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Sphaeropsidones, Phytotoxic Dimedone Methyl Ethers Produced by *Diplodia cupressi*: A Structure−Activity Relationship Study

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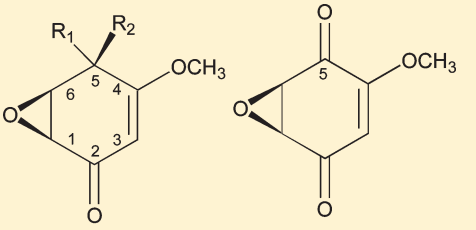
ABSTRACT: Sphaeropsidone and episphaeropsidone are two phytotoxic dimedone methyl ethers produced by *Diplodia cupressi*, the causal agent of a canker disease of cypress in the Mediterranean area. In this study, eight derivatives obtained by chemical modifications and two natural analogues were assayed for phytotoxic and antifungal activities, and a structure−activity relationship was examined. Each compound was tested on nonhost plants and on five fungal pathogenic species belonging to the genus *Phytophthora*. The results provide insights into structure−activity relationships within these compounds. It was found that the hydroxy group at C-5, the absolute C-5 configuration, the epoxy group, and the C-2 carbonyl group appear to be structural features important in conferring biological activity. The conversion of sphaeropsidone into the corresponding 1,4-dione derivative led to a compound showing greater antifungal activity than its precursor. This finding could be useful in devising new natural fungicides for practical application in agriculture.



The fungi associated with canker disease of the Italian cypress (*Cupressus sempervirens* L.) and other species of *Cupressus* in the Mediterranean area belong to the genera *Diplodia*, *Pestalotiopsis*, and *Seiridium*.[1−6] They are found to induce similar but different forms of canker, causing heavy losses in cypress plantations with forestry and ornamental uses, thus altering of the typical landscape of some regions of Central Italy and other countries of the Mediterranean basin. Moreover, Italian species are also very important from an economic point of view considering the noteworthy loss in the nursery industry and the ability to obtain precious cypress wood.[2]

Fungal species associated with cypress canker are able to produce *in vitro* several phytotoxic metabolites of different chemical nature. These metabolites could contribute to developing a rapid, simple, and specific method for disease diagnosis that could be useful in fungal taxonomy.[7] Butenolides, sesquiterpenes, 14-macrolides, and isobenzofuranones were isolated from *in vitro* culture of three *Seiridium* species,[8] while from *Sphaeropsis* and *Diplodia* rearranged pimarane diterpenoids, butanolides, and pyridiones were isolated.[7] In particular, *Diplodia cupressi* A.J.L. Phillips & A. Alves produced all known sphaeropsidins A−F[9−13] along with sphaeropsidones (**1** and **2**, Figure 1),[14] chlorosphaeropsidone, and its 6-epimer (**3** and **4**, Figure 1).[15] While the relationship between the structure and biological activity among sphaeropsidins was extensively studied,[13] only preliminary phytotoxic and antifungal activities were investigated for sphaeropsidones due to the limited amount of both dimedone methyl ethers isolated from the fungal culture filtrates. The absolute configuration, first assigned by a comparison of CD properties of the phytototoxins with those of other related natural cyclohexene oxides, was revised, as depicted in **1**, by the application of the time-dependent density functional theory (TDDFT) calculation of the optical rotation.[16] Consequently, those of episphaeropsidone, chlorosphaeropsidone, and its 6-epimer, assigned by CD correlation with sphaeropsidone, were also revised as depicted in **2**, **3**, and **4**, respectively.

In a preliminary study, we examined a strain of *Diplodia cupressi*, from the Centraalbureau voor Schimmelcultures (CBS), that appeared to be a good producer of both sphaeropsidone and episphaeropsidone. This allowed us to have adequate amounts of crystalline **1** and oily **2** and to investigate the biological activities of both sphaeropsidones as well as that of chlorosphaeropsidone (**3**) and its 6-epimer (**4**), purified from the most polar component of the culture filtrates organic extract.

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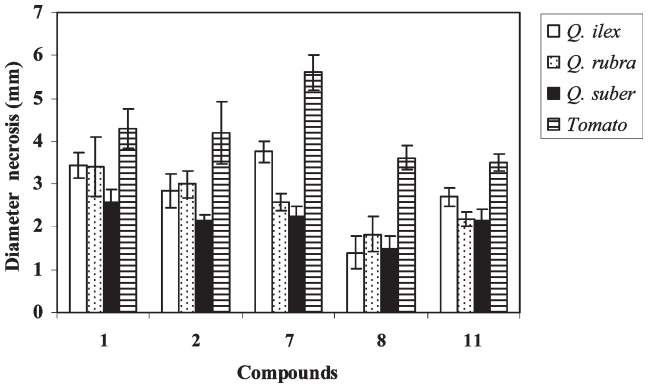
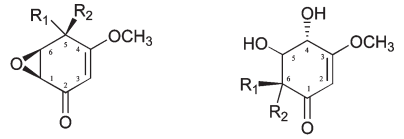


Figure 1. Phytotoxicity of sphaeropsidones (**1**, **2**), their natural analogues **3** and **4**, and derivatives **5**−**12** evaluated at 0.4 mg/mL on four plant species using a leaf puncture assay (7 days after application). Derivatives **3**, **4**, **5**, **6**, **9**, **10**, and **12** did not show any visible symptoms in this bioassay. Bars indicate standard error (±).



