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preliminary communication

Deracemization of (*RS*)-1-[(4-Methylselanyl)Phenyl]Ethanol and (*RS*)-1-[(4-Ethylselanyl)Phenyl]Ethanol by Strains of *Aspergillus terreus*

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Summary

The fungal strains *Aspergillus terreus* URM 3371 and *A. terreus* CCT 4083, isolated in Brazil, catalysed the deracemization of (*RS*)-1-[(4-methylselanyl)phenyl]ethanol (**1**) and (*RS*)-1-[(4-ethylselanyl)phenyl]ethanol (**2**). Different mass of whole fungal cells (1–5 g), pH values (4 and 7), biotransformation temperature (20 and 32 °C) and additives (ethanol, butanol, propanol and cyclohexanol) were employed in attempt to improve product yield and selectivity. The *A. terreus* strain URM 3371 transformed (*RS*)-**1** into (+)-(*R*)-**1** with high enantiomeric excess (e.e.≥98 %), good conversion (≥98 %) and acceptable yield (53 %).

Key words: Aspergillus terreus, biotransformation, chiral organoseleno alcohols, fungal cells, deracemization

Introduction

The search for new enzymatic systems with application in the synthesis of chiral compounds continues to attract considerable interest (*1*). The screening of microorganisms for novel biocatalytic activities may be facilitated by using whole cells rather than isolated enzymatic systems (*2*).

Organoselenium compounds have been widely employed in synthetic transformations, and various enantioselective methodologies involving organoseleno intermediates have been developed (*3*,*4*). In view of this fact, we became interested in the application of biocatalysis in the synthesis of enantiopure chiral organoseleno compounds. Recently we have reported the biocatalytic preparation of chiral organoseleno alcohols by the asymmetric reduction of organoseleno acetophenones by various fungal strains isolated in Brazil (*5*) and by *Daucus carota* roots (*6*). Moreover, the efficient enzymatic resolution of (*RS*)-β-hydroxyselenides in organic media has been promoted by several lipases (*7*,*8*).

In previous work we employed whole cells of the fungus *Aspergillus terreus* to catalyse the deracemization of several phenylethanol derivatives (*9*,*10*). In the present work we report the biotransformation of (*RS*)-1-[(4-methylselanyl)phenyl]ethanol (**1**), (*RS*)-1-[(4-ethylselanyl)phenyl]ethanol (**2**) and (*RS*)-1-[(4-phenylselanyl) phenyl]-ethanol (**3**) by whole cells of two strains of *A. terreus* under different conditions (pH, temperature, additives, fungal cell mass; Scheme 1).

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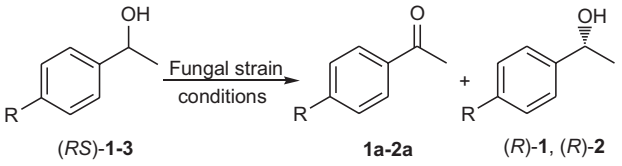

(*RS*)-**1-3** **1a-2a** (*R*)-**1**, (*R*)-**2**

R = SeMe (**1**)
SeEt (**2**)
SePh (**3**)

Fungal strains: *A. terreus* URM 3371 and *A. terreus* CCT 4083

Scheme 1. Deracemization of (*RS*)-organoseleno alcohols by whole cells of *Aspergillus terreus*

Materials and Methods

General methods

^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker model DPX300 spectrophotometer operating at 300.1 and 75.5 MHz, respectively, with samples dissolved in $CDCl_3$ and with tetramethylsilane (TMS) as internal standard.

Chemical syntheses were monitored by TLC analysis using aluminium-backed silica gel 60 F_{254} layers (Merck) eluted with hexane and ethyl acetate; analytes were visualized by spraying with *p*-anisaldehyde/sulphuric acid reagent and heating at approx. 120 °C for 1 min. Conversion yields and enantiomeric excesses (e.e.) of enzyme-catalysed reactions were determined using a Shimadzu GC-17A (FID) gas chromatograph equipped with a Chirasil-Dex CB β-cyclodextrin (25 m×0.25 mm i.d.) fused silica capillary column.

