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The Antifungal Activity of Widdrol and Its Biotransformation by *Colletotrichum gloeosporioides* (penz.) Penz. & Sacc. and *Botrytis cinerea* Pers.: Fr.

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Widdrol (**1**) was tested against the necrotrophic plant pathogens *Botrytis cinerea* and *Colletotrichum gloeosporioides.* While **1** was found to be inactive against *C. gloeosporioides*, it showed a selective and effective control of *B. cinerea*, significantly inhibiting the mycelial growth of the fungus at concentrations of 100 ppm and above. In addition, the biotransformation of **1** by both fungi was studied. Incubation with *C. gloeosporioides* and *B. cinerea* afforded four and one biotransformation products (**2**−**6**), respectively. Biotransformation with *C. gloeosporioides* was highly regioselective, yielding for the most part oxidation products at C-10: 10-oxowiddrol (**2**), 10β-hydroxywiddrol (**3**), 10α-hydroxywiddrol (**4**), and 14α-hydroxywiddrol (**5**). The structures of all products were determined on the basis of their spectroscopic data, including coupling constants, two-dimensional NMR analysis (heteronuclear multiple quantum coherence, heteronuclear multiple bond correlation, and nuclear Overhauser enhancement spectroscopy), and nuclear Overhauser effect. The biotransformation products were then tested against *B. cinerea* and found to be inactive. These results shed further light on the structural modifications, which may be necessary to develop selective fungal control agents against *B. cinerea*.

KEYWORDS: Biotransformation; widdrol; *Botrytis cinerea*; *Colleotrichum gloeosporioides*; antifungal activity

INTRODUCTION

While increased resistance to commercially available agrochemicals has led to a need for new fungicides, the desire for safer and more effective agrochemicals with reduced environmental and/or mammalian toxicity also remains a high priority. Essential to fulfilling both of these needs is the identification of new lead candidates that possess high levels of desirable biological activities, reduced levels of unwanted toxicities, new structural types, and perhaps different modes of action in order to avoid the problem of cross-resistance that is so prevalent with currently used agrochemicals (*1*). In this context, natural product-based fungicides offer advantages in that they have both unique modes of action and low mammalian toxicity. An additional benefit is their ability to decompose rapidly, thereby reducing the risk to the environment.

In our ongoing attempt to identify natural fungicides as alternatives to conventional synthetic agrochemicals, we carried out a preliminary screening for biological activity on plants native to Cuba. In this way, *Juniperus lucayana* Britton, a plant belonging to the family Cupressaceae that only grows in the Antilles, was found to be very active against the phytopathogenic fungus *Botrytis cinerea* Pers.: Fr. Indeed, numerous biological activities have previously been attributed to the *Juniperus* species. The acaricidal and insecticidal activities of *Juniperus occidentalis* and *Juniperus virginiana* have been reported (*2*), while *Juniperus procera* extracts have been shown to be potential antitermite agents (*3*). To our knowledge, however, before our investigation, no fungicidal studies had been carried out on this species. Thus, after the preliminary screening, we conducted a bioassay-guided fractionation of *J. lucayana* Britton and isolated and identified widdrol (**1**) as one of the major components responsible for the antifungal activity of this plant; **1** had been previously isolated from other species of *Juniperus* (*4*). On the other hand, studies on the structure−activity relationships of the substrates that inhibited the growth of *B. cinerea* and their relationships to the botryanes, characteristic metabolites produced by the fungus, revealed structural similarities between them (*5*, *6*). In particular, the distance between the hydrophobic geminal dimethyl group and the hydrophilic

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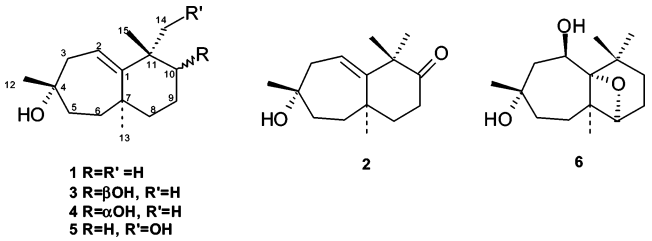

