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Biotransformation of Bioactive Isocaryolanes by *Botrytis cinerea*

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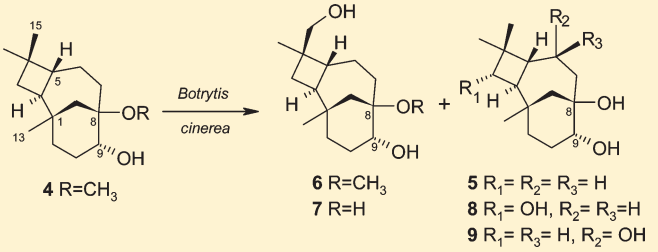
Supporting Information

ABSTRACT: The metabolism of the fungistatic agent (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**) by the fungus *Botrytis cinerea* has been investigated. Biotransformation of compound **4** yielded compounds **5** and **6**−**9**. No dihydrobotrydial is observed after 4 days of incubation of compound **4**. Separate biotransformation of (8*R*,9*R*)-isocaryolane-8,9-diol (**5**) yielded compounds **7**−**11**. The evaluation of the fungistatic activity against *B. cinerea* of compounds **4**, **5**, and **6** is reported. (4*R*,8*R*,9*R*)-8-Methoxyisocaryolane-9,15-diol (**6**), a major metabolite of (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**), shows a much reduced biological activity when compared with the parent compound. Isocaryolane derivatives **6**−**11** are described for the first time.

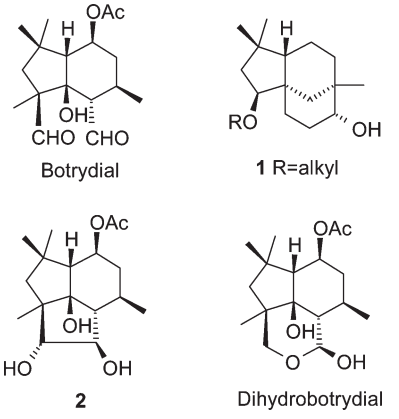


The fungus *Botrytis cinerea* is a serious plant pathogen[1] that has developed resistance to different classes of commercial fungicides, including the tubulin biosynthesis inhibiting methyl benzimidazole carbamates (MBC), quinone "outside" inhibitors (QoI) such as anilinopyrimidines and dicarboximides, the sterol biosynthesis C14-demethylase (SBI) inhibiting phtalimides, and phthalonitriles.[2] Consequently, there is interest in the development of antifungal agents with novel modes of action to combat resistant strains, compatible with the use of biological crop protection agents and harmless to the crop, farmer, consumer, and the environment.[3]

Part of the interaction of *B. cinerea* with a host plant involves the action of the low molecular weight phytotoxin botrydial on the plant.[4] Inhibition of the production of this phytotoxin can lead to a decrease in the symptoms of the disease.[5] Furthermore, there is evidence that the production of botrydial during the idiophase of fungal development has a limiting effect on fungal growth.[6] Therefore, actions aimed at disturbing the delicate balance of secondary metabolites produced by the fungus can potentially lead to the control of the phytopathogen.

A number of sesquiterpene derivatives have been studied as inhibitors of the growth of *B. cinerea*, including compounds with caryophyllane,[7] clovane,[8] ginsane,[9] patchoulane,[9b,10] isoprobotryane,[9b,11] 1-epiprobotryane,[12] norprobotryane (norpresilphiperfolane),[13] cedrane,[9b] globulane,[9b] daucane,[14] and eremophilane[15] skeletons. Some of these studies included the analysis of the hydroxylation and detoxification of the compounds by *B. cinerea*.[7−10,13−15]

For instance, 2-alkoxyclovan-9α-ol derivatives (**1**) present a structural similarity to the proposed key intermediate (**2**)[16] in the biosynthesis[17] of the phytotoxic[18] botryane metabolites produced by the fungus *B. cinerea*, and they have been shown to inhibit the growth of this fungus.[8a,b] Activity is correlated with the nature of the side chain at C-2.[8c] These 2-alkoxyclovan-9α-ol derivatives are metabolized by *B. cinerea* by cleavage of the above-mentioned chain attached at C-2.[8]



Isocariolane skeleton compounds are obtained by cyclization of a caryophylladienol derivative, obtained in turn from caryophyllene oxide.[19] The combination of activity evaluation of isocaryolane compounds against *B. cinerea* and analysis of metabolism of isocaryolane skeleton compounds by *B. cinerea* would help explain the way the fungus detoxifies this class of compounds and, in turn, help to develop these compounds as antifungal leads. We present here the biotransformation by the fungus *B. cinerea* of (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**) and

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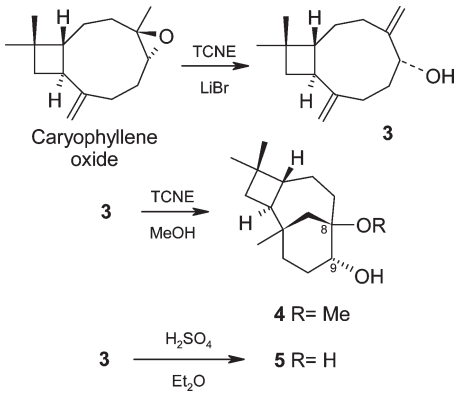
Scheme 1



(8*R*,9*R*)-isocaryolane-8,9-diol (**5**) and the evaluation of their activity against *B. cinerea*.

