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Biotransformation of β-hydroxyphenyl selenides, diphenyldiselenide and benzeneseleninic acid by whole cells of *Aspergillus terreus*

Carlos E. Da Costa [a], João V. Comasseto [a], Iracema H.-S. Crusius [c], Leandro H. Andrade [a], André L.M. Porto [a,b,*]

[a] *Laboratório de Química Fina e Biocatálise, Instituto de Química, Universidade de São Paulo-USP, Av. Prof. Lineu Preste 748, CEP 05508-900, São Paulo, SP, Brazil*
[b] *Instituto de Química de São Carlos, Universidade de São Paulo, Av. Trabalhador São-carlense 400, CEP 13566-590, São Carlos, SP, Brazil*
[c] *Seção de Micologia e Liquenologia, Av. Miguel Estéfano 3687, CEP 04301-902, São Paulo, SP, Brazil*

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Abstract

Aspergillus terreus CCT 3320 and *A. terreus* URM 3571 catalysed the biotransformations of organic β-hydroxyphenyl selenides through oxidation and methylation reactions. The kinetic resolution of (*RS*)-1-(phenylseleno)-2-propanol (**1**) *via* enantioselective oxidation produced (+)-(*S*)-**1** in high enantiomeric excess (>99%) and in a yield of 50% as determined by product isolation. Oxidation of the *R*-enantiomer of **1**, followed by elimination of the propyl moiety and subsequent methylation of the presumed intermediate, led to the formation of methylphenyl-selenide, which was isolated in a yield of 40%. Whole cells of *A. terreus* also biocatalysed transformations of diphenyldiselenide, benzeneseleninic acid, (*RS*)-1-(phenylseleno)-2-pentanol and (*RS*)-1-(phenylseleno)-3-methyl-2-butanol, but not of (*RS*)-1-(phenylseleno)-2-phenyl-methanol. This is the first report of the biomethylation of organoselenium compounds by whole cells of *A. terreus*.

Keywords: Biomethylation; Biotransformation; Selenides; *Aspergillus terreus*; Whole fungal cells

1. Introduction

Whilst selenium is an essential micronutrient for bacteria, algae, fish, animals and humans, it can be toxic at high concentrations. Selenosis syndrome has been described in various species of birds and animals, particularly those inhabiting areas where natural selenium exists [1–3]. Contamination of humans by selenium is, however, quite rare although the prevalence of selenosis is high in certain areas of China and has given rise to significant numbers of fatalities.

In the environment, selenium exists either in an inorganic form, as selenide (Se^{2-}), selenium (Se°), selenite (Se^{4+}) and selenate (Se^{6+}), or as organics containing selenium-carbon bonds. Organoselenium compounds, which may be volatile or non-volatile, include methylated derivatives, seleno-amino acids and seleno-proteins [3]. Soils can contain high levels of natural selenium, such as those from the San Luis Drain area of Central California [4], and various plants have the capacity to accumulate significant quantities of organoselenides. The Brazil nut (*Bertholletia excelsa*), for example, growing on selenium-rich soils can contain such high levels of selenium [5] that ingestion of just 6–8 kernels (*ca.* 28 g) may exceed the tolerable upper intake level of selenium of 400 mcg/day.

The bioremediation of contaminated soils containing inorganic forms of selenium has been extensively studied. Typically micro-organisms have been employed to volatilise the inorganic contaminant [6,7,4], although [8] demonstrated the application of both micro-organisms and plants in the biomethylation of inorganic selenium and tellurium to yield volatile derivatives. Various species of bacteria, fungi and yeast are reportedly able to reduce selenite and selenate producing methylated products and selenium. Recently, however, we have isolated new microbial strains from the Brazilian rain forest and have demonstrated their application in biocatalytic reduction [9,10]

* Corresponding author at: Laboratório de Química Fina e Biocatálise, Instituto de Química, Universidade de São Paulo-USP, Av. Prof. Lineu Preste 748, CEP 05508-900, São Paulo, SP, Brazil. Tel.: +55 11 3091 2287; fax: +55 3815 5579.
E-mail address: almporto@iqsc.usp.br (A.L.M. Porto).

