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In Vitro Antibacterial Activity of Sphaeropsidins and Chemical Derivatives toward *Xanthomonas oryzae* pv. *oryzae*, the Causal Agent of Rice Bacterial Blight

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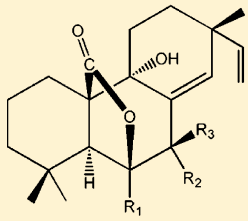
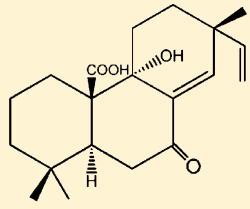
ABSTRACT: Sphaeropsidin A, the main phytotoxin produced by *Diplodia cupressi*, as well as the two natural analogues sphaeropsidins B and C and 14 derivatives obtained by chemical modifications were assayed for antibacterial activity against *Xanthomonas oryzae* pv. *oryzae*, *Pseudomonas fuscovaginae*, and *Burkholderia glumae*, the causal agents of severe bacterial rice diseases. The results showed a strong and specific activity of sphaeropsidin A against *X. oryzae* pv. *oryzae*, while no activity was observed against the other two pathogens. The results of structure−activity relationship studies showed that structural features important to impart this antibacterial activity are the presence of the C-7 carbonyl group and the hemiketalic lactone functionality. The C-13 vinyl group, the double bond of ring C, and/or the tertiary C-9 hydroxy group, as well as the pimarane arrangement of the tricylic carbon skeleton, were also important for the antibacterial activity. These findings may be useful in designing novel compounds for practical applications in agriculture.



1 R_1=OH, R_2+R_3=O
2 R_1=R_3=OH, R_2=H



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Fungal and bacterial diseases cause economic losses worldwide due to crop damage. Among the pathogens associated with rice diseases, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the causal agent of bacterial blight. This disease is one of the most serious bacterial diseases in many of the rice-growing regions of the world together with the bacterial leaf streak induced by *Xanthomonas oryzae* pv. *oryzicola*.[1] The most common method to control these diseases is the application of bacteriocides and in particular bismerthiazol.[2,3] However, *Xoo* can develop resistance to this compound,[4,5] as was observed for streptomycin, the antibiotic introduced for crop protection in 1955 for controlling plant diseases caused by Gram-negative bacteria. Other important bacterial pathogens of rice are *Pseudomonas fuscovaginae*, responsible for sheath brown root[6] and grain rot, and *Burkholderia glumae*, the causal agent of sheath rot and seedling rot. The symptoms induced also depend on the rice growth stage.[7,8] Currently, there are few control methods available for bacterial rice diseases, including chemical and biological methods and the use of resistant cultivars and lines.[8]

The use of synthetic agrochemicals in the control of plant microbial pathogens carries the risk of causing unacceptable environmental damage such as ill effects on soil health, health hazard to humans, toxicity to useful nontarget animals, and environmental pollution.[9] The use of natural products could be a way to reduce this risk, and consequently, the interest in their possible application in biocontrol has recently increased. For example, oryzalexins A−D[10−12] and momilactones A and B,[13] phytoalexins produced by rice (*Oryza sativa* L.) as a consequence of the attack of *Pyricularia orizae*, are compounds with potential application in the control of plant diseases. It has been demonstrated that the phytoalexins have inhibitory activity against spore germination and the germ tube of the rice fungal pathogen *P. oryzae*, which is the causal agent of rice blast disease.[13] Studies on the mode of action of oryzalexin D were also carried out.[14]

Oryzalexins and momilactones, which belong to the pimaradiene diterpene group, are closely related to sphaeropsidins A−F, which are phytotoxins produced by *Diplodia cupressi*, the causal agent of the canker of cypress in the Mediterranean basin.[15] These toxins showed not only phytotoxic activity on host and nonhost plants but also antifungal activity against some phytopathogenic fungi.[16] Interestingly, sphaeropsidins are structurally closely related to both oryzalexins and momilactones.