RESULTS AND DISCUSSION

The chemical transformations carried out for the preparation of isocaryolanes **4** and **5** are summarized in Scheme 1. (8*R*,-9*R*)-8-Methoxyisocaryolan-9-ol (**4**) was obtained from (1*R*,-5*R*,9*S*)-caryophylla-4(12),8(13)-dien-5-ol (**3**) by treatment with tetracyanoethylene (TCNE) in methanol.[19] In turn, (1*R*,5*R*,9*S*)-caryophylla-4(12),8(13)-dien-5-ol (**3**) was obtained from caryophyllene oxide by isomerization with TCNE and LiBr in acetone.[20] (8*R*,9*R*)-Isocaryolane-8,9-diol (**5**) was obtained by treatment of (1*R*,5*R*,9*S*)-caryophylla-4(12),8(13)-dien-5-ol (**3**) with sulfuric acid in diethyl ether.[19b]

The substrates (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**) and (8*R*,9*R*)-isocaryolane-8,9-diol (**5**) were incubated separately with *B. cinerea* for 4−16 days on a surface culture. In every culture, a certain amount of the characteristic metabolite of *B. cinerea*, dihydrobotrydial,[21] was expected. The separate feeding of **4** and **5** to liquid cultures of *B. cinerea* did not suppress the production of botryane metabolites (except in the case of **4** after 4 days of incubation). No dihydrobotrydial could be detected either in the neutral or in the acidic fractions of the extract of the broth of a feeding of **4** after 4 days of incubation. The metabolites that were isolated (Scheme 2) have been tabulated (see Table 1) and quantified for each culture (see Experimental Section). The metabolites were identified by their ^{1}H and ^{13}C NMR spectra, combined with 1D and 2D NMR techniques, and by comparison with authentic samples, where appropriate.

Compound **6** was obtained as a yellow oil. HREIMS gave an ion peak at *m*/*z* 268.2029, consistent with the formula $C_{16}H_{28}O_3$. The ^{13}C NMR spectrum showed resonances at δ_C 80.2, 73.3, and 72.0 ppm (C, CH, and CH_2 carbons, respectively), which is in agreement with the presence of three oxygenated functions on the molecule. The ^{1}H NMR spectrum showed signals at δ_H 3.55, 3.33, and 3.21, which were assigned to H-9β, H_2-15β, and -OCH_3, respectively. A series of NOE effects between signals at δ_H 3.33 (H_2-15β) and 1.94 (H-5β) and between δ_H 3.33 (H_2-15β) and 1.44 (H-3β), on one hand, and between signals δ_H 1.44 (H-3β) and 0.81 (H_3-13), on the other, supported the assignment of CH_2OH as CH_2OH-15β and allowed the assignation of the structure of this compound as (4*R*,8*R*,9*R*)-8-methoxyisocaryolane-9,15-diol (**6**).

Compound **7** possessed ^{1}H NMR signals at δ_H 3.50 and 3.35, which were assigned to H-9β and H_2-15β, respectively. In the ^{13}C NMR spectrum there were signals at δ_C 76.5, 79.1, and 72.1 ppm, which is in agreement with the presence of three oxygenated functions on the molecule. On the other hand, a carbon resonance is lacking when compared with compound **6**. This observation is consistent with the formula $C_{15}H_{26}O_3$, determined by HREIMS. These data, together with an analysis of 2D-NMR, led to the proposal of the structure of compound **7** as (4*R*,8*R*,9*R*)-isocaryolane-8,9,15-triol.

Compounds **8**, **9**, and **10** showed some common spectroscopic and spectrometric features. All displayed an absorption in their IR spectrum that is consistent with the presence of at least one hydroxyl group. Their HREIMS gave the formula $C_{15}H_{26}O_3$. The ^{13}C NMR spectrum of each displayed signals for three carbons bearing oxygenated functions; these were δ_C 76.5 (C, C-8), 78.9 (CH, C-9), and 75.6 (CH, C-3) ppm for compound **8**, δ_C 76.6 (C, C-8), 78.8 (CH, C-9), and 70.8 (CH, C-6) ppm for compound **9**, and δ_C 76.4 (C, C-8), 79.6 (CH, C-9), and 82.7 (CH, C-5) ppm for compound **10**. Furthermore, the comparison of ^{13}C NMR data also shows that two of the above-mentioned carbons resonate at very similar positions in compounds **8**, **9**, and **10** (those signals assigned to C-8 and C-9).

