

First Asymmetric Reduction of Isatin by Marine-Derived Fungi

Willian G. Birolli,^a Irlon M. Ferreira,^{a,b} David E. Q. Jimenez,^a Bianca N. M. Silva,^c
 Bárbara V. Silva,^c Angelo C. Pinto^{c,†} and André L. M. Porto^{*a}

^aLaboratório de Química Orgânica e Biocatálise, Instituto de Química de São Carlos, Universidade de São Paulo, Av. João Dagnone, Ed. Química Ambiental, J. Santa Angelina, 1100, 13563-120 São Carlos-SP, Brazil

^bGrupo de Biocatálise e Biotransformação em Química Orgânica, Colegiado de Química, Universidade Federal do Amapá, Rod. JK, KM 2, 68902-280 Macapá-AP, Brazil

^cInstituto de Química, Universidade Federal do Rio de Janeiro, 21949-900 Rio de Janeiro-RJ, Brazil

In this study, whole cells of marine-derived fungi were used to reduce isatin (1*H*-indole-2,3-dione) to dioxindole (3-hydroxyindolin-2-one) for 7 days at 32 °C. The screening showed that several strains could reduce isatin and produce the enantioenriched dioxindole. The best conversions were obtained by *Cladosporium* sp. CBMAI 1237 and *Westerdykella* sp. CBMAI 1679, however, the best enantiomeric excess was obtained only by *Aspergillus sydowii* CBMAI 935 (66% ee). In conclusion, marine-derived fungi show potential for asymmetric and chemoselective reduction of isatin (1*H*-indole-2,3-dione).

Keywords: whole cells, marine fungi, biocatalysis, chemoselective reaction

Introduction

Isatin (1*H*-indole-2,3-dione) and its derivatives were found in fungi, plants, animals and even in humans as a metabolic derivative of adrenaline.¹ It was first synthesized by Erdmann and Laurent in 1840 when these researchers reacted indigo with nitric and chromic acids.² In addition, diverse pharmacological properties were reported for isatin and its derivatives including anticancer, antioxidant, anti-histaminic, antiviral, anti-inflammatory, anti-Parkinson's, antidiabetic, antiallergic, antimalarial and antimicrobial activities.^{3,4}

The compound 3-hydroxyindolin-2-one is a derivative of the reduction of isatin and showed pharmacological potential to antiallergic, anti-inflammatory and anticancer

activities.⁴ In addition, they have been found in many bioactive natural products, such as convolutamydine A,⁵ flustraminol A⁶ and donaxaridine⁷ (Figure 1).

Few studies in the literature have focused on the biotransformation of isatin derivatives by whole cells. One example is the stereoselective reduction of 1*H*-indole-2,3-dione and analogues by *Candida parapsilosis*, which gives *R*-alcohol in good yields.⁸ More recently, the biotransformation of indole was carried out by a newly isolated KK10, member of the genus *Cupriavidus* that oxidize the *N*-heterocyclic ring of indole and ring cleavage through *N*-formylanthranilic acid.⁹ In another work, a biphenyl dioxygenase was cloned from *Dyella ginsengisoli* LA-4 and expressed in *Escherichia coli* for the biotransformation of indole to indigo.¹⁰

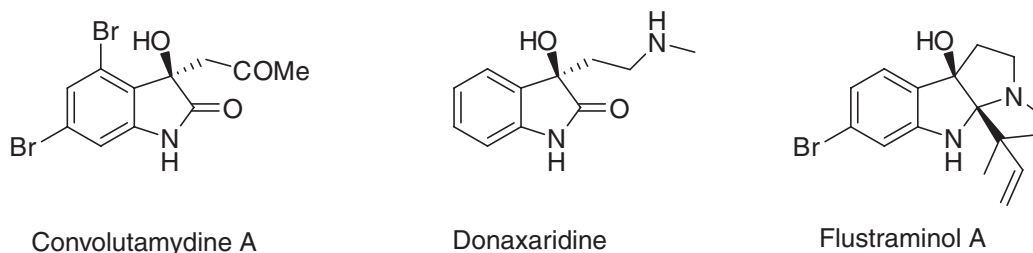


Figure 1. Examples of biologically active 3-substituted-3-hydroxyl-2-oxindoles.

*e-mail: alporto@iqsc.usp.br

†This paper is dedicated to the memory of our wonderful Professor Angelo da Cunha Pinto.

