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Lentisone, a New Phytotoxic Anthraquinone Produced by *Ascochyta lentis*, the Causal Agent of Ascochyta Blight in *Lens culinaris*

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Supporting Information

ABSTRACT: An aggressive isolate of *Ascochyta lentis* obtained from lentil (*Lens culinaris* L.) produced various metabolites in vitro. The metabolites were isolated from the culture filtrates and characterized by spectroscopic, chemical, and optical methods. A new phytotoxic anthraquinone, named lentisone, was isolated and characterized as (1*S**,2*S**,3*S**)-1,2,3,8-tetrahydroxy-1,2,3,4-tetrahydro-6-methylanthraquinone together with the well-known pachybasin (1-hydroxy-3-methylanthraquinone), tyrosol, and pseurotin A. Lentisone, tyrosol, and pseurotin A were phytotoxic to lentil, with lentisone the most toxic of all. The toxicity of these compounds is light-dependent. Finally, lentisone was also found to be phytotoxic to chickpea, pea, and faba bean, with toxicity in the latter higher than in any other tested legume, including lentil.

KEYWORDS: *Ascochyta lentis, Lens culinaris, phytotoxins, anthraquinones, lentisone*

INTRODUCTION

Lentil (*Lens culinaris* L.) is one of the oldest legume crops mainly grown as a valuable protein-rich food for both humans and livestock.[1] Among the several biotic stresses lentil can encounter, Ascochyta blight stands out as one of the most destructive.[2] The pathogen responsible is the necrotrophic fungus *Ascochyta lentis*, which is present in nearly all lentil cultivation areas. Its symptoms are necrotic lesions on leaflets, stems, pods, and seeds, and when these lesions coalesce, they may cause serious damage.[3]

Fungi belonging to the genus *Ascochyta* are causal agents of diseases of both wild plants and crops. They infect the above-ground parts of plants, manifesting as necrotic spots and light gray to tan lesions on leaves and stems.[4] The ability of many of these pathogens to produce phytotoxic metabolites has been ascertained, and their involvement in symptom appearance has been discussed elsewhere.[5−7] Several potent phytotoxins have been isolated from *Ascochyta* species that were pathogenic to some crops and weeds. Pyrenolide A and ascochytin[5] and ascaulitoxin, its aglycone, and *trans*-4-aminoproline[8−10] were isolated, respectively, from *Ascochyta hyalospora* and *Ascochyta caulina*, both pathogens of lamb's quarters (*Chenopodium album*). Ascosonchine has been isolated as the main phytotoxin produced by *Ascochyta sonchi* and proposed as a mycoherbicide for the biocontrol of the weed *Sonchus arvensis*.[11] Papyracillic acid and more recently the new derivative of salicylic aldehyde, called agropyrenol, were isolated from *Ascochyta agropyrina* var. *nana*, a pathogen of *Elytrigia repens*.[12,13] Ascochitine and ascosalitoxin were isolated from *Ascochyta fabae* and *Ascochyta pisi* pathogens of pea.[14] Solanapyrones A−C were isolated from *Ascochyta rabiei*, the causal agent of Ascochyta blight of chickpea.[15] The main phytotoxin identified as pinolidoxin[16] and three of its closely related metabolites[6] were produced in solid culture by Ascochyta *pinodes*. This fungus has been reclassified as (telemorph *Dimydella pinodes*) and has exerted serious constraints on pea production worldwide.[17] Recently, pinolidoxin, a new nonenolide called pinolide, and herbarumin II and its 2-epimer[7] were produced from a very aggressive strain of the same fungus isolated in Spain and grown in liquid culture.

Therefore, the objective of this work was to identify metabolites produced by *Ascochyta lentis*, which possess phytotoxic activity on the host plant (*Lens culinaris* L.).

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were measured in MeOH, unless otherwise noted, on a Jasco (Tokyo, Japan) polarimeter, whereas the ECD spectra were recorded on a JASCO J-815 spectrometer in MeOH. IR spectra were recorded as deposit glass film on a Spectrum One FT-IR spectrometer (Perkin-Elmer (Norwalk, CT, USA), and UV spectra were measured in MeCN on a Perkin-Elmer Lambda 23 UV−vis spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 600 or 400 MHz and at 125 or 100 MHz, respectively, in $CDCl_3$, unless otherwise noted, on Bruker (Karlsruhe, Germany) spectrometers. The same solvent was used as internal standard. Carbon multiplicities were determined by distortionless enhancement by polarization transfer (DEPT) spectra. DEPT, correlation spectroscopy (COSY)-45, heteronuclear single-quantum coherence (HSQC), heteronuclear multiple-bond correlation

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(HMBC), and nuclear Overhauser enhancement spectroscopy (NOESY) experiments were performed using standard Bruker microprograms. HRESI MS spectra were recorded on Thermo LTQ Velos (Thermo Fisher Scientific, Bremen, Germany); ESI and APCI MS spectra were recorded on a 6120 quadrupole LC-MS instrument from Agilent Technologies (Milan, Italy). Analytical and preparative thin layer chromatography (TLC) were performed on silica gel, Kieselgel 60, F_{254} plates, 0.25 and 0.5 mm, respectively (Merck, Darmstadt, Germany). The spots were visualized either by exposure to UV radiation (254 nm) 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. Column chromatography was performed on a silica gel column, Kieselgel 60, 0.063−0.200 mm (Merck).

