

Antifungal Activity and Biotransformation of Diisophorone by *Botrytis cinerea*

MOURAD DAOUBI,[†] ATHINA DELIGEORGOPOULOU,[‡]
 ANTONIO J. MACÍAS-SÁNCHEZ,[†] ROSARIO HERNÁNDEZ-GALÁN,[†]
 PETER B. HITCHCOCK,[‡] JAMES R. HANSON,[‡] AND ISIDRO G. COLLADO^{*,†}

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apartado 40, 11510 Puerto Real, Cádiz, Spain, and The School of Chemistry, Physics and Environmental Science, University of Sussex, Brighton, Sussex BN1 9QJ, United Kingdom

Diisophorone (**1**) was tested against two strains of the necrotrophic plant pathogen *Botrytis cinerea*. Fungal sensitivity varied according to the strain. *B. cinerea* 2100 was more sensitive than *B. cinerea* UCA992: its mycelial growth was significantly inhibited at concentrations of 50 ppm and above. Although diisophorone (**1**) showed an effective control of *B. cinerea*, a detoxification mechanism was present. The detoxification of racemic diisophorone (**1**) by *B. cinerea* was investigated. Incubation with two strains of *B. cinerea* gave one and four biotransformation products (**2–5**), respectively. Their structures were established as the known 8 β -hydroxydiisophorone (**2**), 6 α -hydroxydiisophorone (**3**), 6 β -hydroxydiisophorone (**4**) and 8 β ,14 β -dihydroxydiisophorone (**5**) on the basis of their spectroscopic data, including two-dimensional NMR analysis [heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), and nuclear Overhauser enhancement spectroscopy (NOESY)] and an X-ray crystallographic study.

KEYWORDS: Biotransformation; diisophorone; *Botrytis cinerea*; antifungal activity

INTRODUCTION

Botrytis species are serious plant pathogens, which are implicated in many diseases of flowers, fruits, and vegetables. In particular, *B. cinerea* attacks economically important crops such as lettuces, carrots, tobacco, strawberries, and grapes (1, 2). The rapid development of tolerance to commercial fungicides by *B. cinerea* has led to an increase in the quantities of these compounds that have to be used with the consequent additional problems of persistence and serious economic damage arising from the decreased quality of wines produced from treated grapes (3).

Studies on structure–activity relationships of substrates that inhibited the growth of *B. cinerea* and their relationships to the botryane metabolites produced by the fungus revealed structural similarities between them (4). In particular, the distance between the hydrophobic geminal dimethyl group and the hydrophilic hydroxyl group, as well as the distance between the dimethyl group and the carbon bearing the oxygen function, seem to mimic the analogous distances found in botryanes.

Continuing our research program directed toward the rational design of fungicides for use against fungal infections of commercial crops, we have tested the antifungal properties of diisophorone (**1**) (Figure 1). Racemic diisophorone (**1**) (5, 6)

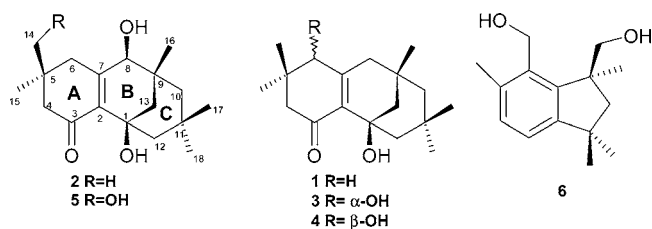


Figure 1. Structures of compounds 1–6.

is a readily available compound, which possesses the key distance between the hydrophobic geminal dimethyl group and the hydrophilic hydroxyl group found in sesquiterpenoid phytoalexins and in the major secondary metabolites of *B. cinerea*. Diisophorone (**1**) is a very interesting compound, as it can be synthesized easily and cheaply in excellent yield. In addition, it has been shown to be able to significantly inhibit the growth of the pathogenic fungus *Aspergillus niger* (7). The microbial transformation of diisophorone (**1**) by use of the plant pathogen *Aspergillus niger* as a biocatalyst caused monohydroxylation in positions 8 α , 10 and 17 α (7). We have investigated the antifungal activity and biotransformation of diisophorone (**1**) by *B. cinerea*, in order to find new compounds that may be targets for future study as fungal inhibitory agents.