Marine-derived organisms are sources of diverse enzymes suitable for the biotransformation or biocatalytic reactions of organic compounds.¹¹ This enzymatic versatility is essential for the adaptation and robustness of their metabolism to extreme conditions observed in the ocean, such as low temperatures, extreme variation of pH conditions, high salt concentration, high pressure and changes in ocean currents.¹² In addition, several marine-derived fungi have been applied in the bioreduction of iodoacetophenones¹³ and azido-ketones,¹⁴ biohydrogenation of chalcones^{15,16} as well as α,β - and $\alpha,\beta,\gamma,\delta$ -unsaturated ketones,¹⁷ and ene-reduction of Knoevenagel compounds.¹⁸ In this study, the bioreduction of isatin **1** by whole cells of marine-derived fungi was performed to produce enantio-enriched 3-hydroxyindolin-2-one **2**.

Experimental

Reagents, solvents, salts, and culture media

Sodium sulfate (99%) was purchased from Labsynth. High-performance liquid chromatography (HPLC) solvents (hexane and isopropyl alcohol) were purchased from Panreac. Ethanol was purchased from Tedia Brazil. The other reagents (chloral hydrate, hydroxylamine sulfate, aniline, hydrochloric and sulfuric acids) were purchased from Vetec, and were used without further purification. The salts used to produce artificial seawater were purchased from Vetec and Synth. Malt extract was obtained from Acumedia. Deuterated chloroform was purchased from Cambridge Isotope Laboratories.

Synthesis of indoline-2,3-dione (**1**)

The synthesis of **1** was based on method of Sandmeyer modified by Marvel and Hiers.^{19,20}

First step: synthesis of isonitrosoacetanilide

A mixture of sodium sulfate (130 g, 0.915 mol), distilled water (120 mL), chloral hydrate ($C_2H_3Cl_3O_2$) (18 g, 0.109 mol) and hydroxylamine sulfate ($(NH_2OH)_2 \cdot H_2SO_4$) (13 g, 0.079 mol) were dissolved in 50 mL of distilled water. Subsequently, aniline (0.9 mL, 0.1 mol) and concentrated

hydrochloric acid (8.6 mL) were added to 60 mL of distilled water and heated in a round bottom flask to 70 °C. Next, 100 mL of ethyl alcohol was added to the reaction medium and kept under magnetic stirring and refluxed for about 4 hours. After this period, the mixture was poured into an ice bath, vacuum filtered and washed with distilled water. A beige solid (isonitrosoacetanilide) was obtained in 80% yield. The product was identified by spectroscopic data (1H and ^{13}C nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC-MS), see Supplementary Information).

Second step: synthesis of indoline-2,3-dione (**1**)

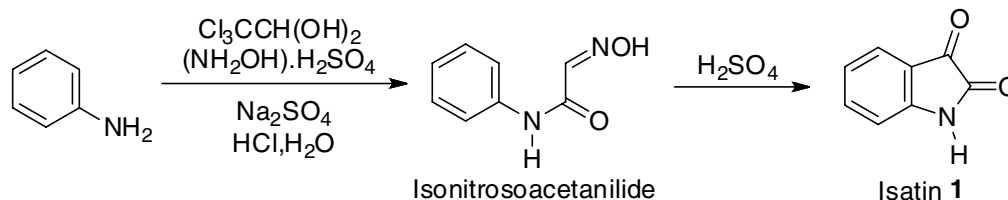
A round bottom flask was charged with isonitrosoacetanilide (3 g, 18.3 mmol) and sulfuric acid (9 mL), which was slowly added in portions over 15 min. After the addition, the mixture was magnetically stirred at room temperature for an additional 10 minutes. The reaction mixture was cooled to room temperature and subsequently poured on ice. The solid product was isolated by vacuum filtration, and extensively washed with ice-water (3×200 mL). An orange-red crystalline solid was obtained in 78% yield. The product was identified by spectroscopic data (1H NMR, ^{13}C NMR and GC-MS, see Supplementary Information). Scheme 1 shows a synthesis of isatin **1** from aniline (first and second steps).

Synthesis of standard racemic alcohol **2**

Ethanol (40 mL) and isatin **1** (10 mmol, 1.47 g) were added to a 25 mL flask equipped with a magnetic stirrer and a PARR hydrogenator at 35 psi. Subsequently, the mixture was stirred for 1 h at room temperature, filtered, and the solvent was evaporated under reduced pressure. A beige solid was obtained in 92% yield. The compound did not require further purification. The product was identified by spectroscopic data (1H NMR, ^{13}C NMR and GC-MS, see Supplementary Information).