Scheme 2

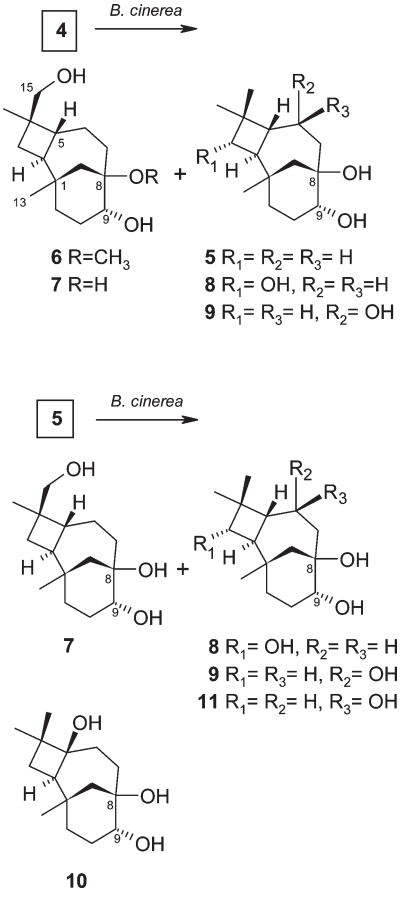


Table 1. Metabolites of Isocaryolanes by *B. cinerea*

substrate	metabolites
4[a]	DHB, **5**, **6**, **7**, **8**, **9**
5	DHB, **7**, **8**, **9**, **10**, **11**

[a] No dihydrobotrydial (DHB) was observed after 4 days of incubation.

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On the other hand, there are significant differences in the NMR spectra of compounds **8**, **9**, and **10**, including an additional ^{13}C signal originating from a carbon bearing an oxygen, which can be assigned to different carbons in each compound. Position assignment and the stereochemistry of this oxygenated function were accomplished by a combination of 1D and 2D homo- and heterocorrelated NMR experiments.

The ^1H NMR spectrum in compound **8** showed signals at δ_H 3.50 and 3.40, which were assigned to H-9β and H-3β, respectively. A series of NOE effects between signals at δ_H 1.44 (H-3β) and 3.33 (H$_3$-15β) and between δ_H 1.44 (H-3β) and 1.94 (H-5β) supported the assignment of CHC*H*OH as H-3β and confirmed the structure of this compound as (3*S*,8*R*,9*R*)-isocaryolane-3,8,9-triol (**8**).

The ^1H NMR spectrum in **10** showed signals at δ_H 2.39, 1.82, 1.49, 1.09, 0.98, and 0.94, which were assigned to H-2α, H-3α, H-3β, H$_3$-14α, H$_3$-15β, and H$_3$-13, respectively. The position and stereochemistry of the hydroxyl group at C-5 have been confirmed by NOE experiments involving the above-described signals (Figure 1). This, in turn, confirmed the structure of this compound as (2*R*,5*S*,8*R*,9*R*)-isocaryolane-5,8,9-triol (**10**).

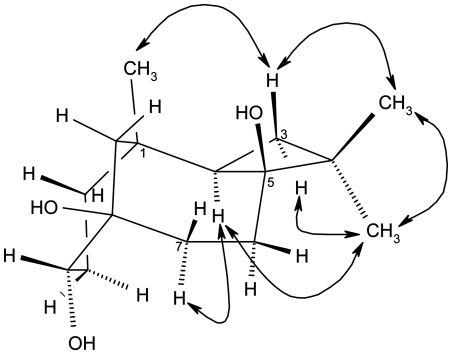

Figure 1. Selected NOE for compound **10**.

Compounds **9** and **11** display a very similar signal pattern in their ^{13}C NMR (Table 3), where resonances at δ_C 70.8 (*C*H) and 69.5 (*C*H), respectively, can be assigned in both cases to C-6. The ^1H NMR spectrum of compound **11** showed signals at δ_H 4.10 and 3.47, which were assigned to H-6α and H-9β, respectively. Nuclear Overhauser effects observed between δ_H 4.10 (H-6α) and both 1.95 (H-2α) and 1.029 (H$_3$-14α) supported the assignment of the β-stereochemistry to the hydroxyl group at C-6 in compound **11** and, in turn, the assignment of the α-stereochemistry to the hydroxyl group at C-6 in compound **9**. Therefore, the structure of these metabolites was established as (6*R*,8*S*,9*R*)-isocaryolane-6,8,9-triol (**9**) and (6*S*,8*S*,9*R*)-isocaryolane-6,8,9-triol (**11**).

