Sapinofuranones A and B, Two New 2(3H)-Dihydrofuranones Produced by *Sphaeropsis sapinea*, a Common Pathogen of Conifers†

Antonio Evidente,*,‡ Lorenzo Sparapano,§ Olga Fierro,‡ Giovanni Bruno,§ and Andrea Motta⊥

Dipartimento di Scienze Chimico-Agrarie, Università di Napoli Federico II, via Università 100, 80055 Portici, Italy, Dipartimento di Patologia Vegetale, Università di Bari, via Amendola 165/A, 70126 Bari, Italy, and Istituto per la Chimica di Molecole di Interesse Biologico del CNR, via Toiano 6, 80072 Arco Felice, Italy

Received July 23, 1998

Two new 5-substituted dihydrofuranones, named sapinofuranones A and B (**1** and **2**), were isolated from liquid cultures of *Sphaeropsis sapinea*, a phytopathogenic fungus causing a wide range of disease symptoms on conifers. A fungal strain isolated from *Cupressus macrocarpa* produced both **1** and **2** at concentrations higher than those produced by the strain isolated from *Cupressus sempervirens*. Bioassay of **1** and **2** gave epinasty and brown discoloration on petioles of tomato leaves, sapwood stain on inner cortical tissues of the stem of cypress seedlings, and yellowing and needle blight on pine seedlings. Compounds **1** and **2** were characterized, using spectroscopic and chemical methods, as two new 4-[(*2Z*,*4E*)-1-hydroxy-2,4-hexadienyl]butan-4-olides, which are epimers at C-1 of the side chain. The absolute stereochemistry of this chiral center, determined by application of the Mosher's method, proved to be *S* and *R* in **1** and **2**, respectively.

The taxonomy of *Sphaeropsis sapinea* (Fr.:Fr.) Dyko & Sutton (Sphaeropsidales), an opportunistic pathogen of more than 30 species of *Pinus* in 25 countries,[1] has been the subject of considerable confusion and conflicting reports. The pathogen occurs in coniferous forests throughout the world and has been associated with significant economic damage in exotic plantations in New Zealand, Australia, and South Africa.[2,3] *S. sapinea* also occurs in the central and eastern United States causing severe damage on both native and introduced species. In Michigan, Minnesota, and Wisconsin, two morphotypes of the pathogen were recognized.[4] Isolates of the A morphotype were aggressive on both red pine and jack pine, but B morphotype isolates caused severe symptoms only on jack pine.[5] More recently, confirmation of two distinct populations of *S. sapinea* in the North Central United States was carried out by random amplified polymorphic DNA markers (RAPDs).[6] These observations opened a reconsideration of the nature of the host−pathogen interaction.

To contribute to understanding their physiology we have undertaken a study on the production and identification of secondary metabolites produced by *Sphaeropsis* and *Diplodia* species. Usually species belonging to the order of Sphaeropsidales produce *in vivo* and *in vitro* toxic substances.[7] Furthermore, on the basis of the metabolic behavior of the fungi, the metabolites can be used to better characterize biologically *S. sapinea* f. sp. *cupressi* from some strains of *S. sapinea* isolated from infected cypress trees.

Recently, morphological, physiological, pathogenic, and epidemiological studies on *S. sapinea*, *S. sapinea* f. sp. *cupressi*, and *Diplodia mutila* suggested that great morphological and physiological variability exists among different isolates of the fungal *taxa* examined.[8,9] This paper describes the isolation and chemical and biological characterization of the main toxic metabolites produced by three strains of *S. sapinea*, isolated from infected cypress trees, which proved to be chemically different from those produced *in vitro* by *S. sapinea* f. sp. *cupressi* and *D. mutila* isolated from the same host plants.

Results and Discussion

Organic extracts of culture filtrates of all isolates of *S. sapinea* showing a high phytotoxic activity on both the host and non-host plants were purified by a combination of column and TLC chromatography to give two main metabolites (**1** and **2**), named sapinofuranones A and B. Strain D-50, isolated from *C. macrocarpa*, produced *in vitro* a concentration of both sapinofuranones (**1**, 63.3 mg/L; **2**, 20 mg/L) higher than those recovered from strains D-54 (**1**, 16.7 mg/L; **2**, 8.3 mg/L) and D-55 (**1**, 9.3 mg/L; **2**, 6.3 mg/L) isolated from *C. sempervirens*.