Fungal Strain, Culture Medium, and Growth Conditions. A monoconidial isolate of *A. lentis* originally isolated from infected cultivated lentil tissue collected in the field in Germany and deposited at the Collection of the Institute for Sustainable Agriculture, CSIC, Cordoba, Spain, n. Al-64-9, was grown in V8 medium (200 mL V8/L and 30 g agar/L at pH 5.5) for 14 days at 20 °C under a cycle of 12 h of darkness and 12 h of exposure to visible light + near-UV radiation, until a carpet of sporulating mycelium was clearly visible. A spore suspension (1×10^6 spores/mL) was obtained from these cultures and added to 250 mL flasks (1 mL per flask) containing 100 mL of a modified Czapek−Dox medium (5% glucose, 0.1% yeast extract, 0.05% K_2HPO_4, 0.2% $NaNO_3$, 0.05% $MgSO_4{\cdot}7H_2O$, and 0.001% $FeSO_4{\cdot}7H_2O$). These liquid cultures were incubated for 21 days at 20 °C in the dark on an orbital shaker at 150 rpm. The content of the flasks was then centrifuged at 5000 *g*, and the supernatant was filtered and lyophilized.

Extraction and Purification of Metabolites from *A. lentis* Culture Filtrates. The lyophilized culture filtrates (3.5 L) were dissolved in $^1/_{10}$ of initial volume with distilled water and extracted with EtOAc (4 × 400 mL). The organic extracts were combined, dried (Na_2SO_4), and evaporated under reduced pressure to yield a solid brown residue (500.5 mg). The residue tested at concentrations of 5 mg/mL as described below was found to be phytotoxic against lentil, and it was then submitted to bioassay-guided fractionation through column chromatography (750 mm × 30 mm) on silica gel, eluted with 1 L of $CHCl_3$/*i*PrOH (9:1, v/v). Eleven homogeneous fraction groups, as detected by TLC analysis using the same solvent, were collected and screened for their phytotoxic activity. The residue (67.2 mg) of the second fraction was further purified by CC on silica gel, eluted with EtOAc/*n*-hexane (3:7, v/v), yielding a homogeneous orange amorphous solid, **2** (R_f 0.55, eluent EtOAc/*n*-hexane (3:7, v/v) 9.1 mg, 2.5 mg/L). The residue (71.8 mg) of the third fraction containing the main metabolite was further purified by TLC on silica gel, eluted with $CHCl_3$/*i*PrOH (95:5, v/v), yielding a white homogeneous amorphous solid **5** (R_f 0.42, eluent $CHCl_3$/*i*PrOH (95:5, v/v), 12.8 mg, 3.7 mg/L). The residue (29.7 mg) of the fifth fraction was further purified by TLC on silica gel, eluted with $CHCl_3$/*i*PrOH (88:12, v/v), yielding a homogeneous amorphous solid, **3** (R_f 0.24, eluent $CHCl_3$/*i*-PrOH (95:5, v/v), 1.1 mg, 0.3 mg/L). The residue (37.5 mg) of the seventh fraction was further purified by TLC on silica gel, eluted with the $CHCl_3$/*i*PrOH (85:15, v/v), yielding a homogeneous yellow amorphous solid, **1** (R_f 0.33, $CHCl_3$/*i*PrOH (85:15, v/v) 4.5 mg, 1.3 mg/L), which was named lentisone due to its being a new phytotoxic anthraquinone.

Lentisone (1): IR ν_{max}, 3390, 1664, 1639, 1615, 1272 cm^{-1}; UV λ_{max} nm (log ε), 420 (3.41), 274 (3.84), 250 (3.91), 214; ^1H and ^{13}C NMR spectra, see Table 1; HRESI MS (+) spectrum, 313.0697 [$C_{15}H_{14}NaO_6$, calcd 313.0688, M + Na]$^+$, 285 [M + Na − CO]$^+$; ESI MS (−) spectrum, *m/z* 289 [M − H]$^-$.

Pachybasin (2): IR ν_{max}, 1671, 1636, 1590, 1480, 1367, 1277 cm^{-1}; UV λ_{max} nm (log ε), 404 (3.41), 329 (3.12), 280 (sh), 259 (4.04), 246 (4.02), 224 (3.96) [lit.[18] UV λ_{max} (MeOH), 402 (log ε = 3.7), 327, 249; IR ν_{max} ($CHCl_3$) cm^{-1} 1675, 1637, 1590, 1451, 1359, 1274, 1220, 864, 756, 718; lit.[19] UV (MeOH) λ_{max} nm, 224, 238, 243, 258, 326 and 403; IR (KBr) ν_{max} cm^{-1}, 3440 (OH), 2924, 1677 (C═O), 1638 (chelated C═O), 1588, 1457, 1306, 1218, 1065, and 706]; ^1H and ^{13}C NMR spectra, very similar to those previously reported;[18,19] ESI MS (+), *m/z* 261 [M + Na]$^+$, 239 [M + H]$^+$; APCI MS (+), *m/z* 239 [M + H]$^+$.

Table 1. ^1H and ^{13}C NMR Data of Lentisone (1) in CD_3OD[a,b]

position	δC[c]	δH (*J* in Hz)	HMBC
1	67.3 d	4.90 d (3.1)	H-2
2	73.3 d	4.03 dd (3.1, 2.3)	H-1, H_2-4
3	66.0 d	4.15 ddd (9.0, 5.7, 2.3)	H_2-4
4	28.2 t	2.97 dd (19.3, 5.7)	H-2
		2.54 dd (19.3, 9.0)	
4a	146.1 s		H_2-4
5	121.0 d	7.12 br s	H-7, Me-11
6	149.5 s		Me-11
7	124.7 d	7.47 br s	H-5, Me-11
8	162.7 s		H-7
8a	114.2 s		H-5, H-7
9a	142.0 s		H-1, H-2
9	190.0 s		
10	185.5 s		H-4A, H-5
10a	133.2 s		
Me	22.1 (q)	2.45 s	H-5, H-7

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

Tyrosol (3): ^1H NMR was similar to data reported;[20] ESIMS (+), *m/z* 161 [M + Na]$^+$; ESIMS (−), *m/z* 137 [M − H]$^-$.