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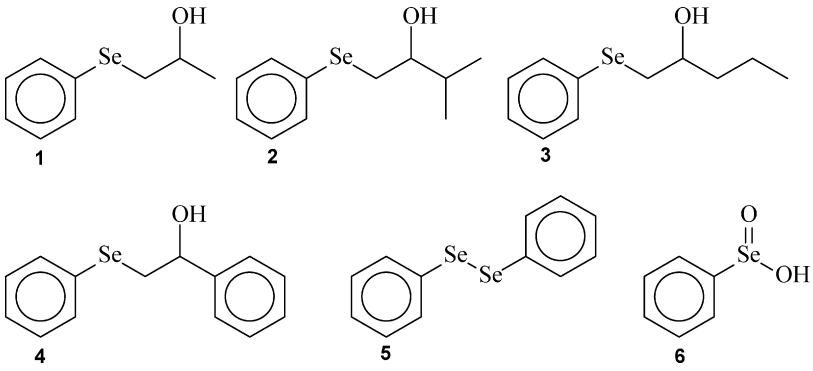


Fig. 1. The structures of organoselenium compounds **1**–**6** employed as substrates in biotransformation studies using whole cells of *A. terreus*.

and chemo-enzymatic resolution [11] of selenium compounds. In the present paper, we describe studies on the biomethylation and biotransformation of the organoselenium compounds **1**–**6** (Fig. 1) using whole cells of the fungal strains *Aspergillus terreus* CCT 3320 and *A. terreus* URM 3571 that have been isolated from Brazilian Forests.

2. Experimental

2.1. General methods

Optical rotation values were measured using a Jasco DIP-378 polarimeter with 1 dm cuvette and are reported with reference to the sodium D-line. The absolute configurations of 1-(phenylseleno)-2-propanol (**1**) were determined by comparison of the measured sign of optical rotation sign with the literature values for (+)-(*S*)-**1** of $[\alpha]_D^{20}$ + 34.0° (*ca.* 0.55, $CHCl_3$; enantiomeric excess 99%) and $[\alpha]_D^{20}$ + 56.0° (*ca.* 2.00, CH_2Cl_2; enantiomeric excess 99%) [11]. The absolute configurations of 1-(phenylseleno)-2-pentanol (**2**) and 1-(phenylseleno)-3-methyl-2-butanol (**3**) were compared with the literature [11].

Compound purification was carried out by column chromatography over silica gel (230–400 mesh) eluted with mixtures of *n*-hexane and ethyl acetate (100:0, 90:10, and 80:20). The column effluent was monitored by TLC on pre-coated silica gel 60 F_{254} layers (aluminium-backed: Merck) eluted with *n*-hexane/ethyl acetate (8:2) and visualised by spraying with *p*-anisaldehyde/sulphuric acid reagent and heating at *ca.* 120 °C. Products of the enzyme-catalysed reactions and their enantiomeric excesses were determined using a Shimadzu model GC-17A (FID) gas chromatograph equipped with a Supelco® Alfa Dex™ 120 chiral capillary column (30 m × 0.25 mm × 0.25 μm; packed with α-cyclodextrin), Beta Dex™ 120 (30 m × 0.25 mm × 0.25 μm; packed with β-cyclodextrin) and Gamma Dex™ 120 (30 m × 0.25 mm × 0.25 μm; packed with γ-cyclodextrin). The chromatographic conditions were: oven temperature 135 °C; run time 45 min; injector temperature 200 °C; detector temperature 220 °C; injector split ratio 1:20; and hydrogen carrier gas at a pressure of 100 kPa. Under these conditions, the retention time of (*S*)-**1** was 37.34 min and that of (*R*)-**1** was 38.35 min. Conditions for the GC analysis of compounds **2** and **3** were as reported earlier [11].