MATERIALS AND METHODS

Chemical Analysis. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Mattson Genesis

* Corresponding author: Tel 34 956 016371; fax 34 956 016193; e-mail isidro.gonzalez@uca.es.

[†] Universidad de Cádiz.

[‡] University of Sussex.

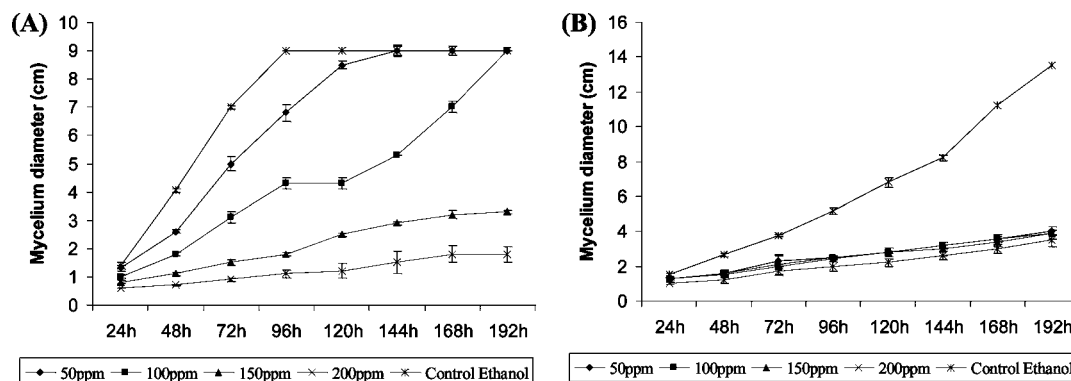


Figure 2. Antifungal effect of diisophorone on in vitro grown (A) *B. cinerea* UCA992 and (B) *B. cinerea* 2100. Results are shown as medium values of three replicates of micelium diameter; bar = \pm SD.

Table 1. ^1H NMR (400 MHz) of Biotransformation Products (2–5)

^1H	2	3	4	5
4 α	2.29 (d, $J = 16.2$ Hz)	2.47 (d, $J = 16.1$ Hz)	2.17 (d, $J = 16.4$ Hz)	2.23 (dd, $J = 16.4$ and 1.7 Hz)
4 β	2.33 (dd, $J = 16.2$ and 1.2 Hz)	2.21 (d, $J = 16.2$ Hz)	2.41 (d, $J = 16.4$ Hz)	2.46 (d, $J = 16.4$ Hz)
6 α	2.08 (d, $J = 18.25$ Hz)	3.84 (br s)		2.38 (d, $J = 18.3$ Hz)
6 β	2.71 (dd, $J = 18.25$ and 1.2 Hz)		3.94 (br s)	2.58 (dd, $J = 18.3$ and 1.7 Hz)
8 α	3.47 (d, $J = 6.61$ Hz)	2.07 (d, $J = 20.7$ Hz)	2.07 (d, $J = 20.9$ Hz)	
8 β		2.59 (d, $J = 20.7$ Hz)	2.53 (d, $J = 20.7$ Hz)	3.48 (br s)
10 α	1.28 (m)	1.33 (d, $J = 14.2$ Hz)	1.26 (d, $J = 14.2$ Hz)	1.29 (m)
10 β	1.28 (m)	1.17 (d, $J = 14.2$ Hz)	1.20 (d, $J = 14.2$ Hz)	1.29 (m)
12 α	1.80 (br d, $J = 13.2$ Hz)	1.87 (br dd, $J = 13.2$ and 1.5 Hz)	1.82 (td, $J = 13.4$ and 1.8 Hz)	1.80 (br d, $J = 13.5$ Hz)
12 β	1.44 (d, $J = 13.2$ Hz)	1.43 (d, $J = 13.2$ Hz)	1.44 (d, $J = 13.4$ Hz)	1.43 (d, $J = 13.5$ Hz)
13 α	1.20 (d, $J = 12.5$ Hz)	1.29 (dd, $J = 11.9$ and 1.5 Hz)	1.29 (brd, $J = 11.9$ and 1.5 Hz)	1.20 (dd, $J = 12.2$ and 1.2 Hz)
13 β	1.76 (td, $J = 12.5$ and 1.73 Hz)	1.52 (td, $J = 11.9$ and 2.4 Hz)	1.58 (td, $J = 11.9$ and 2.4 Hz)	1.78 (td, $J = 12.2$ and 1.7 Hz)
14	1.06	1.04	1.00	3.43 and 3.40 (d, $J = 10.7$ Hz)
15	1.06	1.00	1.09	1.06
16	1.05	1.03	1.04	1.02
17	0.93	0.90	0.91	0.93
18	0.69	0.83	0.72	0.69
OH	5.31	5.23	5.35	5.33