The antifungal properties of compounds **4**, **5**, and **6** were determined against the growth of *B. cinerea* using the poisoned food technique.[22] The commercial fungicide Euparen was used as a standard for comparison in this test. Several levels of inhibition were observed. Maximal inhibition was shown for compound **4**, and it was found to decrease in the sequence **4** > **5** > **6**.

In the transformation of (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**) by *B. cinerea*, the methoxyl group appears to be metabolized in a concurrent way with a hydroxylation of C-15β. No other position seems to be functionalized prior to removal of the methoxyl group, as no compounds have been observed with both the hydroxyl group at C-6 or C-3 and a methoxyl group at C-8.

Compound **6** shows very little activity compared to compounds **4** and **5**, so the metabolism of (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**) to yield **6** seems an effective mechanism of

Table 2. ^1H NMR Spectroscopic Data for 6−11

	6	7	8	9	10	11
position	δ_H[a] (mult, *J* in Hz)	δ_H[a] (mult, *J* in Hz)	δ_H[b] (mult, *J* in Hz)	δ_H[c] (mult, *J* in Hz)	δ_H[a] (mult, *J* in Hz)	δ_H[b] (mult, *J* in Hz)
1						
2	α: 2.17 (m)	α: 2.15, ddd (12.0, 10.8, 8.4)	α: 1.87−1.78 (m)	α: 1.99−1.88 (m)	α: 2.39, dd (12.0, 8.1)	α: 1.95 (m)
3	α: 1.35 (m)	α: 1.42−1.36 (m)	β: 3.40, d (8.8)	a: 1.46, t (8.8)	α: 1.82, dd (12.0, 9.0)	a: 1.46, dd (10.4, 9.2)
	β: 1.44, dd (9.6, 7.8)	β: 1.48−1.42 (m)		b: 1.40−1.30 (m)	β: 1.49, dd (9.0, 8.1)	b: 1.35, t (10.4)
4						
5	β: 1.94 (m)	β: 1.92, ddd (12.0, 7.8, 6.0)	β: 1.23−1.16 (m)	β: 1.75−1.65 (m)		β: 1.78−1.74 (m)
6	a: 1.65 (m)	a: 1.64 (m)	a: 1.76−1.62 (m)	β: 3.79, dt (8.2, 4.2)	a: 1.93−1.86 (m)	α: 4.10 (m)
	b: 1.58−1.52 (m)	b: 1.48−1.42 (m)	b: 1.43 (m)		b: 1.59−1.53 (m)	
7	a: 1.91−1.86 (m)	α: 1.98, ddd (15.6, 11.4, 4.0)	α: 2.03, ddd (14.8, 9.2, 4.0)	a: 2.26, dd (14.4, 4.0)	α: 1.93−1.86 (m)	a: 2.10, dd (15.4, 3.2)
	b: 1.58−1.52 (m)	β: 1.42−1.36 (m)	β: 1.38−1.30 (m)	b: 1.40−1.30 (m)	β: 1.59−1.53 (m)	b: 1.74−1.68 (m)
8						
9	β: 3.55 dd (13.2, 5.4)	β: 3.50 dd (12.0, 4.8)	β: 3.50, dd (11.6, 5.6)	β: 3.38, dd (11.6, 5.2)	β: 3.52 (11.4, 5.4)	β: 3.47, dd (12.0, 5.6)
10	α: 1.75 dq (13.2, 4.6)	α: 1.77−1.70 (m)	a, b: 1.88−1.72 (m)	a, b: 1.75−1.55 (m)	a: 1.93−1.86 (m)	a: 1.86−1.80 (m)
	β: 1.85−1.80 (m)	β: 1.84 (m)			b: 1.74−1.72 (m)	b: 1.66−1.60 (m)
11	α: 1.46−1.38 (m)	α: 1.48−1.42 (m)	a: 1.53−1.47 (m)	a: 1.40−1.30 (m)	a: 1.41−1.38 (m)	a: 1.43−1.38 (m)
	β: 1.22 dt (13.2, 4.8)	β: 1.26, dt (13.2, 4.8)	b: 1.38−1.30 (m)	b: 1.30−1.20 (m)	b: 1.15, dt (13.2, 4.8)	b: 1.27, dt (13.2, 4.8)
12	a: 1.91−1.86 (m)	a: 1.77−1.70 (m)	a: 1.76−1.62 (m)	a: 1.99−1.88 (m)	a: 2.62, dd (12.6, 3.0)	a: 2.04, dd (13.2, 3.2)
	b: 0.90, d (12.6)	b: 1.01, d (13.2)	b: 1.03, d (13.2)	b: 1.05, d (13.6)	b: 0.97, d (12.6)	b: 1.10, d (13.2)
13	0.81 (s)	0.81 (s)	0.94 (s)	0.81 (s)	0.94 (s)	0.83 (s)
14α	1.01 (s)	1.03 (s)	0.80 (s)	1.12 (s)	1.09 (s)	1.03 (s)
15β	3.33 (s)	3.35 (s)	1.02 (s)	1.08 (s)	0.98 (s)	1.09 (s)
−OC$\underline{H_3}$	3.21 (s)					

[a] Acquired in $CDCl_3$ (600 MHz). [b] Acquired in $CDCl_3$ (400 MHz). [c] Acquired in CD_3OD (400 MHz).