Figure 1. Structures of compounds **1**–**6**.

hydroxyl group seems to mimic the analogous distances found in botryanes (*5*). Furthermore, the basic skeleton of **1** possesses structural similarities with the clovanes, natural compounds that have previously been shown to have a specific action against *B. cinerea* (*5*, *7*). On the basis of these similarities, in this paper, we study the antifungal activity and biotransformation of this compound by *B. cinerea* and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and discuss the repercussions of the results on the search for 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 spectrophotometer, series FTIR. ^{1}H and ^{13}C NMR measurements were obtained on Varian Inova 400 NMR and 600 MHz spectrometers with $SiMe_4$ as the internal reference. Mass spectra were recorded on gas chromatography/mass spectrometry Agilent-LR and Finnigan MAT95 S instruments. High-performance liquid chromatography (HPLC) was performed with a Hitachi/Merck L-6270 apparatus equipped with an 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. Silica gel (Merck) was used for column chromatography. Purification by means of HPLC was accomplished using a silica gel column Hibar 60, 7 m, 1 cm wide, and 25 cm long. All solvents used were freshly distilled.

Microorganism and Antifungal Assay. The cultures of *B. cinerea* and *C. gloeosporioides* employed in this work, *Bc* 2100 and *Cg* 20122, respectively, were obtained from the Colección Española de Cultivos Tipos (CECT), Universidad de Valencia, Facultad de Biología, Spain, where a culture of these strains is deposited. Bioassays consisted of measuring the 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 [61 g/L of glucose (20 g)−malt (20 g)−peptone (1 g)−agar (20 g), pH 6.5−7.0] for *B. cinerea* or a PDB medium (4.0 g of potato starch and 20.0 g of dextrose per liter) for *C. gloeosporioides*. The final ethanol concentration was identical in both control and treated cultures. The medium was poured into sterile plastic Petri dishes measuring 13 cm in diameter, and then, a mycelial disc of fungus cut from an actively growing culture and measuring 1.5 cm in diameter was placed in the center of the agar plate. Inhibition of radial growth was measured for 6 days (**Figure 2**). Inhibition of growth 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 replications of colony diameters [± standard deviation (SD)]. The commercial fungicide dichlofluanid was used throughout as a standard agent for comparison.

Isolation of Widdrol (1). Ethanolic extract (52.8 g) of wood of *J. lucayana* Britton (870 g) was fractionated by column chromatography using different solvent systems [*n*-hexane, *n*-hexane/ethyl acetate (AcOEt) 10−30%, AcOEt, $CHCl_3$, $CHCl_3$−MeOH 30−50%, and MeOH] yielding 11 fractions (F1−11). Purification of fraction F6 was carried out by means of column chromatography on silica gel, eluting with *n*-hexane:AcOEt mixtures containing increasing percentages of AcOEt to give 13 fractions (F'1−13), one of which (F'5) was further purified by HPLC (hexane:AcOEt 7%; 3.5 mL min^{-1}). The majority

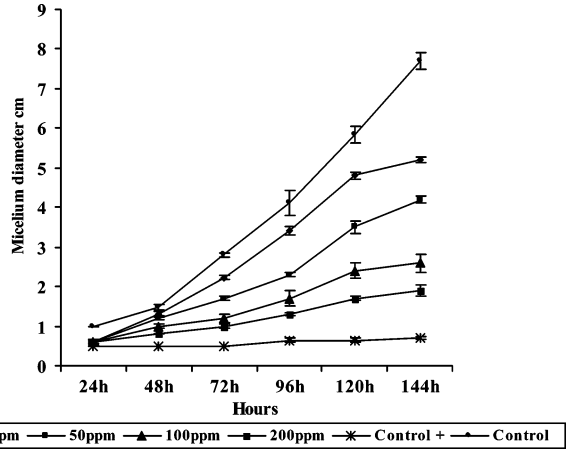

Figure 2. Antifungal effect of widdrol (**1**) on in vitro growth of *B. cinerea* 2100. Results are shown as mean values of mycelium diameter from three replications; bar = ±SD.

compound was purified again by HPLC (*n*-hexane:CH_2Cl_2 70%; 3.5 mL min^{-1}) to afford 651 mg of **1** (*8*).

