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ORIGINAL ARTICLE

Biotransformation of α-Bromoacetophenones by the Marine Fungus *Aspergillus sydowii*

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Abstract The biotransformation reactions of α-bromoacetophenone (**1**), *p*-bromo-α-bromoacetophenone (**2**), and *p*-nitro-α-bromoacetophenone (**3**) by whole cells of the marine fungus *Aspergillus sydowii* Ce19 have been investigated. Fungal cells that had been grown in artificial sea water medium containing a high concentration of chloride ions (1.20 M) catalysed the biotransformation of **1** to 2-bromo-1-phenylethanol **4** (56%), together with the α-chlorohydrin **7** (9%), 1-phenylethan-1,2-diol **9** (26%), acetophenone **10** (4%) and phenylethanol **11** (5%) identified by GC-MS analysis. In addition, it was observed that the enzymatic reaction was accompanied by the spontaneous debromination of **1** to yield α-chloroacetophenone **5** (9%) and α-hydroxyacetophenone **6** (18%) identified by GC-FID analysis. When **2** and **3** were employed as substrates, various biotransformation products were detected but the formation of halohydrins was not observed. It is concluded that marine fungus *A. sydowii* Ce19 presents potential for the biotransformations of bromoacetophenone derivatives.

Keywords Marine fungus · *Aspergillus sydowii* · α-Bromoacetophenone · Biotransformation

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Introduction

Halohydrins are valuable intermediates in the synthesis of various important pharmaceutical products, including β-receptor agonists and bronchodilators (Patel et al. 1998; Wei et al. 1998; Antunes et al. 2004; Lagos et al. 2004; Zhu et al. 2005), and novel methods for their preparation are of considerable interest. In this context, a number of reports have recently become available concerning the application of biocatalysts, especially in the form of whole cells of microorganisms, to the reduction of ketones to yield chiral alcohols with high enantioselectivity (Goswami et al. 2000, 2001; Chartrain et al. 2002; Rodrigues et al. 2004; Goldberg et al. 2007). Although the microbial reduction of prochiral acetophenone-derivatives generally affords alcohols in good yield, the stereochemical course of the reaction may be influenced by the group linked to the carbonyl group as well as by selective oxidation of the alcohol formed or by selective reduction of the prochiral ketone.

Few studies have been carried out relating to enzymatic methods for the asymmetric reduction of α-haloketones, and in most cases the enantioselectivity of the reaction was found to be modest. Thus, α-bromoacetophenone was reduced to (*R*)-2-bromo-1-phenylethanol by *Rhodotorula rubra* in a moderate yield of 70% and with 61% enantiomeric excess (ee; De Carvalho et al. 1991).

Additionally, baker's yeast (*Saccharomyces cerevisiae*) is able to reduce α-fluoro- and α-chloro-acetophenones into halohydrins in reasonable chemical and optical yields (Aleixo et al. 1993; Basavaiah et al. 2006). Additionally, several chemical synthetic methodologies for the production of bromohydrins have been described (Basavaiah et al. 2006, 2007). However, these studies do not describe the transformation of bromohydrins in other products, which has been obtained in excellent yields and high optical purities.

As a part of an ongoing programme aimed at screening microorganisms found in the tropical rainforests of Brazil for their potential use as biocatalysts in organic chemistry (Cagnon et al. 1999; Assis et al. 2007; Piovan et al. 2007), we have recently demonstrated the reduction of α-bromoacetophenones by terrestrial fungi (Andrade et al. 2006). In the present study, the biotransformation reactions of substituted α-bromoacetophenones catalysed by whole cells of the marine fungus *Aspergillus sydowii* Ce19 have been investigated.