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Table 3. ^{13}C NMR Data ($CDCl_3$, 100 MHz) for Compounds 4−11

	4	5	6[a]	7[a]	8	9[b]	10[a]	11
position	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	32.7, C	33.4, C	32.9, C	33.3, C	32.9, C	33.6, C	33.4, C	33.6, C
2	36.5, CH	38.0, CH	36.3, CH	37.4, CH	50.4, CH	40.1, CH	39.1, CH	38.4, CH
3	35.4, CH_2	36.0, CH_2	30.2, CH_2	30.4, CH_2	75.6, CH	37.2, CH_2	34.3, CH_2	35.5, CH_2
4	34.8, C	34.0, C	39.8, C	39.8, C	40.9, C	35.5, C	41.9, C	35.1, C
5	43.9, CH	45.2, CH	38.5, CH	39.6, CH	37.3, CH	54.4, CH	82.7, C	53.7, CH
6	21.6, CH_2	20.8, CH_2	21.9, CH_2	20.8, CH_2	20.1, CH_2	70.8, CH	30.5, CH_2	69.5, CH
7	28.8, CH_2	32.8, CH_2	28.9, CH_2	32.4, CH_2	33.0, CH_2	43,9 CH_2	32.0, CH_2	40.2, CH_2
8	80.3, C	77.0, C	80.2, C	76.5, C	76.5, C	76.6, C	76.4, C	77.0, C
9	76.2, CH	79.3, CH	76.3, CH	79.1, CH	78.9, CH	78.8, CH	79.6, CH	78.4, CH
10	27.3, CH_2	28.6, CH_2	27.3, CH_2	28.4, CH_2	28.4, CH_2	29.5, CH_2	28.5, CH_2	27.9, CH_2
11	36.6, CH_2	37.7, CH_2	36.4,CH_2	37.2, CH_2	38.8, CH_2	39.9, CH_2	37.9, CH_2	37.0, CH_2
12	41.9, CH_2	48.3, CH_2	41.8, CH_2	48.0, CH_2	48.2, CH_2	49.2, CH_2	46.2, CH_2	47.8, CH_2
13	26.1, CH_3	26.2, CH_3	26.1, CH_3	25.8, CH_3	25.4, CH_3	27.2, CH_3	26.6, CH_3	26.0, CH_3
14	20.7, CH_3	20.9, CH_3	16.2, CH_3	16.1, CH_3	14.8, CH_3	21.3, CH_3	23.7, CH_3	21.3, CH_3
15	30.6, CH_3	30.8, CH_3	72.0, CH_2	72.1, CH_2	28.1, CH_3	31.8, CH_3	21.9, CH_3	31.1, CH_3
$-OCH_3$	49.0, CH_3		49.2, CH_3					

[a] 150 MHz. [b] Acquired in CD_3OD.

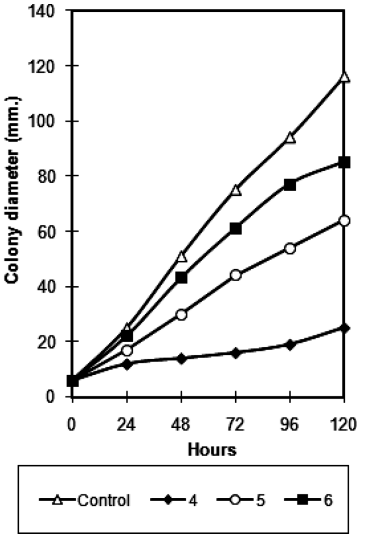

Figure 2. Comparison of fungal growth inhibition (*B. cinerea*) among compounds **4**, **5**, and **6** (100 ppm dose).

detoxification of compound **4**. On the other hand, the metabolism of the methoxyisocaryolanol **4** to yield diol **5** is a less effective way of detoxification of compound **4**, as (8*R*,9*R*)-isocaryolane-8,9-diol (**5**) still possesses a moderate level of antifungal activity (see Figure 2). It is possible that further metabolism of compound **5** by the fungus, which is consistent with the range of hydroxylated compounds detected in the biotransformations of the isocaryolanes **4** and **5** by *B. cinerea* and with the variations in the observed levels of compound **5** in the extract, occurs.

Further oxidation or epimerization at C-9 on the isocaryolane skeleton does not occur in the metabolism of either compound **4** or compound **5**. This suggests that the hydroxyl group at this position is interacting with a binding site in the enzymatic complex responsible for hydroxylations.