Marine-derived fungi

The marine-derived fungi were isolated from marine sponges at São Sebastião, coast of São Paulo State,



Scheme 1. Synthesis of isatin **1** employing Sandmeyer's method.

Brazil, by Roberto G. S. Berlinck (Instituto de Química de São Carlos, Universidade de São Paulo, IQSC-USP) and purified at the Department of Ecology and Aquatic Microbiology by Mirna H. R. Selegim (Universidade Federal de São Carlos, UFSCar).

Strains of marine-derived fungi were identified at the Chemical, Biological and Agricultural Multidisciplinary Research Center (CPQBA/UNICAMP, Brazil) and deposited in the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI, <http://www.cpqba.unicamp.br/colecoes/2015-06-10-19-50-09.html>). The isolated strains were deposited as *Mucor racemosus* CBMAI 847, *Penicillium miczynskii* CBMAI 930, *Aspergillus sydowii* CBMAI 935, *Cladosporium* sp. CBMAI 1237, *Acremonium* sp. CBMAI 1676, *Trichoderma harzianum* CBMAI 1677, *Westerdykella* sp. CBMAI 1679, *Aspergillus* sp. CBMAI 1829, *Fusarium* sp. CBMAI 1830, and *Trichoderma* sp. CBMAI 932.

Microorganisms cultivation in solid culture medium

The marine-derived strains were cultivated in malt 2% medium composed of malt extract (20 g L⁻¹) and agar (20 g L⁻¹) in solid medium with artificial seawater (ASW). The ASW composition was: CaCl₂·2H₂O (1.3 g L⁻¹), MgCl₂·6H₂O (9.68 g L⁻¹), KCl (0.61 g L⁻¹), NaCl (30.0 g L⁻¹), Na₂HPO₄ (0.014 mg L⁻¹), Na₂SO₄ (3.47 g L⁻¹), NaHCO₃ (0.17 g L⁻¹), KBr (0.1 g L⁻¹), SrCl₂·6H₂O (0.040 g L⁻¹) and H₃BO₃ (0.030 g L⁻¹).¹⁵ The pH was adjusted to 8 with a solution of KOH (0.1 mol L⁻¹), and the culture medium was sterilized in an autoclave (121 °C, 20 min, 1.5 kPa). The strains were preserved in a refrigerator at 4 °C.

Bioreduction of isatin **1** by whole cells of marine-derived fungi in liquid culture medium of malt extract

An initial screening with 10 fungal strains was performed for the biotransformation of isatin **1** to dioxindole **2**. The microorganisms were grown in a Petri dish on a solid medium of 2% malt extract for 5 days. Small disks with 5 mm diameter from the mycelial colony were transferred to 125 mL Erlenmeyer flasks containing 50 mL of 2% malt liquid medium. The flasks were incubated in an orbital shaker (130 rpm, 32 °C) for 5 days. After that, 50 mg (0.35 mmol) of **1** dissolved in 100 µL of DMSO was added to each culture, and the reaction was incubated in an orbital shaker over 7 days (130 rpm, 32 °C). After the reaction period, the culture was filtered with a Buchner apparatus, and the mycelial mass obtained was suspended in 50 mL of water and ethyl acetate (1:1). The mixture was

vigorously stirred magnetically for 30 minutes and filtered again with a Buchner funnel.

The supernatant was extracted over three steps with ethyl acetate. The organic phase was evaporated under vacuum, dried with anhydrous sodium sulfate and dissolved in a 10 mL volumetric flask with ethyl acetate HPLC grade. The screening of isatin **1** bioreduction of used GC-MS analysis to evaluate the strain efficiency.

Bioreduction of isatin **1** by whole cells of *Acremonium* sp. CBMAI 1676 and *Aspergillus* sp. CBMAI 1829 in phosphate buffer solution

The mycelia of the fungi *Acremonium* sp. CBMAI 1676 and *Aspergillus* sp. CBMAI 1829 were harvested by Buchner filtration and suspended in 100 mL phosphate buffer solution (Na₂HPO₄/KH₂PO₄, pH = 7; 0.1 mol L⁻¹) in Erlenmeyer flasks (250 mL). The biocatalytic reductions were carried out with 5.0 g (wet weight) of mycelium and 50 mg (0.35 mmol) of isatin **1**, which was added in dimethylsulfoxide solution (100 µL). The reactions were incubated in an orbital shaker at 32 °C and 130 rpm and monitored with GC-MS analysis after 3, 6 and 9 days. The obtained extracts were purified using flash column chromatography (CC) over silica gel eluting with *n*-hexane and ethyl acetate (1:1). The enantiomeric excesses (*ee*) were measured by chiral HPLC analysis.