When assayed on host and non-host plants, **1** and **2** showed more toxic activity on inner bark tissues than on external ones. Phytotoxicity was observed both on herbaceous non-host plants such as tomato, and on host plants such as cypress and pine. Symptoms appeared on severed twigs of the cypress and pine species or on cuttings of the herbaceous plants, after toxin absorption and after injection of a toxic solution into the cortical tissues of 2-year-old pine and 3-year-old cypress trees. Browning or yellowing was observed on severed twigs of cypress and pine, respectively. Sapwood-stain-like symptoms were observed on young active zone wood contiguous to cambium of cypress seedlings, while a severe withering of needles appeared on 2-year old pine seedlings. Tomato plants were also affected by both compounds: epinasty and brown discoloration appeared on petiols and leaves.

As deduced from HREIMS, **1** had a molecular formula of $C_{10}H_{14}O_3$. From this it was possible to deduce the presence of four unsaturations or rings. IR bands typical of γ-lactone and diene groups were observed together with that of at least one hydroxy group.[10] A conjugated diene system was indicated by a characteristic UV absorption maximum at 228 nm.[11] These structural features were consistent with the signal patterns observed in ^{1}H and ^{13}C NMR spectra (Table 1). The ^{1}H NMR spectrum showed four

* To whom correspondence should be addressed Tel. +39-81-7885224. Fax: +39-81-7755130. E-mail: evidente@unina.it.
† Dedicated to the memory of Prof. G. Randazzo.
‡ Dipartimento di Scienze Chimico-Agrarie.
§ Dipartimento di Patologia Vegetale.
⊥ Istituto per la Chimica di Molecole di Interesse Biologico. This institute is part of the "Istituto Nazionale per la Chimica dei Sistemi Biologici".

10.1021/np980318t CCC: $18.00
Published on Web 01/26/1999

Table 1. ^{1}H and ^{13}C NMR Spectral Data of Sapinofuranones A and B (**1** and **2**)[a]

	1[b]			**2**		
C	δ[c]	^{1}Hδ	J (Hz)	δ[c]	^{1}Hδ	J (Hz)
1	177.4 (s)			177.0 (s)		
2	28.5 (t)	2.60 (m)		28.4 (t)	2.61 (m)	
		2.50			2.52	
3	21.4 (t)	2.20 (m)		23.7 (t)	2.22 (m)	
					2.09 (m)	
4	82.2 (d)	4.51 (dt)	(7.2, 3.1)	82.8 (d)	4.45 (dt)	(7.1, 5.5)
5	68.7 (d)	4.88 (dd)	(8.4, 3.1)	70.1 (d)	4.80 (dd)	(10.2, 5.5)
6	123.7 (d)	5.20 (dd)	(11.0, 8.4)	123.2 (d)	5.31 (dd)	(11.0, 10.2)
7	133.1 (d)	6.15 (dd)	(11.2, 11.0)	133.0 (d)	6.19 (dd)	(11.8, 11.0)
8	126.0 (d)	6.30 (ddq)	(13.7, 11.2, 1.8)	126.0 (d)	6.36 (br dd)	(13.8, 11.8)
9	133.8 (d)	5.82 (dq)	(13.7, 6.8)	134.0 (d)	5.86 (dq)	(13.8, 6.7)
10	18.3 (q)	1.80 (dd)	(6.8, 1.8)	18.4 (q)	1.82 (dd)	(6.7, 1.3)

[a] The chemical shifts are in δ values (ppm) from TMS. [b] 2D ^{1}H$-^1$H (COSY) and 2D ^{13}C$-^1$H (HMQC) NMR experiments delineated the correlations of all protons and the corresponding carbons. [c] Multiplicities determined by DEPT spectra.