The aim of this study was to investigate the potential antibacterial activity of sphaeropsidin A against several bacterial rice pathogens and to identify which structural features are essential for this biological activity. For this reason, 14 derivatives (**4**−**17**, Scheme 1−3) were prepared by chemical transformation of the

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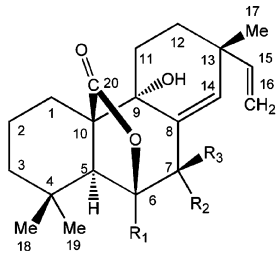
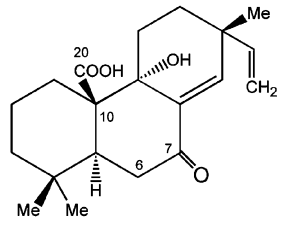


1 R_1=OH, R_2+R_3=O
2 R_1=R_3=OH, R_2=H



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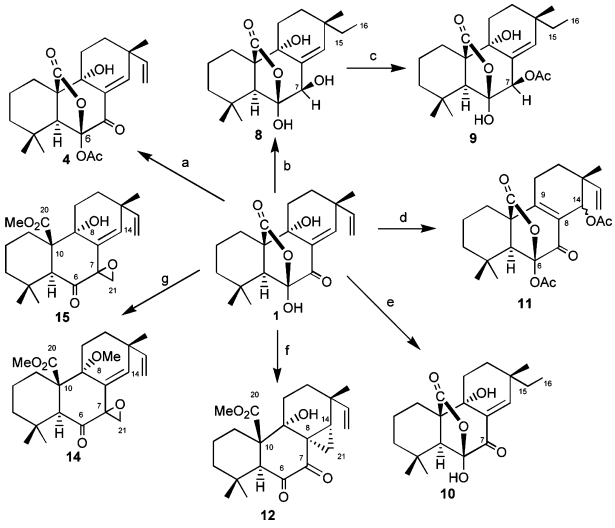
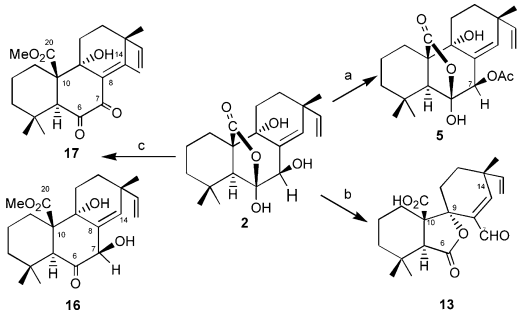
Scheme 1[a]



[a]Reagents and conditions: (a) Ac_2O, pyridine, p-TSA, rt, 12 h; (b) H_2, PtO_2, MeOH, rt, 18 h; (c) Ac_2O, pyridine, 25 °C, 3 h; (d) Fritz and Schenk reagent, EtOAc, 0 °C, 2 h; (e) H_2, PtO_2, MeOH, rt, 15 h; (f) CH_2N_2, MeOH, rt, 18 h; (g) CH_2N_2, MeOH, rt, 24 h.

Scheme 2[a]



[a]Reagents and conditions: (a) Ac_2O, pyridine, rt, 4 h; (b) $NaIO_4$, MeOH, rt, 2 h; (c) CH_2N_2, MeOH, rt, 4 h.

functionalities present in sphaeropsidins A, B, and C (**1**–**3**) and also tested in comparison with the natural sphaeropsidins against three rice bacterial pathogens.

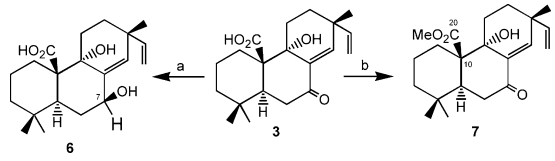
RESULTS AND DISCUSSION

In order to assess the structure–function relationship in antibacterial activity, several derivatives of sphaeropsidins A, B, and C were synthesized. By acetylation, sphaeropsidin A (**1**) was converted into the 6-*O*-acetyl derivative (**4**, Scheme 1), while with the Fritz and Schenk reagent,[17] used to acetylate the hindered C-9 hydroxy group, **1** was converted into the 6-*O*-acetyl-14-acetoxy derivative (**11**, Scheme 1). The catalytic hydrogenation of **1** generated the derivatives **8** and **10** (Scheme 1), i.e., saturation of the C-13 vinyl group for **10** and also the reduction of the C-7 carbonyl group for **8**. Acetylation of **8** gave the corresponding 7-*O*-acetyl derivative (**9**, Scheme 1). Finally, by reaction with diazomethane, sphaeropsidin A (**1**) was converted into the known derivative **12** and the new compounds **14** and **15** (Scheme 1). All these three derivatives showed the opening of the hemiketal lactone ring, the conversion of the carboxy group into the corresponding methyl ester, and the conversion of the C-6 hemiketal group into the corresponding carbonyl group.