Pseurotin A (5): $[\alpha]_D^{25}$ −2.5 (*c* 0.4); CD (*c* 1.04 × 10^{-3} M) λ_{max} nm ($\Delta\varepsilon$), 233 (−3.63), 250.8 (+5.03), 278.2 (−13.3) 314.2 (+3.83), 350 (−1.42); UV λ_{max} nm (log ε), 278 (4.40), 254 (4.57); IR ν_{max}, 3390, 1731, 1700, 1680, 1625, 1447, 1101 1712 cm^{-1} (lit.[21] $[\alpha]_D^{25}$ −2.5 (*c* 0.1 MeOH); UV (MeOH) λ_{max} (nm; log ε), 206 (3.98), 252 (3.93) 280 (2.77)); CD (MeOH) λ_{max} (nm; $\Delta\varepsilon$), 206.8 (+30.5), 228 (−8.1), 250.4 (+10.0), 277.7 (−25.2), 308.1 (+3.8), 312.5 (+7.1), 350.4 (−3.0); IR (KBr cm^{-1}) ν_{max}, 3211, 1725, 1705, 1632, 1440, 1079, 1041, 1017, 807, 666; ^1H and ^{13}C NMR were very similar to those previously reported;[21] HRESIMS (+) spectrum, *m/z* 885 [2M + Na]$^+$, 470 [M + K]$^+$, 454.1469 [$C_{22}H_{25}NO_8Na$, calcd 454.1478, M + Na]$^+$, 432.1652 [$C_{22}H_{26}NO_8$, calcd 432.1658, M + H]$^+$.

1-*O*-Acetylpachybasin (4). Pachybasin **2** (1.4 mg) was acetylated with pyridine (20 μL) and Ac_2O (20 μL) at room temperature for 12 h. The reaction was stopped by the addition of MeOH, and an azeotrope formed by the addition of benzene was evaporated in an N_2 stream. The solid residue (1.6 mg) was purified by TLC on silica gel, eluent $CHCl_3$, yielding derivative **4** as a yellow homogeneous solid (1.2 mg, R_f 0.61). **4**: IR ν_{max}, 1772, 1673, 1608, 1594, 1447, 1367, 1197 cm^{-1}; UV λ_{max} nm (log ε), 334 (3.68), 273 (sh), 256 (4.56), 206 (4.47); ^1H NMR δ 8.5 (1H, m, H-8), 8.25 (1H, m, H-5), 7.78 (2H, m, H-6 and H-7), 8.09 (1H, br s, H-4), 7.24 (1H, br s, H-2), 2.52 (3H, s, MeCO), 2.50 (3H, s, CH_3); ESIMS (+), *m/z* 583 [2M + Na]$^+$, 303 [M + Na]$^+$, 281 [M + H]$^+$, 239 [M + H − CH_2CO]$^+$; APCI (+), *m/z* 281 [M + H]$^+$.

Crystal Structure Determination of 1-*O*-Acetylpachybasin (4). Yellow, block-shaped single crystals of **4** were obtained by slow evaporation of a EtOAc/*n*-hexane (1:3) solution at ambient temperature. X-ray data collection was performed at 298 K on a Bruker-Nonius Kappa CCD diffractometer equipped with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å, CCD rotation images, thick slices, φ and ω scans to fill asymmetric unit). Unit cell parameters were obtained from a least-squares fit of the θ angles of 51 reflections in the range 4.174° ≤ θ ≤ 17.941°. A semiempirical absorption correction (multiscan, SADABS) was applied. The structure was solved by direct methods using the SIR97 package[22] and refined by the full matrix least-squares method on F^2 against all independent measured reflections using the SHELX97 software package.[23] All non-hydrogen atoms were refined anisotropically. H

atoms were placed in calculated positions and refined according to a riding model (C−H in the 0.93−0.96 Å range; Uiso(H) = 1.2·Ueq (C_{Ar}), Uiso(H) = 1.5Ueq (C_{Me}) of the carrier atom). The final refinement converged to R_1 = 0.0642, wR_2 = 0.1400 for 1199 observed reflections having $I > 2\sigma(I)$. Minimum and maximum residual electronic densities were −0.183 and 0.207 eÅ^{-3}. Crystal data for **4**: empirical formula, $C_{17}H_{12}O_4$; formula weight, 280.28 g mol^{-1}; crystal system, monoclinic; space group, $C2/c$; unit cell dimensions, a = 32.8420(11), b = 4.989(2), c = 16.679(9) Å, β = 100.12(3)°; reflections collected/unique, 8315/2795 [R(int) = 0.0757]; data/restraints/parameters, 2795/0/192; goodness of fit on F^2, 1.048; final R indices [$I > 2\sigma(I)$], R_1 = 0.0642, wR_2 = 0.1400; R indices (all data), R_1 = 0.1805, wR_2 = 0.1996; largest difference peak and hole (e/Å^3), 0.207 and −0.183. Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 928742. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (international) +44-1223/336-033.

Bioassays. Three different experiments were carried out. In the first, the effect of the isolated metabolites on *L. culinaris* was assessed. In the second, the influence of light on phytotoxicity was investigated. Finally, the phytotoxicity of lentisone on different cultivated legumes was compared.

Phytotoxic Activity. Five plants of *L. culinaris* (genotype S8) were grown in 1 L plastic pots filled with a mixture of sand and peat and grown for 5 weeks (until they had 15 expanded leaves) in a growth chamber at 20 °C with a photoperiod of 14 h of visible light (150 μmol/m^2/s photon flux density) and 10 h of darkness. The leaflets of the 10th leaf of each plant were then detached, and three small cuts (2 mm) were performed on the epidermis of their adaxial surface. The leaflets were then placed onto a square Petri dish (15 × 15 cm) containing water/agar (0.4% w/v) medium, their adaxial surface facing up. Samples were dissolved in MeOH and brought up to a final concentration of 1 mg/mL; the concentration of MeOH was 5% v/v, which proved to be nonphytotoxic to *L. culinaris*. A drop of each sample (10 μL) was placed onto one leaflet (one sample per leaflet of each plant). Because each leaflet from a single plant was considered a replication, there were five replications for each sample. The dish was then placed in a laminar flow chamber to facilitate drying of the droplets; once they were completely dried, the dish was covered with its lid and kept in darkness at 20 °C for 48 h, after which it was exposed to a photoperiod of 14 h of visible light (150 μmol/m^2/s photon flux density) and 10 h of darkness. Evaluation was made as a measure of the area of lesions surrounding the site of application of the drops; this area was corrected with a visual estimation of tissue damage expressed as a percentage (0% being green tissue with no damage at all and 100% complete necrotic tissue). It was recorded every 24 h after sample application until a total of four measurements had been made. Analyses of variance for studied factors were carried out; means were compared by Tukey tests. All statistical analyses were performed using Statistix 8 (Analytical Software, Tallahassee, FL, USA).