Analyses

GC-MS analysis

GC-MS analyses were performed in a Shimadzu GC2010plus coupled to a mass selective detector (Shimadzu MS2010plus) in electron ionization (EI, 70 eV) mode. The GC-MS was equipped with a chromatography column type DB5 (30 m × 0.25 mm × 0.25 µm, J&W Scientific). The conditions were: oven temperature started at 90 °C for 4 min, increased to 280 °C at 6 °C min⁻¹ and held for 6 min; injector and interface temperature was maintained at 250 °C; splitless 1 µL injection; helium was used as the carrier gas at a constant flow 0.75 mL min⁻¹, and the run time was 30 min. The scan range was *m/z* 40-500. Retention times for the indoline-2,3-dione **1** and 3-hydroxyindolin-2-one **2** were 19.5 and 17.5 min, respectively.

HPLC analysis

High pressure liquid chromatography analyses were conducted in Shimadzu high pressure liquid chromatographic system composed of LC-20AT pump, DGU-20A5 degasser, SIL-20AHT sampler, SPD-M20A UV-VIS detector operating in 248 nm, CTO-20A column oven and CBM-20A controller. The column was a Chiracel

OD-H column (0.46 × 25 cm; 5 μm), and the elution was carried out in isocratic mode with hexane and isopropanol (85:15) at a flow rate of 0.5 mL min⁻¹. The total analysis time was 35 min. The retention time for the (*R*)-enantiomer **2** was 9.2 min and 9.8 min for the (*S*)-enantiomer **2**.

¹H and ¹³C NMR analysis

NMR experiments used a Bruker 200 or 500 MHz spectrometer with CDCl₃ and DMSO-*d*₆ as solvents, and TMS as the internal standard unless otherwise noted. The chemical shifts are given in ppm and coupling constants (*J*) in Hz.

Optical rotation analysis

Optical rotation was measured in methanol with a JASCO P2000 polarimeter equipped with a Na lamp (589 nm) and a 1 dm cuvette. The absolute configurations for the enantiomerically enriched compound **2** were determined by comparing their specific optical rotation values with the corresponding values reported in the literature.

The optical rotation of compound **2**: for *Acremonium* sp. CBMAI 1676 (Table 2), (–)-(*S*)-3-hydroxyindolin-2-one **2**, [α]_D²⁵ –14.6 (*c* 0.4, methanol, 52% *ee*); Lit.²¹ [α]_D²⁵ –35.5 (*c* 2.1, acetone, 99% *ee*). For *Westerdykella* sp. CBMAI 1679 (Table 1), (+)-(*R*)-3-hydroxyindolin-2-one **2**, [α]_D²⁵ +5.3 (*c* 0.4, methanol, 18% *ee*); Lit.⁸ [α]_D²⁵ +7 (*c* 1.0, methanol, > 99% *ee*).

Results and Discussion

Screening of marine-derived fungi for reduction of isatin **1**

The screening of marine-derived fungi was carried out with GC-MS analyses considering the conversion of isatin **1** to dioxindole **2** (Table 1, entries 1-10). The strains *M. racemosus* CBMAI 847, *A. sydowii* CBMAI 935 and *Fusarium* sp. CBMAI 1830 had low conversions of 2, 9 and 4%, respectively. They did not convert the substrate efficiently and thus are not good biocatalysts for this compound (Table 1, entries 1-3). However, a good enantiomeric excess was observed with whole cells of *A. sydowii* CBMAI 935 (Table 1, entry 2), which showed the best enantioselectivity in the reduction of isatin **1** to (*R*)-3-hydroxyindolin-2-one **2** (66% *ee*).

T. harzianum CBMAI 1677, *Cladosporium* sp. CBMAI 1237 and *Westerdykella* sp. CBMAI 1679 showed better conversion to dioxindole **2**, but low enantiomeric excess (1-18% *ee*) (Table 1, entries 4-6). Whereas *P. miczynskii* CBMAI 930, *Trichoderma* sp. CBMAI 932, *Aspergillus* sp. CBMAI 1829 and *Acremonium* sp. CBMAI 1676 showed modest selectivities (42-48% *ee*) (Table 1, entries 7-10).

The 3-hydroxyindolin-2-one **2** with *R* or *S*-configuration was obtained by bioreduction of isatin **1**, but the structure of isatin **1** is not predictable via the empirical Prelog rule²² due to the attack of the hydride ion by the carbonyl group. Thus the enantioenriched alcohol with *R* or *S*-configuration was obtained according to the employed microorganism strain.