The C-7 carbonyl group in the methyl ester of **1** was susceptible to methylene insertion by diazomethane to generate the corresponding 1,1-disubstitued oxirane ring in **14** and **15**. The C-8–C-14 double bond was similarly susceptible to methylene insertion to generate **12**. Because of steric hindrance, the presence of the C-9 *O*-methyl group in **14** may be explained as resulting from solvolysis (MeOH) of the allylic hydroxy group. The structure of **14** was supported by the fragmentation ions observed in the EIMS spectrum at *m*/*z* 374, 356, and 342, which are generated from the molecular ion at 388 by the loss of CH_2, CH_3OH, and both, respectively.

Acetylation of sphaeropsidin B (**2**) gave the 7-*O*-acetyl derivative (**5**, Scheme 2), preserving the hemiketal hydroxy group at C-6. Treatment of **2** with diazomethane yielded the new derivatives **16** and **17** (Scheme 2), which, like the sphaeropsidin A derivatives **12**–**14**, showed opening of the hemiketal lactone and the conversions of the carboxy group into the corresponding methyl ester and the C-6 hemiketal functionality into the corresponding carbonyl group. Furthermore, derivative **17** also showed the methylene insertion into the olefinic C(14)–H bond, generating the corresponding vinylic methyl group. Compound **2**, in turn, was converted by $NaIO_4$ oxidation into the oxidized derivative **13** (Scheme 2), which showed marked modification of the B ring, while the A and C rings appeared practically unaltered. In particular, derivative **13** exhibited the opening of the hemiketal lactone, producing a carboxylic group at C-10, and the cleavage of the C-6–C-7 bond. C-6 was oxidized into a hydroxycarbonyl group, which formed a γ-lactone functionality with the C-9 hydroxy group, while the carbonyl group at C-7 appeared as a formyl group conjugated with the C-8–C-14 double bond. These noteworthy structural modifications resulted in substantial disruption of the tricyclic pimarane system. Treatment of sphaeropsidin C (**3**) with diazomethane afforded the corresponding methyl ester **7** (Scheme 3), while stereoselective reduction with $NaBH_4$, as was observed in the conversion of **1** into **2**,[18,19] gave the 7-hydroxy derivative **6** (Scheme 3).

Scheme 3[a]



[a]Reagents and conditions: (a) $NaBH_4$, MeOH, rt, 0.5 h; (b) CH_2N_2, MeOH, rt, 1 h.

The derivatives **4**–**17**, in comparison to the natural sphaeropsidins A–C (**1**–**3**), were assayed for their antibacterial

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activity against *Burkholderia glumae*, *Pseudomonas fuscovaginae*, and *Xanthomonas oryzae* pv. *oryzae*, by growing the three pathogens in top agar media and exposing them to 25 and 100 μg of sphaeropsidin A pipetted into a well. The growth inhibition zone around the well was taken as a measure to assess the antibacterial activity.

On *B. glumae* and *P. fuscovaginae,* sphaeropsidin A (**1**) showed weak antagonistic activity, while sphaeropsidins B and C and derivatives **4**–**17** were inactive. On *Xoo*, on the other hand, sphaeropsidin A displayed strong antibacterial activity (Figure 1).

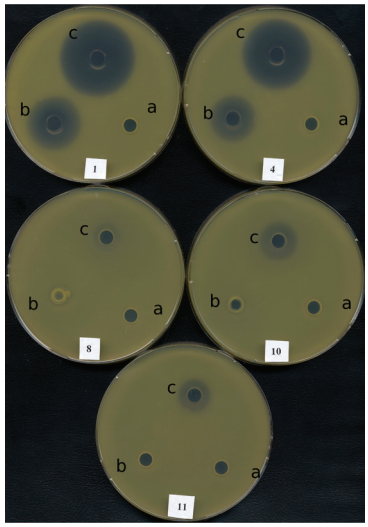

Figure 1. Petri dishes showing the growth inhibition of *Xanthomonas oryzae* pv. *oryzae* by sphaeropsidin A (**1**) and derivatives **4**, **8**, **10**, and **11**. On each Petri dish: (a) negative control (50 μL of DMSO); (b and c) 25 and 100 μg of sphaeropsidin A or its derivatives.

Table 1 and Figure 1 depict the results using sphaeropsidins B and C, which appear to be naturally modified analogues of **1**, and of derivatives **4**–**17** compared with that of sphaeropsidin A. Interestingly, only the monoacetyl derivative **4** of sphaeropsidin A showed the same strong activity of **1**, while the diacetyl (**11**) and dihydro (**10**) derivatives of sphaeropsidin A showed a reduced activity, and all natural and hemisynthesized compounds were practically inactive. When ampicillin was used as a positive control, the activities of compounds **1** and **4** were, respectively, 49% and 39% of the activity of ampicillin (Table 2).