signals typical of olefinic protons belonging to trans- and cis-disubstituted double bonds of a diene system.[12] In particular, the doublet of double quartets at δ 6.30 (H-8) was coupled in the COSY spectrum,[13] with H-9 of the trans-disubstituted double bond (δ 5.82) being also coupled with a terminal methyl group (CH_3-10), which resonated at δ 1.80 with typical *cis*-allylic coupling to H-8.[12] The latter also correlated with H-7 of the cis-disubstituted double bond resonating at δ 6.15, which in turn was coupled with H-6 appearing at δ 5.20. The multiplicity of H-6 was due to further coupling with the H-5 of an adjacent secondary hydroxylated carbon. This resonated at δ 4.88 and was also coupled with H-4 of another oxygenated secondary carbon, which in turn appeared at δ 4.51 and was finally coupled with the protons of an adjacent methylene group (CH_2-3). The methylene protons resonated as complex systems at δ 2.60 and 2.50, being further coupled with the protons of another adjacent methylene group (CH_2-2) appearing as a very complex system centered at δ 2.20. The chemical shifts of the protons of the two methylene groups as well as that of H-4 were in good agreement with the values reported for γ-lactone derivatives.[12] The presence of this partial structure in **1** was consistent with two residual unsaturations calculated considering the diene system and the very typical γ-lactone shift at δ 177.4 (C-1)[14] observed in its ^{13}C NMR spectrum for the carbonyl group. As also expected ^{13}C-chemical shifts measured for the hydroxylated secondary carbon closing the butanolide ring (C-4) and the two methylene groups (C-2 and C-3) agreed with the values reported for γ-lactone derivatives.[14] In addition to the four doublets typical of the carbons of the diene system, the ^{13}C NMR spectrum also showed those of the other secondary hydroxylated carbon (C-5) and the methyl group (C-10) at δ 68.7 and 18.3, respectively.

On the basis of these findings and considering the molecular formula of $C_{10}H_{14}O_3$, sapinofuranone A (**1**) proved to be a new 5-substituted-dihydro-2(3H)furanone bearing a 1-hydroxy-2,4-hexadienyl side chain at C-4. This structure was supported by HREIMS of **1**, which showed fragmentations typical of a substituted γ-lactone derivative having a hydroxy group.[12,15] The molecular ion $[M]^+$ at *m/z* 182, losing, in succession, H_2O and Me, generated ions at *m/z* 164 ($C_{10}H_{12}O_2$) and 149; while alternative loss of the side chain ($C_6H_9O_1$) produced the ion (base peak) at *m/z* 85. The hydroxylated hexadienyl side chain generated the ion at *m/z* 97 ($C_6H_9O_1$), which, by loss of H_2O, yielded *m/z* 79.

The structure of **1** was further confirmed by preparation of its corresponding 5-*O*-acetyl derivative (**3**). The ^{1}H NMR of **3** differed from that of **1** only in the downfield shift ($\Delta\delta$ 0.96) of H-5, which appeared as a multiplet, being overlapped with the signal of H-9 (δ 5.84), and in the acetyl singlet at δ 2.07. Its EIMS showed the expected molecular ion $[M]^+$ at *m/z* 224 and ions generated by consecutive losses of CH_2CO and the side chain ($C_6H_9O_1$) (*m/z* 182 and 85). Alternative loss of HOAc from the molecular ion produced the ion at *m/z* 164. Moreover, the acetylated side chain yielded a significant ion at *m/z* 139, which, by consecutive losses of CH_2CO and H_2O, generated ions at *m/z* 97 (base peak) and 79, respectively. Therefore, sapinofuranone A is 4-[(2*Z*,4*E*)-1-hydroxy-2,4-hexadienyl]butan-4-olide (**1**).

Table 2. ^{1}H and ^{13}C NMR Data of the (*R*)-(+)- and (*S*)-(−)-α-Methoxy-α-trifluorophenylacetate (MTPA) Esters of Sapinofuranone A (**4** and **5**) and B (**6** and **7**)[a]

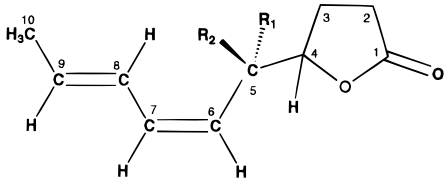
compound	Ph	OCH_3	H-4	H-5	H-6
4[b]	7.50−7.36 m	3.54 s	4.56 dt	6.19 dd	5.17 dd
5[b]	7.51−7.36 m	3.50 s	4.62 dt	6.12 dd	5.01 dd
6[c]	7.50−7.40 m	3.56 s	4.62 dt	5.90 m[d]	5.07 dd
7[c]	7.54−7.40 m	3.55 s	4.58 dt	5.93 m[d]	5.30 dd

[a] Chemical shifts are in δ values (ppm) from TMS. [b,c] The other proton resonances were very close to those of **1** and **2**, respectively. [c] J (Hz) **4, 5**: 3,4=7.3; 4,5=3.2; 5,6=9.5; 6.7=10.2; **6,7**: 3,4=7.2; 4,5=6.0; 5,6=10.2; 6,7=11.1. [d] This signal overlapped that of H-9.