Materials and Methods

General Methods

α-Bromoacetophenone (**1**), *p*-bromo-α-bromoacetophenone (**2**) and *p*-nitro-α-bromoacetophenone (**3**) were purchased from Sigma-Aldrich. All manipulations involving the fungus *A. sydowii* Ce19 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 biotransformation experiments. Extracts of reaction mixtures were analysed using a Hewlett Packard model HP-5890 (FID) gas chromatograph equipped with a Varian CP-Chirasil-DEX CB column (25 m×0.25 mm i.d.; 0.39 μm). The chromatographic conditions were: oven temperature initially at 120°C and increased to 165°C at 2°C/min; run time 32.5 min; injector temperature 200°C; detector temperature 200°C; injector split ratio 1:20; and carrier gas hydrogen at a pressure of 60 kPa. The enantiomeric excesses of 2-bromo-1-phenylethanol (**4**) were determined by GC analyses based on retention times of 15.0 and 15.6 min for the (*S*)- and (*R*)-enantiomers, respectively. Gas chromatography–mass spectrometry: a Shimadzu GC2010plus gas chromatography system coupled to a mass selective detector (Shimadzu MS2010plus) in electron ionisation (EI) mode was used. The GC-MS oven was fitted with a DB5 fused silica column (J&W Scientific 30m × 0.25mm × 0.25). The oven temperature was programmed from 50°C to 250°C at an increasing rate of 5°C/min. The injector and detector temperatures were maintained at 200°C; injector split ratio was 1:20 and helium was used as the carrier gas at a pressure of 60 kPa. The identities of the compounds (Figs. 1 and 2) were identified by GC and GC-MS analysis. The structures were confirmed

