.53 (m, 1H), 1.95–2.06 (m, 2H), 1.83–1.88 (m, 1H), 1.64–1.77 (m, 3H), 1.59–1.62 (m, 1H), 1.36–1.45 (m, 1H). ^{13}C NMR (125 MHz $CDCl_3$) δ 134.4, 129.2, 127.6, 68.3, 53.6, 32.5, 33.4, 29.1, 25.2, 25.2, 20.9. IR (film) ν/cm^{-1} 3429, 2991, 2855, 1758, 1476, 1442, 1066, 740, 693. HRMS (ESI) $[M+Na]^+$ calcd for $C_{12}H_{16}ONaSe$: 279,0265. C, 56.47; H, 6.32. Found: $C_{12}H_{16}ONaSe$: 279.0254.

4.5. Characterisation of 2-(phenylseleno)cyclohexanone 1

Yellow oil; isolated yield 48%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.5 (m, 2H), 7.2 (m, 3H), 3.9 (t, 1H), 2.9 (m, 1H), 2.3–1.7 (m, 7H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 207.8, 134.6, 129.2 (2C), 128.1 (2C), 51.6, 38.5, 34.0, 26.8, 22.9. IR (film) ν/cm^{-1} 3054, 2940, 2859, 1700. HRMS (ESI) $[M+Na]^+$ calcd for $C_{12}H_{14}ONaSe$: 277,0196. C, 56.92; H, 5.57. Found: $C_{12}H_{14}ONaSe$ 277.0099; $[M+1]^+$ calcd for $C_{12}H_{14}OSe$: 255.021. Found: 255.0281.

4.6. Selenoxide *syn* elimination of *cis*-1a

To a 25 mL one necked round bottomed flask was added *cis*-alcohol **1a** (77 mg, 0.3 mmol), 0.05 mL of H_2O_2 (30% v/v) and 10 mL of CH_2Cl_2. The reaction mixture was stirred for 20 min at 35 °C, after which, the reaction was extracted with ethyl acetate (2 × 10 mL). The combined organic phases were dried over magnesium sulfate and then filtered. The organic solvent was evaporated under reduced pressure and the residue purified by silica gel column chromatography using hexane/ethyl acetate (8:2) as eluent. $[\alpha]_D^{25} = +105.7$ (*c* 0.51, $CHCl_3$).[18] Yield: 20 mg (68%). The ^{1}H NMR and ^{13}C NMR spectra of cyclohex-2-en-1-ol was in agreement with that reported in the literature.[20]

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