Sapinofuranone B (**2**) showed the same molecular formula, $C_{10}H_{14}O_3$, and gave ^{1}H NMR, ^{13}C NMR, HREIMS, IR, and UV spectra very similar to those of **1**, indicating that **1** and **2** were isomers. Compounds **1** and **2** gave different optical rotations and different chromatographic behaviors in two solvent systems. Therefore, it was hypothesized that **2** was a diastereomer of **1**. Comparison of the coupling constants of **1** and **2**, showed that the couplings ($J_{4,5}$ = 3.1 and $J_{5,6}$ = 8.4 Hz and $J_{4,5}$ = 5.5 and $J_{5,6}$ = 10.2 Hz) between H-5 and both H-6 and H-4 in **1** and **2**, respectively, were the only substantial differences. These differences indicated that **2** was the C-5 epimer of **1**.

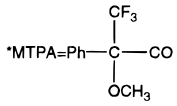
This conclusion was in agreement with the opposite absolute configuration determined at C-5 for both metabolites using Mosher's method.[16,17] Sapinofuranone A (**1**) was converted to the diastereomeric (*R*)-(+)-α-methoxy-α-trifluorophenylacetate (MTPA) (**4**) and the (*S*)-(−)-MTPA (**5**) esters; accurate ^{1}H NMR studies were carried out on both derivatives. Comparison of the ^{1}H NMR data of **4** with those of **5** (Table 2) showed a downfield shift ($\Delta\delta$ 0.16) of H-6 along with an upfield shift ($\Delta\delta$ 0.06) of H-4. These data, in conjunction with the literature data,[16,17] allowed assignment of the *S*-configuration at C-5 in **1**. Whereas, comparing the ^{1}H NMR data of the (*R*)-(+)-MTPA (**6**) and (*S*)-(−)-MTPA (**7**) esters (Table 2) obtained from **2**, an upfield shift

($\Delta\delta$ 0.23) of H-6 along with a downfield shift ($\Delta\delta$ 0.04) of H-4 was observed. On the basis of the same reference data[16,17] it was possible to assign the *R*-configuration at C-5 in **2**, which is opposite to that of **1** and therefore in agreement with their epimeric nature as found by the inspection of their NMR data.



1 R_1=OH, R_2=H
2 R_1=H, R_2=OH
3 R_1=OAc, R_2=H

4 R_1=O-*R*-(+)-MTPA*, R_2=H
5 R_1=O-*S*-(-)-MTPA*, R_2=H
6 R_1=H, R_2=O-*R*-(+)-MTPA*
7 R_1=H, R_2=O-*S*-(-)-MTPA*



The butanolide nature of **1** and **2** is rare in natural products.[18] These compounds are closely related to butenolides and tetronic acids, which are well known as plant, fungal, and lichen metabolites that also exhibit interesting biological activities.[19] Among these are the seiridins, which are the 3,4-dialkylbutenolides isolated from culture filtrates of three species of *Seiridium* that produce canker disease on cypress.[20]

Experimental Section

General Experimental Procedures. Optical rotations were measured in $CDCl_3$ on a Perkin−Elmer 243B polarimeter; IR and UV spectra were determined neat and in MeOH, respectively, on a Perkin−Elmer IR FT-1720X spectrometer and a Perkin−Elmer Lamda 3B spectrophotometer. ^{1}H and ^{13}C NMR were recorded at 500 or 250 and at 125 or 62.5 MHz, respectively, in $CDCl_3$, on DRX 500 and AM 250 Bruker spectrometers. The same solvent was used as internal standard. Carbon multiplicities were determined by DEPT spectra.[14] DEPT, COSY-45, HMQC,[21] and HMBC[22] NMR experiments were performed using Bruker microprograms. EIMS and HREIMS were taken at 70 eV on a Fisons Trio-2000 and a Fisons ProSpec spectrometer, respectively. Analytical and preparative TLC were performed on Si gel (Merck, Kieselgel, 60 F_{254}, 0.25 and 0.50 mm, respectively) or on reversed-phase (Whatman, KC18 F_{254}, 0.20 mm) plates; the spots were visualized by exposure to UV and/or by spraying with 10% H_2SO_4 in MeOH and then with 5% phosphomolybdic acid in MeOH, followed by heating at 110 °C for 10 min. Column chromatography was performed on Si gel (Merck, Kieselgel, 60, 0.063−0.20 mm).

