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Highly enantioselective reduction of the C–C double bond of *N*-phenyl-2-methyl- and *N*-phenyl-2,3-dimethyl- maleimides by fungal strains

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ABSTRACT

The C—C double bond of non-substituted and substituted maleimides were hydrogenated to the corresponding succinimides by fungal strains. 2- and 2,3-methylated-phenyl-maleimides were enantioselectively reduced to (*R*)-*N*-phenyl-3-methylsuccinimide and to *trans*-(*R*,*R*)-*N*-phenyl-2,3-dimethylsuccinimide respectively by *Aspergillus niger, A. flavus* and *A. fumigatus* (conversion 96 to 99%), *Fusarium graminearum* and *Penicillium sp* (conversion: 37 and 39%), with 99% ee.

1. Introduction

The production of chiral structures from easily obtained compounds is highly useful[1] not only in organic synthesis,[2] but also in pharmaceutical and agrochemical fields, because of the vast differences in biological activity of stereoisomers (enantiomers, diastereomers, etc.).[3–6]

Chiral products obtained through bioconversions offer the advantage of employing a renewable and natural source of reagents and avoiding the use of solvents while operating at room temperature.[1] Among the different catalysts, fungi are capable of catalyzing chemical reactions of diverse types and accepting a wide range of substrates. They also produce an abundant biomass and are simple to use.

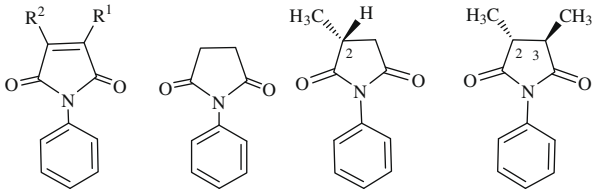
Over the course of our studies devoted to generating new bioactive compounds through fungal catalysis, we studied the reactivity of non-*N*-substituted and substituted *N*-phenyl maleimides and the propensity of fungal strains for the enantioface discrimination of *N*-phenyl-2-methyl and *N*-phenyl-2,3-dimethylmaleimides. Some of these structures have been demonstrated to be good substrates for biocatalysis with cultured plants cells.[7–9]

2. Results and discussion

N-Phenyl maleimides **1–2** obtained through reported procedures[10,11] (125 mg each) in DMSO (5 mL) were poured into flasks containing the fungal biomass in 250 mL of culture media.[12] The reaction mixtures were incubated at 30 °C for 72 h on an orbital shaker (150 rpm). After this time, the mixtures were filtered, and the aqueous phases were bulked and extracted with ethyl acetate. The organic phases were dried over Na_2SO_4 and the products were analyzed by TLC and by GC.[13]

The results show that among the 15 strains tested, none produced any changes in maleimide **2**. In contrast, the three *Aspergillus* spp. (*Aspergillus flavus, Aspergillus fumigatus,* and *Aspergillus niger*) caused the reduction of the C–C double bond of maleimide **1** to afford *N*-phenyl succinimide **5** with conversion rates ranging from 92% to 96% (Table 1).[14,15]

It is clear from these results that the strains of *Aspergillus* spp. have a high capacity for reducing the C–C double bond of *N*-phenyl-2,3-non-substituted maleimides but not of *N*-phenyl-2,3-dichloromaleimides.



1, $R^1 = R^2 = H$ **5** **6** **7**
2, $R^1 = R^2 = Cl$
3, $R^1 = CH_3$; $R^2 = H$
4, $R^1 = R^2 = CH_3$

In a second set of experiments, *N*-phenyl-2-methylmaleimide **3** and *N*-phenyl-2,3-dimethylmaleimide **4**, obtained by reported procedures,[16,17] were submitted to the whole panel of fungal strains under conditions that were the same as those used for maleimides **1** and **2**. Compound **3** possesses a prochiral center at C-2 and com-

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Table 1
Biotransformation of *N*-phenylmaleimide **1**, *N*-phenyl-2-methylmaleimide **3**, and *N*-phenyl-2,3-dimethylmaleimide **4** by filamentous fungi and yeasts.