spectrophotometer, series FTIR (Fourier transform infrared). ^1H and ^{13}C NMR measurements were obtained on Varian Gemini 300 and Varian Unity 400 NMR spectrometers with SiMe_4 as the internal reference. Mass spectra were recorded on Fisons MD800 and Finnigan MAT95 S instruments. High-performance liquid chromatography (HPLC) was performed with a Hitachi/Merck L-6270 apparatus equipped with a UV/vis detector (L 4250) and a differential refractometer detector (RI-71). Thin-layer chromatography (TLC) was performed on Merck Kiesegel 60 F_{254} , 0.2 mm thick (catalog no. 1.05554.0001). Silica gel (Merck) was used for column chromatography. Purification by means of HPLC was accomplished on a 25×1 cm Hibar 60 silica gel column. All solvents used were freshly distilled.

Microorganism and Antifungal Assay. The culture of *B. cinerea* UCA992 employed in this work was obtained from grapes of Domecq vineyard, Jerez de la Frontera, Cádiz, Spain. This culture of *B. cinerea* is deposited in the Universidad de Cadiz, Facultad de Ciencias Mycological Herbarium Collection (UCA). *B. cinerea* 2100 comes from the Centro Español de Cultivos Tipos (CECT), Universidad de Valencia, Facultad de Biología, Spain, where a culture of this strain is deposited.

Bioassays were performed by measuring inhibition of radial growth on an agar medium in a Petri dish. The test compound was dissolved in ethanol to give final compound concentrations ranging from 50 to 200 mg/L. Solutions of the test compound were added to a glucose–malt–peptone–agar medium [61 g/L glucose (20 g)–malt (20 g)–peptone (1 g)–agar (20 g), pH 6.5–7.0]. The final ethanol concentration was identical in both control and treated cultures. The medium was poured into sterile plastic Petri dishes measuring 13.5 cm in diameter, and a 1.5 cm diameter mycelial disk of *B. cinerea* cut from an actively growing culture was placed in the center of the agar plate. Inhibition of radial growth was measured for 8 days (Figure 2). Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the negative control. The assays were carried out in triplicate, and

the results are shown as mean values of three replicates of colony diameters (\pm SD). The commercial fungicide dichlofluanid was used throughout as a standard agent for comparison. The IC_{50} value was determined by the linear regression of the probit of the test fungus percentage inhibition and the log of the studied compound concentrations. The IC_{50} was the average of three replications.

Biotransformation of Diisophorone (1) with *B. cinerea* UCA992.

B. cinerea UCA992 was grown for 3 days on a shake culture of Czapek–Dox medium (70 mL per 250 mL flask) comprising (per liter of distilled water) glucose (40 g), yeast extract (1 g), potassium dihydrogen phosphate (5 g), sodium nitrate (2 g), magnesium sulfate (0.5 g), ferrous sulfate (10 mg), and zinc sulfate (5 mg). The pH was adjusted to 7.0. Diisophorone (1) (250 mg) in ethanol (24 mL) was then evenly distributed among 24 flasks and incubated with *B. cinerea* for 10 days. The mycelium was filtered and washed with water and ethyl acetate. Sodium chloride was added to the filtrate and the mixture was incubated for 25 min. The filtrate was acidified to pH 2 and extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, water, and brine and then dried over sodium sulfate.