Fungal Isolates. The *S. sapinea* strains used in this study were collected from *Cupressus* species from various locations in the Apulia region of southern of Italy. Isolate D-50 was obtained from infected cortical tissues of *C. macrocarpa* Hartw., whereas isolates D-54 and D-55 were from *C. sempervirens* L. Each culture originated from a single conidium. Each isolate was grown on potato−sucrose (2%)−agar medium (PSA) in Petri dishes and then transferred to slants containing the same substrate, at 25 °C for 2 weeks. Monoconidial isolates (numbered: D-50, D-54, and D-55, respectively) were maintained at 5 °C in the fungal collection of the "Dipartimento di Patologia Vegetale, Università di Bari," Italy.

Production, Extraction, and Purification of Sapinofuranones. Isolates D-50, D-54, and D-55 of *S. sapinea* were grown in stationary culture in 1-L Roux flasks containing 150 mL of a modified Czapek medium with 2% corn meal (pH 5.7). Each flask was seeded with 5 mL of a suspension of three culture tubes (10-day-old cultures) in 50 mL of sterile medium. The flasks were incubated at 25 °C for 30 days in the dark. At harvest, the mycelium mat was removed by filtration on Myracloth (Calbiochem). The culture filtrates (3 L each; D-50, pH 6.0; D-54, pH 6.3, and D-55, pH 7.0) were acidified to pH 4 with 2 M of HCl and extracted with EtOAc (4 × 1.5 L). The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give red-brown oil residues (D-50, 942 mg; D-54, 458 mg, and D-55, 1060 mg) having high phytotoxic activity. TLC analysis on Si gel, eluent $CHCl_3$−*i*-PrOH (95:5), of these showed a main metabolite at R_f 0.44. The crude residue (942 mg) from the D-50 isolate was chromatographed by column chromatography eluted with the same solvent system to afford 13 fractions, of which 1, 2, 5, 7, and 9−12 showed phytotoxic activity. The residue (361 mg) from fraction 5, containing the major metabolite, was a mixture of two metabolites by TLC on Si gel and reversed-phase, using petroleum ether−Me_2CO (7:3) and H_2O−EtOH (7:3) as solvent systems, respectively. The latter was separated by preparative reversed-phase TLC, eluent H_2O−EtOH (7:3), to give sapinofuranones A (**1**, 190 mg, 63.3 mg/L) and B (**2**, 60 mg, 20 mg/L), respectively, both homogeneous oils [R_f 0.44, 0.47, and 0.43 and R_f 0.44, 0.34, and 0.48, by Si gel and reversed-phase TLC, eluent systems $CHCl_3$−*i*-PrOH (95:5), petroleum ether−Me_2CO (7:3), and H_2O−EtOH (7:3), respectively]. When the same procedure was applied to the organic crude extract (0.458 and 1.060 g) of culture filtrates of isolates D-54 and D-55, **1** and **2** were obtained at lower concentrations (16.7 and 8.3 mg/L and 9.3 and 6.3 mg/L, respectively) in comparison to the isolate D-50.

Sapinofuranone A (1): colorless oil; $[\alpha]^{25}_D$ +65.2° (*c* 1.3); UV λ_{max} nm (log ϵ) 228 (4.16); IR ν_{max} 3415, 1762, 1655 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1. HMBC correlations H-2/C-1, C-4; H-2′/C-1, C-4; H-3/ C-1, C-4, C-5; H-3′/ C-1, C-4, C-5; H-6/ C-7, C-8; H-7/C-5, C-9; H-8/C-10; H-9/ C-6, C-7, C-8; H-10/C-6, C-8, C-9. HREIMS *m/z* (rel int.) 182 [M]$^+$ (3), 167 (0.5), 164.0827 (calcd for $C_{10}H_{12}O_2$, 164.0837) (42) [M − H_2O]$^+$, 149 (5), 97.0670 (calcd for $C_6H_9O_1$, 97.0653) (85) [$C_6H_9O_1$ (side chain)]$^+$, 85 (100), 79 (14).