Widdrol (1). White solid, mp 89.7−90.9 °C, lit (*8*) 98 °C; $[\alpha]_D^{20}$ = +98.2° (*c* 0.675, $CHCl_3$), lit (*8*) 105°; 98% ee. IR (film): ν_{max} 3309, 2964, 2924, 1134, 1116, 1098 cm^{-1}. ^{1}H and ^{13}C NMR data: **Tables 1** and **2**. EIMS *m/z*: 222 (M^+, 11), 207 (M^+-Me, 2), 204 (M^+-H_2O, 6), 164 (9), 151 (100).

Biotransformation of Widdrol (1) with *C. gloeosporioides* 20122. *C. gloeosporioides* was grown on an agar−malta−peptone medium in a Petri dish. A 10 day old mycelium was used to inoculate 11 (500 mL) flasks, each containing 250 mL of Czapek−Dox medium: glucose (50 g), yeast extract (1 g), KH_2PO_4 (5 g), $NaNO_3$ (2 g), $MgSO_4$ (0.5 g), and $FeSO_4$ (10 mg) per liter of distilled water. The pH was adjusted to 7.0 with aqueous NaOH. The flasks were incubated for 3 days at 25 °C with stirring at 180 rpm; the mycelium was then filtered and transferred into 9 (500 mL) flasks containing 250 mL of Czapek−Dox medium (with only 25 g of glucose) and 100 mg L^{-1} of **1**. The remaining two flasks constituted the control. Three days after inoculation, the mycelium was filtered and the fermentation broth was extracted three times with ethyl acetate. The extract was dried over anhydrous sodium sulfate, and the solvent was then evaporated. Fractionation of the extract (508 mg) was carried out by means of column chromatography on silica gel (SiCC); the column was eluted with petroleum ether: ethyl acetate (6:4), ethyl acetate, and methanol to give 14 fractions (F1−14), seven of which contained products that were not present in the control. Final purification of fraction F8 was carried out by means of analytical HPLC (hexane:acetone 70:30, 1.2 mL min^{-1}) to afford 10-oxowiddrol (**2**) (5 mg, 2.1%) and 10*β*-hydroxywiddrol (**3**) (6 mg, 2.5%). TLC revealed that fractions F9−11 contained the same majority product. These fractions were thus purified together by means of analytical HPLC (hexane:acetone 70:30, 1.2 mL min^{-1}) to afford 10α-hydroxywiddrol (**4**) (77 mg, 32%). Purification of F12 by means of column chromatography with increasing concentrations of acetone in hexane led to the isolation of 14α-hydroxywiddrol (**5**, 31 mg), which was also obtained (8.6 mg) upon purification of fractions F13 and F14 with analytical HPLC (hexane:acetone 70:30, 1.2 mL min^{-1}).

10-Oxowiddrol (2). Melting point, 83.0−85.0 °C; $[\alpha]_D^{20}$ = +29.3° (*c* 0.058, $CHCl_3$). IR (film): ν_{max} 3391, 2930, 1706 cm^{-1}. ^{1}H and ^{13}C NMR data: **Tables 1** and **2**. EIMS *m/z*: 236 (M^+, 10), 221 (M^+-15, 6), 203 (8), 175 (14), 165 (78), 43 (100). HREIMS (*m/z*) M^+ calcd for $C_{15}H_{24}O_2$, 236.1776; found, 236.1773.

10*β*-Hydroxywiddrol (3). Melting point, 111−113 °C; $[\alpha]_D^{20}$ = +271.4° (*c* 0.042, $CHCl_3$). IR (film): ν_{max} 3443, 2930, 1636 cm^{-1}. ^{1}H and ^{13}C NMR data: **Tables 1** and **2**. EIMS *m/z*: 238 (M^+, 0.2), 220 (M^+-18, 3), 205 (2), 149 (100). HREIMS (*m/z*) M^+ calcd for $C_{15}H_{26}O_2$, 238.1933; found, 238.1936.