As mentioned above, in almost every incubation of **4** and **5** with *B. cinerea*, a certain amount of the characteristic metabolite of *B. cinerea*, dihydrobotrydial,[21] was produced. Only in the feeding of **4** after 4 days of incubation could no dihydrobotrydial be detected either in the neutral or in the acidic fractions of the extract of the broth. An interaction with botryane metabolism seems to be taking place, which should be further explored.

In conclusion, (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**) and (8*R*,9*R*)-isocaryolane-8,9-diol (**5**) show an interesting activity profile against *B. cinerea*. They undergo metabolism by the fungus, by a combination of methoxyl group removal (for compound **4**) and hydroxylation (for both compounds). Further development of this class of compounds as antifungal leads should take into account these observations in order to prevent the fungus from readily detoxifying improved antifungal compounds based on the isocaryolane sesquiterpene skeleton.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured with a Reichert-Jung Kofler block and are uncorrected. Optical rotations were determined with a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrophotometer. ^1H and ^{13}C NMR measurements were obtained on Varian INOVA 400 and INOVA 600 NMR spectrometers with $SiMe_4$ as internal reference. NMR assignments were made by a combination of 1D and 2D techniques and by comparison with those made for previously described compounds, where appropriate. Mass spectra were recorded on a Finnigan Voyager spectrometer at 70 eV. High-resolution mass spectra were recorded on a Micromass Autospec spectrometer at 70 eV. 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). TLC was performed on Merck Kiesegel 60 F_{254}, 0.2 mm thick. Silica gel (Merck) was used for column chromatography. Purification by HPLC was accomplished using a Si gel column (LiChrospher Si 60, 10 μm, 1 cm wide, 25 cm long).

Microorganism and Antifungal Assays. The culture of *Botrytis cinerea* employed in this work, *B. cinerea* (UCA 992), was obtained from

grapes from the Domecq vineyard, Jerez de la Frontera, Cádiz, Spain. This culture of *B. cinerea* has been deposited at the Mycological Herbarium Collection (UCA), Facultad de Ciencias, Universidad de Cádiz. Bioassays were performed by measuring radial growth on agar medium in a Petri dish in the presence of test compound. Test compounds were dissolved in EtOH to give a final compound concentration in the culture medium of 50 to 200 mg L^{-1}. Solutions of test compounds were added to glucose-malt-peptone-agar medium (61 g of glucose-malt-peptone-agar per L, pH 6.5−7.0). The final EtOH concentration was identical in both the control and treated cultures. The medium was poured in 6 or 9 cm diameter sterile plastic Petri dishes, and a 5 mm diameter mycelia disk of *B. cinerea* cut from an actively growing culture was placed in the center of the agar plate. Radial growth was measured for six days. Every concentration was evaluated in triplicate.

General Culture Conditions. *B. cinerea* (UCA 992) was grown on surface culture in Roux bottles at 25 °C for 4 days on a Czapek-Dox medium (150 mL per flask) comprising (per L 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 substrate dissolved in ethanol was added to each flask, and the fermentation continued for a further period (see below). The mycelium was filtered and washed with brine and EtOAc. The broth was saturated with sodium chloride, acidified (pH 2), and extracted with EtOAc. The extracts were separated into acidic and neutral fractions with aqueous sodium hydrogen carbonate. The acid fraction was recovered in EtOAc. The extracts were dried over sodium sulfate, the solvent was evaporated, and the residues were chromatographed on silica gel in a gradient mixture of petroleum ether−EtOAc of increasing polarity. The acidic fractions were methylated with diazomethane prior to chromatography. Chromatography of the acidic fractions yielded inseparable mixtures.