1 R_1=H, R_2=OH

2 R_1=OH, R_2=H

3 R_1=Cl, R_2=H

4 R_1=H, R_2=Cl

In order to obtain information on the structure−activity relationship of sphaeropsidones, eight derivatives (**5**−**12**) were prepared by chemical transformation of the functionalities present in **1** and **2**. The aim of the present work was to identify which structural features are essential for the biological activities of these compounds, in order not only to understand their mechanism of action on plants and their true role in pathogenesis but also to generate new compounds with potential application in agriculture.

RESULTS AND DISCUSSION

In this study the phytotoxic and antifungal activity of eight derivatives was evaluated in comparison to sphaeropsidone (**1**). The structural features of **1** and those of the naturally occurring episphaeropsidone (**2**) provide evidence for considering the latter as a naturally modified analogue of **1**. Furthermore, two natural analogues of sphaeropsidone, as well as chlorosphaeropsidone (**3**) and its 6-epimer (**4**), were also bioassayed in comparison to **1** and **2**.

The structures assigned to the new derivatives of sphaeropsidone (**5**, **7**−**10**) and episphaeropsidone (**11** and **12**) were determined by comparing their UV, IR, ^{1}H and ^{13}C NMR, and EI or ESIMS spectra with those of **1** and **2**,[14] respectively. In particular, the structures assigned to derivatives **9** and **10** were also supported by the couplings observed in their COSY, HSQC, HMBC, and NOESY spectra.

By acetylation, sphaeropsidone (**1**) (Scheme 1) was converted into the corresponding 5-*O*-acetylderivative (**5**) and into the 2,4,5-triacetylanisole (**6**). This latter showed strong structural modification relative to **1**, with the acetylation of the same hydroxy group at C-5 but also the aromatization of the cyclohexenone ring with the acetylation or acetoxylation of the generated nucleophilic oxygens and/or carbons.

The allylic hydroxy group at C-5 was oxidized with activated MnO_2 (Scheme 1), yielding the corresponding 1,4-dione derivative (**7**), in which the cyclohexene ring lost conformational flexibility, having only the epoxy oxygen projecting from the plane. The same derivative was also obtained by oxidation using Corey's reagent.[17]

The stereoselective epoxy ring opening that converted **1** into the corresponding bromohydrin (**8**) was performed with dilithium tetrabromonickelate,[18] a source of "soft" bromide, which also provide a "hard" but mild electrophile (i.e., Li^+). The stereochemistry of the bromohydrin **8** was deduced by the coupling constants measured in its ^{1}H NMR spectrum and by its agreement with the mechanism of reaction that provides the nucleophilic attack of bromide at the less hindered C-1 and from the α-side opposite the epoxy ring.[18] Consequently, the bromohydrin **8** assumes a half-chair conformation with the C-6 hydroxy group assuming a *β*-orientation. The values of 6.0 and 3.5 Hz measured in the ^{1}H NMR spectrum for the coupling constants of H-6 with H-1 and H-5, respectively, justify the location of H-1 as *β*-pseudoaxial, H-5 as α-pseudoaxial, and H-6 as α-pseudoequatorial.[19] This configuration is also in agreement with a Dreiding model of **8**.

Reduction with $NaBH_4$ (Scheme 1) converted **1** into the corresponding 1,4-diol derivative (**9**), which differed from sphaeropsidone only by the reduction of the α,*β*-unsaturated carbonyl group with consequent increase of the cyclohexene ring flexibility. The structure of this derivative was confirmed by NMR studies (COSY, HSQC, HMBC, and NOESY). The C-2 stereochemistry was deduced by the coupling observed in the ^{1}H and NOESY NMR spectra. In particular, the values of 3.5 and 3.1 Hz for the coupling of H-6 with both H-1 and H-5, respectively, as expected, were similar to those observed in **1**. The small constant (<1 Hz) observed for the coupling between H-1 and H-12 showed an H-2 α-pseudoaxial and its geminal hydroxy group *β*-pseudoequatorial orientation.[19] This stereochemistry at C-2 is also in agreement with the mechanism of reduction, with the nucleophilic attack of $NaBH_4$ at this carbon at the less hindered α-side opposite the epoxy ring. The configuration at C-2 was also confirmed by the absence of a coupling between H-1 and H-2 in the NOESY spectrum, while, as expected, a clear effect was observed between the methoxy group and H-3, and by an inspection of a Dreiding model of **9**.