The strains with satisfactory conversion to dioxindole **2** and good enantioselectivity were *Aspergillus* sp. CBMAI 1829 and *Acremonium* sp. CBMAI 1676. These fungi were selected for further reduction experiments (Table 1, entries 9-10).

Bioreduction of isatin **1** by *Acremonium* sp. CBMAI 1676 and *Aspergillus* sp. CBMAI 1829 in malt 2% medium

The strains *Acremonium* sp. CBMAI 1676 and *Aspergillus* sp. CBMAI 1829 were employed in reactions containing 100 mL of liquid medium (experiments with 50 mL of liquid culture medium were used in the screening) for 3, 6 and 9 days (Table 2).

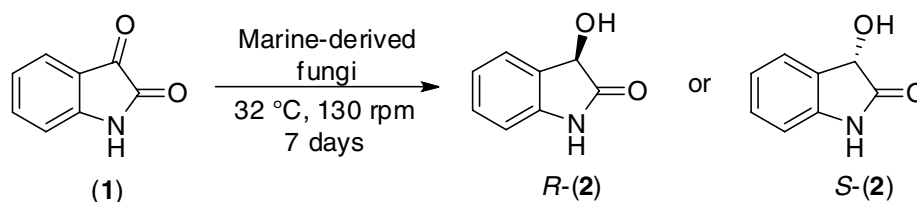
Aspergillus sp. CBMAI 1829 had conversion values of 39, 34, 19% for 3, 6 and 9 days, respectively (Table 2, entries 1-3). Same pattern was observed for the reduction by the strain *Acremonium* sp. CBMAI 1676, which showed conversions of 35, 30 and 26% for 3, 6 and 9 days of reaction, respectively (Table 2, entries 4-6). Thus, the conversion decreased during the reaction with *Aspergillus* sp. CBMAI 1829 and *Acremonium* sp. CBMAI 1676 proving that dioxindole **2** is produced by the reduction of isatin **1**. However, it is also consumed by these strains and consequently reducing the yield. This probably happens because the biotransformation of **2** by these strains is faster than isatin **1** reduction at this point of the reaction.

The enantiomeric excess of **2** increases to 42 and 52% after 9 days of reaction for *Aspergillus* sp. CBMAI 1829 and *Acremonium* sp. CBMAI 1676, respectively (Table 2, entries 3 and 6). This increase in the *ee* of **2** can be explained by an enantioselective biotransformation of this compound to another product.

The dioxindole **2** produced in the 3 days reactions was isolated by CC and 37% yield with enantiomeric excess of 38% for *Aspergillus* sp. CBMAI 1829, and 26% yield with 32% *ee* for *Acremonium* sp. CBMAI 1676 (Table 2, entries 1 and 4).

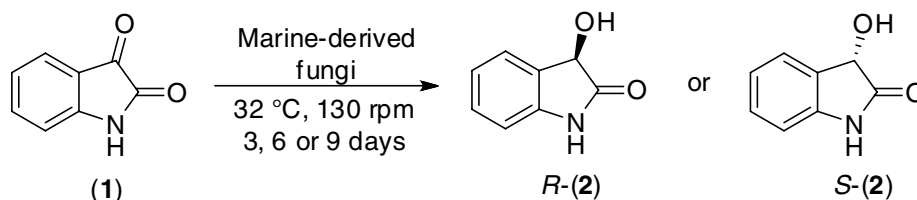
Bioreduction of isatin **1** by resting cells of *Acremonium* sp. CBMAI 1676 and *Aspergillus* sp. CBMAI 1829 in phosphate buffer

The reactions employing growing cells showed moderate biotransformation of **1** to product **2**. Therefore,

Table 1. Screening of marine-derived strains for reduction of isatin **1** to dioxindole **2**

entry	Fungi strains	Conversion ^a / %	ee ^b / %	AC
1	<i>M. racemosus</i> CBMAI 847	2	30	<i>S</i>
2	<i>A. sydowii</i> CBMAI 935	9	66	<i>R</i>
3	<i>Fusarium</i> sp. CBMAI 1830	4	5	<i>S</i>
4	<i>T. harzianum</i> CBMAI 1677	70	1	<i>R</i>
5	<i>Cladosporium</i> sp. CBMAI 1237	89	4	<i>S</i>
6	<i>Westerdykella</i> sp. CBMAI 1679	89	18	<i>R</i>
7	<i>P. miczynskii</i> CBMAI 930	83	42	<i>S</i>
8	<i>Trichoderma</i> sp. CBMAI 932	72	45	<i>S</i>
9	<i>Aspergillus</i> sp. CBMAI 1829	67	46	<i>S</i>
10	<i>Acremonium</i> sp. CBMAI 1676	63	48	<i>S</i>

^aAnalysis obtained by GC-MS; ^banalysis obtained by HPLC-UV (254 nm) using a Chiracel® OD-H chiral column. *ee*: enantiomeric excess; AC: absolute configuration. GC-MS and HPLC analyses are shown in Supplementary Information.