Sapinofuranone B (2): colorless oil; $[\alpha]^{25}_D$ −18.9° (*c* 0.57); UV λ_{max} nm (log ϵ) 226 (4.13); IR ν_{max} 3447, 1773, 1654 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1. HREIMS *m/z* (rel int.) 182 [M]$^+$ (0.2), 167 (0.3), 164.0849 (calcd for $C_{10}H_{12}O_2$, 164.0837) (42) [M − H_2O]$^+$, 149 (1), 97 (100), 85 (61), 79 (14).

5-*O*-Acetylsapinofuranone A (3): sapinofuranone A (**1**, 6 mg) was acetylated with pyridine (300 μL) and Ac_2O (300 μL) at room temperature overnight. The oily residue, left by the reaction workup, was purified by preparative TLC on Si gel (petroleum ether−Me_2CO, 7:3) to give **3** as a homogeneous compound (7 mg): UV λ_{max} nm (log ϵ) 228 (4.15); IR ν_{max} 1776, 1736, 1654 cm^{-1}; ^{1}H NMR differed from that of **1** in the following signal systems δ 5.84 (2H, m, H-5 and H-9), 2.07 (3H, s, MeCO); EIMS *m/z* (rel int.) 224 [M]$^+$ (2), 182 (3), 164 (5), 153 (12), 139 (24), 97 (100), 85 (99), 79 (23).

(*R*)-(+)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of Sapinofuranone A (4). (*R*)-(+)-MTPA-Cl (20 μL) was added to sapinofuranone A (**1**, 9 mg), dissolved in dry pyridine (200 μL). The mixture was allowed to stand at room temperature. After 1 h, the reaction was complete, and MeOH was added. The pyridine was removed by a N_2 stream. The residue was purified by preparative TLC on Si gel (petroleum ether−Me_2CO, 7:3) affording **4** as an oil (10.2 mg): UV λ_{max} nm (log ϵ) 229 (4.18); IR ν_{max} 1781, 1756, 1655, 1603 cm^{-1}; ^{1}H NMR is reported in Table 2; EIMS *m/z* (rel int.) 398 [M]$^+$ (11), 313 (13), 285 (4), 189 (100), 181 (21), 164 (99), 85 (99), 77 (99), 65 (40).

(*S*)-(−)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of Sapinofuranone A (5). (*S*)−(−)-MTPA-Cl (20 μL) was added to sapinofuranone A (**1**, 9 mg), dissolved in dry pyridine (200 μL). The reaction was carried out under the same conditions used for preparing **4** from **1**. Purification of the crude residue by preparative TLC on Si gel (petroleum ether−Me_2CO, 7:3) yielded **5** as an oil (10.4 mg): UV, IR, and EIMS were very similar to those of **4**; ^{1}H NMR is reported in Table 2.

(*R*)-(+)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of Sapinofuranone B (6). The ester derivative **6** was obtained from **2** (3.6 mg) as described for preparing **4** from **1**. The pure oil **6** (4.5 mg) had UV, IR, and EIMS very similar to those of **4**; ^{1}H NMR is reported in Table 2.

(*S*)-(−)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of Sapinofuranone B (7). The ester derivative **7** was obtained from **2** (3.2 mg) as described for preparing **5** from **1**. The pure oil **7** (3.2 mg) had UV, IR, and EIMS very similar to that of **4**; ^{1}H NMR is reported in Table 2.

Toxin Bioassays. Culture filtrates, their chromatographic fractions, and pure substances were assayed for phytotoxicity using severed twigs of cypress (*C. sempervirens* var. *pyramidalis*, *C. macrocarpa* var. *lambertiana*, and *C. arizonica*) and pine (*Pinus halepensis* L., *P. radiata* L.). For the experiments, the apical parts of the twigs, approximately 12 cm long, were used. The cuttings were taken from young cypress and pine seedlings (3 years old and 2 years old, respectively) grown in the greenhouse at 25−27 °C and 60−70% relative humidity. During the bioassay, the severed twigs were maintained in a growth chamber at relatively low relative humidity (60%), temperature (23 °C), and light (150 mmol × m^{-2} × s^{-1}). The phytotoxicity of both **1** and **2** was also tested on a nonhost herbaceous plant (tomato: *Lycopersicon esculentum* L. var. Marmande). Seedlings of tomato were grown in a growth chamber at 25 °C and 70−80% relative humidity exposed to a luminous flux of 400 mmol × m^{-2} × s^{-1} with a 12 h photoperiod. Cuttings were taken from 21-day-old seedlings. The pure substances were tested at concentrations of 0.1−0.2 mg × mL^{-1} on cypress and pine and 0.05−0.1 mg × mL^{-1} on tomato cuttings, respectively. The toxicity of these solutions was evaluated by placing the test plant parts (cypress and pine cuttings for 96 h and tomato cuttings for 48 h) in the assay solution (3 mL) and then transferring them to distilled H_2O. Symptoms developed within 2 days on tomato and within 21 days on cypress and pine. A solution of **1** and **2** (0.1 mg × mL^{-1}) was also injected into the cortical tissue of 3-year-old cypress seedlings or into 2-year-old pine seedlings, at a distance of 25 cm from the apex.