Effect of Light. Plant material and application of metabolites were managed in a similar way as in the previous experiment; only lentisone, pseurotin A, and tyrosol were tested. In this case, two leaves were detached from each plant, and each was placed in a different Petri dish with water/agar (four replications). After the metabolites were applied and the droplets dried, both dishes were kept at 20 °C, one of them in darkness and the other exposed to a photoperiod of 14 h of visible light (150 μmol/m^2/s photon flux density) and 10 h of darkness. Evaluation and statistical analyses were carried out as previously described.

Phytotoxicity of Lentisone on Different Legumes. Lentisone was tested simultaneously on chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.), and lentil *L. culinaris*. Plant material and application of metabolites experiments were carried out as previously described. Four replications per species were taken. After the metabolites were applied and the droplets dried, dishes were kept at 20 °C, exposed to a photoperiod of 14 h of visible light (150 μmol/m^2/s photon flux density) and 10 h of darkness. Evaluation and statistical analyses were performed as in previous experiments.

RESULTS AND DISCUSSION

The liquid culture filtrates of *A. lentis* were exhaustively extracted. The organic extract, showing high phytotoxic activity on the host plant, was purified by combined column and TLC chromatography, guided by bioassay, affording four different metabolites **1**−**3** and **5** (Figure 1).

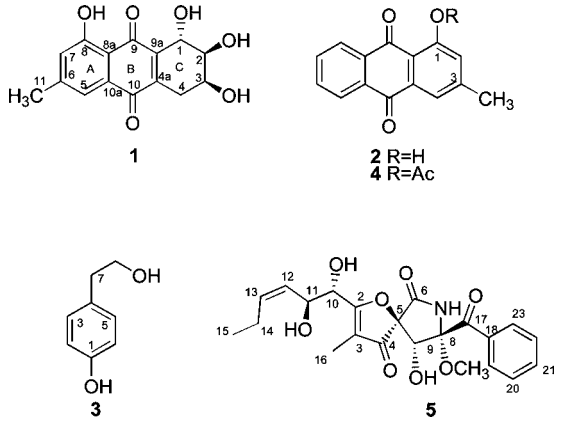

Figure 1. Structures of lentisone **1**, pachybasin **2**, its 1-*O*-acetyl derivative **4**, tyrosol **3**, and pseurotin A **5** isolated from *A. lentis*.

Preliminary NMR investigation, both ^{1}H and ^{13}C NMR spectra, showed that at least two metabolites (**1** and **2**) are closely related anthraquinones. These were consistent with the typical bands for hydroxy and carbonyl groups and absorption maxima observed in their IR and UV spectra.[24,25]

Investigation of its ^{1}H and ^{13}C NMR spectra (Table 1) proved that **1** contains a hydroxylated and methylated tetrahydroanthracenone skeleton and that it is a new metabolite, to which the name of lentisone **1** was assigned (Figure 1). Lentisone has a molecular weight of 290 due to its molecular formula of $C_{15}H_{14}O_6$ deduced from its HRESI MS spectrum and consistent with nine hydrogen deficiencies. First inspection of the ^{1}H NMR spectrum showed the presence of two broad singlets at δ 7.47 (H-7) and 7.12 (H-5), which is typical of two meta-coupled aromatic protons, as also confirmed by the COSY spectrum,[26] the singlet of a methyl group at δ 2.45[27] and protons for three secondary hydroxylated and one methylene carbon. Inspection of its ^{13}C NMR spectrum showed the presence of 15 carbons, which, considering the methyl carbon, are typical of an anthraquinone ring system with the two carbonyl groups present at δ 190.0 and 185.5 (C-9 and C-10). These results suggest that **1** is a tetrahydroxylated and methylated tetrahydroanthraquinone. Indeed, the couplings observed in the HMBC spectrum[26] between O═C-10 and both H-5 and H-4A permits the quinone B ring to be joined to the 1,2,3,5-tetrasubstituted aromatic A ring bearing the two coupled H-5 and H-7 aromatic protons and the methyl group (Me-11) at C-6. The A ring also showed a hydroxy group at C-8 as proved by the typical shift value of δ 114.2 observed in the ^{13}C NMR spectrum for the adjacent quaternary carbon C-8a.[28] As expected, C-8a, which is one of the bridgehead carbons of the A/B ring junction, coupled in the same HMBC spectrum with both H-5 and H-7, as well as the hydroxylated C-8, observed at δ 162.7 coupled with H-7. Similarly, C-5 and C-7, at δ 121.0 and 124.7, coupled