Table 2. Bioreduction of isatin **1** by *Acremonium* sp. CBMAI 1676 and *Aspergillus* sp. CBMAI 1829 in malt 2% medium

entry	time / days	Conversion ^a / %	ee ^b / %	AC
<i>Aspergillus</i> sp. CBMAI 1829				
1	3	39 (37%) ^c	38	<i>S</i>
2	6	34	40	<i>S</i>
3	9	19	42	<i>S</i>
<i>Acremonium</i> sp. CBMAI 1676				
4	3	35 (26%) ^c	32	<i>S</i>
5	6	30	50	<i>S</i>
6	9	26	52	<i>S</i>

^aAnalysis obtained by GC-MS; ^banalysis obtained by HPLC-UV (254 nm) using a Chiracel® OD-H chiral column; ^cyield isolated. *ee*: enantiomeric excess; AC: absolute configuration. GC-MS and HPLC analyses are shown in Supplementary Information.

reactions with resting cells were performed in phosphate buffer (Na₂HPO₄/KH₂PO₄, pH = 7; 0.1 mol L⁻¹) to prevent possible secondary reactions.

The formation of **2** was not detected by TLC analyses. Thus, the samples were analysed by GC-MS and HPLC-UV equipped with chiral column. For the fungus *Acremonium* sp. CBMAI 1676, the conversions were 2, 5 and 1% for 3, 6 and

9 days, respectively, showing that the product **2** was practically consumed after 9 days of reaction (Table 3, entries 4-6). Thus we suspected that isatin **1** was continuously reduced as confirmed by the decrease in the isatin peak area over time (25 × 10⁶, 20 × 10⁶ and 2 × 10⁶ a.u.) for 3, 6 and 9 days of reaction, respectively. However, product **2** remained in low yield because it was also biotransformed by the fungi cells.

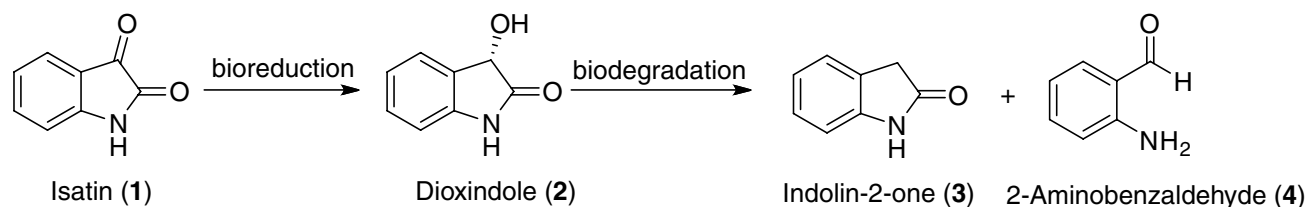
The conversion of **1** to **2** increased over time with *Aspergillus* sp. CBMAI 1829 to 2, 4 and 8% for 3, 6 and 9 days reactions, respectively (Table 3, entries 1-3). However, during reaction the peak area of isatin **1** reduced from 24×10^6 to 6×10^6 a.u., but the area of **2** remained at ca. 0.5×10^6 a.u. (which corresponds to a concentration so low that the product could not be detected by TLC analyses). Therefore, while the conversion of isatin **1** increases over time, the reaction yield of **2** remains low because the biodegradation process consumes both compounds.

The yield for dioxindole **2** was low for both strains showing that the use of resting cells in buffer solution was not an appropriate methodology for reduction of isatin **1**, because these conditions increased product **2** via reduction (biodegradation). The indolin-2-one (**3**) and 2-aminobenzaldehyde (**4**) were identified by GC-MS based on their high similarity with the mass spectra library NIST 21, 93 and 95%, respectively (Supplementary Information). In addition, a biotransformation pathway was proposed (Scheme 2).