Optical rotations were determined using a Jasco DIP-378 polarimeter with a 1-dm cuvette and were measured with reference to the sodium D-line. The absolute configuration of (+)-(*R*)-**1** was determined by comparison of its optical rotation ($[\alpha]_D^{20}$=+44.4° (*c* 1.4, $CHCl_3$; e.e.>98 %) with that reported in the literature for (–)-(*S*)-**1** ($[\alpha]_D^{20}$=–56.8°; *c* 0.47, $CHCl_3$; e.e.=96 %) (*5*). The absolute configuration of **2** was determined by comparison of the chiral chromatograms with the literature data (*5*).

GC-MS analyses were performed on a Shimadzu QP 5050A instrument equipped with a J&W Scientific DB-5 capillary column (30 m×0.25 mm i.d.; 0.25 μm) employing helium as the carrier gas.

*Synthesis of the racemic alcohols **1–3***

The racemic organoseleno alcohols **1–3** were obtained by reduction of the corresponding organoseleno acetophenones with sodium borohydride in methanol, as previously described by Andrade *et al.* (*5*). The organoseleno acetophenones were prepared by methods described by Omori *et al.* (*8*).

Fungal cultures

The fungal strains employed were *A. terreus* CCT 4083 (isolated by L. Pfenning in August 1994 from pasture soil in Belém, PA, Brazil) and *A. terreus* URM 3371 (isolated in 1993 from the rhizosphere soil of *Vernonia herbaceae* in São Paulo, SP, Brazil). A slant culture was used to inoculate 1 L of sterilised malt extract medium (Oxoid, 20 g/L) contained in a 2-litre Erlenmeyer flask, which was incubated on an orbital shaker (Tecnal TE-421 or Superohm G-25; rotational speed 160 rpm) for 96 h at 32 °C. Cells were harvested by vacuum filtration and washed under sterile conditions in laminar flow cabinet.

Small scale biotransformation reactions

Samples (5 mg) of **1–3**, dissolved in 200 μL of an additive (ethanol, propanol, butanol or cyclohexanol), were introduced under sterile conditions into the Erlenmeyer flasks (125 mL) containing suspensions of washed, wet cells of *A. terreus* (1–5 g, see Tables 1–3) in 50 mL of 0.1 M phosphate buffer (pH=4 or 7) (*11*). The mixtures were incubated on an orbital shaker (rotational speed 160 rpm) at 32 °C, and the progress of the reaction was monitored at 3, 5 and 10 days by collecting samples of 1 or 2 mL. These samples were extracted by stirring with ethyl acetate (0.5 mL), and aliquots (4 μL) of the organic phase were analysed by gas chromatography-flame ionization detector (GC-FID) using a fused silica chiral capillary column. The products of the deracemization were compared with the racemic mixture of organoseleno alcohols obtained by chemical reduction of the respective organoseleno ketones.

Semi-preparative scale deracemization reactions

An aliquot (100 μL) of (*RS*)-1-[(4-methylselanyl)phenyl]ethanol (**1**), dissolved in 600 μL of additive (ethanol), was introduced under sterile conditions into an Erlenmeyer flask (500 mL) containing a suspension of washed, wet cells of *A. terreus* URM 3371 (15 g) in 200 mL of phosphate buffer (pH=4). The mixture was incubated on an orbital shaker (rotational speed 160 rpm) at 32 °C until the substrate had been completely consumed (18 days). Following incubation, the cell suspension was filtered and the cells were washed with ethyl acetate (4×100 mL). The organic phases were combined, dried over $MgSO_4$, and the solvent removed under vacuum. The residue was purified by column chromatography on silica gel (230–400 mesh) eluted with mixtures of hexane and ethyl acetate (9:1 and 8:2) to afford (+)-(*R*)-**1**.