Biotransformation of (8*R*,9*R*)-8-Methoxyisocaryolan-9-ol (4) by *B. cinerea*. Compound **4** (360 mg) was distributed between 24 flasks of *B. cinerea*, and the fermentation grown for a further 4, 7, 9, and 16 days. Chromatography of the neutral fraction (4 days, 6 flasks) gave (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**) (18 mg), (8*R*,9*R*)-isocaryolane-8,9-diol (**5**) (16 mg), (4*R*,8*R*,9*R*)-8-methoxyisocaryolane-9,15-diol (**6**) (15 mg), (3*S*,8*R*,9*R*)-isocaryolane-3,8,9-triol (**8**) (5 mg), (4*R*,8*R*,9*R*)-isocaryolane-8,9,15-triol (**7**) (7 mg), and (6*R*,8*S*,9*R*)-isocaryolane-6,8,9-triol (**9**) (2 mg), and no dihydrobotrydial was detected. Chromatography of the neutral fraction (7 days, 6 flasks) gave (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**) (13 mg), (8*R*,9*R*)-isocaryolane-8,9-diol (**5**) (19 mg), (4*R*,8*R*,9*R*)-8-methoxyisocaryolane-9,15-diol (**6**) (19 mg), (3*S*,8*R*,9*R*)-isocaryolane-3,8,9-triol (**8**) (2 mg), (4*R*,8*R*,9*R*)-isocaryolane-8,9,15-triol (**7**) (4 mg), (6*R*,8*S*,9*R*)-isocaryolane-6,8,9-triol (**9**) (<1 mg), and dihydrobotrydial (3 mg). Chromatography of the neutral fraction (9 days, 6 flasks) gave (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**) (11 mg), (8*R*,9*R*)-isocaryolane-8,9-diol (**5**) (16 mg), (4*R*,8*R*,9*R*)-8-methoxyisocayolane-9,15-diol (**6**) (17 mg), (3*S*,8*R*,9*R*)-isocaryolane-3,8,9-triol (**8**) (2 mg), (4*R*,8*R*,9*R*)-isocaryolane-8,9,15-triol (**7**) (4 mg), (6*R*,8*S*,9*R*)-isocaryolane-6,8,9-triol (**9**) (2 mg), and dihydrobotrydial (10 mg). Chromatography of the neutral fraction (16 days, 6 flasks) gave (8*R*,9*R*)-8-methoxyisocaryolan-9-ol (**4**) (3 mg), (8*R*,9*R*)-isocaryolane-8,9-diol (**5**) (11 mg), (4*R*,8*R*,9*R*)-8-methoxyisocaryolane-9,15-diol (**6**) (17 mg), (3*S*,8*R*,9*R*)-isocaryolane-3,8,9-triol (**8**) (3 mg), (4*R*,8*R*,9*R*)-isocaryolane-8,9,15-triol (**7**) (8 mg), (6*R*,8*S*,9*R*)-isocaryolane-6,8,9-triol (**9**) (5 mg), and dihydrobotrydial (26 mg).

Biotransformation of (8*R*,9*R*)-Isocaryolane-8,9-diol (5) by *B. cinerea*. Compound **5** (180 mg) was distributed over 12 flasks of *B. cinerea*, and the fermentation grown for a further 4 and 9 days. Chromatography of the neutral fraction (4 days, 6 flasks) gave (8*R*,9*R*)-isocaryolane-8,9-diol (**5**) (22 mg), (3*S*,8*R*,9*R*)-isocaryolane-3,8,9-triol (**8**) (8 mg), (4*R*,8*R*,9*R*)-isocaryolane-8,9,15-triol (**7**) (4 mg), (6*R*,8*S*,9*R*)-isocaryolane-6,8,9-triol (**9**) (8 mg), (6*S*,8*S*,9*R*)-isocaryolane-6,8,9-triol (**11**) (<1 mg), (2*R*,5*S*,8*R*,9*R*)-isocaryolane-5,8,9-triol (**10**) (2 mg), and dihydrobotrydial (32 mg). Chromatography of the neutral fraction (9 days, 6 flasks) gave (8*R*,9*R*)-isocaryolane-8,9-diol (**5**) (20 mg), (3*S*,8*R*,9*R*)-isocaryolane-3,8,9-triol (**8**) (4 mg), (4*R*,8*R*,9*R*)-isocaryolane-8,9,15-triol (**7**) (2 mg), (6*R*,8*S*,9*R*)-isocaryolane-6,8,9-triol (**9**) (15 mg), (6*S*,8*S*,9*R*)-isocaryolane-6,8,9-triol (**11**) (<1 mg), (2*R*,5*S*,8*R*,9*R*)-isocaryolane-5,8,9-triol (**10**) (2 mg), and dihydrobotrydial (35 mg).

*(4R,8R,9R)-8-Methoxyisocaryolane-9,15-diol (**6**):* oil; $[\alpha]^{25}_{D}$ −5 (*c* 0.22, $CHCl_3$); IR (film) ν_{max} 3421, 2945, 1461, 1364, 1064 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) (see Table 2); ^{13}C NMR ($CDCl_3$, 150 MHz) (see Table 3); HMBC (selected correlations) C_1 → H-3α, H-5*β*, H-12a/H-12b, H_3-13; C_4 → H-3*β*, H_3-14α, H_2-15*β*; C_8 → −O*CH*$_3$, H-7a/H-7b, H-9*β*, H-12a/H-12b; EIMS *m/z* 268 $[M]^+$ (2), 253 (2), 237 (2), 218 (2), 211 (4), 210 (15), 209 (100), 191, (10), 141 (8), 137 (8), 109 (15), 81 (10), 55 (8); HREIMS *m/z* 268.2029 $[M]^+$ (calcd for $C_{16}H_{28}O_3$, 268.2038).