The catalytic hydrogenation of **1** (Scheme 1) produced a tetrahydro derivative (**10**). The structure of this derivative was determined by extensive NMR (COSY, HSQC, HMBC, and NOESY) and MS spectra. The configuration was deduced, as for **9**, by the coupling observed in the ^{1}H NMR and NOESY spectra. In particular, considering that **9** assumes a half-chair conformation, the configuration at C-4 was assigned by considering the mechanism of reaction but essentially by the absence of any coupling in the NOESY spectrum between the methoxy protons and H-5, while as expected coupling was observed between H-4 and both H_2-3 and between H-1 and both H_2-6 and H-5 and H-6. These results, together with the value measured in the ^{1}H NMR spectrum for the coupling constants (4.8 and 4.0 Hz) of H-4 with both H_2-3 and the absence of its coupling with H-5, showed that H-4 was α-pseudoaxially oriented and its geminal methoxy group *β*-pseudoequatorial.[19] Similarly, the low values observed for the coupling of both H-1 and H-5 with H_2-6 placed H-1 and H-5

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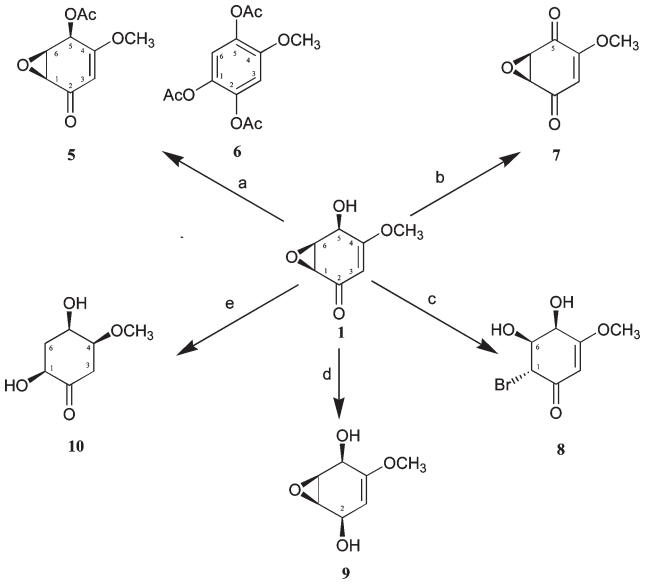
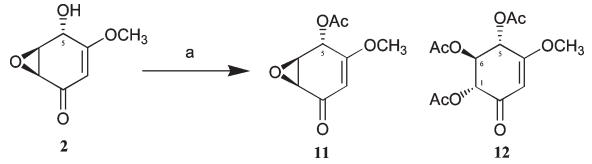
Scheme 1. Syntesis of 5−10[a]



[a] Reagents and conditions: (a) Ac_2O, pyridine, 80 °C; (b) MnO_2, CH_2Cl_2, rt; (c) Li_2NiBr_4, THF, rt; (d) $NaBH_4$, MeOH, rt; (e) H_2, Pd 10%, MeOH, rt.

Scheme 2. Synthesis of 11 and 12[a]



[a] Reagents and conditions: (a) Ac_2O, NaOAc, 80 °C.

α-pseudoequatorial and α-pseudoaxial, respectively, and consequently their geminal hydroxy group β-pseudoaxial and β-pseudoequatorial.[19] This stereochemistry is also in agreement with a Dreiding model of **10**.

Derivative **10** showed both the reduction of the olefinic double bond and the selective reductive opening of the epoxy ring, resulting in the 2,4-dihydroxycyclohexanone, which should assume a half-chair conformation.

Finally, acetylation of episphaeropsidone (Scheme 2) yielded the corresponding 5-*O*-acetyl derivative (**11**), which is the 5-epimer of **5**, and the triacetyl derivative (**12**). The latter, following acetylation of the C-5 hydroxy group as in **11**, was probably generated by cleavage of the epoxy ring via nucleophlic attack of the acetoxy group at the less hindered C-1 from the α-side and subsequent acetylation of the resulting anionic oxygen at C-6. The configuration of **12**, deduced from the coupling constants, confirmed such a hypothesized reaction mechanism.

Two bioassays were used to investigate the phytotoxic activity of sphaeropsidones (**1**, **2**), their natural analogues **3** and **4**, and derivatives **5**−**12** as described in the Experimental Section. In the leaf puncture bioassay, the phytotoxicity was evaluated for *Quercus ilex*, *Q. rubra*, *Q. suber*, and tomato leaves. The toxicity data in Figure 1 show that both sphaeropsidone (**1**) and its epimer (**2**), at the concentration used, had remarkable toxicity, causing necrotic lesions to leaves of all species tested. Among the sphaeropsidone (**1**) derivatives, only compounds **7** and **8** preserved phytotoxicity to different extents, whereas a significant decrease and/or a complete loss of activity was observed for the derivatives **3**, **4**, **5**, **6**, **9**, **10**, and **12**. In particular, the 1,4-dione derivative (**7**) showed the same strong activity as shown by **1** on all tested plant species. The derivative **8** was moderately toxic (necrosis diameter <2 mm for all species tested except for tomato leaves). Compound **11**, the monoacetyl derivative of episphaeropsidone (**2**), exhibited a modest decrease in phytotoxicity in comparison to **2**, whereas its triacetyl derivative (**12**) was inactive in the bioassay.

In the tomato cuttings bioassay, compounds **1**, **2**, **7**, and **11** were active. The cuttings treated with 0.1 mg/mL sphaeropsidone (**1**) and derivative **7** showed complete wilting (leaves and stems) within 48 h of application. Furthermore, symptoms of phytotoxicity (stewing on stem) were also observed with derivative **7** at concentration of 0.025 mg/mL. At the same concentration, sphaeropsidone (**1**) and its epimer (**2**) were inactive. Episphaeropsidone (**2**) appeared to be less toxic than **1** in this bioassay. The tomato cuttings showed wilting symptoms (at first only on the stem) after four days at a concentration of 0.1 mg/mL. Derivative **11** showed stewing on the stem at 0.1 mg/mL. None of the other compounds caused any visible symptoms in this bioassay at the highest concentration used.