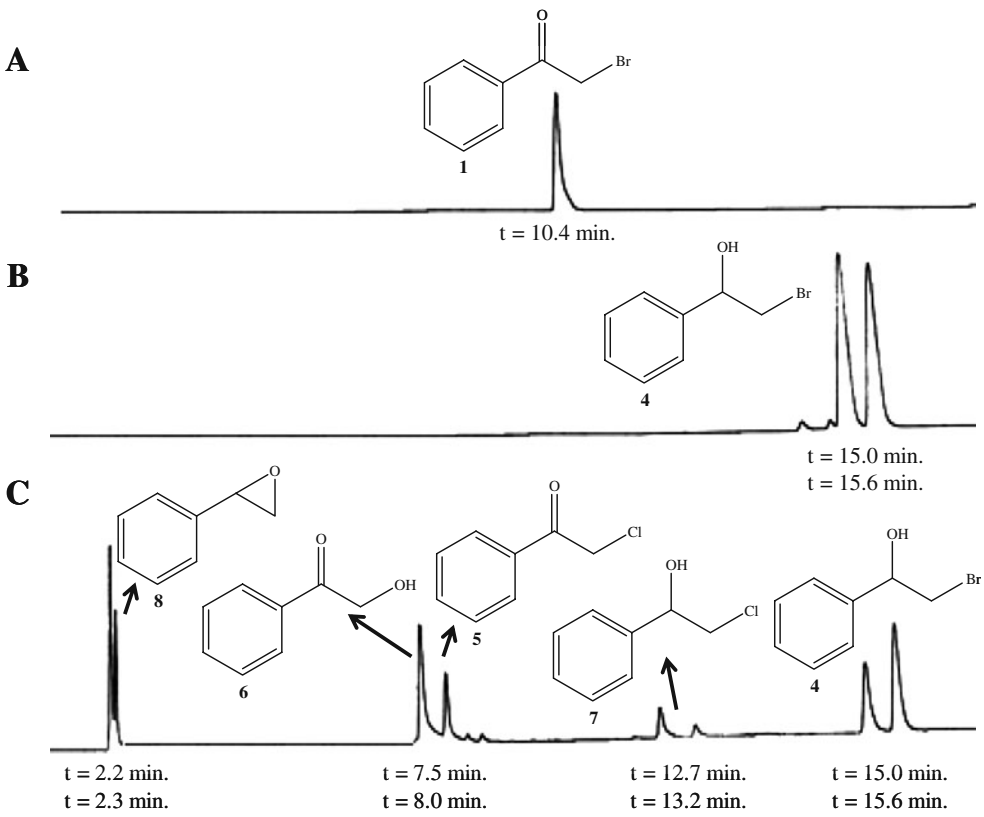


Fig. 1 GC-FID chromatograms of **a** α-bromoacetophenone (**1**), **b** (±)-2-bromo-1-phenylethanol (**4**), and **c** total extract of the reaction mixture following incubation of **1** with *A. sydowii* Ce19 (5 g of wet whole cells; 100 mL of phosphate buffer; 50 mg of **1**; 100 μL of DMSO; incubation conditions—150 rpm, 32°C, 3 days). Concentrations: **4** (41%), **5** (9%), **6** (18%), **7** (7%) and **8** (25%)

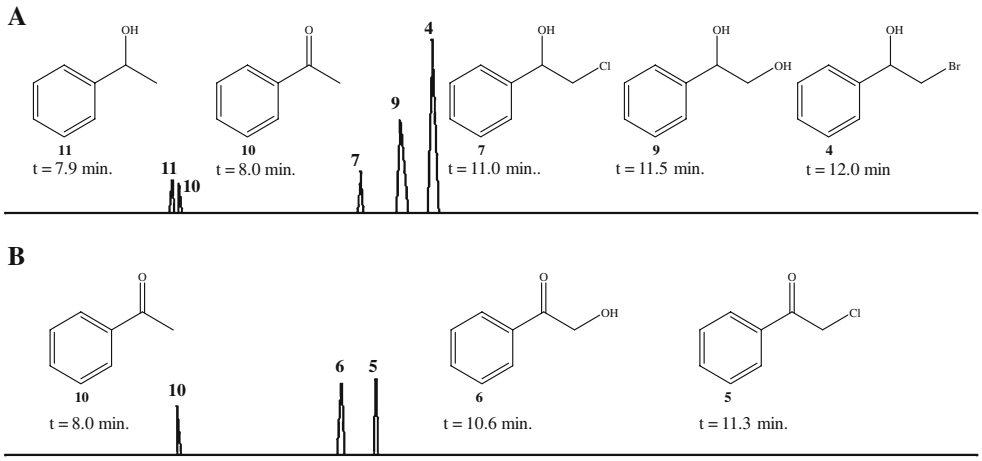


Fig. 2 GC-MS TIC chromatograms of **a** the biotransformation products of α-bromoacetophenone (**1**) with whole cells of *A. sydowii* Ce19 (5 g of wet whole cells; 100 mL of buffer phosphate; 50 mg of **1**; 100 μL of DMSO; incubation conditions - 150 rpm, 32°C, 3 days). Concentrations: **4** (56%), **7** (9%), **9** (26%), **10** (4%) and **11** (5%). (**b**) total extract of the reaction mixture following incubation of α-bromoacetophenone (**1**) in filtered culture medium of *A. sydowii* Ce19 (100 mL filtered culture medium; 50 mg of **1**; 100 μL of DMSO; incubation conditions—150 rpm, 32°C, 3 days). Concentrations: **5** (32%), **6** (52%), **10** (16%)

after comparison with Mass Spectral Database (CLASS-5000/WILEY) and by injection of authentic standards. Reaction products were purified by column chromatography over silica gel (230–400 mesh) eluted with mixtures of *n*-hexane and EtOAc (9:1 and 8:2). Column effluents were monitored by TLC using aluminium-backed pre-coated silica gel 60 F_{254} layers (Sorbent Technologies) eluted with *n*-hexane:EtOAc (8:2).

Culture of *A. sydowii* Ce19

The marine fungus *A. sydowii* Ce19 was isolated from a specimen of the sponge *Chelonaplysylla erecta* collected off the coast at São Sebastião, São Paulo, Brazil, by R.G.S. B. Isolation of the fungus was performed at the Centro de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA) Universidade Estadual de Campinas (UNICAMP), Brazil (http://www.cpqba.unicam.br/cpqba_folder_ing.pdf).