*(4R,8R,9R)-Isocaryolane-8,9,15-triol (**7**):* yellow oil; $[\alpha]^{25}_{D}$ −6 (*c* 0.33, $CHCl_3$); IR (film) ν_{max} 3379, 2937, 1456, 1380, 1049 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) (see Table 2); ^{13}C NMR ($CDCl_3$, 150 MHz) (see Table 3); HMBC (selected correlations) C_1 → H-3α, H-5*β*, H-12a/H-12b, H_3-13; C_4 → H-2α, H-3α/ H-3*β*, H-5*β* H_3-14α, H_2-15*β*; C_8 → −O*CH*$_3$, H-7a/H-7b, H-12a/H-12b; EIMS *m/z* 254 $[M]^+$ (0.7), 236 (3), 218 (3), 196 (23), 195 (100), 177 (36), 149 (17), 135 (25), 127 (34), 123 (69), 109 (55), 81 (61), 55 (41); HREIMS *m/z* 254.1904 $[M]^+$ (calcd for $C_{15}H_{26}O_3$, 254.1882).

*(3S,8R,9R)-Isocaryolane-3,8,9-triol (**8**):* yellow oil; $[\alpha]^{25}_{D}$ −11 (*c* 0.33, $CHCl_3$); IR (film) ν_{max} 3378, 2947, 1461, 1366, 1058 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) (see Table 2); ^{13}C NMR ($CDCl_3$, 100 MHz) (see Table 3); EIMS *m/z* 254 $[M]^+$ (0.2), 236 (5), 218 (50), 195 (47), 177 (36), 161 (17), 147 (18), 136 (26), 123 (79), 109 (71), 95 (75) 84 (100), 69 (82) 55 (77), 41 (75); HREIMS *m/z* 254.1860 $[M]^+$ (calcd for $C_{15}H_{26}O_3$, 254.1882).

*(6R,8S,9R)-Isocaryolane-6,8,9-triol (**9**):* amorphous solid; $[\alpha]^{25}_{D}$ +7 (*c* 0.41, MeOH); IR (film) ν_{max} 3419, 2952, 1459, 1350, 1055 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) (see Table 2); ^{13}C NMR (CD_3OD, 100 MHz) (see Table 3); HMBC (selected correlations) C_1 → H-2α, H-12a/H-12b, H_3-13; C_4 → H-5*β* H_3-14α, H_3-15*β*; C_8 → H-6*β*, H-9*β*, H-7a/H-7b, H-12a/H-12b; EIMS *m/z* 254 $[M]^+$ (1), 236 (5), 221 (3), 218 (4), 195 (100), 179 (22), 177 (20), 149 (15), 135 (18), 121 (22), 109 (48), 107 (25), 95 (35) 81 (30), 67 (18) 55 (28); HREIMS *m/z* 254.1881 $[M]^+$ (calcd for $C_{15}H_{26}O_3$, 254.1882).

*(2R,5S,8R,9R)-Isocaryolane-5,8,9-triol (**10**):* yellow oil; $[\alpha]^{25}_{D}$ +4 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3423, 2933, 1461, 1340, 1060 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) (see Table 2); ^{13}C NMR ($CDCl_3$, 150 MHz) (see Table 3); HMBC (selected correlations) C_1 → H-2α, H_3-13; C_4 → H-6a, H_3-14α, H_3-15*β*; C_8 → H-9*β*, H-7a/H-7b, H-12a/H-12b; EIMS *m/z* 254 $[M]^+$ (1), 236 (8), 218 (9), 198 (10), 195 (12), 180 (11), 165 (5), 151 (7), 140 (10), 139 (100), 123 (22), 109 (22), 107 (15), 98 (17), 97 (18), 81 (16), 55 (28); HREIMS *m/z* 254.1878 $[M]^+$ (calcd for $C_{15}H_{26}O_3$, 254.1882).

*(6S,8S,9R)-Isocaryolane-6,8,9-triol (**11**):* needles ($CHCl_3$); 113−115 °C; $[\alpha]^{25}_{D}$ +9 (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 3368, 2905, 1430, 1380, 1052 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) (see Table 2); ^{13}C NMR ($CDCl_3$, 100 MHz) (see Table 3); HMBC (selected correlations) C_1 → H_3-13; C_4 → H-3a/H-3b, H_3-14α, H_3-15*β*; C_5 → H-2α, H_3-14α, H_3-15*β*; C_6 → H-5*β*; EIMS *m/z* 254 $[M]^+$ (2), 236 (8), 221 (5), 218 (6), 196 (12), 195 (100), 177 (23), 149 (18), 135 (18), 121 (22), 109 (48), 91 (35), 81 (32), 55 (31); HREIMS *m/z* 236.1777 $[M - 18]^+$ (calcd for $C_{15}H_{24}O_2$, 236.1776).

■ ASSOCIATED CONTENT

Supporting Information. ^1H and ^{13}C NMR spectra of compounds **6**−**11**. This information is available free of charge via the Internet at http://pubs.acs.org.

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