10α-Hydroxywiddrol (4). Melting point, 106−108 °C; $[\alpha]_D^{20}$ = +328.6° (*c* 0.021, $CHCl_3$). IR (film): ν_{max} 3390, 2929, 1632, 1063 cm^{-1}. ^{1}H and ^{13}C NMR data: **Tables 1** and **2**. EIMS *m/z*: 238 (M^+,

Table 1. ^{1}H NMR (400 MHz) of Widdrol (**1**) and Its Biotransformation Products (**2**–**6**)

^{1}H	1	2	3	4	5	6
2	5.49 (dd, J = 6.0, 9.0 Hz)	5.59 (dd, J = 5.3, 9.5 Hz)	5.60 (dd, J = 10.7, 6.1 Hz)	5.52 (dd, J = 8.8, 5.8 Hz)	5.49 (dd, J = 8.8, 6.2 Hz)	4.60 (dd, J = 6.4, 1.5 Hz)
3α	2.48 (dd, J = 6.0, 13.6 Hz)	2.59 (dd, J = 5.3, 14.1 Hz)	2.51 (dd, J = 13.8, 6.1 Hz)	2.51 (dd, J = 13.7, 5.8 Hz)	2.57 (dd, J = 13.8, 6.2 Hz)	2.03 (dd, J = 15.1, 1.5 Hz)
3β	1.98 (dd, J = 9.0, 13.6 Hz)	2.13 (m superimposed)	2.00 (dd, J = 13.8, 10.7 Hz)	2.02 (dd, J = 13.7, 8.8 Hz)	2.06 (dd, J = 13.8, 8.8 Hz)	1.94 (dd, J = 15.1, 6.4 Hz)
5	1.68–1.61 (m superimposed)	1.96 (t, J = 13.6 Hz); 1.75 (bdd, J = 7.1, 13.6 Hz)	1.53 (m, superimposed)	1.67 (m, superimposed)	1.67 (m)	1.92 (m, superimposed); 1.74 (m, superimposed)
6	1.68–1.61 and 1.51–1.34 (m superimposed)	1.62 (m)	1.53 (m. superimposed), 1.34 (m)	1.67 (m. superimposed), 1.40 (m)	1.38 (m)	1.34 (m, 2H)
8α	1.28–1.21 (m superimposed)	1.56 (m, superimposed)	1.23 (dt, J = 13.6, 3.3 Hz)	1.08 (m)	1.25 (m, superimposed with H-12)	
8β	1.51–1.34 (m superimposed)	2.09 (m, superimposed)	1.78 (ddd, J = 13.6, 13.3, 3.8 Hz)	1.92 (dt, J = 13.5, 5.1 Hz)	1.61–1.41 (m, superimposed)	4.26 (t, J = 5.9 Hz)
9	1.68–1.61 and 1.51–1.34 (m superimposed)	2.50 (m)	1.71 (m), 1.57 (m)	2.09 (m), 1.58 (m)	1.61–1.41 (m, superimposed)	1.89 (ddd, J = 11.8, 6.1, 2.3 Hz); 1.42 (m, superimposed)
10	1.51–1.34 and 1.28–1.21 (m superimposed)		3.27 (dd, J = 10.7, 4.9 Hz)	3.51 (t, J = 4.3 Hz)	α 1.61–1.41 (m, superimposed); β 1.31 (ddd, J = 13.7, 12.1, 4.7 Hz)	1.46 (m, superimposed); 1.72 (ddd, J = 11.8, 6.1, 2.3 Hz)
12β	1.20 (s)	1.26 (s)	1.21 (s)	1.22 (s)	1.23 (s)	1.21 (s)
13α	1.18 (s)	1.24 (s)[a]	1.21 (s)	1.21 (s)	1.16 (s)	1.46 (s)
14α	1.07 (s)[a]	1.23 (s)[a]	1.15 (s)	1.13 (s)[a]	3.53 and 3.23 (d, J = 10.6 Hz)	1.02 (s)
15β	1.06 (s)[a]	1.16 (s)	1.04 (s)	1.12 (s)[a]	1.04	1.41 (s)

[a] Interchangeable signals.