Acknowledgment. This investigation was supported by grants from the Italian Ministry of University and Scientific Technological Research. The authors also thank the "Centro di Metodologie Chimico-Fisiche" dell'Università di Napoli Federico II and Dr. O. Taglialatela of the "Centro Interdipartimentale di Analisi Strumentale" dell'Università di Napoli Federico II for NMR and HREIMS spectra, respectively. Contribution no. 173 (DISCA). We are also grateful to Mr. L. Scarola for technical assistance in performing the bioassays. The mass spectra were provided by the "Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli Federico II". The assistance of the staff is gratefully aknowledged.

References and Notes

(1) Swart, W. J.; Wingfield, M. J. *Plant Dis.* **1991**, *75*, 761−766.
(2) Chou, C. K. *New Zealand J. For. Sci.* **1976**, *6*, 72−79.
(3) Zwolinski, J. B.; Swart, W. J.; Wingfield, M. J. *Eur. J. For. Path.* **1990**, *20*, 405−411.
(4) Palmer, M. A.; Stewart, E. L.; Wingfield, M. J. *Phytopathology* **1987**, *77*, 944−948.
(5) Stanosz, G. R.; Smith, D. R.; Guthmiller, M. A.; Stanosz, J. C. *Mycologia* **1997**, *89*, 525−530
(6) Smith, D. R.; Stanosz, G. R. *Phytopathology* **1995**, *85*, 699−704
(7) Uspenskaya, G. A.; Reshetnikova, I. A. *Mikol. Fitopatol.* **1975**, *9*, 355−360.
(8) Frisullo S.; Bruno, G.; Lops, F.; Sparapano, L. *Petria* **1997**, *7*, 141−158.
(9) Frisullo, S.; Bruno, G.; Lops, F.; Sparapano, L. *Proc. Congr. Mediterr. Phytopathol. Union*, **10th**, Montpellier (France) **1997**, 201−205.
(10) Nakanishi, K.; Solomon, P. H. *Infrared Absorption Spectroscopy*, 2nd ed. Holden Day: Oakland, 1977; pp 17−44.
(11) Scott A. I. *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon: Oxford, 1964; pp 45−52.
(12) Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tables of Spectral Data for Structure Determination of Organic Compounds*; Fresenius, W., Huber, J. F. K., Pungor, E., Rechnitz, G. A., Simon, W., West, T. S., Eds.; Springer: Berlin, 1983; pp H55, H145, H205, H210, M220.
(13) Bax, A.; Freeman, R. *J. Magn. Res.* **1981**, *44*, 542−561.
(14) Breitmaier, E.; Völter, W. *Carbon-13 NMR Spectroscopy*; VCH: Weinheim, 1987; pp 80−84, 192−194, 206−213, 276−281.
(15) Porter, Q. N. *Mass Spectrometry of Heterocyclic Compounds*; John Wiley & Sons: New York, 1985; pp 262−266.
(16) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543−2549.
(17) Dale, J. A.; Mosher, H. S.*J. Am. Chem. Soc.* **1973**, *95*, 512−519.
(18) Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Academic: London, 1983; pp 367−383, 468−484, 530−532.
(19) Dean, F. M. *Naturally Occurring Oxygen Ring Compounds*; Butterworth: London, 1963; pp 53−81.
(20) Evidente, A.; Sparapano, L. *J. Nat. Prod.* **1994**, *57*, 1720−1725 (and references therein).
(21) Bax, A.; Ikura, M.; Kay, L. E.; Torchia, D. A.; Tschudin, R. *J. Magn. Res.* **1990**, *86*, 304−318.
(22) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.

NP980318T