The solvent was evaporated in a vacuum to give a residue that was chromatographed on silica. Elution with 10% ethyl acetate in light petroleum gave unchanged diisophorone (1) (80 mg), which was identified by its ^1H NMR spectrum.

Elution with 15% ethyl acetate gave 2 (30 mg) as a solid, which was recrystallized from ethyl acetate as white needles, mp 119–121 $^{\circ}\text{C}$ (lit. mp 121–123 $^{\circ}\text{C}$); $[\alpha]_{\text{D}}^{25} 3.9^{\circ}$ (c 1.0, CHCl_3); IR (Nujol) ν_{max} 3443, 1690, 1639 cm^{-1} ; ^1H and ^{13}C NMR data, Tables 1 and 2; EIMS m/z 292 (M^+ , 6), 221 (100), 179 (30), 137 (30); HREIMS (m/z) M^+ calcd. for $\text{C}_{18}\text{H}_{28}\text{O}_3$, 292.2038; found, 292.2038.

Crystal Data and Structure Determination of Compound 2.

$\text{C}_{18}\text{H}_{28}\text{O}_3$, M_r 292.40, orthorhombic, space group *Pccn* (no. 56), $a = 22.7961(17)$ Å, $b = 19.2527(11)$ Å, $c = 7.6170(4)$ Å, $\alpha = \beta = \gamma =$

Table 2. ^{13}C NMR (100 MHz) of Biotransformation Products (2–5)

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
2	71.5	136.2	202.8	49.7	37.2	41.2	155.4	76.2	32.6	52.2	31.2	50.6	42.8	27.8	29.8	30.1	37.5	27.0
3	72.0	136.8	200.9	48.9	37.9	76.2	157.4	43.1	32.3	52.6	31.8	50.6	46.5	23.2	26.9	33.2	37.5	28.9
4	71.8	136.3	199.8	50.9	38.2	77.4	159.3	41.8	32.2	52.4	31.6	50.2	46.1	20.1	27.3	32.9	37.5	28.4
5	71.5	136.2	202.8	46.8	37.3	36.6	155.1	76.0	37.3	50.4	31.2	49.5	40.9	70.7	21.5	27.6	37.2	29.4

90° , $V = 3343.0$ (4) \AA^3 , $Z = 8$, $D_c = 1.16$ mg m^{-3} , $F(000) = 1280$, $\lambda = 0.71073$ \AA , $\mu = 0.08$ mm^{-1} . Data were collected from a crystal of size $0.40 \times 0.05 \times 0.05$ mm^3 on an Enraf-Nonius CAD4 diffractometer. A total of 10 827 reflections were collected for $3.85 < \theta < 22.99^\circ$ and $-25 \leq h \leq 22$, $-21 \leq k \leq 21$, $-8 \leq l \leq 7$. There were 2315 independent reflections, and 1258 reflections with $I > 2\sigma(I)$ were used in the refinement. There was no crystal decay, and no absorption correction was applied. The structure was solved by direct methods with SHELXL-97 (9) for the refinement. The non-hydrogen atoms were refined anisotropically by full-matrix least-squares on F^2 . The hydrogen atoms were placed in calculated positions and added to the refinement as fixed isotropic contribution. The final R indices were $R_1 = 0.063$, $wR_2 = 0.144$, and R indices (all data) were $R_1 = 0.096$, $wR_2 = 0.160$. The goodness of fit on F^2 was 1.068, and the largest peak on the final difference map was 0.21 e \AA^{-3} . Crystallographic data of **2** including crystal data and structure refinement, atomic coordinates, equivalent isotropic displacement parameters, and bond lengths and angles have been sent to the Cambridge Crystallographic Data Centre.