Table 1. Growth Inhibition by Sphaeropsidins (1–3) and Derivatives (4–17) on *Xanthomonas oryzae* pv. *oryzae*

compound	inhibition diameter (mm)[a]	concentration (M)[b]	inhibition diameter (mm)	concentration (M)
1	18.3	1.5×10^{-3}	25.5	6.0×10^{-3}
2	ni[c]	1.5×10^{-3}	ni	6.0×10^{-3}
3	ni	1.5×10^{-3}	ni	6.0×10^{-3}
4	15.8	1.3×10^{-3}	20.7	5.2×10^{-3}
5	ni	1.0×10^{-3}	ni	4.0×10^{-3}
6	ni	1.5×10^{-3}	ni	6.0×10^{-3}
7	ni	1.5×10^{-3}	ni	6.0×10^{-3}
8	ni	1.5×10^{-3}	8.9	6.0×10^{-3}
9	ni	1.3×10^{-3}	ni	5.2×10^{-3}
10	ni	1.5×10^{-3}	11.6	6.0×10^{-3}
11	ni	0.5×10^{-3}	15.3	2.0×10^{-3}
12	ni	1.3×10^{-3}	ni	5.2×10^{-3}
13	ni	1.5×10^{-3}	ni	6.0×10^{-3}
14	ni	1.3×10^{-3}	ni	5.2×10^{-3}
15	ni	1.3×10^{-3}	ni	5.2×10^{-3}
16	ni	1.4×10^{-3}	ni	5.6×10^{-3}
17	ni	1.4×10^{-3}	ni	5.6×10^{-3}

[a]The inhibition of growth around the wells was measured in mm (4 mm well diameter included). [b]Amount assayed in 50 μL of DMSO. The negative control was represented by 50 μL of DMSO [c]ni = no inhibition.

Table 2. Inhibition of *Xanthomonas oryzae* pv. *oryzae* Growth in a Petri Dish Assay Using Sphaeropsidin A (1) and Derivatives 4, 8, 10, and 11[a]

compound	concentration (M)	zone of inhibition diameter (mm)	% inhibition[b]
1	6.0×10^{-3}	25.5 ± 7.0	49.0
4	5.2×10^{-3}	20.7 ± 5.6	39.8
8	6.0×10^{-3}	nd[c]	nd
10	6.0×10^{-3}	11.6 ± 2.1	22.3
11	2.0×10^{-3}	15.3 ± 2.5	29.4
ampicillin	5.7×10^{-3}	52.0 ± 4.0	100

[a]The experiment was repeated three times, and the standard deviation calculated. The compounds not reported in this table were unable to inhibit the growth. [b]Percentage of inhibition is calculated considering the inhibition zone given by ampicillin (positive control) as 100%. [c]nd = not detected.

In order to confirm these results and collect more information about the bacteriostatic or bactericidal activity of the sphaeropsidins, the growth inhibition experiment was performed also in liquid medium, and *Xoo* growth was measured 6 and 24 h after the inoculation. The result obtained on Petri dishes was confirmed except for compound **8**, whose activity was not detected in liquid culture. Moreover, the results suggested that compounds **1** and **4** also have bactericidal activity because the cfu/mL after 24 h is lower than after 6 h (Figures 2 and 3). When the bactericidal activity of compound

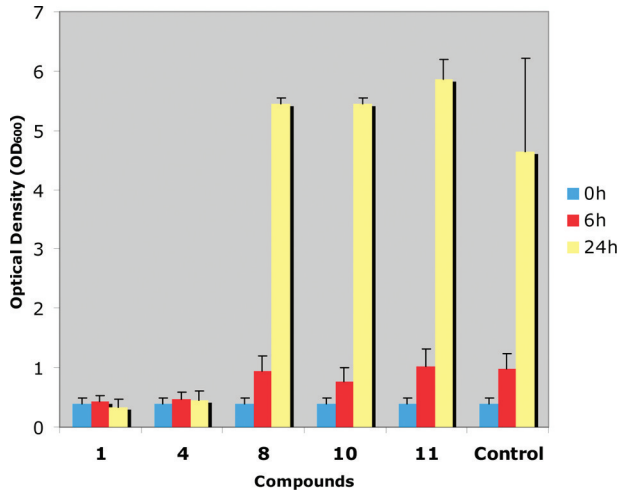

Figure 2. Growth inhibition of *Xanthomonas oryzae* pv. *oryzae* by sphaeropsidin A (**1**) and derivatives **4**, **8**, **10**, and **11** calculated by measuring the optical density of the cultures 6 and 24 h after dilution of an overnight culture (initial OD_{600} = 0.4). The compounds were tested in a concentration range of $(0.5–1.5) \times 10^{-4}$ M. The experiment was repeated three times, and the standard deviation calculated. The control is a *Xoo* culture grown without any spheropsidin derivative.