These results allowed us to speculate on the structure−activity relationships and on some structural features determining the phytotoxic activity shown by each dimedone methyl ether.

The epoxy ring seems to be somehow associated with the activity, even if it is not alone sufficient to produce the phytotoxic effects. The marked decrease and/or the complete lack of activity for derivatives **3**, **4**, **6**, **10**, and **12** supports this result. Considering that some compounds preserving the epoxy ring were inactive, it is reasonable to suppose that other features of the molecule, other than the epoxy ring, could be important.

The oxidation of the C-5 allylic hydroxy group leading to **7** seems to enhance, albeit slightly, the bioactivity of **1**, whereas the stereoselective epoxy ring-opening obtained by converting **1** into the corresponding bromohydrin (**8**) reduces the bioactivity of **1**. The activity of **7** can probably be ascribed to its quinonoid nature, and thus to its greater reactivity with nucleophiles compared to the α,β-unsaturated carbonyl group of **1**. The persistent, although reduced, toxicity of **8** is probably due to its conversion *in vivo* into **1** by an S_N2 nucleophilic oxirane-forming process via the C-6 OH group and the bromide ion being a good leaving group. This probably did not occur in **3**, as the chloride ion is a poor nucleofuge compared to the bromide ion. However, in this case it generated an α-oxirane ring. On the other hand, **4** is not suitable for an S_N2 nucleophilic substitution.

Both the reduction of the C-2 carbonyl group (**9**) and the reduction of the olefinic double bond with the selective reductive opening of the epoxy ring (**10**) led to a complete loss of activity. The lack of toxicity exhibited by derivative **9** underlines the important role of the C-2 carbonyl group in the biological activity.

Furthermore, not all compounds (see derivatives **3**, **4**, **5**, **9**, and **12**) preserving the olefinic Δ^3 double bond were active. This result could suggest that this feature is probably not essential for activity. However, compounds **3**, **4**, **5**, **9**, and **12** also showed the opening of the oxirane ring and/or the modification of other structural features important for toxicity.

A marked loss of activity was observed with the conversion, by acetylation, of **1** in derivative **5** and **6**. It is interesting to note that 5-*O*-acetylsphaeropsidone (**5**) was inactive compared to its

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Table 1. Isolates and Hosts of the *Phytophthora* Species Used in the Antifungal Assay[a]

species	strains	host
P. cambivora	Ph 041	sweet chestnut
P. cinnamomi	Ph 001	holm oak
P. gonopodyides	Ph 038	sweet chestnut
P. nicotianae	Ph 002	mirtle
P. pseudosyringae	Ph 043	sweet chestnut

[a] All fungal pathogens tested are available in the collection of the Dipartimento di Protezione delle Piante, University of Sassari, Italy.

epimer (**11**), indicating the importance of the absolute configuration at this position for the interaction of the molecule with its corresponding target site. The activity of 5-*O*-acetylepisphaeropsidone is probably due to its hydrolysis *in vivo* into **2**, which occurs more easily compared to that of **5** into **1** since the acetyl group in **11** is located at the less hindered α-side opposite to the epoxy group.

Similar SAR trends were also observed for antifungal activity, and the structural requirements for sphaeropsidone (**1**) seem to be the same as those needed for its phytotoxicity. Sphaeropsidones **1** and **2**, their natural analogues **3** and **4**, and derivatives

Table 2. Percentage Growth Inhibition by Sphaeropsidones (1, 2), Their Natural Analogues 3 and 4, and Derivatives 5−12 Tested on Five Plant Pathogenic Fungi Belonging to the Genus *Phytophthora* after 4−7 Days of Treatment[a]

		fungal species				
compound	concentration (mg/plug)	*Phytophthora cambivora*	*Phytophthora cinnamomi*	*Phytophthora gonopodyides*	*Phytophthora nicotianae*	*Phytophthora pseudosyringae*
1	0.2	100.0	100.0	100.0	100.0	100.0
	0.1	64.1	73.3	50.5	79.8	100.0
	0.05	48.9	64.5	24.1	61.5	64.2
2	0.2	70.5	72.8	82.6	61.8	100.0
	0.1	41.3	39.8	10.7	43.1	39.9
	0.05	27.3	21.1	n.i.	25.1	22.4
3	0.2	ni	20.3	ni	ni	12.3
	0.1	nt	nt	nt	nt	nt
	0.05	nt	nt	nt	nt	nt
4	0.2	10.4	23.3	ni	14.1	13.4
	0.1	nt	nt	nt	nt	nt
	0.05	nt	nt	nt	nt	nt
5	0.2	26.1	49.7	24.1	33.9	100.0
	0.1	nt	nt	nt	nt	23.1
	0.05	nt	nt	nt	nt	11.9
6	0.2	13.7	28.0	15.9	16.5	15.8
	0.1	nt	nt	nt	nt	nt
	0.05	nt	nt	nt	nt	nt
7	0.2	100.0	100.0	100.0	100.0	100.0
	0.1	100.0	100.0	100.0	100.0	100.0
	0.05	100.0	100.0	12.9	100.0	100.0
8	0.2	44.2	61.5	21.8	53.2	74.6
	0.1	nt	nt	nt	nt	62.7
	0.05	nt	nt	nt	nt	39.9
9	0.2	n.i.	n.i.	n.i..	n.i.	n.i.
	0.1	nt	nt	nt	nt	nt
	0.05	nt	nt	nt	nt	nt
10	0.2	ni	ni	ni	ni	ni
	0.1	nt	nt	nt	nt	nt
	0.05	nt	nt	nt	nt	nt
11	0.2	100.0	100.0	100.0	100.0	100.0
	0.1	82.5	100.00	57.2	100.0	100.0
	0.05	30.0	57.7	14.8	57.2	42.9
12	0.2	n.i.	20.8	ni	16.5	ni
	0.1	nt	nt	nt	nt	nt
	0.05	nt	nt	nt	nt	nt
mefenoxam	0.2	84.1	100.0	47.0	100.0	100.0
	0.1	81.9	100.0	43.3	100.0	100.0
	0.05	78.3	100.0	37.6	100.0	100.0