GC–MS analyses were performed using a Shimadzu model QP 5050A GC–MS equipped with a J & W Scientific DB-5 capillary column (30 m × 0.25 mm; 0.25 μm) with helium as the carrier gas. Biotransformation products were identified by comparison of their GC–MS data with those available in the CLASS-5000/Wiley-Shimadzu Mass Spectral Database supplied with the instrument.

2.2. Synthesis of racemic alcohols

The general procedure described by Costa et al. [11] for the preparation of the racemic alcohols **1**–**4** was employed.

2.3. Fungal cultures

The fungal strains studied were *A. terreus* CCT 3320 (isolated by D.S. Attili in September 1993 from the soil of the Atlantic rain forest in Peruíbe, SP, Brazil) and *A. terreus* URM 3571 (isolated in 1996 from reservoir waters in the north-eastern region of Brazil). Slant cultures were used to inoculate 1 L of sterilised malt extract medium (oxoid: 20 g/L) contained in a 2 L Erlenmeyer flask, which was incubated on a orbital shaker (Tecnal TE-421 or Superohm G-25; rotational speed 160 rpm) for 72–96 h at 32 °C [12]. Cells were harvested by vacuum filtration under sterile conditions (Veco model VLFS-12 laminar flow cabinet).

2.4. Standard procedure for small scale biotransformation reactions

The substrates, β-hydroxyphenyl selenides **1**–**4** (20 μL), diphenyldiselenide **5** (20 mg) and benzeneseleninic acid **6** (20 mg), were added under sterile conditions to separate 250 mL Erlenmeyer flasks each containing 50 mL of a suspension of washed, wet cells of *A. terreus* (1–5 g) in phosphate buffer [13]. The mixtures were incubated on an orbital shaker (rotational speed 160 rpm) at 32 °C, and the progress of the reaction was monitored at the times shown in Tables 1–3 by collecting 2 mL portions. These samples were extracted by stirring with ethyl acetate (0.5–1.0 mL), and aliquots (4 μL) of the organic phase were analysed by GC–FID on a fused silica chiral capillary column. The chromatographic properties of the products of the biocatalysed reactions were com-

Table 1
The biotransformation of 20 μL of (*RS*)-1-(phenylseleno)-2-propanol (**1**) by whole cells of two strains of *A. terreus*

Entry	Cell mass (g)	Time (days)	*Aspergillus terreus* CCT 3320			*Aspergillus terreus* URM 3571		
			7 (%)[a]	(*S*)-**1** (%)[a]	Enantiomeric excess of (*S*)-**1** (%)	**7** (%)[a]	(*S*)-**1** (%)[a]	Enantiomeric excess of (*S*)-**1** (%)
1	1	3	1	97	10	0.6	99	*98*
2		5	1.5	95	31	1.5	98	>99
3	2	3	2.5	93	8	25	75	>99
4		5	3.5	92	83	85.5	0	–
5	3	3	5.8	89	32	1.4	93	99
6		5	50.6	49	≥98	100	0	–
7	4	3	10.5	85	63	6	86	88
8		5	67	33	≥98	100	0	–
9	5	3	25	71	74	10	87	91
10		5	100	–	–	100	–	–

[a] Conversion determined by CG-FID.

Table 2
The semi-preparative biotransformation of 100 μL of (*RS*)-1-(phenylseleno)-2-propanol (**1**) by whole cells of *A. terreus* URM 3571 (5 g)

Entry	Time (days)	**7** (%)[a]	(*S*)-**1** (%)[a]	Enantiomeric excess of (*S*)-**1** (%)
1	3	0.6	88	32
2	5	0.8	99	≥98
3[b]	6	3	94	≥99
4	7	14	78	>99

[a] Conversion determined by CG-FID.
[b] Yields determined by semi-preparative isolation of products: (+)-(*S*)-**1** 50%; phenylmethyl-selenide (**7**) 40%.

pared with those of racemic mixtures obtained by chemical synthesis.