*Chromatographic conditions employed in the GC separation of the enantiomers of the chiral organoseleno phenylethanols **1–3***

Chromatographic conditions were: injector temperature 220 °C; split ratio 1:20; detector temperature 220 °C; hydrogen carrier gas at 100 kPa and oven temperature program: (*RS*)-1-[(4-methylselanyl)phenyl]ethanol (**1**) – GC conditions: 150 °C for 30 min, then rising to 180 °C at 1 °C/min; retention time: *R*-isomer 17 min, *S*-isomer 18 min; (*RS*)-1-[(4-ethylselanyl)phenyl]ethanol (**2**) – GC conditions: 150 °C for 30 min, then rising to 180 °C at 1 °C/min; retention time: *R*-isomer 13 min, *S*-isomer 14 min; and (*RS*)-1-[(4-phenylselanyl)phenyl]ethanol (**3**) – GC conditions: 150 to 180 °C at 2 °C/min, retention time: *R*-isomer 34 min, *S*-isomer 35 min.

Results and Discussion

Microbial deracemizations of the organoseleno phenyl alcohols (*RS*)-**1**, (*RS*)-**2**, and (*RS*)-**3** (Scheme 1) were studied using buffer solutions containing whole cells of *A. terreus* URM 3371 and *A. terreus* CCT 4083. The reactions were performed varying the experimental conditions (pH, temperature, mass of fungal cells and additives). Initially, the reactions were carried out by incubating the (*RS*)-organoseleno alcohols **1–3** with 1–5 g of whole cells of *A. terreus* URM 3371 at pH=4 and at 32 °C in the presence of ethanol as an additive for a reaction period of up to 10 days. For all experimental conditions investigated, the compound (*RS*)-**3** was inactive when in contact with the whole cells of *A. terreus*. The most efficient deracemization was achieved with compound (*RS*)-**1**. When 2–5 g of whole fungal cells were employed, (+)-(*R*)-**1** was obtained in high conversion rate and selectivity (entries 6–15 for compound **1** in Table 1 and Fig. 1). In contrast, (*RS*)-**2** was not efficiently deracemized by *A. terreus* URM 3371 under the employed conditions, the organoseleno acetophenone **2a** being the main product (entries 6, 8–9, 11–12, 14 and 15 for compound **2** in Table 1), while (*S*)-**2** was obtained only with modest selectivity.

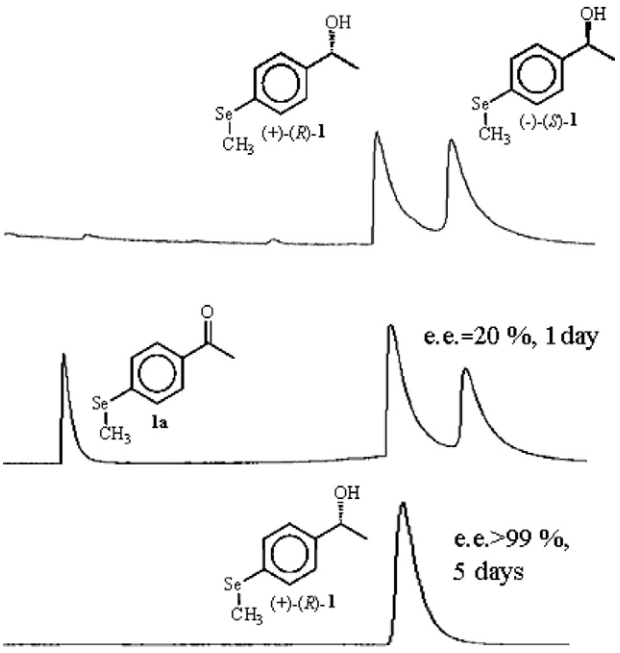

Fig 1. GC chromatograms showing the progress of the deracemization of (*RS*)-1-[(4-methylselanyl)phenyl]ethanol (**1**) by whole cells of *Aspergillus terreus* URM 3371 (2 g) at pH=4 and 32 °C; e.e. enantiomeric excess