0.8), 220 (M^+-18, 3), 205 (2), 149 (100). HREIMS (*m/z*) M^+ calcd for $C_{15}H_{26}O_2$, 238.1933; found, 238.1980.

14α-Hydroxywiddrol (5). Melting point, 98−100 °C; $[\alpha]_D^{20}$ = +130° (*c* 0.06, $CHCl_3$). IR (film): ν_{max} 3443, 1636 cm^{-1}. ^{1}H and ^{13}C NMR data: **Tables 1** and **2**. EIMS *m/z*: 238 (M^+, 0.4), 208 (M^+-CH_2O, 20), 189 (72), 147 (61), 43 (100). HREIMS (*m/z*) M^+ calcd for $C_{15}H_{26}O_2$, 238.1933; found, 238.1959.

Oxidation of 10α-Hydroxywiddrol (4). A 5.5 mg (0.023 mmol) amount of 10α-hydroxywiddrol (**4**) dissolved in 700 μL of CH_2Cl_2 was treated with 5.45 mg (0.0253 mmol) of pyridiniun chlorochromate (PCC) and the reaction mixture was stirred at room temperature for 5 h. Evaporation of the solvent under reduced pressure and purification by means of column chromatography with first CH_2Cl_2 and then AcOEt led to the isolation of a compound whose spectroscopic data were identical to those of compound **2**.

Biotransformation of 1 with *B. cinerea* 2100. *B. cinerea* was grown on an agar−tomato medium (500 mL of sterilized natural tomato juice, 20 g of agar per 500 mL of water) in a Petri dish for 12 days, after which spores were recovered to inoculate 11 flasks containing the previously described medium. In the same manner as described above, *B. cinerea* was inoculated with 100 mg L^{-1} of **1**. Five days after inoculation, the fermentation broth was extracted with ethyl acetate to afford 552.8 mg of crude material, which was then chromatographed on silica gel. Elution with increasing concentrations (10%) of ethyl acetate in light petroleum gave ten fractions (F1−10). F7 was purified further by means of column chromatography; in this case, elution with increasing concentrations of acetone in hexane yielded nine fractions (F7-1−9), one of which, F7-5, was finally purified with the same procedure to give compound **6** (6.3 mg, 2.5%).

Compound 6. Melting point, 90−92 °C; $[\alpha]_D^{20}$ = +2.86° (*c* 0.07, $CHCl_3$). IR (film): ν_{max} 3443, 2959 cm^{-1}. ^{1}H and ^{13}C NMR data: **Tables 1** and **2**. EIMS *m/z*: 254 (M^+, 6), 236 (M^+-H_2O, 20), 221 (M^+-H_2O-CH_3, 2), 167 (11), 138 (19), 43 (100). HREIMS (*m/z*) M^+ calcd for $C_{15}H_{26}O_3$, 254.1881; found, 254.1896.

RESULTS AND DISCUSSION

The antifungal properties of **1**, previously isolated from *J. lucayana* Britton as described in the Materials and Methods, against *B. cinerea* and *C. gloeosporioides* were tested with the "poisoned food technique" (*9*). While the compound proved to be inactive against *C. gloeosporioides*, at concentrations of 100 ppm and above, it significantly inhibited the mycelial growth of *B. cinerea* (**Figure 2**). Thus, after 6 days, fungus growth was inhibited by 60% at 100 ppm and by 70.7% at 200 ppm. These results showed that **1** has a significant inhibitory effect on *B. cinerea*; nevertheless, a detoxification mechanism would be present. In order to study this mechanism, we subjected **1** to biotransformation studies.

Because the antifungal effect was completely different for *C. gloeosporioides* and *B. cinerea*, we studied the biotransformation of **1** with both fungi, subjecting all of the biotransformation products to antifungal assays.

Compound **1** was first incubated with *C. gloeosporioides* for 3 days in accordance with a methodology previously described by our group (*10*). The medium was extracted with ethyl acetate and purified by means of chromatography to yield four biotransformation products.