Fungal spp.	Voucher sp. N^0	Substrate										
		1			**3**				**4**			
		Prod	Conv (%)	ee (%)	Prod	Conv (%)	ee (%)	Conf	Prod	Conv (%)	ee (%)	Conf
Alternaria alternata	CEREMIC 172-02	—	—	—	—	—	—	—	—	—	—	—
Aspergillus flavus	ATCC 9170	**5**	92	—	**6**	98	>99	*R*	**7**	99	>99	(*R*,*R*)
Aspergillus fumigatus	ATCC 26934	**5**	96	—	**6**	99	>99	*R*	**7**	99	>99	(*R*,*R*)
Aspergillus niger	ATCC 9029	**5**	94	—	**6**	96	>99	*R*	**7**	96	>99	(*R*,*R*)
Fusarium graminearum	CEREMIC 170-02	—	—	—	—	—	—	—	**7**	37	>99	(*R*,*R*)
Geotrichum candidum	CEREMIC 116-71	—	—	—	—	—	—	—	—	—	—	—
Mucor circinelloides	CEREMIC 128-09	—	—	—	—	—	—	—	—	—	—	—
Neurospora crassa	ATCC 9279	—	—	—	—	—	—	—	—	—	—	—
Penicillium spp	CEREMIC 129-09	—	—	—	—	—	—	—	**7**	39	>99	(*R*,*R*)
Rhizopus oryzae	CEREMIC 130-09	—	—	—	—	—	—	—	—	—	—	—
Rhodotorula rubra	CEREMIC 131-09	—	—	—	—	—	—	—	—	—	—	—
Saccharomyces cerevisiae	ATCC 9763	—	—	—	—	—	—	—	—	—	—	—
Schizosaccharomyces pombe	CEREMIC 132-09	—	—	—	—	—	—	—	—	—	—	—
Sclerotium bataticola	CEREMIC 173-02	—	—	—	—	—	—	—	—	—	—	—
Trichosporon cutaneum	CEREMIC 133-09	—	—	—	—	—	—	—	—	—	—	—

Time of reaction: 3 days.
Prod: Biotransformation product; Conv.: conversion rate; ee: enantiomeric excess; Conf: absolute configuration; CEREMIC: Reference Center in Mycology, National University of Rosario, Suipacha 531, Rosario 2000, Argentina; ATCC: American Type Culture Collection (Rockville, USA).

pound **4** has two prochiral centers at C-2 and C-3, giving us the possibility to examine the ability of fungal strains to discriminate between enantiotopic faces and also to determine the *syn*- or *anti*-hydrogenation of the double bond.

The results showed (Table 1) that after 72-h incubation, maleimide **3** was reduced to (*R*)-(+)-*N*-phenyl-3-methylsuccinimide **6**[14,18] (conversion rate ranging from 96% to 99%)[15] by the three *Aspergillus* spp. with a 99% ee. The enantiomeric excesses were determined by ^{1}H NMR spectroscopy using europium tris [3-(heptafluoropropylhydroxymethylen)-(+)-camphorate [Eu(hfc)$_3$] as a chiral shift reagent.[19,20] The absolute configuration of (+)-**6** was determined by comparison of the sign of the specific rotation with previous reports.[7,8] In turn, maleimide **4** was reduced only to *trans*-(*R*,*R*)-(+)-*N*-phenyl-2,3-dimethylsuccinimide **7**[9,21,22] by all *Aspergillus* spp. (conversion rates = 96–99%) and also by *Fusarium graminearum* and *Penicillium* sp. (37% and 39%, respectively).[15] The production of only *trans N*-phenyl-2,3-dimethylmaleimides is indicative of an *anti*-addition of the H to the double bond of maleimides by fungi, a clear difference from the *cis*-product obtained by catalytic hydrogenation of the double bond. In all cases, the ee was >99%.[19]

The diastereo- and enantioselective production of pure (+)-**7** clearly show that *Aspergillus* strains possess a high ability not only for reducing double bonds, but also for discriminating the enantiotopic faces of dimethylated maleimides. *F. graminearum* and *Penicillium* sp. showed a lower capacity for reducing the double bonds of **4,** but showed the same stereoselectivity.

Our results with the filamentous fungi of the genera *Aspergillus*, *Fusarium*, and *Penicillium* are similar to those obtained by Hirata et al.[7–9] with a cultured suspension of plant cells. They have recently demonstrated that *Nicotiana tabacum*, *Catharanthus roseus, Parthenocissus tricuspidata,* and *Cynechococcus* sp. possess the capacity to hydrogenate the double bond of maleimide **1** affording phenylsuccinimide **5** in 0.5–7 days with *Cynechococcus* sp. being the most efficient plant spp. (0.5 days).[7,8] In turn, *N. tabacum, Cyinechococcus* sp. along with *Marchantia polymorpha* showed the ability to hydrogenate the double bond of the pro-chiral *N*-phenyl-2-methylmaleimide **3** to afford (*R*)-*N*-phenyl-3-methylsuccinimide **6** with a 99% ee.[8] In turn, *M. polymorpha* enantioselectively hydrogenated *N*-phenyl-2,3-dimethylmaleimide **4** to yield *trans*-(*R*, *R*)-*N*-phenyl-2,3-dimethylsuccinimide **7** with 99% ee.[9]

A comparison of the results obtained with phenylmaleimides **1–4** suggests that electron-withdrawing substituents on the 2,3-positions (maleimide **2**) prevent the hydrogenation of the double bond by fungi, while hydrogen atoms or donor substituents at the same positions (compounds **1**, **3**, and **4**) render the maleimides highly susceptible to the reduction of the double C–C bond by fungi.