In the absence of nutrients, fungal cells usually employ new metabolic pathways for obtention of different carbon and nitrogen sources.²³ The isatin **1** was consumed, and **2**, **3** or **4** did not accumulate during the reaction, only small amounts of these substances were observed by GC-MS analyses. Therefore compounds **1** and **2** were biodegraded for use as nitrogen and carbon sources by the fungi cells, which prevents accumulation of any biotransformation product.

One of the most common metabolic pathways in the biotransformation of isatin **1** is the action of an isatin hydrolase, which is a manganese-dependent enzyme that catalyzes the hydrolysis of the highly conjugated and heterocyclic compound isatin **1** (*H*-indole-2,3-dione) to isatinic acid (2-(2-aminophenyl)-2-oxoacetate).^{24,25}

In the literature, the reduction of isatin **1** used isolated enzymes such as a hamster monomeric carbonyl reductase and human carbonyl reductases CBR1 and CBR3.²⁶ However, the products were not isolated, and the enantiomeric excesses were not determined. This approach might offer higher yields in the reduction of isatin **1**.



Scheme 2. Proposed biotransformation of isatin **1** by the marine-derived fungi *Acremonium* sp. CBMAI 1676 and *Aspergillus* sp. CBMAI 1829 in phosphate buffer.

Table 3. Bioreduction of isatin by *Acremonium* sp. CBMAI 1676 and *Aspergillus* sp. CBMAI 1829 in phosphate buffer

entry	time / days	Isatin 1 peak area ($\times 10^6$ a.u.) ^a	Dioxindole 2 peak area ($\times 10^6$ a.u.) ^a	Conversion ^a / %	<i>ee</i> ^b / %	AC
<i>Aspergillus</i> sp. CBMAI 1829						
1	3	24	0.5	2	29	<i>S</i>
2	6	19	0.8	4	11	<i>S</i>
3	9	6	0.5	8	11	<i>S</i>
<i>Acremonium</i> sp. CBMAI 1676						
4	3	25	0.5	2	11	<i>S</i>
5	6	20	1	5	9	<i>S</i>
6	9	2	0.1	1	9	<i>S</i>

^aObtained by GC-MS; ^bobtained by HPLC-UV with a Chiracel® OD-H chiral column. *ee*: enantiomeric excess; AC: absolute configuration.

Conclusions

In conclusion, we report the use of whole mycelia of marine-derived fungi for the biocatalytic reduction of isatin **1** into the respective dioxindole **2** with satisfactory conversion (2-89%) and moderate enantiomeric excesses (up to 62% *ee*) in mild and environmentally friendly conditions. There was a conversion decrease during the reaction for *Aspergillus* sp. CBMAI 1829 and *Acremonium* sp. CBMAI 1676, which proves that a biodegradation process occurs concomitantly with the biotransformation of these compounds by fungi. This consequently reduces the reaction yield.

Supplementary Information

Supplementary data are available free of charge at <http://jbcs.s bq.org.br>.

Acknowledgments

D. E. Q. J. thanks to CAPES, I. M. F. and W. G. B. thank to CNPq for their scholarships, respectively. The authors thank to CNPq and FAPESP for financial support. A. L. M. P. thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Proc. No. 2012/18605-3). B. N. M. S. thanks the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support.