[a] ni = no inhibition; nt = not tested.

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5−**12** were assayed on five fungal species belonging to the genus *Phytophthora* (Table 1), which are destructive pathogens of forest trees and shrubs. As shown in Table 2, all fungal species were highly sensitive to sphaeropsidone (**1**) at 0.2 mg/plug. At the lowest concentration assayed (0.05 mg/plug) **1** was still active against all fungi, with the exception of *P. gonopodyides*, which was inhibited less than 25%. Episphaeropsidone (**2**) had a weaker effect compared to sphaeropsidone (**1**), emphasizing once more the role of the absolute configuration at C-5 in the expression of bioactivities.

The results showed that most of the modifications made to **1** led to a loss of toxicity toward fungal species tested. Among the natural analogues and derivatives **3**−**12**, compounds **3**, **4**, **6**, **9**, **10**, and **12** were pratically ineffective against all fungi tested even at the highest concentration assayed. Compound **5** inhibited less than 50% of the mycelial growth of *P. nicotianae*, *P. cinnamomi*, *P. gonopodyides*, and *P. cambivora* at 0.2 mg/plug; it was more active against *P. pseudosyringae*, at 0.2 mg/plug (100% inhibition). Derivative **7** completely inhibited the mycelial growth of fungi assayed even at the lowest concentration, except for *P. gonopodyides*. Derivative **8** displayed significant activity toward *P. pseudosyringae*. Compound **11** caused a marked reduction of mycelial growth of all fungi except for *P. gonopodyides* even at the lowest concentration. It is interesting to highlight that the only structural change in **1** resulting in an increase of its bioactivity was the oxidation of the C-5 allylic hydroxy group.

In summary, 10 compounds, **3**−**12**, either natural or synthetic analogues of sphaeropsidones, were evaluated for their phytotoxic and antifungal activities in comparison to their precursors in order to estabilish SAR. The *in vitro* phytotoxic and antifungal data reported here indicated which specific structural features are related to the toxicity of the sphaeropsidones. The C-5 hydroxy group, the epoxy ring, and the C-2 carbonyl group, along with the C-5 absolute configuration, could be pointed out as important structural features. In fact, modifications of the C-5 hydroxy group, such as acetylation, the reduction of the C-2 carbonyl group, and opening of the epoxy ring, led to compounds that were much less active and/or inactive in comparison to **1**. The role of the α,β-double bond remains to be defined by preparing a derivative with only one modification in **1**. Finally, among the synthesized derivatives, compound **7** was found more effective than sphaeropsidone (**1**) in inhibiting mycelial growth of invasive pathogens on a global scale such as *Phytophthora* species. Its antifungal activity *in vitro* was shown to be similar to the synthetic fungicide (mefenoxam) commonly employed for control of diseases caused by oomycetes (Table 2).[20] This encourages us to continue studies aimed at generating further compounds that are suitable for development of alternative strategies for management of these pathogens, also in light of the development of resistance in *Phytophthora* species to the phenylamides.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured in $CHCl_3$ solution on a Jasco P-1010 digital polarimeter, IR spectra were recorded as glassy film on a Perkin-Elmer Spectrum One FT-IR spectrometer, and UV spectra were taken in MeCN solution on a Perkin-Elmer Lambda 25 UV/vis spectrophotometer. ^1H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively, in $CDCl_3$ on Bruker spectrometers, unless otherwise noted. The same solvent was used as internal standard. Carbon multiplicities were determined by DEPT spectra.[21] DEPT, COSY-45, HSQC, HMBC, and NOESY experiments[21] were performed using Bruker microprograms. ESIMS spectra were recorded on a Waters Micromass Q-TOF Micro spectrometer; EIMS spectra were taken at 70 eV on a QP 5050 Shimadzu spectrometer. Analytical and preparative TLC were performed on Si gel (Merck, Kieselgel 60 F_{254}, 0.25 and 0.50 mm, respectively) plates; the spots were visualized by exposure to UV light and/or by spraying first with 10% H_2SO_4 in MeOH and then with 5% phosphomolybdic acid in EtOH, followed by heating at 110 °C for 10 min. CC: Si gel (Merck, Kieselgel 60, 0.063−0.200 mm).

Fungal Strain. *D. cupressi* used in this study was purchased from Centraalbureau voor Schimmelcultures of Baarn (Netherland), Strain 261.85 CBS.