In the light of these preliminary results, the biotransformation of (*RS*)-**1** and (*RS*)-**2** was performed at 32 °C using the two fungal strains, *A. terreus* URM 3371 and *A. terreus* CCT 4083, in buffer solutions at pH=4 and 7 (Table 2). The most efficient conversions were obtained with *A. terreus* URM 3371 using (*RS*)-**1** as substrate (entries 1–6 for substrate **1** in Table 2). Under these conditions, (*RS*)-**1** was deracemized to yield (+)-(*R*)-**1** in high optical purity (e.e.≥98 %) at both pH, 4 and 7. Appreciably lower deracemization of (*RS*)-**1** was obtained using *A. terreus* CCT 4083, particularly under neutral conditions (entries 10–12 for substrate **1** in Table 2), the major product being the *p*-(organoseleno)acetophenone **1a**. The microbial deracemization of (*RS*)-**2** by *A. terreus* URM 3371 led to the formation of (*S*)-**2** with low selectivity

Table 1. Deracemization of (*RS*)-1-[(4-methylselanyl)phenyl]ethanol (**1**) and (*RS*)-1-[(4-ethylselanyl)phenyl]ethanol (**2**) by whole cells of *Aspergillus terreus* URM 3371 at pH=4 and 32 °C in the presence of 200 μL of ethanol

Entry	*m*(fungal cell)/g	*t*/ day	Reaction products with 5 mg of compound **1**				Reaction products with 5 mg of compound **2**			
			1a	**1**			**2a**	**2**		
			c^a/%	c^a/%	e.e./%	A.C.	c^a/%	c^a/%	e.e./%	A.C.
1		3	–	–	–	–	12	88	17	*S*
2	1	5	15	85	41	*R*	48	52	20	*S*
3		10	19	81	56	*R*	26	74	10	*S*
4		3	42	58	47	*R*	63	37	19	*S*
5	2	5	72	28	98	*R*	44	56	18	*S*
6		10	17	83	98	*R*	89	11	30	*S*
7		3	11	89	30	*R*	40	60	2	*S*
8	3	5	11	89	99	*R*	74	26	42	*S*
9		10	0	98	99	*R*	79	21	60	*S*
10		3	0	98	98	*R*	55	45	14	*S*
11	4	5	0	98	98	*R*	72	28	9	*S*
12		10	0	98	98	*R*	81	19	53	*S*
13		3	0	98	99	*R*	57	43	28	*S*
14	5	5	0	98	99	*R*	70	30	9	*S*
15		10	0	98	99	*R*	98	0	0	–

[a]concentration determined by GC analysis
e.e.=enantiomeric excess
A.C.=absolute configuration

Table 2. Deracemization of (*RS*)-1-[(4-methylselanyl)phenyl]ethanol (**1**) and (*RS*)-1-[(4-ethylselanyl)phenyl]ethanol (**2**) by whole cells (2 g) of *Aspergillus terreus* at 32 °C in the presence of 200 μL of ethanol

Entry	pH	*t*/ day	Reaction products with 5 mg of compound **1**				Reaction products with 5 mg of compound **2**			
			1a	**1**			**2a**	**2**		
			c^a/%	c^a/%	e.e./%	A.C.	c^a/%	c^a/%	e.e./%	A.C.
Aspergillus terreus URM 3371										
1		3	0	98	99	*R*	43	57	13	*S*
2	4	5	8	92	99	*R*	62	38	38	*S*
3		10	0	99	99	*R*	82	18	18	*S*
4		3	40	60	56	*R*	65	35	44	*S*
5	7	5	0	98	99	*R*	77	23	24	*S*
6		10	0	98	99	*R*	99	0	0	–
Aspergillus terreus CCT 4083										
7		3	45	55	56	*R*	33	67	2	*R*
8	4	5	60	40	98	*R*	70	30	46	*S*
9		10	81	19	98	*R*	88	12	99	*S*
10		3	75	25	99	*R*	99	0	0	–
11	7	5	80	20	65	*R*	90	10	12	*R*
12		10	82	28	49	*R*	98	0	0	–