2.5. Standard procedure for semi-preparative scale biotransformation reactions

Filtered, wet cells of *A. terreus* URM 3571 (5 g) were suspended in 200 mL of phosphate buffer in a 500 mL Erlenmeyer flask, and an aliquot (100 μL) of **1** was added under sterile conditions. The mixture was incubated on an orbital shaker (rotational speed 160 rpm) at 32 °C and the progress of the reaction was monitored by GC–FID (Table 2). After an appropriate incubation time, the reaction mixture was filtered, the aqueous phase extracted with ethyl acetate, the yellow organic phase dried over $MgSO_4$ and the solvent removed under vacuum. The residue was purified by silica gel column chromatography to yield the desired alcohol **1** and the biomethylated compound **7**, the identity of the latter being confirmed from its mass spectral data.

3. Results and discussion

The microbial biotransformation of (*RS*)-1-(phenylseleno)-2-propanol (**1**) was studied using whole cells of *A. terreus* strains CCT 3320 and *A. terreus* URM 3571, and the results obtained are presented in Tables 1 and 2. Previously we have observed that the quantity of resting fungal cells employed can influence the course of the reaction. Thus, in order to determine the most appropriate conditions for the enzymatic reaction, the mass of fungal cells present was varied between 1 and 5 g, and the formation of product was studied with respect to time (Table 1). For *A. terreus* strain URM 3571, the highest rate of biotransformation of (*RS*)-**1** was achieved when 1 g of whole fungal cells was employed (Table 1, entries 1 and 2). Under these conditions the reaction was rapid (Fig. 2) and (*S*)-**1** was obtained in high enantiomeric excess (>99%) and in good conversion (>98%). However, the enantioselectivity and production of the desired chiral seleno-alcohol **1** decreased with increasing mass of whole fungal cells used (Table 1). Thus, in the presence of 2–5 g of whole cells of *A. terreus* URM 3571, methylphenyl-selenide (**7**) was formed exclusively after 5 days of incubation (Table 1, entries 4, 6, 8 and 10).

The biotransformation of (*RS*)-**1** was also performed on a semi-preparative scale with 100 μL of substrate and 5 g of whole

Table 3
The biotransformation of 20 μL of (*RS*)-1-(phenylseleno)-2-pentanol (**2**) by whole cells of *A. terreus* CCT 3320[a]

Entry	Cell mass (g)	Time (days)	(*S*)-**2** (%)[b]	Enantiomeric excess of (*S*)-**2** (%)
1	1	3	95	7
2		5	95	12
3		7	97	12
4		10	96	13
5	2	3	96	19
6		5	95	30
7		7	96	30
8		14	96	42
9	3	3	94	20
10		5	93	26
11		10	96	48
12	4	3	94	33
13		5	95	57
14		10	94	76
15		14	94	81
16	5	3	95	47
17		5	95	90
18		10	94	92
19		14	96	95

[a] Formation of methylphenyl-selenide (**7**) detected by GC–MS analysis as a minor product.
[b] Conversion determined by CG-FID.