Biotransformation of Diisophorone with *B. cinerea* 2100. *B. cinerea* 2100 was inoculated in 45 flasks containing the previously described medium. The flasks were incubated at 25°C and stirred at 180 rpm for 3 days; the mycelium was then filtered and transferred into 40 500-mL flasks containing 200 mL of Czapek-Dox medium (without glucose). The substrate (400 mg) dissolved in ethanol was divided evenly among 35 flasks and the fermentation continued for a further period of 3–4 days. The remaining five flasks were used as the control. The mycelium was filtered, and the broth was saturated with sodium chloride and extracted three times with ethyl acetate. The extract was dried over anhydrous sodium sulfate and the solvent was evaporated. Fractionation of the extract (455 mg) was carried out by means of column chromatography on silica gel (SiCC), eluting with increasing concentrations of ethyl acetate in light petroleum ether to give nine fractions F1–F9. Final purification of fraction F2 was carried out by means of semipreparative HPLC on a 25×1 cm LiChrospher Si60 (10 μm) silica gel column, a differential refractometer detector, and hexane/ethyl acetate 73:27 (3.5 mL/min) as eluent to afford 8 β -hydroxydiisophorone (**2**; 6 mg). Compounds **3** and **4** were obtained after further purification of fraction F6 by means of column chromatography with CHCl_3 as eluent to afford 6 α -hydroxydiisophorone (**3**; 48 mg) and 6 β -hydroxydiisophorone (**4**; 55 mg). Final purification of products from fraction F9 by means of semipreparative HPLC on a 25×1 cm LiChrospher Si60 (10 μm) silica gel column, a differential refractometer detector, and hexane/ethyl acetate 75:25 (3.5 mL/min) as eluent led to the isolation of 8 β ,14 β -dihydroxydiisophorone (**5**; 2 mg) in addition to a small amount of a known compound from the fungus, 11-hydroxydehydrobotrydienediol (**6**) (10).

8 β -Hydroxydiisophorone (2). Amorphous solid, $[\alpha]_{\text{D}}^{26} +32.6^\circ$ (c 0.38, CHCl_3).

6 α -Hydroxydiisophorone (3). Amorphous solid, $[\alpha]_{\text{D}}^{26} +140.2^\circ$ (c 0.38, CHCl_3); IR (film) ν_{max} 3404, 2948, 2912, 2859, 1639 cm^{-1} ; ^1H and ^{13}C NMR data, **Tables 1** and **2**; EIMS m/z 292 (M^+ , 2), 274 ($\text{M}^+ - 18$, 4), 221 (93), 203 (100), 175 (44); HREIMS (m/z) M^+ calcd. for $\text{C}_{18}\text{H}_{28}\text{O}_3$, 292.2038; found, 292.2075.

6 β -Hydroxydiisophorone (4). Amorphous solid, $[\alpha]_{\text{D}}^{26} -91.0^\circ$ (c 1.28, CHCl_3); IR (film) ν_{max} 3404, 2948, 2912, 2859, 1642 cm^{-1} ; ^1H and ^{13}C NMR data, **Tables 2** and **3**; EIMS m/z 292 (M^+ , 4), 274 ($\text{M}^+ - 18$, 2), 221 (99), 203 (100), 175 (53); HREIMS (m/z) M^+ calcd. for $\text{C}_{18}\text{H}_{28}\text{O}_3$, 292.2038; found, 292.2065.

8 β ,14 β -Dihydroxydiisophorone (5). Amorphous solid, $[\alpha]_{\text{D}}^{26} +53^\circ$ (c 0.085, CHCl_3); IR (film) ν_{max} 3405, 2956, 1643 cm^{-1} ; ^1H and ^{13}C NMR data, **Tables 1** and **2**; EIMS m/z 308 (M^+ , 15), 293 ($\text{M}^+ - 15$,

5), 290 ($\text{M}^+ - 18$, 3) 277 (3), 237 (100), 219 (60); HREIMS (m/z) M^+ calcd. for $\text{C}_{18}\text{H}_{28}\text{O}_4$, 318.1988; found, 318.1991.