Extraction and Isolation of Sphaeropsidones. The fungus was grown in 2 L Erlenmeyer flasks containing 400 mL of modified Czapek medium supplemented with 2% corn meal (pH 5.7). Each flask was seeded with 5 mL of a mycelia suspension and then incubated at 25 °C for 4 weeks in darkness. The culture filtrates (15 L) were acidified and extracted with EtOAc as previously reported.[14] The organic extract, obtained as a brown-red oil (9.2 g), having a high phytotoxic activity, was chromatographed on a silica gel column eluted with $CHCl_3$−*i*-PrOH (19:1), affording nine groups of homogeneous fractions. The residues (3.6 g) of fractions 4−7 were combined and further purified by a silica gel column, eluted with $CHCl_3$−*i*-PrOH (9:1), yielding six groups of homogeneous fractions. The residue of fraction 3 was crystallized from EtOAc−*n*-hexane (1:5), yielding sphaeropsidone (**1**, R_f 0.40, 2.3 g, 153.3 mg/L) as white needles. The mother liquors were further purified by silica gel CC, eluted with petroleum ether−acetone (7:3), affording episphaeropsidone (**2**, R_f 0.53, 725 mg, 48.3 mg/L) as a homogeneous oil. The residue (775.5 mg) of fraction 8 from the first column containing two more polar metabolites (R_f 0.21 and 0.14) was purified by silica gel CC, eluted with $CHCl_3$−*i*-PrOH (9:1), to afford five groups of homogeneous fractions. The residues (150 and 102.4 mg) of fractions 3 and 4 were independently purified by two further steps of preparative TLC on silica gel, using $CHCl_3$−*i*-PrOH (9:1) and petroleum ether−acetone (7:3), to give chlorosphaeropsidone and epichlorosphaeropsidone (**3** and **4**, 80 and 57 mg, 5.3 and 3.8 mg/L, respectively) as homogeneous oils.

Acetylation of Sphaeropsidone. To sphaeropsidone (**1**, 21.2 mg), dissolved in pyridine (240 μL), was added under stirring Ac_2O (240 μL). The reaction was carried out at 80 °C for 30 min. The reaction was stopped by addition of MeOH and evaporation of the mixture by an N_2 stream. The residual crude oil was purified by preparative TLC, using EtOAc−*n*-hexane (6:4) for elution, to yield both derivatives **5** and **6** (7.9 and 11.4 mg, respectively) as homogeneous compounds. Derivative **5**: $[\alpha]^{25}_D$ −20.7 (*c* 0.2); IR ν_{max} 1747, 1668, 1616 cm^{-1}; UV λ_{max} nm (log ε) 251 (3.81); ^1H NMR, δ 5.96 (1H, d, *J* = 3.1 Hz, H-5), 5.31 (1H, d, *J* = 1.6 Hz, H-3), 3.77 (1H, dd, *J* = 3.9 and 3.1 Hz, H-6), 3.72 (3H, s, OMe), 3.51 (dd, *J* = 3.9 and 1.6 Hz, H-1), 2.24 (3H, s, MeCO); EIMS (rel int) *m/z* 198 $[M]^+$ (10), 156 $[M - CH_2CO]^+$ (50), 141 $[M - CH_2CO - Me]^+$ (17), 127 $[M - CH_2CO - HCO]^+$ (75), 113 $[M - CH_2CO - Me - CO]^+$ (24), 111 (88), 69 (100). Derivative **6**: IR ν_{max} 1769 cm^{-1}; UV λ_{max} nm (log ε) 277 (3.42), 222 (shoulder); ^1H NMR, δ 6.94 and 6.79 (1H each, s, H-3 and H-6), 3.80 (3H, s, OMe), 2.29, 2.28, and 2.25 (3H each, s, three MeCO); ^{13}C NMR, δ 168.3, 168.1, and 168.0 (s, three C═O), 149.2 (*s*, C-5), 139.9, 137.0, and 134.8 (s, C-1, C-2, and C-4), 117.8 (d, C-3), 107.4 (d, C-6) 56.3 (q, MeO), 20.5 (q, three MeCO); EIMS (rel int) *m/z* 282 $[M]^+$ (34), 267 $[M - Me]^+$ (10), 240 $[M - CH_2CO]^+$ (54), 198 $[M - 2\times CH_2CO]^+$ (81), 156 $[M - 3\times CH_2CO]^+$ (100), 141 $[M - 3\times CH_2CO - Me]^+$ (94), 127 $[M - 3\times CH_2CO - HCO]^+$ (28), 113 $[M - 3\times CH_2CO - Me - CO]^+$ (15), 69 (25), 43 (44).

Oxidation of Sphaeropsidone with MnO_2. Sphaeropsidone (**1**, 15.3 mg) was dissolved in dry CH_2Cl_2 (300 μL) and oxidized with MnO_2 (15 mg) under stirring at room temperature. After 1 h the starting

compound was converted and the reaction stopped by filtration. The residue was washed with a small volume of CH_2Cl_2, which then was evaporated under reduced pressure. The oily residue (12.5 mg) was purified by preparative TLC, using $CHCl_3$−*i*-PrOH (9:1) for elution, to give the derivative **7** as a homogeneous solid (9.3 mg): $[\alpha]^{25}_D$ −94.8 (*c* 0.2); IR ν_{max} 1713, 1668, 1598 cm^{-1}; UV λ_{max} nm (log ε) 291 (3.38), 229 (shoulder); ^{1}H NMR, δ 5.77 (1H, d, *J* = 1.8 Hz, H-3), 3.86 (1H, d, *J* = 3.6 Hz, H-6), 3.80 (1H, dd, *J* = 3.6 and 1.8 Hz, H-1), 3.78 (3H, s, OMe); ESIMS (+) *m*/*z* 193 $[M + K]^+$, 177 $[M + Na]^+$, 155 $[M + H]^+$.