References

- Silva, J. F. M.; Garden, S. J.; Pinto, A. C.; *J. Braz. Chem. Soc.* **2001**, *12*, 273; Erdmann, O. L.; *J. Prakt. Chem.* **1840**, *19*, 321; Laurent, A.; *Annu. Rev. Chim. Phys.* **1840**, *3*, 393.
- Silva, B. V.; *J. Braz. Chem. Soc.* **2013**, *24*, 707.
- Khan, F. A.; Maalik, A.; Noor, T.; Zaidi, A.; Farooq, U.; Bukhari, S. M.; *Trop. J. Pharm. Res.* **2015**, *14*, 1937.
- Vine, K. L.; Matesic, L.; Locke, J. M.; Ranson, M.; Skropeta, D.; *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 397; Cerchiaro, G.; Ferreira, A. M. C.; *J. Braz. Chem. Soc.* **2006**, *17*, 1473; Motzer, R. J.; Michaelson, M. D.; Redman, B. G.; Hudes, G. R.; Wilding, G.; Figlin, R. A.; Ginsberg, M. S.; Kim, S. T.; Baum, C. M.; DePrimo, S. E.; Li, J. Z.; Bello, C. L.; Theuer, C. P.; George, D. J.; Rini, B. I.; *J. Clin. Oncol.* **2006**, *24*, 16; Silva, B. V.; Ribeiro, N. M.; Vargas, M. D.; Lanznaster, M.; Carneiro, J. W. M.; Krogh, R.; Andricopulo, A. D.; Dias, L. C.; Pinto, A. C.; *Dalton Trans.* **2010**, *39*, 7338.
- Zhang, H.; Kamano, Y.; Ichihara, Y.; Kizu, H.; Komiyama, K.; Itokawa, H.; Pettit, G. R.; *Tetrahedron* **1995**, *51*, 5523; Fernandes, P. D.; Zardo, R. S.; Figueiredo, G. S.; Silva, B. V.; Pinto, A. C.; *Life Sci.* **2014**, *116*, 16.
- Carle, J. S.; Christophersen, C.; *J. Org. Chem.* **1981**, *46*, 3440.
- Rasmussen, H. B.; MacLeod, J. K.; *J. Nat. Prod.* **1997**, *60*, 1152.
- Hata, H.; Shimizu, S.; Hattori, S.; Yamada, H.; *J. Org. Chem.* **1990**, *55*, 4377.
- Fukuoka, K.; Tanaka, K.; Ozeki, Y.; Kanaly, R. A.; *Int. Biodeterior. Biodegrad.* **2015**, *97*, 13.
- Qu, Y.; Xu, B.; Zhang, X.; Ma, Q.; Zhou, H.; Kong, C.; Zhang, Z.; Zhou, J.; *Biochem. Eng. J.* **2013**, *72*, 54.
- Birolli, W. G.; Ferreira, I. M.; Alvarenga, N.; Santos, D. A.; Matos, I. L.; Comasseto, J. V.; Porto, A. L. M.; *Biotechnol. Adv.* **2015**, *33*, 481.
- Trincone, A.; *Mar. Drugs* **2011**, *9*, 478.
- Rocha, L. C.; Luiz, R. F.; Rosset, I. G.; Raminelli, C.; Seleglim, M. H. R.; Sette, L. D.; Porto, A. L. M.; *Tetrahedron: Asymmetry* **2012**, *14*, 396.
- Rocha, L. C.; Seleglim, M. H. R.; Comasseto, J. V.; Porto, A. L. M.; *Mar. Biotechnol.* **2015**, *17*, 736.
- Ferreira, I. M.; Vasconcellos, S. P.; Cruz, J. B.; Comasseto, J. V.; Porto, A. L. M.; Rocha, L. C.; *Biocatal. Agric. Biotechnol.* **2015**, *4*, 144.
- Ferreira, I. M.; Rocha, L. C.; Yoshioka, S. A.; Nitschke, M.; Jeller, A. H.; Pizzuti, L.; Seleglim, M. H. R.; Porto, A. L. M.; *Biocatal. Agric. Biotechnol.* **2014**, *3*, 358.
- Ferreira, I. M.; Meira, E. B.; Rosset, I. G.; Porto, A. L. M.; *J. Mol. Catal. B: Enzym.* **2015**, *115*, 59.
- Jimenez, D. E. Q.; Ferreira, I. M.; Birolli, W. G.; Fonseca, L. P.; Porto, A. L. M.; *Tetrahedron* DOI: 10.1016/j.tet.2016.02.014.
- Garden, S.; Torres, J.; Ferreira, A.; Silva, R.; Pinto, A.; *Tetrahedron Lett.* **1997**, *38*, 1501.
- Sandmeyer, T.; *Helv. Chim. Acta* **1919**, *2*, 234.
- McKenzie, A.; Stewart, P.; *J. Chem. Soc.* **1935**, 104.
- Prelog, V.; *J. Mol. Catal.* **1976**, *1*, 159.
- Tortora, G. J.; Funke, B. R.; Case, C. L.; *Microbiology: An Introduction*, 10th ed.; Pearson Education: New Jersey, USA, 2010.
- Sommer, T.; Bjerregaard-Andersen, K.; Simensen, S. M.; Jensen, J. K.; Jochimsen, B.; Riss, P. J.; Etzerodt, M.; Morth, J. P.; *ACS Chem. Neurosci.* **2015**, *6*, 1353.
- Bjerregaard-Andersen, K.; Sommer, T.; Jensen, J. K.; Jochimsen, B.; Etzerodt, M.; Morth, J. P.; *J. Biol. Chem.* **2014**, *289*, 21351.
- El-Hawari, Y.; Favia, A. D.; Pilka, E. S.; Kisiela, M.; Oppermann, U.; Martin, H.-J.; Maser, E.; *Chem. Biol. Interact.* **2009**, *178*, 234.

Submitted: July 14, 2016

Published online: September 14, 2016

FAPESP has sponsored the publication of this article.