Culture Media

Artificial sea water contained (per litre): $CaCl_2{\cdot}2H_2O$ (1.36 g), $MgCl_2{\cdot}6H_2O$ (9.68 g), KCl (0.61 g), NaCl (30.0 g), Na_2HPO_4 (0.014 mg), Na_2SO_4 (3.47 g), $NaHCO_3$ (0.17 g), KBr (0.1 g), $SrCl_2.6H_2O$ (0.040 g) and H_3BO_3 (0.030 g). Stock cultures of the marine microorganism were stored on solid culture medium (malt extract 30 g/L, agar 15 g/L and soya peptone 3 g/L in artificial sea water adjusted to pH 8 by the addition of 3 M KOH) in Petri dishes maintained at room temperature or at 4°C in the refrigerator. For liquid cultures, the medium described above was employed but without agar.

Biotransformation of α-Bromoacetophenones **1**–**3** by Marine Fungus *A. sydowii* Ce19

Small slices of solid medium (0.5×0.5 cm) bearing mycelia of *A. sydowii* were cut from the stock solid culture and inoculated into liquid culture medium (1 L) contained in 2-L Erlenmeyer flasks. The fungal mycelia were incubated at 32°C for 5 days on an orbital shaker (150 rpm) and harvested by Buchner filtration. The biocatalytic reactions were carried out in 250-mL Erlenmeyer flasks containing 5.0 g (wet weight) of mycelia and 50 mg of α-bromoacetophenone substrate **1**–**3** (dissolved in 100 μL of DMSO) and 100 mL of 0.1 M Na_2HPO_4/KH_2PO_4 buffer (pH 7). The reaction mixture was incubated for 1–4 days on an orbital shaker at 32°C (see Table 1). The progress of the reaction was assessed each day by collecting 2 mL of samples from the mixture, extracting with EtOAc (1.0 mL) and centrifuging at 6,000 rpm for 6 min. Extracts were subsequently analysed by GC-FID and GC-MS. All the biotransformation of α-bromoacetophenones **1**–**3** by marine fungus *A. sydowii* Ce19 were carried out in triplicate.

Synthesis of Standard Racemic Bromohydrin **4**

A standard of racemic **4** was obtained by reduction of the corresponding bromoketone **1** (100 mg; 0.54 mmol) with $NaBH_4$ (80 mg; 2 mmol) and methanol (10 mL; Pavia et al.

Table 1 Biotransformation of α-bromoacetophenone (**1**) by whole cells of marine fungus *Aspergillus sydowii* Ce19

Reaction time (h)	Content of **4** (%)[a]	Enantiomeric excess ee of **4** (%)	Absolute configuration of **4**
2	100	55	*R*
4	100	53	*R*
6	100	55	*R*
10	100	54	*R*
24	100	32	*R*
72	100	22	*R*
120	100	16	*R*

Reaction conditions: 5 g (wet wt) mycelia; 50 mg (0.25 mmol) of **1**, DMSO (100 μL) in phosphate buffer (pH 7) incubated at 32°C on an orbital shaker (150 rpm)

[a] Concentration determined by chiral GC-FID analysis

1999). The spectroscopy data (^{1}H-NMR, MS and IR) of **4** were in agreement with those reported in the literature (Goswami et al. 2000).