Conversion of Sphaeropsidone (1) into the Corresponding 5,6-Bromohydrin (8). Sphaeropsidone (**1**, 15 mg) dissolved in dry THF (1.5 mL) was treated with a solution of 0.4 M Li_2NiBr_4 in dry THF (1.5 mL) prepared according to Dawe et al., 1984.[18] The reaction was performed at room temperature with stirring in the dark. After 30 min **1** was completely transformed and the reaction was stopped by addition of phosphate buffer at pH = 7.5 (15 mL). The aqueous solution was extracted with CH_2Cl_2. The organic extracts were combined, washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to give a crude residue. The latter was purified by preparative TLC, using $CHCl_3$−*i*-PrOH (9:1) for elution, to give **8** as a homogeneous compound (13 mg): $[\alpha]^{25}_D$ +44.8 (*c* 0.2); IR ν_{max} 3392 1653, 1599 cm^{-1}; UV λ_{max} nm (log ε) 253 (3.08) nm; ^{1}H NMR, δ 5.46 (1H, s, H-3), 4.74 (1H, d, *J* = 3.5 Hz, H-5), 4.54 (1H, d, *J* = 6.0 Hz, H-1), 4.32 (1H, dd, *J* = 6.0 and 3.5 Hz, H-6), 3.72 (3H, s, OMe); ^{13}C NMR ($CDCl_3$+methanol-d_4, 1:10 v/v) δ 191.0 (s, C-2), 174.3 (s, C-4), 99.2 (d, C-3), 72.1 (d, C-6), 67.1 (d, C-5), 56.6 (q, OMe), 49.7 (d, C-1); EIMS *m*/*z* (%): 238 $[M + 2]^+$ (0.4), 236 $[M]^+$ (0.5), 220 $[M + 2 - H_2O]^+$ (3.9), 218 $[M - H_2O]^+$ (4.2), 156 $[M - HBr]^+$ (13), 139 $[M - HBr - OH]^+$ (83), 114 $[M - HBr - OH - Me]^+$ (100), 86 (29), 58 (64), 43 (72).

$NaBH_4$ Reduction of Sphaeropsidone. Sphaeropsidone (**1**, 30 mg) in MeOH (15 mL) was reduced with $NaBH_4$ (30 mg) under stirring at room temperature for 30 min. The mixture was neutralized with 0.1 M HCl, extracted with CH_2Cl_2 (3 × 30 mL), and dried (Na_2SO_4). The oily residue was purified by preparative TLC, using $CHCl_3$−*i*-PrOH (9:1) for elution, to give the derivative **9** as white needles (28.2 mg). Derivative **9**: $[\alpha]^{25}_D$ +50.2 (*c* 0.5); IR ν_{max} 3392, 1668 cm^{-1}; UV λ_{max} nm (log ε) < 220; ^{1}H NMR, δ 4.55 (br d, *J* = 11.0 Hz, H-2), 4.50 (br s, H-3), 4.42 (br s, H-5), 3.64 (dd, *J* = 3.5 and 3.1 Hz, H-6), 3.60 (br s, H-1), 3.59 (s, OMe), 2.54 (d, *J* = 4.4 Hz, HO-C(5)), 1.89 (d, *J* = 11 Hz, HO-C(2)); ^{13}C NMR δ 153.0 (s, C-4), 95.0 (d, C-3), 65.6 (d, C-2), 65.0 (d, C-5), 54.9 (each d, C-1 and C-6), 54.8 (s, OMe); ESIMS (+) *m*/*z* 197 $[M + K]^+$, 181 $[M + Na]^+$, 159 $[M + H]^+$.

Hydrogenation of Sphaeropsidone. Sphaeropsidone (**1**, 20 mg), dissolved in MeOH (1.5 mL), was added to a presaturated suspension of Pd (10%) in MeOH (1.5 mL). Hydrogenation was carried out at room temperature and atmospheric pressure with continuous stirring. After 2 h, the reaction was stopped by filtration and the clear solution evaporated under an N_2 stream. The residue was purified by preparative TLC, using $CHCl_3$−*i*-PrOH (9:1) for elution, to yield derivative **10** (8.3 mg) as a homogeneous compound. Derivative **10**: $[\alpha]^{25}_D$ +42 (*c* 0.2); IR ν_{max} 3392, 1709 cm^{-1}; UV λ_{max} nm (log ε) < 220; ^{1}H NMR, δ 4.20 (br s, H-5), 4.04 (br s, H-1), 3.71 (dd, *J* = 4.8 and 4.0 Hz, H-4), 3.42 (3H, s, OMe), 2.78 (br d, *J* = 14.5, H-6A), 2.75 (br d, *J* = 14.5 Hz, H-6B), 2.62 (dd, *J* = 14.5 and 4.8 Hz, H-3A), 2.55 (br d, *J* = 14.5 and 4.0 Hz, H-3B); ^{13}C NMR, δ 205.7 (s, C-2), 79.8 (d, C-4), 70.3 (d, C-1), 60.6 (d, C-5), 57.3 (q, OMe), 46.2 (t, C-6), 41.7 (t, C-3); ESIMS (+) *m*/*z* 199 $[M + K]^+$, 183 $[M + Na]^+$, 161 $[M + H]^+$.