[a]concentration determined by GC analysis
e.e.=enantiomeric excess
A.C.=absolute configuration

and was accompanied, under both acidic and neutral conditions, by high levels of conversion to the ketone **2a** (Table 2). With *A. terreus* CCT 4083, a low degree of deracemization of (*RS*)-**2** was observed at both pH (4 and 7), and **2a** was the major product (entries 7–12 for substrate **2** in Table 2).

The deracemization of (*RS*)-**1–2** was performed using *A. terreus* URM 3371 in the presence of other additives, namely propanol, butanol, and cyclohexanol, and at different temperatures (20 and 32 °C). Under these conditions the deracemization of alcohol (*RS*)-**2** was not efficient, the organoseleno acetophenone **2a** being observed in high concentration (Table 2). Under the same conditions (*RS*)-**1** gave (+)-(*R*)-**1**, but with low enantioselectivity in most cases (Table 3). It is concluded that the presence of the additives promoted preferentially the oxidation of the organoseleno phenylethanols to the corresponding acetophenones.

Since the most efficient deracemization of (*RS*)-organoseleno alcohols was obtained when whole cells of *A. terreus* URM 3371 were used with (*RS*)-**1** as substrate, the reaction was carried out on a preparative scale in which 15 g of wet fungal cells were incubated at 32 °C, pH=4.0, with 100 μL of **1** and 600 μL of ethanol. After 18 days, (+)-(*R*)-1-[(4-methylselanyl)phenyl]ethanol could be isolated in 53 % yield (0.0163 mmol/g of biomass) and the product exhibited an e.e.>98 %.

Table 3. Deracemization of (*RS*)-1-[(4-methylselanyl)phenyl]ethanol (**1**) by whole cells (2 g) of *Aspergillus terreus* URM 3371 at pH=4 in the presence of 200 μL of butanol, propanol or cyclohexanol

Entry	Additive	*t*/day	Products of the reaction at 32 °C[a]				Products of the reaction at 20 °C[a]			
			1a	**1**			**1a**	**1**		
			c^b/%	c^b/%	e.e./%	A.C.	c^b/%	c^b/%	e.e./%	A.C.
1		3	37	67	47	*R*	14	86	12	*R*
2	Butanol	5	46	54	25	*R*	28	72	23	*R*
3		10	45	55	99	*R*	59	41	56	*R*
4		3	43	57	17	*R*	–	–	–	–
5	Propanol	5	27	73	32	*R*	–	98	54	*R*
6		10	–	–	–	–	17	83	51	*R*
7		3	–	98	4	*R*	–	98	8	*R*
8	Cyclo-hexanol	5	–	98	17	*R*	–	98	6	*R*
9		10	–	–	–	–	–	98	55	*R*

[a]The mass of the substrate **1** employed was 5 mg
[b]concentration determined by GC analysis
e.e.=enantiomeric excess
A.C.=absolute configuration

Conclusions

This study demonstrated that fungal strains of *A. terreus*, isolated from different provenances in Brazil, offer a potential for deracemization of (*RS*)-organoseleno alcohols. Under specific conditions, whole cells of *Aspergillus terreus* catalysed the deracemization of (*RS*)-1-[(4--methylselanyl)phenyl]ethanol (**1**) to give an acceptable yield of (+)-(*R*)-**1** with high enantiomeric excess (>98 %). The deracemization reactions were sensitive to pH, acidic medium providing the best conversion. The presence of additives promoted mainly the oxidation of the secondary alcohols.

Acknowledgements

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