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with both H-7 and Me-11 and H-5 and Me-11, respectively. As expected, Me-11 resonated at δ 22.1 and coupled with both H-5 and H-7. The other bridgehead carbon (C-10a) of the A/B ring junction resonated at a typical δ value of 133.2.[28] On the basis of the couplings observed in the HMBC spectrum between C-4a with H_2-4 and C-9a with both H-1 and H-2, the residual C ring was joined to the quinone B ring through these two bridgehead carbons and resonated at δ 142.0 (C-9a) and 146.1 (C-4a). H-1 resonated in the ^{1}H NMR spectrum as a doublet (J = 3.1 Hz) at δ 4.90, a typical shift value of a proton of secondary hydroxylated carbon (C-1), and coupled in the COSY spectrum with the proton (H-2) of another adjacent secondary hydroxylated carbon (C-2), observed as a double doublet (J = 3.1 and 2.3 Hz) at δ 4.03. The latter (H-2), in turn, coupled with the proton (H-3) of the third secondary hydroxylated carbon observed at δ 4.15 as a doublet of double doublets (J = 9.0, 5.7, and 2.3 Hz), being also coupled with the protons of the adjacent methylene group (H_2C-4). Finally, these two latter protons (H_2C-4) appeared as two double doublets of an AB part of an ABX system (J = 19.3 and 5.7 Hz and J = 19.3 and 9.0 Hz) at δ 2.97 and 2.54, respectively.[27] Therefore, the C ring system appeared to be a 3,4,5-trihydroxycyclohexene. The coupling observed in the HSQC spectrum allowed us to assign the chemical shift values of δ 67.3, 73.3, 66.0, and 28.2, respectively, to C-1, C-2, C-3, and C-4 and to assign to lentisone the structure of a 1,2,3,8-tetrahydroxy-6-methyl-1,2,3,4-tetrahydroanthraquinone, **1**.

The structure **1** assigned to lentisone was confirmed by all other couplings observed in the HMBC spectrum (Table 1) and by the data of its HRESI MS spectrum. Indeed, the latter, besides the sodium cluster $[M + Na]^+$ at m/z 313.0697, showed the ion at m/z 285 generated by loss of CO. Finally, when the ESI MS spectrum was recorded in negative ion mode, the pseudomolecular ion $[M - H]^-$ was observed at m/z 289.

The relative stereochemistry of lentisone was deduced from the vicinal couplings measured for the proton of the C ring in the ^{1}H NMR spectrum (Table 1). Given that this ring assumes a pseudochair conformation, H-4B at δ 2.54 and H-3 ($J_{3,4B}$ = 9.0 Hz) should be *anti*-pseudoaxially positioned, whereas H-4A and H-2 ($J_{3,4A}$ = 5.7 Hz and $J_{3,2}$ = 2.3 Hz) should be *cis*-pseudoequatorially positioned. Finally, H-1 ($J_{1,2}$ = 3.1 Hz) could be pseudoaxially or pseudoequatorially positioned.[27,29] H-1 was equatorially positioned on the basis of the lack of correlation with H-2 in the NOESY spectrum,[26] whereas, as expected, the correlations between H-3 with both H-2 and H-4A were observed. Lentisone can be formulated as (1*S**,2*S**,3*S**)-1,2,3,8-tetrahydroxy-1,2,3,4-tetrahydro-6-methylanthraquinone. It appears to be the 1-epimer of pleosporone, which is a metabolite with modest antibacterial activities and is isolated from a pleosporalean ascomycete, an endophytic fungus isolated from *Anthyllis vulneraria* (Fabaceae) to discover antibiotics by using antisense technology.[30]

Metabolite **2** was identified as the known 1-hydroxy-3-methylanthraquinone by comparing its spectroscopic properties (IR, UV, ^{1}H and ^{13}C NMR) with those already reported in the literature.[18] It was first isolated together with the other related anthraquinone, chrysophanol, and called pachybasin from *Trichoderma aureoviride*.[18] Assignment of all the protons and corresponding carbons was based on the couplings observed in the COSY, HSQC, and HMBC spectra. The same data agree with those subsequently reported when pachybasin was isolated together with two already known 1,6- and 1,7-dihydroxy-3-methyl analogues, the new one, 1,7-dihydroxy-3-hydroxymethyl, and the two hexahydroanthraquinone derivatives, dendryols E and F, from *Phoma sorghina*, found in association with *Tithonia diversifolia* (Asteraceae).[19] Pachybasin was also isolated together with three new and two already known anthraquinone derivatives from the marine fungus *Halorosellinia* sp., an ascomycete isolated from decaying wood in Mai Po, Hong Kong, and a salt lake in the Bahamas.[31] Identification of pachybasin was also confirmed by the data of its ESI and APCI MS spectra, which showed the sodium cluster $[M + Na]^+$ and the pseudomolecular ion $[M + H]^+$ at m/z 261 and 239, respectively.

By the usual acetylation procedure carried out with pyridine and acetic anhydride, pachybasin was converted into the corresponding 1-*O*-acetyl derivative **4** (Figure 1). As expected, the ^{1}H NMR of **4** differed from that of **2** only in the absence of the singlet of the hydrogen-bonded phenolic proton at δ 12.58 and in the presence of the singlet of the acetyl group at δ 2.52. Its ESI MS spectrum showed the sodium dimer $[2M + Na]^+$, the sodium cluster $[M + Na]^+$, and the pseudomolecular ion $[M + H]^+$ at m/z 583, 303, and 281, respectively. The pseudomolecular ion from the significant loss of the ketene residue CH_2CO generated the ion at m/z 239.

The monoacetyl derivative **4** gave yellow block single crystals by slow evaporation of a EtOAc/*n*-hexane (1:3) solution. The crystals were suitable for X-ray analysis. An ORTEP overview of the molecule is shown in Figure SI1 in the Supporting Information.

Compound **4** crystallizes in the monoclinic C 2/c space group with one molecule in the asymmetric unit. All bond lengths and angles are in the normal range and in agreement with data reported for similar compounds.[32,33] The molecule consists of an anthraquinone skeleton substituted at positions C1 and C3 by an acetyl and a methyl group, respectively. The anthraquinone ring system is substantially planar, with the angle between the two benzene mean planes being 3.3(3)°. The acetyl moiety at C1 is almost perpendicular to the anthraquinone ring system as confirmed by the torsion angle around C1−O3 bond (C9a−C1−O3−C11 = 83.2(4)°). The crystal packing is dominated by regular van der Waals interactions and weak C_{Ar}−H···O=$COCH_3$ interactions that generate a herringbone packing of molecules (C7−H···O4^i: 0.93, 2.661 Å, ∠ 132.6(3)°, i = −x, −y + 1, z + 1/2). Molecules are stacked at about 3.3 Å, forming layers along the **b** axis (Supporting Information, Figure SI1bis). A substantial orthogonality can be observed between molecules of adjacent layers (the angle between mean planes of adjacent anthraquinone groups is 85.39°).