The HREIMS of **2** showed a molecular ion corresponding to the molecular formula $C_{15}H_{24}O_2$. The IR spectrum possessed absorption bands at 3391, 2930, and 1706 cm^{-1}, indicating that a hydroxyl and a carbonyl group were present. The absence in the ^{13}C NMR of a methylene signal at δ19 ppm, characteristic of C-9 in the widdrol skeleton, indicated that the carbonyl group was located in the cyclohexane ring. Correlation observed in the HMBC experiment between the carbonyl group signal at δ 215.07 ppm a methyl group signal at δ 1.23 ppm and two methylene protons, which were correlated between themselves

Table 2. ^{13}C NMR (100 and 150* MHz) of Widdrol (**1**) and Its Biotransformation Products (**2**–**6**)

C	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15
1	154.28	117.68	39.81	72.99	37.95	39.40	39.81	41.63	18.53	40.00	36.79	28.44	26.66	31.94	32.88
2	152.11	120.31	40.31	72.15	39.15	37.79	39.00	35.95	34.60	215.07	50.64	28.97	26.75	27.41	23.25
3	152.05	119.70	39.35	73.08	37.54	39.17		34.58	39.03	76.94	42.73	28. 79	27.64	27.77	24.36
4	152.00	120.41	40.02	72.50	38.19	39.24		35.23	39.67	75.90	42.17	28. 87	26.00	27.80	30.20
5	150.00	122.40	39.24	72.94	37.62	39.50		41.56	18.30	35.70	42.12	28. 90	26.20	69.13	28.01
6*	92.00	71.42	42.05	74.88	36.77	40.52	47.77	74.33	51.32	48.22	46.38	32.51	29.90	25.99	33.16

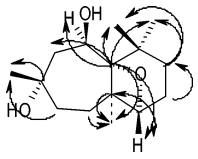

Figure 3. HMBC correlations for compound **6**.

in the $^1H-^1H$ COSY experiment, situated the carbonyl group on C-10 or C-8. The structure of compound **2** was definitively assigned through chemical correlation with compound **4**.

Compounds **3** (6.2 mg, 2.6%) and **4** (77 mg, 32%) were both monohydroxylated derivatives of **1** as deduced from their HREIMS data, which were found to be 238.1936 and 238.1980, respectively. This indicated that the molecular formula for both compounds was $C_{15}H_{26}O_2$. The ^1H NMR spectra of both products showed a new secondary alcohol resonance at δ 3.27 and 3.51 ppm, respectively, suggesting the introduction of a new hydroxyl group at one of the methylene groups in the widdrol molecule. This was corroborated by the presence of a low-field hydroxyl-bearing methine carbon signal at δ_C 76.94 and 75.90 ppm. The absence of a signal at δ 19 ppm in the ^{13}C NMR spectrum, characteristic of C-9 in the widdrol skeleton, indicated that the hydroxyl group was located on the cyclohexane ring in both compounds. Correlations observed in the HMBC experiments unequivocally determined the location of the hydroxyl group to be on C-10 in both compounds. Likewise, correlations between the methine carbon signals at δ 76.94 and 75.90 and two methyl group signals at δ 1.15 and 1.04 and 1.13 and 1.12 ppm, respectively, both of which were correlated with a quaternary carbon at δ 42.73 and 42.17 ppm, assigned to C-11, were observed. The stereochemistry of the hydroxyl group in compounds **3** and **4** was determined considering the minimum energy conformation and the coupling constants observed for H-10. Signal corresponding to H-10 in compound **4** appeared as a triplet ($J = 4.3$ Hz), indicating an α-disposition for the hydroxyl group. This assignment was consistent with the correlations shown in the NOE experiments. Correlations observed between H-2 and both methyl groups on C-11 (H-14 and H-15) determined the preferred pseudochair conformation, where the equatorial disposition of H-14 minimizes 1,3-diaxial interactions with the methyl group on C-7. On this conformation, H-10 must be in equatorial and the hydroxyl group in axial; this disposition was corroborated for the correlation observed in the NOE experiment between H-10 and H-14, H-15, and both protons on C-9. One of the principal differences observed in the ^1H NMR spectra of compounds **3** and **4** was the coupling pattern of the hydroxylic proton, which in **3** showed a double doublet signal ($J = 10.7$ and 4.9 Hz) indicating an axial disposition and therefore an α-orientation for the proton and a β-orientation for the hydroxyl group.