RESULTS AND DISCUSSION

The antifungal properties of **1** against *B. cinerea* were determined by the “poisoned food technique” (11). Diisophorone (**1**) inhibited the mycelial growth of *B. cinerea*; however, fungal sensitivity varied according to the strain (**Figure 2**). *B. cinerea* 2100 was the most sensitive, and its mycelial growth was significantly inhibited at concentrations of 50 ppm and above. Depending on the concentration of **1**, the fungus retained an inhibition percentage of 69–80% after 5 days. In contrast, the growth of the other strain, UCA992, was not significantly inhibited up to 100 ppm. The inhibition percentage after 5 days was only 6% at 50 ppm but it reached 76% and 92% at 150 and 200 ppm, respectively. The IC_{50} values calculated for *B. cinerea* 2100 and UCA992 strains were 70 and 89 ppm after 5 days, respectively.

These results showed good control of *B. cinerea* by diisophorone (**1**). Nevertheless, a detoxification mechanism would be present. To study it, diisophorone (**1**) was subjected to biotransformation studies. Because the antifungal effect was different, we studied the biotransformation of **1** with both strains.

Diisophorone (**1**) was incubated with *B. cinerea* UCA992 for 10 days. The medium was extracted with ethyl acetate and purified by means of column chromatography. Apart from unchanged diisophorone (**1**), the only eluted metabolite from the column was 8 β -hydroxydiisophorone (**2**). The recovered starting material showed $[\alpha]_{\text{D}}^{26} -18.6$ (c 0.32, CHCl_3), indicating that the fungus exhibited preference for (+)-diisophorone in the biotransformation. The HREIMS of **2** showed a molecular ion corresponding to the molecular formula $\text{C}_{18}\text{H}_{28}\text{O}_3$. The IR spectrum possessed absorption bands at 1639, 1690, and 3443 cm^{-1} , confirming that the alkene, carbonyl moieties, and hydroxyl groups were present. The ^1H NMR spectrum of the product showed a new secondary alcohol resonance at δ 3.46 ppm (1H, s), suggesting the introduction of a new hydroxyl group at one of the methylene groups in the diisophorone molecule. This was further indicated by a low-field hydroxyl-bearing methine carbon signal at δ_{C} 76.2 ppm. The position of newly introduced hydroxyl was established by comparing the ^{13}C NMR spectrum of **2** with that of diisophorone (**7**). The methylene signal at δ 46.3 ppm assigned to C-8 in **1** was not present in the ^{13}C NMR spectrum of **2**, indicating that the hydroxyl group was inserted at C-8. The β stereochemistry of the alcohol was established when irradiation of the proton geminal to the C-8 at δ 3.46 ppm gave 2.9% nuclear Overhauser enhancement (NOE) to the signal at δ 2.08 ppm, which was assigned to the proton α at position C-6. An X-ray crystal structure of the compound confirmed the structure proposed for **2** (**Figure 3**). All other physical and spectroscopic data were in agreement with those described in the literature for 8 β -hydroxydiisophorone (**2**) (8).

Diisophorone (**1**, 400 mg) was incubated with a second strain, *B. cinerea* 2100, following methodology previously described by our group (13). Four compounds (**2–5**) that were not present

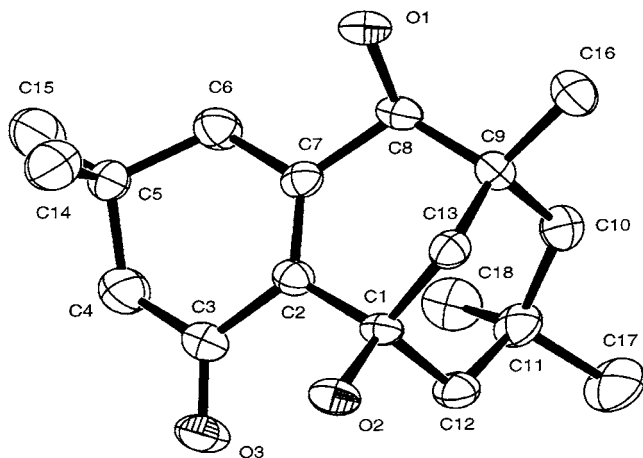


Figure 3. X-ray molecular structure of compound **2** (ORTEP drawing).