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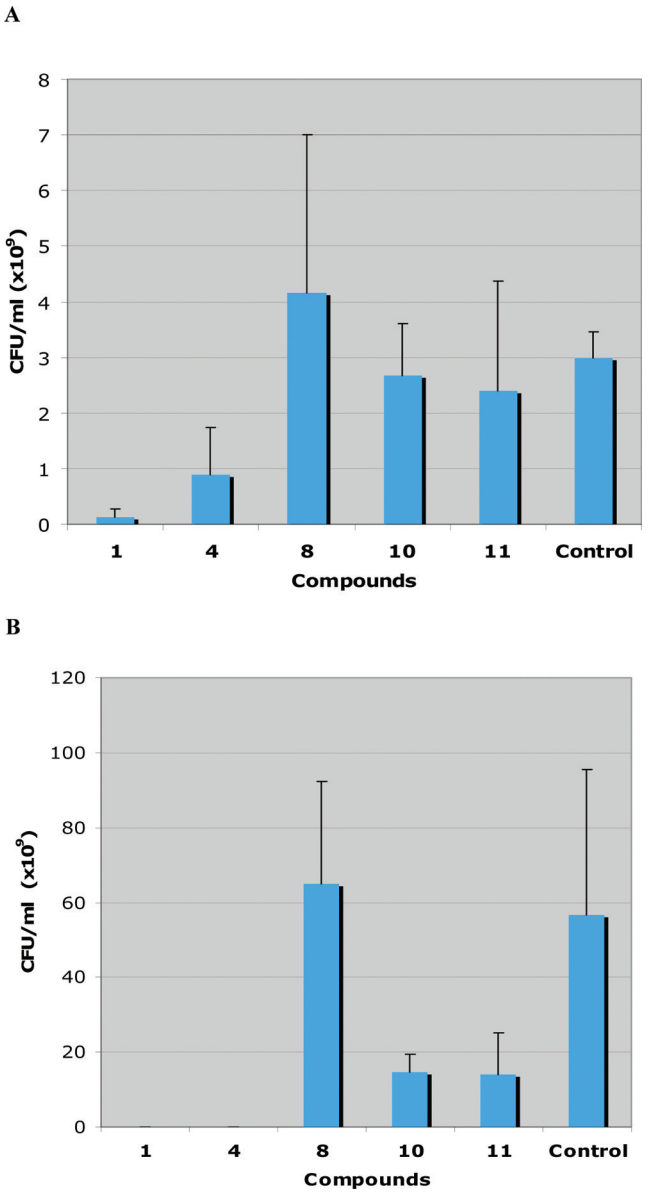

Figure 3. Growth inhibition of *Xanthomonas oryzae* pv. *oryzae* by sphaeropsidin A (**1**) and derivatives **4**, **8**, **10**, and **11** estimated by measuring the colony forming units (cfu/mL) after 6 h (A) and 24 h (B). The compounds were tested in a concentration range of (0.5–1.5) × 10^{-4} M. The experiment was repeated three times, and the standard deviation calculated. The control is a *Xoo* culture grown without any sphaeropsidin derivative.

1 was estimated, an approximately 1:1 000 000 survival rate was found (data not shown).

This result could be explained as, according to a known lethal mechanism,[20] **4** was probably in vivo hydrolyzed to **1**. This result also agrees with a reduced activity observed testing the diacetyl derivative **11**, in which probably the same mechanism operates, i.e., hydrolysis of the C-6 acetyl group. However, it could be that the C-14 acetyl group was not hydrolyzed as well, while a role could be played by the shift of the double bond from C-8–C-14 to C-8–C-9, which also affects the stereochemistry of the B/C ring junction and, hence, the conformational freedom of the two rings. This derivative lacks of the C-9 hydroxy group, which is modified as an *O*-methyl ether in derivative **14**. Also important for the activity appeared to be the presence of a C-7 carbonyl group, as shown by the total loss of activity of sphaeropsidin B and its 7-*O*-acetyl derivative (**5**).