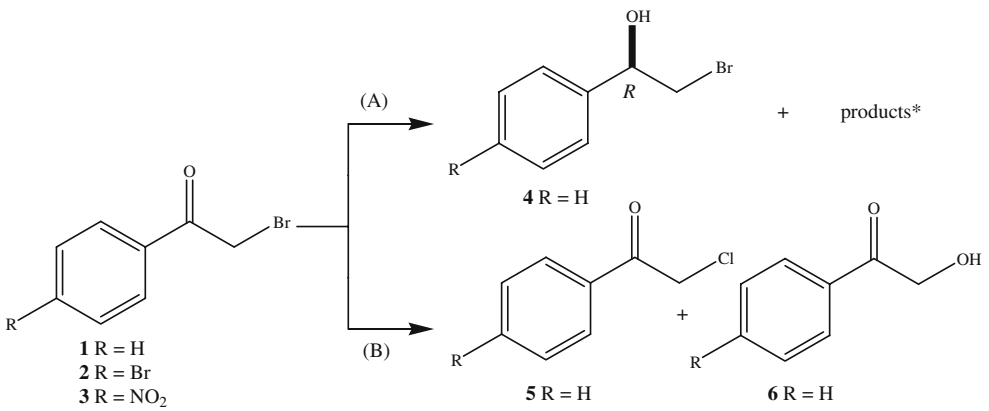
Results and Discussion

Recently, the asymmetric reduction, catalysed by whole cells of different marine fungi, of α-chloroacetophenone (**5**) to 2-chloro-1-phenylethanol (**7**) in good conversion and moderate enantioselectivities (17–66% ee) has been demonstrated (Rocha 2008, Rocha et al. 2009). In an attempt to improve the efficiency of this method, the biotransformations of the prochiral α-bromoacetophenone (**1**), *p*-bromo-α-bromoacetophenone (**2**) and *p*-nitro-α-bromoacetophenone (**3**) by the marine fungus *A. sydowii* Ce19 were investigated. The microorganism was grown in artificial sea water medium containing a high concentration of chloride ions (1.20 M), and the biocatalytic reactions were performed with 5 g of humid whole fungal cells and 50 mg (0.27 mmol) of α-bromoacetophenone (**1**) as substrate. In further experiments, each substrate was incubated with filtered culture medium, in the absence of fungal cells, under the same reaction conditions.

Resting fungal cells promoted the rapid reduction of α-bromoacetophenone (**1**) to form the bromohydrin **4** in high conversion (Table 1). However, the optical purity of the **4** was very moderate (55% ee) after 2 h of reaction and decreased throughout the incubation attaining a value of 16% after 120 h of reaction. In this case, racemisation occurred possibly due to several redox reactions. The absolute configuration was assigned by comparison of the sign of $[\alpha]_D^{25}$ determined for the (*R*)-bromohydrin **4**, i.e. −5.80° (*c* 0.2, $CHCl_3$; 55% ee), with that reported in the literature (Goswami et al. 2000).

The total extraction of the medium containing whole cells was carried out after 3 days of reaction with **1,** and subsequent GC analysis allowed the identification of other reaction products (Fig. 1; Scheme 1). The following compounds were identified: the bromohydrin **4** (41%; 22% ee), the α-chloroketone **5** (9%), α-hydroxyacetophenone (**6**; 18%), the chlorohydrin **7** (7%; 50% ee) and the oxirane **8** (25%; 30% ee). The halohydrins **4** and **7** were obtained through enzymatic reduction, whilst compounds **5**, **6** and **8** were produced by co-occurring (possibly by non-enzymatic) reactions. The identities of compounds **4**–**8** were determined from GC retention times and by injection of authentic standards.

In order to confirm the identities of the products, extracts obtained following incubation of **1** for 3 days with whole fungal cells of *A. sydowii* Ce19 or with filtered culture medium (in the absence of fungal cells) were analysed by GC-MS (Fig. 2). The results showed that the main product **4** was formed in 56% by enzymatic reduction, together with



Scheme 1 (*A*) Biotransformation of α-bromoacetophenones **1 - 3** by mycelia of *A. sydowii* Ce19 that had been cultured in artificial sea water. (*B*) Products obtained following reaction of α-bromoacetophenone (**1**) with artificial sea water. *Products (See Figs. 1 and 2)

the α-chlorohydrin **7** (9%), 1-phenylethan-1,2-diol **9** (26%), acetophenone **10** (4%) and phenylethanol **11** (5%), (Fig. 2a).