Compound **4** was chemically correlated with **2** through chemical oxidation. Treatment of alcohol **4** with PCC in CH_2Cl_2 for 3 h at room temperature led to a product with spectroscopic data identical to those of compound **2**. In addition, 38.6 mg (16%) of compound **5** was isolated. The absence of a methyl group signal in the ^1H and ^{13}C NMR spectra of **5**, as well as the appearance of hydroxymethyl resonances (δ_H 3.53 and 3.23; δ_C 69.13 ppm), suggest that **5** is hydroxylated on the *gem*-dimethyl group at C-11. The stereochemistry of C-11 was determined to be *R* due to the correlations observed in the NOESY experiment between H-14 and H-13, which have an α-disposition in the widdrol skeleton. In addition, correlations were observed between H-12 and H-3β and between H-3 α and H-14.

We then incubated **1** with *B. cinerea* 2100. Apart from the characteristic metabolites of *B. cinerea*, the only metabolite that could be isolated from a complicated mixture of products was compound **6**. Its HREIMS data indicated a molecular ion corresponding to the molecular formula $C_{15}H_{26}O_3$. The IR spectrum possessed an absorption band at 3423 cm^{-1}, confirming the presence of at at least one hydroxyl group. The presence in the ^{13}C NMR of four oxygen-linked carbons at δ 92 .00 (s), 74.88 (s), 74.33 (d), and 71.42 (d) ppm, together with the high chemical shift of the corresponding protons in the ^1H NMR spectrum and the molecular formula, all indicated that a heterocycle was present in the widdrol skeleton. A signal at δ 1.21 ppm was assigned to H-12 by the correlation observed in the HMBC experiment with signals assigned to C-3 and C-5 at δ 42.05 (t) and 36.77 (t), respectively. In addition, the H-12 proton signal showed correlation with a signal at δ 74.88 (s) ppm, and this was then assigned to C-4. Comparison with the characteristic signals of widdrol skeleton corroborated this assignment. Correlations observed between the carbon signal at δ 92 .00 ppm with the rest of the methyl group signals (δ 1.02, 1.41, and 1.46 ppm), along with a signal at δ 4.60 ppm, which was assigned to H-2, and a signal at δ 4.26 ppm all situated the heterocycle ring between carbons 1 and 6 or 8. The chemical shift observed for carbons 9 and 10 indicated that an oxetane ring was located between carbons 1 and 8. These and other correlations observed in the HMBC experiment (**Figure 3**) led us to propose the structure shown in **Figure 1** for compound **6**. The stereochemistry was assigned upon examining the coupling constants of protons H-2 and H-8. H-2, which appeared as a doublet, was thus assigned an α-orientation. In addition, irradiation of the H-2 signal gave an NOE interaction between H-3 α and the methyl group signal at δ 1.02, which was assigned to H-14. The absence of any interaction between the H-2 and the methyl group on C-4, with a β-disposition, corroborated the β-orientation for the hydroxyl group. Moreover, assuming that the oxygen groups on C-1 and C-2 arise from the ring opening of an epoxy group, the oxetane group must be situated on the α-face. This orientation is in agreement with the coupling constant observed for H-8, which appeared as a triplet, thus indicating that it has a β-orientation. Irradiation of the H-8 signal gave an NOE interaction between H-6 and H-9, but no effect was observed for the methyl group on C-7, which has an α-disposition in the widdrol skeleton.

The antifungal properties of these biotransformation products were then tested against *B. cinerea*, and all of the metabolites were found to be inactive at 100 ppm. These results, which

indicate that hydroxylations in these positions on the widdrol skeleton lead to a pronounced decrease in antifungal activity against *B. cinerea*, shed further light on the structural modifications, which may be necessary if **1** is to be developed as an inhibitory agent. Work is now in progress in order to obtain new widdrol derivatives with improved antifungal activity.

Finally, the regio- and stereoselectivity observed in the biotransformation with *C. gloeosporioides*, both of which could probably be improved under optimal conditions, suggest that the biotransformation with this fungus may be a good tool for functionalizing the C-10 position of the widdrol skeleton.

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