Very important for the activity appeared to be the presence of the hemiketal lactone functionality, as shown by the inactivity of sphaeropsidin C and its derivatives **7** and **6**, with the latter also showing the reduction of the C-7 carbonyl group as in **2** and **5**, while in **7** the carboxy group was converted into the corresponding methyl ester. As expected, the derivatives having the latter structural modification showed total loss of activity, like the three (**12**, **14**, and **15**) and the two (**16** and **17**) derivatives obtained, respectively, by reaction of sphaeropsidins A and B with diazomethane. The derivatives **14** and **15** also showed the conversion of the C-7 carbonyl group into the corresponding 1,1-disubstituted oxirane ring, confirming the importance of this structural feature for the activity. Compounds **12** and **17** showed different modifications of the C-8–C-14 double bond, which is converted into the corresponding cyclopropane ring and C-14 methyl derivative, respectively. As described above for derivative **11** the structural modification of the C-8–C-14 double bond could affect the activity.

The vinyl group at C-13 appeared to impart activity, as shown by the reduced activity of the C-7 oxo derivative **10** of sphaeropsidin A, which differs from **1** by saturation of the C-13 vinyl group. As expected, the tetrahydro derivative **8** of **1**, as well as its acetyl derivative (**9**), showed total loss of activity, as it also possesses a reduced C-7 carbonyl group as in **2**, **5**, and **6**. The same result was observed testing derivative **13**, in which the B ring modification resulted in substantial disruption of the tricyclic pimarane system.

The results described above for the structure–activity relationship (SAR) studies agreed with those obtained assaying the three natural sphaeropsidins A–C, as well as the minor sphaeropsidins D–F, and some (seven) of the cited derivatives of **1**–**3** testing their phytotoxicity against both hosts (three species of cypress) and nonhosts (tomato and mung bean).[16] They also agreed with the results obtained by testing the antimycotic activity of the same sphaeropsidins and derivatives against fungal pathogens in forestry (*Seiridium cardinale*, *S. cupressi*, and *S. unicorne*) and agriculture (*Botrytis cinerea*, *Fusarium oxysporum*, *Penicilllium expansum*, *Verticillium dahliae*, and *Phomopsis amygdali*).[16]

In summary, 14 compounds (**4**–**17**), either natural or hemisynthetic analogues of sphaeropsidines, were evaluated for antibacterial activity in comparison to their precursors (**1**–**3**) in order to establish the SAR. The in vitro antibacterial data indicated which specific structural features are related to the toxic properties of sphaeropsidines, such as the presence of the C-7 carbonyl group and the hemiketal lactone. The vinyl group at C-13, the C-ring double bond, and/or the C-9 tertiary hydroxy as well as the pimarane structural feature are important for the activity. The antibacterial activity displayed by sphaeropsidin A and derivatives against *Xoo* could make these compounds potentially useful for practical applications in agriculture.

EXPERIMENTAL SECTION

General Experimental Procedures. IR spectra were recorded as glassy films on a Perkin-Elmer Spectrum One FT-IR spectrometer, and UV spectra were recorded in MeCN solution on a Perkin-Elmer Lambda 25 UV/vis spectrophotometer. ^{1}H NMR spectra were recorded at 400 MHz in $CDCl_3$ on a Bruker spectrometers unless otherwise noted. EI and ESIMS spectra were recorded at 70 eV on a QP 5050 Shimadzu and on a Waters Micromass Q-TOF Micro and Agilent 1100 coupled to a JEOL AccuTOF (JMS-T100LC) instrument, respectively. Analytical and preparative TLC were performed on

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silica gel (Merck, Kieselgel 60 F_{254}, 0.25 and 0.50 mm, respectively) plates; the spots were visualized by exposure to UV light or by spraying with 10% H_2SO_4 in MeOH and then with 5% phosphomolybdic acid in EtOH, followed by heating at 110 °C for 10 min. Column chromatography was performed with silica gel (Merck, Kieselgel 60, 0.063−0.200 mm).

Fungal Strain. *D. cupressi* was purchased from Centraalbureau voor Schimmelcultures of Baarn (The Netherlands), strain 261.85 CBS, and deposited in the collection of Dipartimento di Protezione delle Piante, Università di Sassari, Sassari, Italy.