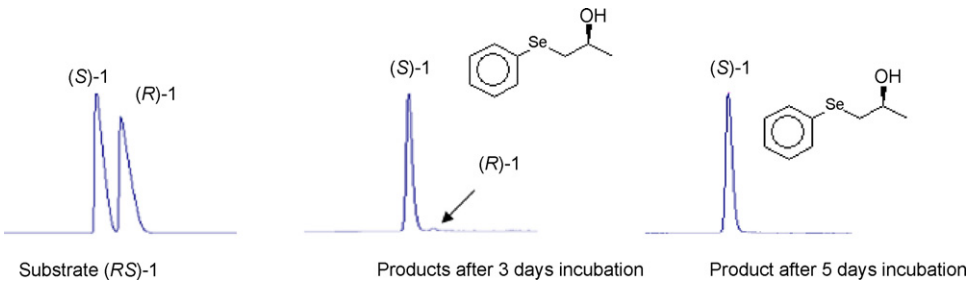
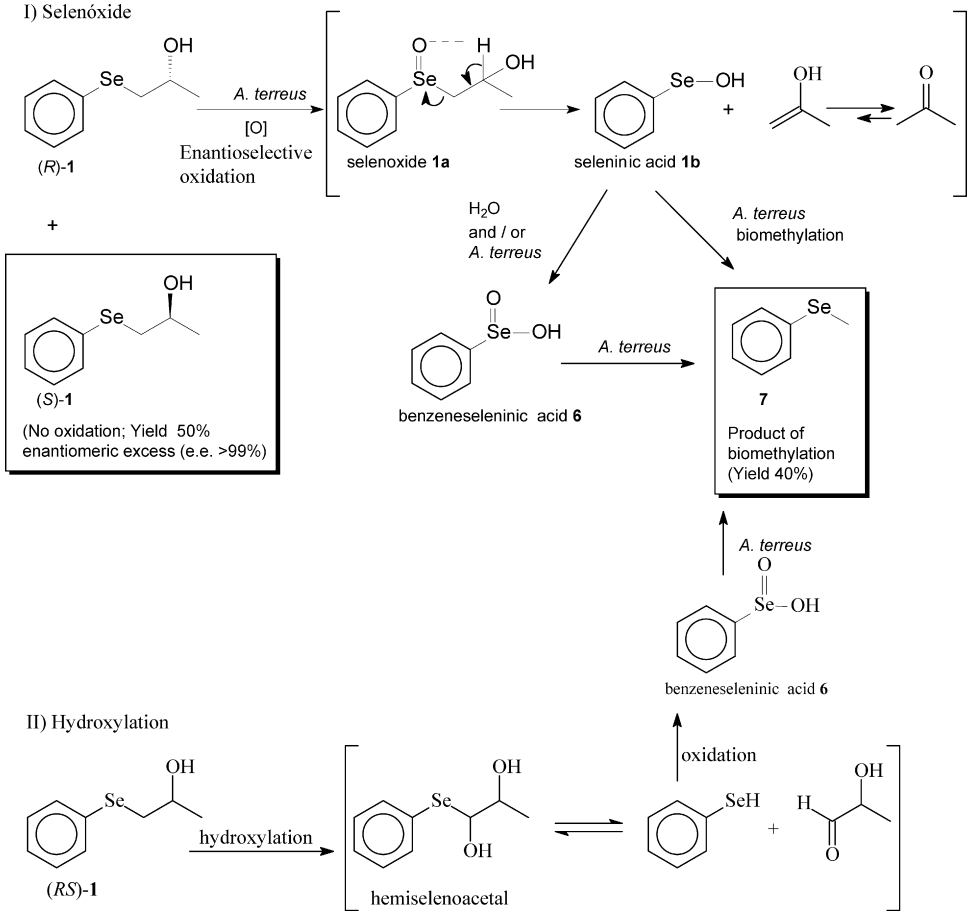


Fig. 2. GC chromatograms showing the kinetic resolution, *via* enantioselective oxidation, of (*RS*)-1-(phenylseleno)-2-propanol **1** (20 μL) by whole cells of *A. terreus* URM 3571 (1 g) at 32 °C and 160 rpm.

cells of *A. terreus* URM 3571. The formation of product was followed by GC–FID analysis and the results are presented in Table 2. Furthermore, when the products were isolated from a 3 days incubation reaction mixture, a 50% yield of (+)-(*S*)-**1** (with an enantiomeric excess of >99%) and a 40% yield of **7** were obtained.