Metabolite **3** was identified as tyrosol on the basis of its ^{1}H NMR spectrum, which was very similar to that previously reported.[20] It also showed the same ESI MS data recorded in both positive and negative ion mode that was recently reported when it was isolated from *Neofusicoccum australe*, a pluvirous pathogen associated with grapevine cordon dieback and branch dieback of Phoenician juniper.[34] From this last fungus, **3** was isolated together with cyclobotryoxide, a new cyclohexenonepoxide, 3-methylcatechol, botryosphaerone D, and (3*S*,4*S*)-3,4,8-trihydroxy-6-methoxy-3,4-dihydro-1-(2*H*)-naphthalenone.[34] Tyrosol is a phytotoxic metabolite produced by plants and fungi.[34]

Metabolite **5** was identified as the already known pseurotin A by comparison of its optical and spectroscopic properties (OR, CD, and IR, UV, ^{1}H and ^{13}C NMR) with those already reported in the literature: it was recently isolated together with

the two diastereomers pseurotin A_1 and A_2 from the marine-derived fungus *Aspergillus fumigatus* in a study to discover antitumor metabolites.[21] The ^1H and ^{13}C NMR data of **5** were completely assigned on the basis of the couplings observed in its COSY, HSQC, and HMBC spectra. Identification of pseurotin A was also supported by the HRESI MS data, which showed the sodium dimer $[2M + Na]^+$, the potassium $[M + K]^+$ and sodium $[M + Na]^+$ clusters, and the pseudomolecular ion $[M + H]^+$ at m/z 885, 470 and 454.1469, and 432.1652, respectively. To unambiguously assign the absolute stereochemistry to **5**, its CD spectrum was recorded and compared with those reported in the literature for pseurotin A and its two diastereomers pseurotins A_1 and A_2.[21] This comparison allowed us to rule out pseurotin A_2. Furthermore, the ^1H NMR and NOESY spectra of **5** were also recorded in DMSO-d_6 and compared to those reported for pseurotin A and its diastereomer pseurotin A_1. In particular, the NOESY spectrum of **5** showed a significant correlation between HO-9 and 8-OMe, whereas in that of pseurotin A_1, in which both groups have a *trans*-stereochemistry, a correlation was instead observed between H-9 and 8-OMe. These results allowed us to rule out pseurotin A_1. The stereochemistry at C-9 was further confirmed by the chemical shift and the coupling constant values measured in the ^1H NMR spectrum of **5** for OH-9 (δ 6.22, d, J = 9.3 Hz), and H-9 (δ 4.41, d, J = 9.3 Hz), which were very similar to those recorded for pseurotin A (OH-9, δ 6.26, d, J = 9.2 Hz and H-9 δ 4.40, d, J = 9.2 Hz) and significantly different from those of pseurotin A_1 (OH-9, δ 6.07, d J = 6.2 Hz and H-9, δ 4.62, d, J = 6.2 Hz).[21]

When assayed against P388, HL-60, A549, and BEL-7402 cell lines, pseurotin A showed a slight cytotoxicity on the HL-60 cell line.[21] First, pseurotin A was previously isolated from *Pseudeurotium ovali*, and its absolute stereochemistry was assigned by X-ray analysis of its 12,13-dibromo derivative.[35] Subsequently, from a soil strain of *A. fumigatus*, pseurotin A was isolated together with fumoquinones A and B and spinulosin, and it showed an effective nematicidal activity against *Bursaphelenchus xylophilus*.[36] However, no antifungal activity was found when it was isolated from *Chaetomium globosum*, an endophytic fungus in *Ginkgo biloba*, and assayed against some phytopathogenic fungi.[37] No antifungal or zootoxic activity was found when **5** was isolated from another strain of *A. fumigatus*, isolated from healthy stem bark of *Melia azedarach*, and was tested against several phytopathogenic fungi and brine shrimps (*Artemia salina*).[38]

Among the four tested samples (lentisone, pachybasin, pseurotin A, and tyrosol), only lentisone, pseurotin A, and tyrosol showed phytotoxicity. Analyses of variance for lesion size at each time showed significant differences by metabolite from 48 h after application (data not shown). Lentisone proved to be the most phytotoxic, being significantly different from pseurotin A and tyrosol in the size of necrotic area until the end of the experiment. The results are presented in Figure 2.

Lentisone is key for killing host cells, which is essential in the process of infection by a necrotrophic plant pathogen such as *A. lentis*. The fungus developed a toxin that is highly toxic for its host. The other two metabolites may add to infection and phytotoxicity, although it cannot be disregarded that they might play other roles in the development of the disease or in fungus development; tyrosol has been related to "quorum sensing" in a human pathogenic fungus *Candida albicans*.[39]

The dramatic increase in phytotoxicity after exposure of the treated leaflets to light led to the suspicion that light might play

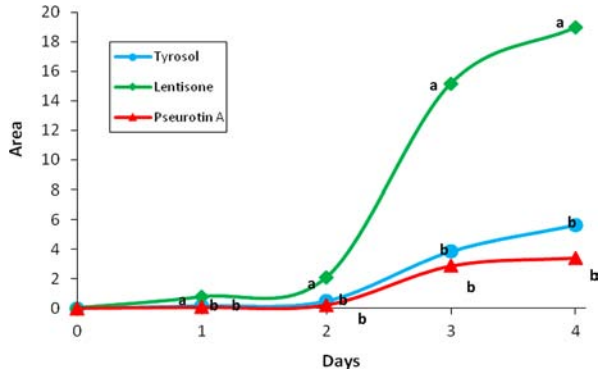

Figure 2. Necrotic areas (mm^2) induced by lentisone, pseurotin A, and tyrosol after treatment of *Lens culinaris* leaves. Values followed by the same letter are not significantly different (Tukey test, $p < 0.05$).