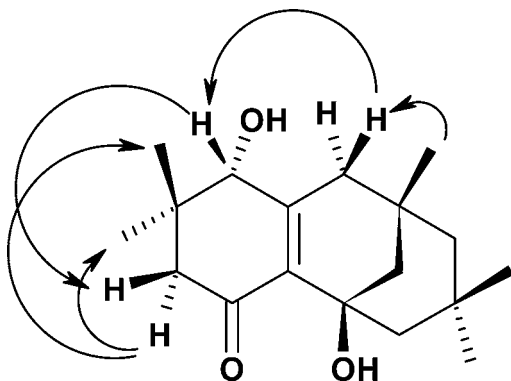


Figure 4. NOESY correlations for compound **3**.

in the control fermentation were detected by TLC. The metabolites were extracted from the medium with ethyl acetate on the third and fourth days. Both extracts were purified by chromatography to yield four biotransformation products in addition to **6**, a natural product produced by *B. cinerea*. The first compound to be isolated was 8 β -hydroxydiisophorone (**2**) (2.6 mg), the only compound obtained in the first experiment.

Compounds **3** (48 mg, 11.34%) and **4** (55 mg, 13%) both of which are monohydroxylated derivatives of **1**, were the major biotransformation products. The exact molecular masses of both compounds were determined by HREIMS and found to be 292.2075 and 292.2065 respectively, confirming the molecular formula, C₁₈H₂₈O₃ for both compounds. A methine proton signal at δ 3.84 and 3.94 ppm, respectively, was correlated in the HMBC experiment with vinylic carbons C-2 and C-7 (δ 136.8, 157.4 and 136.3, 159.3 ppm), a quaternary carbon (δ 37.9 and 38.2, respectively) assigned to C-5, and two methyl signals (δ 23.2, 26.9 and 20.1, 27.3 ppm) corresponding to C-14 and C-15. These results indicated that both compounds were monohydroxylated derivatives of **1** at C-6. The α -stereochemistry of **3** was assigned by NOESY experiment (Figure 4). NOE experiments with compound **4** confirmed the β -disposition of the hydroxyl group at C-6.

Further hydroxylation of compound **2** gave **5**, which was isolated as a colorless amorphous material and displayed a molecular formula of C₁₈H₂₈O₄. The absence of a methyl group signal in the ¹H and ¹³C NMR spectra and the appearance of hydroxymethyl resonances (δ _H 3.40 and 3.43; δ _C 70.7 ppm) suggested that **5** is hydroxylated on the *gem*-dimethyl group at C-5. This was confirmed by the downfield shift of the signal assigned to C-5. The stereochemistry at C-5 was revealed by

the correlations observed in the NOESY experiment, between H-8 and H-15 and between H-16 and H-14.

The antifungal properties of biotransformation products against *B. cinerea* UCA992 were tested, showing that all hydroxylated metabolites are inactive at 100 and 200 ppm. These results confirm that *B. cinerea* has a mechanism to detoxify compound **1** by hydroxylating positions C-6 and C-8.

The metabolites obtained from biotransformation of racemic diisophorone (**1**) by *B. cinerea* were found to be optically active. This result indicated that the microbiological transformation has taken place with one enantiomer but it does not prove that the metabolites belong to the same enantiomeric series. Nevertheless, the specific rotation showed by recovered diisophorone (**1**) in the biotransformation with UCA992 strain indicated that at least an enantioselective biotransformation has taken place. Fungal biodegradation may proceed via a monooxygenase-catalyzed hydroxylation of the C–H bonds to yield compounds **2–5**. The structures of these metabolites suggest compounds that might be targets for future study as inhibitory agents.