The proposed mechanism for the biotransformation of (*RS*)-**1** by *A. terreus* involves a multi-enzymatic sequence of oxidoreduction reactions that leads to the kinetic resolution of the alcohol. In Scheme 1, selenoxide **1a** (I) is initially produced by enzymatic oxidation of (*R*)-**1**, but suffers spontaneous elimination of volatile propanone to produce seleninic acid (**1b**). This intermediate may be transformed into methylphenyl-selenide (**7**) through direct biomethylation catalysed by *A. terreus*, or *via* the formation of benzeneseleninic acid (**6**) (formed by hydration of **1b** or by oxidation catalysed by *A. terreus*) followed by biomethylation. Although the suggested intermediates **1a** and **b** have not been isolated, the finding that whole cells of *A. terreus* CCT 3320 efficiently transformed benzeneseleninic acid (**6**) exclusively into methylphenyl-selenide (**7**) (conversion >90%) supports the proposed mechanism. Moreover, the same whole fungal cells were also able to convert exogenous diphenyldiselenide (**5**) into **7** with high conversion (*ca.* 100%) as determined by GC–FID. A debenzylation (dealkylation) mechanism has been proposed to account for the formation of toluene-*p*-seleninic acid by action of *Aspergillus niger* on



Scheme 1. Proposed mechanistic sequence involved in the enantioselective biotransformation of (*RS*)-1-(phenylseleno)-2-propanol (**1**) by whole cells of *A. terreus*.

benzyl *p*-tolylselenide [14]. Another pathway for the biomethylation can be suggested by hydroxylation (II) of the methylene group adjacent to the selenium atom might be occurring via hemiselenoacetal, this intermediate may be transformed into methylphenyl-selenide (**7**) (Scheme 1). These two alternative mechanisms for fungal metabolism of selenides have been discussed by Holland [15,16].

It has already been demonstrated that strains of *A. terreus* are efficient at oxidation of sulphides [17] and ketones, Baeyer-Villiger reactions [18]. In the present case, the oxidation of the (*RS*)-β-hydroxyphenyl selenide **1**, diphenyldiselenide (**5**) and benzeneseleninic acid (**6**), catalysed by whole cells of *A. terreus* CCT 3320, could have been mediated by flavin-containing monooxygenases [19–21].

The biotransformations of the β-hydroxyphenyl selenides **2**–**4** by whole cells of *A. terreus* were also studied. With (*RS*)-**2** as substrate, *A. terreus* CCT 3320 produced (*S*)-**2** in high enantiomeric excess and in good conversion after 5–14 days of incubation with 5 g of whole fungal cells (Table 3, entries 17–19). On the other hand, *A. terreus* strain URM 3571 did not promote the biotransformation of **2** under any of the conditions tested, and the alcohol substrate was obtained in racemic form.

In contrast to the transformation behaviour of (*RS*)-**1** and (*RS*)-**2**, the biotransformation of (*RS*)-**3** with *A. terreus* strains CCT 3320 and *A. terreus* URM 3571 occurred with degradation of **3** and formation of **7**, whilst (*RS*)-**4** was not efficiently transformed into products by either strain (data not shown).

4. Conclusions

Whole cells of *A. terreus* are efficient in the biomethylation of organoselenium compounds including **1**, **2**, **5** and **6**. Such reactions occur rarely in nature [8], biomethylation being far more common with inorganic selenium substrates. The biotransformation of (*RS*)-β-hydroxyphenyl selenide (**1**) by *A. terreus* URM 3571 occurred with kinetic resolution, *via* oxidation reactions, to produce (+)-(*S*)-**1** with high enantiomeric excess (>99%) and in a yield of 50%. The biomethylated product, methylphenyl-selenide (**7**) was formed through bio-oxidation of the *R*-enantiomer. The high potential of *A. terreus* CCT strain 3320 for application in the biotransformation of organoselenium, reported here for the first time, may be of significant interest in green chemistry.

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