a role in the activity of these toxins. Light is known to influence plant–pathogen interactions, albeit with some exceptions; it is generally acknowledged that lack of light increases infection by pathogens.[40] This may be related in part to other environmental factors such as humidity or temperature, which might be more propitious for disease at night. In contrast, there are examples of phytotoxins that require light to fully produce their effect.[41−43] This influence of light is usually explained by two hypotheses that are not incompatible with one another; either they interfere with the photosynthetic apparatus[41] or they contribute to the generation of reactive oxygen species such as singlet oxygen.[42−44] In the case of *A. lentis*, a significant effect of light on the phytotoxicity for each of the three test metabolites was detected 48 h after their application, and all of them proved to be more phytotoxic when exposed to light (ANOVA results not shown). An example of this is given in Figure 3, and the results are shown in Figure 4. The three compounds, lentisone,

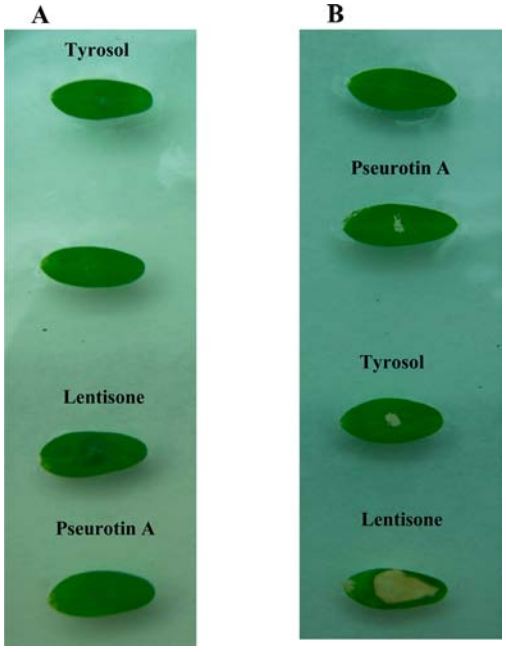

Figure 3. Comparison of leaves kept in darkness (A) and those placed under light (B) at 72 h after application of phytotoxic metabolites. Those exposed to light show clear necrotic lesions, in contrast with those deprived of light. Unlabeled leaflets are the controls (treated with 5% MeOH).

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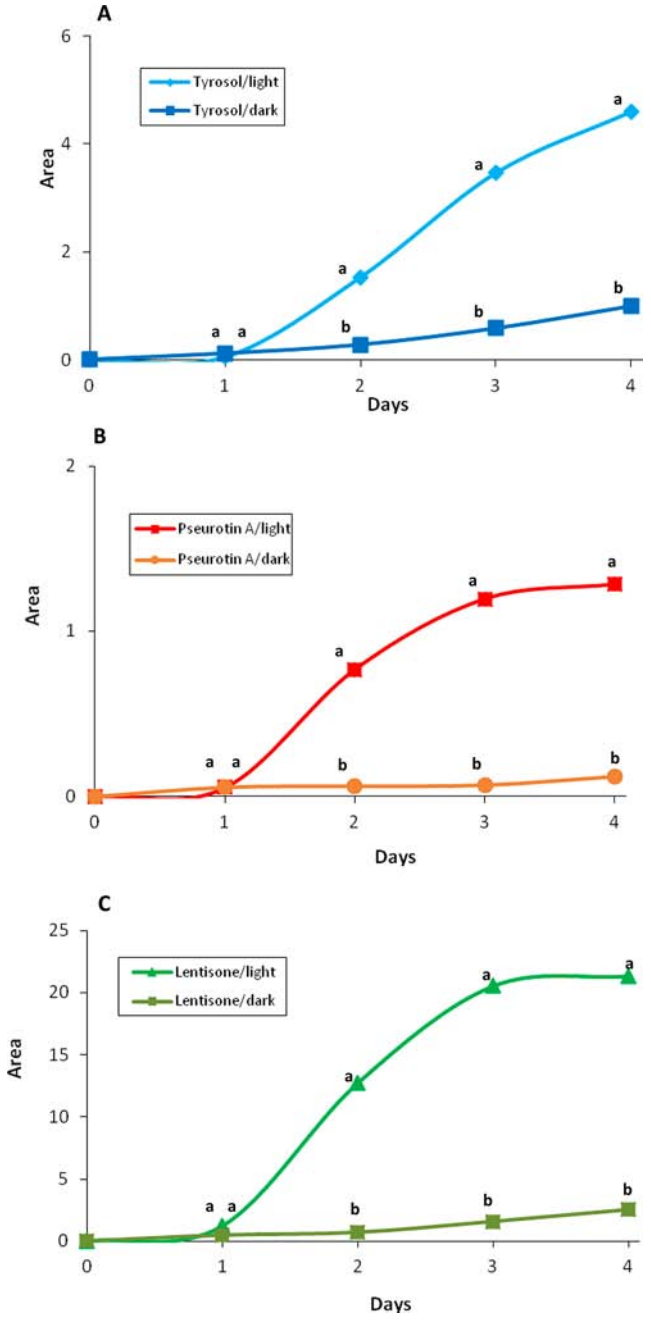

Figure 4. Effect of light on the necrotic area (mm^2) incited by tyrosol (A), pseurotin A (B), and lentisone (C) after treatment of leaves of *Lens culinaris*. Toxicity is significantly higher when plant tissues have been exposed to light than when they have been kept in darkness. Values followed by the same letter are not significantly different (Tukey test, $p < 0.05$).

pseurotin A, and tyrosol, therefore require light to produce their various toxicity effects. This may provide us with a clue toward understanding their mode of action and should be the subject of future studies.