Comparing the products obtained from the biotransformation by *B. cinerea* with those described for the biotransformation by *A. niger* (**7**), we observed a common feature: all monohydroxylations were produced on carbons that were in 1,4 relative positions from the initial hydroxyl group of diisophorone (**1**). This observation seems to indicate that the hydroxyl group must be the point of binding between the substrate and the enzyme. Nevertheless while *A. niger* produces the hydroxylation on ring C, *B. cinerea* does it on ring A.

Supporting Information Available: Crystal data, structure refinement, atomic coordinates, displacement parameters, bond lengths and angles, and hydrogen coordinates for compound **2**. This information is available free of charge via the Internet at <http://pubs.acs.org>.

LITERATURE CITED

- (1) Verhoeff, K. The Infection Process and Host–Pathogen Interactions. In *The Biology of Botrytis*; Coley Smith, J. R., Verhoeff, K., Jarvis, W. R., Eds.; Academic Press: London, 1980; pp 153–175.
- (2) *Botrytis: Biology, Pathology and Control*; Elad, Y., Williamson B., Tudzynsky, P., Delen, N., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2004.
- (3) Staub, T. Fungicide resistance: practical experience with anti-resistance strategies and the role of integrated use. *Annu. Rev. Phytopathol.* **1991**, *29*, 421–42.
- (4) Deligeorgopoulou, A. Sesquiterpenoids and their biotransformation by *Botrytis cinerea*. Ph.D. Thesis, The School of Chemistry, Physics and Environmental Science, University of Sussex, Brighton, Sussex, U.K., 2002; pp 176–177.
- (5) Kabas, G.; Rutz, H. C. The alkali-catalyzed self-condensation of isophorone. *Tetrahedron* **1966**, *22*, 1219–26.
- (6) Kabas, G. Alkali-catalyzed self-condensation of isophorone II. *Chimia* **1967**, *21*, 260–261.
- (7) Kiran, I.; Yildirim, H. N.; Hanson, J. R.; Hitchcock, P. B. The antifungal activity and biotransformation of diisophorone by the fungus *Aspergillus niger*. *J. Chem. Technol. Biotechnol.* **2004**, *79*, 1366–70.
- (8) Furth, B.; Kossanyi, J.; Morizur, J. P. Chemical reactions in the tricyclo[7.3.1.02.7]-2(7)-tridecen-1-ol series. *Bull. Soc. Chim. Fr.* **1967**, 1428–38.
- (9) Sheldrick, G. M.; SHELXS-97, Program for the solution of Crystal Structures, University of Gottingen, Gottingen, Germany, 1997.

- (10) Durán-Patrón, R.; Hernández-Galán, R.; Collado, I. G. Seco-botrytriendiol and related sesquiterpenoids: New phytotoxic metabolites from *Botrytis cinerea*. *J. Nat. Prod.* **2000**, *63*, 182–184.
- (11) Chattannavar, S. N.; Kulkarni, S.; Hegde, R. K. Bioassay of non-systemic fungicides against the wheat pathogen, *Drechslera sativus* (Pam., King and Bakke). *Pesticides* **1985**, *19*, 33–34.
- (12) Patil, I. S.; Kulkarni, S.; Hegde, R. K. Bioassay of fungicides against *Drechslera sorokiniana* (sacc). *Pesticides* **1986**, *20*, 30–31.
- (13) Durán-Patrón, R.; Corrales, E.; Hernández-Galán, R.; Collado, I. G. Biotransformation of caryophyllene oxide by *Botrytis cinerea*. *J. Nat. Prod.* **1999**, *62*, 41–44.

Received for review March 17, 2005. Revised manuscript received May 20, 2005. Accepted May 20, 2005. Financial support from the Spanish Science and Technology Ministry and “Consejería de la Presidencia of Junta de Andalucía” through projects AGL2003-6480-C02-01 and AM19-04 is gratefully acknowledged.

JF050600N