Extraction and Isolation of Sphaeropsidins. The fungus was grown in 2 L Erlenmeyer flasks containing 400 mL of modified Czapek medium supplemented with 2% corn meal (pH 5.7).[21] The culture filtrates (15 L) were acidified and extracted with EtOAc and chromatographed on a silica gel column.[21] In particular, the organic extract, obtained as a brown-red oil (9.2 g) and possessing 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 residue of the second fraction (2.4 g) was crystallized from EtOAc−*n*-hexane (1:5), yielding sphaeropsidin A (**1**, R_f 0.65, 1.6 g). The residue left from the mother liquors and the residue of the third fraction were combined (970 mg) and further purified by a combination of column chromatography and preparative TLC eluted with $CHCl_3$−*i*-PrOH (9:1) to give a further amount of **1** (300 mg, for a total of 126.6 mg/L), sphaeropsidin B (**2**, R_f 0.50, 120 mg, 8 mg/L), and sphaeropsidin C (**3**, R_f 0.43, 63 mg, 4.2 mg/L).

Preparation of Sphaeropsidin A−C Derivatives (4−17). The 6-*O*-acetyl (**4**), 7,*O*,15,16-tetrahydro (**8**), 6-*O*-acetyl-14-acetoxy-9-dehydroxy-$\Delta^{8,9}$ (**11**), and methyl ester cyclopropyl derivative (**12**) of sphaeropsidin A as well as the oxidized derivative of sphaeropsidin B (**13**) and the methyl ester of sphaeropsdin C (**7**) were prepared according to the procedures previously reported.[16] The 7-OH derivative (**6**) of **3** was prepared as previously reported.[19] 7-*O*-Acetylsphaeropsidin B (**5**), 7-*O*-acetyl-7,*O*,15,16-tetrahydrosphaeropsidin A (**9**), and 15,16-dihydrosphaeropsidin A (**10**) were prepared according to the procedure reported elsewhere.[22]

Reaction of Sphaeropsidin A with CH_2N_2. An ethereal solution of CH_2N_2 was added to a solution of **1** (150 mg) in MeOH (15 mL) to obtain a persistent yellow color. The reaction was carried out at room temperature under stirring and was stopped after 24 h by evaporation under an N_2 stream. The crude residue (130 mg) was purified by preparative TLC, using *n*-hexane−EtOAc (7:3), to give 100 mg of the 8,14-methylensphaeropsidin A methyl ester (**12**) and the two new derivatives **14** and **15** (7.4 and 9.6 mg, respectively) as homogeneous compounds. Derivative **14**: IR ν_{max} 1730, 1634 cm^{-1}; UV λ_{max} nm (log ε) < 220 nm; ^1H NMR, see Table 3; ESIMS, *m/z* 427 $[M + K]^+$, 411 $[M + Na]^+$; EIMS, *m/z* (%) 388 $[M]^+$ (1), 374 $[M - CH_2]^+$ (9), 356 $[M - CH_3OH]^+$ (28), 342 $[M - CH_2 - CH_3OH]^+$ (13). Derivative **15**: IR ν_{max}, 3383, 1730, 1633 cm^{-1}; UV λ_{max} nm (log ε) < 220 nm; ^1H NMR, see Table 3; ESIMS, *m/z* 413 $[M + K]^+$, 397 $[M + Na]^+$, 375 $[M + H]^+$.

Reaction of Sphaeropsidin B with CH_2N_2. An ethereal solution of CH_2N_2 was added to a solution of **2** (20 mg) in MeOH (1.5 mL) to obtain a persistent yellow color. The reaction was carried out at room temperature under stirring and was stopped after 4 h by evaporation under an N_2 stream. The crude residue (16 mg) was purified by preparative TLC, using petroleum ether−Me_2CO (8:2), to give derivatives **16** and **17** (8.1 and 6.3 mg, respectively) as homogeneous compounds. Derivative **16**: IR ν_{max}, 3461, 1726, 1665 cm^{-1}, UV λ_{max} nm (log ε) 265 (1.72); ^1H NMR, see Table 3; ESIMS, *m/z* 385 $[M + Na]^+$, 363 $[M + H]^+$. Derivative **17**: IR ν_{max}, 3445, 1715, 1623 cm^{-1}; UV λ_{max} nm (log ε) 281 (3.20); ^1H NMR, see Table 3; ESIMS, *m/z* 413 $[M + K]^+$, 397 $[M + Na]^+$, 375 $[M + H]^+$.