Acetylation of Episphaeropsidone. To episphaeropsidone (**2**, 50 mg), dissolved in Ac_2O (2.5 mL), was added under stirring NaOAc (50 mg). The reaction was carried out at 80 °C for 30 min. The reaction was cooled at room temperature and stopped by addition of H_2O. The mixture was extracted with EtOAc (3 × 20 mL). The extracts were combined, dried (Na_2SO_4), and evaporated under vacuum. The residual crude oil was purified by preparative TLC, using EtOAc−*n*-hexane (6:4) for elution, to yield derivatives **11** and **12** (4.6 and 33.2 mg, respectively) as homogeneous compounds. Derivative **11**: $[\alpha]^{25}_D$ −20.7 (*c* 0.2); IR ν_{max} 1746, 1667, 1621 cm^{-1}; UV λ_{max} nm (log ε) 252 (4.14); ^{1}H NMR, δ 5.91 (1H, dt, *J* = 1.5 and 0.5 Hz, H-5), 5.36 (1H, d, *J* = 1.5 Hz, H-3), 3.71 (3H, s, OMe), 3.62 (1H, dd, *J* = 3.5 and 1.5 Hz, H-6), 3.44 (ddd, *J* = 3.5, 1.5, and 0.5 Hz, H-1), 2.17 (3H, s, MeCO); ESIMS (+) *m*/*z* 237 $[M + K]^+$, 221 $[M + Na]^+$, 199 $[M + H]^+$. Derivative **12**: $[\alpha]^{25}_D$ −40.6 (*c* 0.2); IR ν_{max} 1752, 1681, 1608 cm^{-1}; UV λ_{max} nm (log ε) 289 (3.01), 244 (4.11); ^{1}H NMR, δ 5.91 (dd, *J* = 11.3 and 2.7 Hz, H-6), 5.52 (d, *J* = 2.7 Hz, H-5), 5.43 (d, *J* = 11.3, H-1), 5.30 (s, H-3), 3.77 (3H, s, OMe), 2.17, 2.12, and 2.07 (3H each, s, three MeCO); ESIMS (+) *m*/*z* 339 $[M + K]^+$, 323 $[M + Na]^+$, 301 $[M + H]^+$.

Leaf Puncture Assay. Tomato (*Lycopersicon esculentum* var. Marmande), young cork oak (*Quercus suber*), holm oak (*Q. ilex* L.), and red oak (*Q. rubra* L.) leaves were utilized for this assay. Compounds were dissolved in MeOH and then brought up to the assay concentrations with distilled H_2O (the final content of MeOH was 4%). Each compound was assayed at 0.4 mg/mL. The test solutions (20 μL) were applied on the ad axial side of leaves that had previously been needle punctured. Droplets (20 μL) of MeOH in distilled H_2O (4%) were applied on leaves as control. Each treatment was repeated three times. The leaves were then kept in a moist chamber to prevent the droplets from drying. Leaves were observed daily and scored for symptoms after 1 week. The effects of the toxins on the leaves, consisting in necrotic spots surrounding the puncture, were observed up to 15 days.

Tomato Cutting Assay. Each compound was assayed at four different concentration (0.1, 0.05, 0.025, and 0.01 mg/mL) on tomato cuttings taken from 21-day-old seedlings. Cuttings were placed in the test solutions (3 mL) for 48 h and then transferred to distilled water. Symptoms were visually evaluated up to 7 days.

Antifungal Assays. Sphaeropsidones (**1** and **2**), their natural analogues **3** and **4**, and derivatives **5**−**12** were tested on five fungal species, listed in Table 1, belonging to the genus *Phytophthora* to evaluate their antifungal properties. The antifungal activity was evaluated on carrot agar (CA) as inhibition of the mycelial radial growth. In brief, mycelial plugs (6 mm diameter) were cut from the margin of actively growing 4-day-old colonies using a flamed cork borer. One plug was placed in the center of a 9 cm diameter Petri dish with the mycelia in contact with the medium. Then 20 μL of the test solution at a concentration of 200 μg/plug was applied on top of each plug. As required, highly toxic compounds were tested again at concentrations of 100 and 50 μg/plug. The controls were obtained by applying 20 μL of MeOH. The solvent was evaporated in a laminar flow cabinet, and the plates were incubated at 20 °C for 4−7 days depending on the fungal species. The antifungal activity of the compounds was also compared with metalaxyl-M (mefenoxam; p.a. 43.88%; Syngenta), a synthetic fungicide to which the oomycetes are sensitive. Colony diameters were measured in two perpendicular directions for all treatments. Each treatment consisted of three replicates, and the experiment was repeated twice.

Statistical Analysis. Data from the antifungal activity assay were analyzed by one-way variance analysis (ANOVA) using the XLSTAT software (Addinsoft). $P < 0.05$ was considered statistically significant.

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