Incubation of **1** with filtered culture medium afforded the α-chloroketone **5** (32%) and the α-hydroxyketone **6** (52%), compounds that were formed by substitution of the bromo group of **1** by chloro or hydroxyl groups, and low concentrations of compound **10** (16%; Fig. 2b). The substitution reactions occurred because artificial sea water contains a high concentration of chloride ions (1.2 M). Thus, when **1** was incubated in the presence of artificial sea water and in the absence of any biocatalyst it was spontaneously transformed into **5** (80%) and **6** (20%; Scheme 1). In this way, the experiments with bromoacetophenones **1**–**3** were used only in the whole cells of marine fungus *A. sydowii*.

The transformations of **10** and **11** have been investigated in detail in a study of the reduction of α-iodoacetophenone by baker's yeast, and it appears that these compounds can be formed by a free radical chain process in living systems (De Carvalho et al. 1991; Aleixo et al. 1993). Moreover, freshwater and marine-sediments reportedly catalysed the reduction of α-chloroacetophenone (**5**) to acetophenone (**10**) and phenylethanol (**11**) via electron transfer (Smolen et al. 1999; Zhu et al. 2005). In the present study, therefore, it is possible that *A. sydowii* Ce19 may have catalysed the biotransformation of **1** by a free radical mechanism involving electron transfer to yield products **10** and **11**.

The association between reductase production and the conditions under which the marine microorganisms were cultured has been recently investigated (Rocha 2008, Rocha et al. 2009). When whole cells of *A. sydowii* Ce19 were grown in artificial sea water, they maintained the ability to catalyse the reduction of the carbonyl group of bromoketone **1**. However, fungal cells grown in medium containing low levels of chloride ions did not exhibit this catalytic activity, thus confirming previous findings relating to the enzymatic reduction of α-chloroacetophenone (**5**) (Rocha 2008, Rocha et al. 2009). It is concluded, therefore, that the role of artificial sea water in the growth of the fungus is very important for the formation of the reductases required to catalyse the reduction of bromoketone **1**.

The products obtained following the biotransformation of *p*-bromo-α-bromoacetophenone (**2**) and *p*-nitro-α-bromoacetophenone (**3**) by whole cells of *A. sydowii* Ce19 in phosphate buffer solution were different from those obtained using **1** as substrate. The presence of the α-bromo group, together with the electron-withdrawing effect of the bromo and nitro substituents at the *p*-position of the phenyl ring, were not favourable for the formation of bromohydrins mediated by *A. sydowii* Ce19. Possibly, the electron-withdrawing effect of the bromo and nitro groups at the *para*-position on the aromatic ring led to a dramatic degradation of the substrates/products affording a mixture of compounds.

Thus, although reaction with ketones **2** and **3** produced a mixture of products following enzymatic and/or spontaneous transformations, no halohydrins could be identified by either GC-FID or GC-MS analysis. Additionally, low yields of *p*-bromo- or *p*-nitro-acetophenones were detected by GC-MS. In these conditions, the substrates **2**–**3** were consumed affording a complex mixture of products at low concentrations and of difficult identification.

In conclusion, mycelia of the marine fungus *A. sydowii* Ce19 have been shown to catalyse the bioreduction of the α-bromoketone **1** to the bromohydrin **4** together with other enzymatic and spontaneous reaction products. The substituted α-bromoacetophenones **2** and **3** were not favourable substrates for asymmetric reduction by whole cells of *A. sydowii* Ce19. Whole cells of the marine fungus catalysed the biotransformation of α-bromoacetophenones **1**–**3** affording acetophenone, *p*-bromoacetophenone and *p*-nitroacetophenone at minor concentrations. *A. sydowii* Ce19 therefore presents potential for the biodegradation of bromoacetophenone derivatives.

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