3. Conclusion

In conclusion, we have found in this work that fungi are efficient biocatalysts for the enantioselective hydrogenation of 2- and 2,3-methylated-phenyl-maleimides, constituting new efficient tools for the production of chiral succinimides, which could be asymmetric synthons for organic synthesis or useful biologically active compounds.[9]

The results obtained here are extremely attractive since whole fungal cells are highly advantageous biocatalysts because of their rapid growth in natural and synthetic media, their ease of handling, and simple scale-up. They play a leading role in 'chemoenzymatic syntheses' because of their great diversity which produces a myriad of useful enzymes with catalytic abilities.[23]

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12. Fungi were grown on a plate with agarized Czapek (for *Aspergillus* spp.) or Sabouraud (for the rest of fungal strains) culture medium for 3 days at 30 °C until well sporulated. Cells ($2–5 \times 10^6$) from the surface culture were used to inoculate flasks (one for the bioconversion experiment and the other for control) containing liquid medium having a composition that was the same as that of solid medium in a 2-L erlenmeyer flask. Two inoculated cultures were incubated for 72 h on an orbital shaker (Innova 4000, New Brunswick Sci Co., NJ) at 150 rpm at 30 °C prior to use for bioconversion experiments.
13. Both the bioconversion flask (fungal culture and compound) and the control flask (only fungal culture) were submitted to GC–MS using a Turbo Mass Perkin Elmer chromatograph, equipped with a fused silica gel column (SE-30 25 m–0.22 mm ID) with He as a carrier gas, coupled to a mass selective detector, 0.25 mm film, and had ionization energy of 70 eV with a temperature program of 70–200 °C at 10 °C/min; total time 30 min.
14. The structures of **5** and (±)-**6** were confirmed by direct comparison of GC, MS, and NMR spectral data with those of authentic samples obtained by the catalytic hydrogenation with Pd/C from the **1** and **3**, which were synthesized according to reported procedures.[10,11]
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18. *(+)-(R)-N-Phenyl-2-methylsuccinimide* ***6***: Yield 82%. $[\alpha]_D^{25} = +5.1$ (*c* 0.91, $CHCl_3$) {lit[8] $[\alpha]_D^{25} = +6.6$ (*c* 0.56, $CHCl_3$)}; IR (in $CHCl_3$) 1705 cm^{-1} (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ 1.47 (3H, d, *J* = 7.2 Hz, 2-Me), 2.52 (1H, dd, *J* = 17.7 and 4.5 Hz, 3-Ha), 3.05 (1H, ddq, *J* = 9.3, 4.6, and 7.3 Hz, 2-H), 3.11 (1H, dd, *J* = 17.6 and 9.3 Hz, 3-Hb), 7.29 (2H, d, *J* = 8.3 Hz, 2′-H and 6′-H), 7.39 (1H, t, *J* = 7.4 Hz, 4′-H), 7.47 (2H, t, *J* = 7.7 Hz, 3′-H and 5′-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.9 (Me), 34.9 (CH), 36.7 (CH_2), 126.4 (C-2′ and C-6′), 128.6 (C-4′), 129.2 (C-3′ and C-5′), 132.0 (C-1′), 175.4 (2-C=O), 179.5 (5 C=O).
19. To determine the applicability of the method, racemic (±)-**6** was prepared as described in Ref. 14. The methyl proton signals of (±)-**6** were observed at δ 1.97 (d, *J* = 7.5 Hz) and 1.92 (d, *J* = 7.5 Hz) in the $CDCl_3$ solution of the sample with $Eu(hfc)_3$ (1:1 mol ratio). The signal at δ 1.92 belongs to (+)-**6**. The ee was calculated, for each experiment, from the ratio of relative integral values of both methyl proton signals in the NMR spectrum of the product.
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21. *(+)-(2R,3R)-N-Phenyl-2,3-dimethylsuccinimide* **7**: Yield 69%. $[\alpha]_D^{25} = +39.35$ (*c* 0.36, $CHCl_3$) {lit[9] $[\alpha]_D^{25} = +21.0$ (*c* 0.03, $CHCl_3$)}; IR ($CHCl_3$) 1708 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.46 (6H, d, *J* = 7.2 Hz, 2- and 3-Me), 2.62 (2H, m, 2-H and 3-H), 7.23–7.67 (5H, m, H_{Ar}); ^{13}C NMR ($CDCl_3$) δ 15.1 (2- and 3-Me), 43.3 (C-2 and C-3), 126.4 (C-2′ and 6′), 128.4 (C-1′), 129.1 (C-3′ and 5′), 132.1 (C-4′), 178.4 (C=O in C-1 and 4).
22. Racemic (±)-**7** was prepared from aniline and (±)-*trans*-2,3-dimethylsuccinic anhydride, which in turn was prepared from commercial (±)-2,3-dimethylsuccinic acid (Aldrich Chem. Co.). The methyl proton signals of (±)-**7** were observed at δ 1.74 (d, *J* = 6.6 Hz) and 1.70 (d, *J* = 6.6 Hz) in the $CDCl_3$ solution of the sample with $Eu(hfc)_3$ (1:1 mol ratio). The signal at δ 1.70 belongs to (+)-**7**. The ee was calculated as described in Ref. 19.
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