Antibacterial Assays. Sphaeropsidin A (**1**), its natural analogues sphaeropsidins B and C (**2** and **3**), and their derivatives (**4**, **8**−**12**, **14**, and **15**; **5**, **13**, **16**, and **17**; **6** and **7**, respectively) were tested at a concentration range of (0.5−6.0) × 10^{-3} M against three major bacterial pathogens of rice, *Xanthomonas oryzae* pv. *oryzae* XKK.12 (isolated in Kerala, India),[23] *Burkholderia glumae* AU6208 (clinical isolate highly virulent to rice),[24] and *Pseudomonas fuscovaginae* UPB0736 (isolated in Madagascar).[25] The bacterial isolates, deposited in the collection of the International Centre for Genetic Engineering and Biotechnology, Area Science Park, Padriciano Trieste, Italy, were grown overnight at 30 °C in PY,[23] King's medium,[26] and Luria−Bertani (LB) media, respectively. Each culture was mixed with PSA, KB, and LB top agar (0.75% w/v agar) at 45 °C, respectively, with a ratio of 50 μL of culture to 10 mL of top agar, and poured on top of LB Petri dishes. Petri dishes with 100 μL wells were obtained by using part of a plastic tip on the LB agar before pouring the top agar. As soon as the top agar was solidified, 25 or 100 μg of different compounds was pipetted into the wells. The negative control was represented by 50 μL of DMSO, as this solvent was used to resuspend the compounds. The Petri dishes were incubated at 30 °C for 1−2 days or until the growth in the top agar was evident. The inhibition of growth around the wells was measured in millimeters. The effect of sphaeropsidins and their derivatives on the growth of *X. oryzae* pv. *oryzae* was estimated also in liquid cultures, measuring both the optical density at 600 nm and the colony-forming units per mL (cfu/mL) of cultures grown with and without the different compounds. A 10 mL amount of PY medium with and without the addition of 50 μg/mL ((0.5−1.5) × 10^{-4} M) of different compounds was inoculated with an overnight culture of *Xoo* at a dilution rate estimated to give an initial OD_{600} of 0.4. Samples were taken 6 and 24 h after the inoculation, and the OD_{600} and cfu/mL were measured. The experiments were repeated three times, and the standard deviation was calculated. In order to estimate the bactericidal activity of compound **1**, 100 μL of culture at time 0 and after 24 h were taken, washed twice with PY medium, and plated at different dilution rates to measure the cfu/mL.

Table 3. ^1H NMR Data of Derivatives of Sphaeropsidins A (14 and 15) and B (16 and 17)[a]

	14	15	16	17
position	δ_H, *J* (Hz)	δ_H, *J* (Hz)	δ_H, *J* (Hz)	δ_H, *J* (Hz)
1	2.28 br d (11.0)	2.28 br d (11.0)	2.29 br d (11.6)	2.36 br d (12.6)
1′	1.56 m	1.60 m	1.65 m	1.56 m
2′	1.56 m	1.60 m	1.65 m	1.56 m
2′	1.56 m	1.60 m	1.65 m	1.56 m
3	1.56 m	1.80 m	1.84 m	1.70 m
3′	1.56 m	1.60 m	1.65 m	1.56 m
5	3.02 s	3.85 s	2.94 s	3.13 s
7			4.70 br s	
11	1.38 m	1.38 m	1.32 m	1.41 m
11′	1.29 m	1.29 m	1.24 m	1.28 m
12	1.78 m	1.80 m	1.80 m	2.15 m
12′	1.56 m	1.60 m	1.65 m	1.56 m
14	5.17 br s	5.17 br s	5.85 br s	5.85 br s
15	5.70 dd (17.4, 10.5)	5.70 dd (17.4, 10.59	5.82 dd (17.4, 10.6)	5.82 dd (17.5, 10.7)
16	5.00 dd(17.4, 1.5)	4.91 dd (17.4, 1.5)	5.01 dd (17.4, 1.5)	5.04 dd (17.5, 1.5)
16′	4.99 dd (10.5, 1.5)	4.88 dd (10.5, 1.5)	4.98 dd (10.6, 1.5)	5.01 dd (10.7, 1.5)
17	0.87 s	0.87 s	0.90 s	0.87 s
18[b]	1.15 s	1.15 s	1.14 s	1.25 s
19[b]	1.02	1.02 s	0.95 s	0.97 s
21	2.78 d (6.2)	2.78 d (6.2)		
21′	2.47 d (6.2)	2.47 d (6.2)		
OMe	3.62 s	3.46 s	3.54 s	3.70 s
OMe	3.47 s			
Me-C=C				1.34 s

[a]Chemical shifts are in δ values (ppm) from TMS. [b]These assignments may be reversed.

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