Lentisone is toxic not only to lentil but also to chickpea, pea, and faba bean (Figure 5). As far as we know, no other *Ascochyta* pathogen has developed related compounds. Surprisingly, this toxin specific to *A. lentis* proves more phytotoxic on faba bean than on lentil. Because legume-associated *Ascochyta* fungi are host-specific, each species is only pathogenic to its host and even a cospeciation of *Ascochyta* spp. and their legume hosts

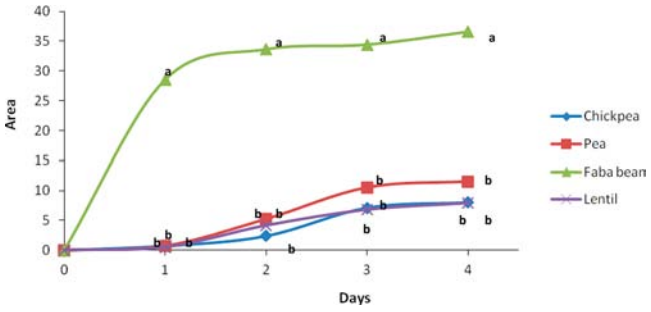

Figure 5. Necrotic area (mm^2) incited by lentisone after treatment of leaves of chickpea, pea, faba bean, and lentil. Values followed by the same letter are not significantly different (Tukey test, $p < 0.05$).

has been suggested.[45] However, *A. lentis* has evolved to produce a toxin that is more harmful to a plant that is not its host, whereas *Ascochyta fabae* has not been able to develop a similar compound.

In conclusion, a new tetrahydroxytetrahydroanthraquinone, called lentisone, and the related known pachybasin, pseurotin A, and tyrosol were isolated from the crude organic extract of the phytotoxic culture filtrates of *A. lentis*. Tyrosol is known to be a plant and fungi phytotoxic metabolite, and pseurotin A is known as a fungal bioactive metabolite, but this is the first report on its phytotoxic activity. Lentisone is the metabolite key for phytotoxicity, and its light dependence should help elucidate its mode of action in future studies. The fact that it is much more phytotoxic to faba bean than to any other tested legume seems to be rather coincidental. Anthraquinones and their derivatives such as **1** and **2** belong to a large group of compounds with different biological activities[46−48] occurring in both plants and fungi. Anthraquinone derivatives were also already reported as fungal phytotoxins[46] such as macrosporin and altersolanols A and C, which we recently isolated together with the pentaketide monoterpenoid nectryapyrone from the culture filtrates of *Phomopsis foeniculi*, the causal agent of umbel browning and stem necrosis of fennel in Bulgaria.[49] Anticancer activity has also been reported for 14 anthraquinone derivatives in part isolated from the above-cited *Halorosellinia* sp. (no. 1403) fungus[31] and from another mangrove endophytic fungus *Guignardia* sp. (no. 4382).[50]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

IR, UV, 1D and 2D ^1H and ^{13}C NMR, and HRESI MS spectra of lentisone (**1**) and Figures SI1 and SI1bis for X-ray of 1-*O*-acetylpachybasin (**4**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ REFERENCES

(1) Graham, P. H.; Vance, C. P. Legumes: importance and constraints to greater use. *Plant Physiol.* **2003**, *131*, 872−877.

(2) Ye, G.; McNeil, D. L.; Hill, G. D. Breeding for resistance to lentil Ascochyta blight. *Plant Breed.* **2002**, *121*, 185−191.

(3) Muehlbauer, F. J.; Chen, W. Resistance to ascochyta blights of cool season food legumes. *Eur. J. Plant Pathol.* **2007**, *119*, 135−141.

(4) Mel'nik, V. A. Taxonomy of the genus *Ascochyta* Lib. *Mikol. Fitopatol.* **1971**, *5*, 15−22.

(5) Venkatasubbaiah, P.; Chilton, W. S. Phytotoxins of *Ascochyta hyalospora*, causal agent of lambsquarters leaf spot. *J. Nat. Prod.* **1992**, *55*, 461−467.

(6) Evidente, A.; Capasso, R.; Abouzeid, A. M. A.; Lanzetta, R.; Vurro, M.; Bottalico, A. Three new toxic pinolidoxins from *Ascochyta pinodes*. *J. Nat. Prod.* **1993**, *56*, 1937−1943.

(7) Cimmino, A.; Andolfi, A.; Fondevilla, S.; Abouzeid, M. A.; Rubiales, D.; Evidente, A. Pinolide, a new nonenolide produced by *Didymella pinodes*, the causal agent of *Ascochyta* blight on *Pisum sativum*. *J. Agric. Food Chem.* **2012**, *60*, 5273−5278.

(8) Evidente, A.; Capasso, R.; Cutignano, A.; Taglialatela-Scafati, O.; Vurro, M.; Zonno, M. C.; Motta, A. Ascaulitoxin, a phytotoxic *bis*-amino acid *N*-glucoside from *Ascochyta caulina*. *Phytochemistry* **1998**, *48*, 1131−1137.

(9) Evidente, A.; Andolfi, A.; Vurro, M.; Zonno, M. C.; Motta, A. *Trans*-4-aminoproline, a phytotoxic metabolite with herbicidal activity produced by *Ascochyta caulina*. *Phytochemistry* **2000**, *53*, 231−237.

(10) Evidente, A.; Andolfi, A.; Vurro, M.; Zonno, M. C. Determination of *Ascochyta caulina* phytotoxins by high-performance anion exchange chromatography and pulsed amperometric detection. *Phytochem. Anal.* **2001**, *12*, 383−387.

(11) Evidente, A.; Andolfi, A.; Abouzeid, M. A.; Vurro, M.; Zonno, M. C.; Motta, A. Ascosonchine, the enol tautomer of 4-pyridylpyruvic acid with herbicidal activity produced by *Ascochyta sonchi*. *Phytochemistry* **2004**, *65*, 475−480.

(12) Evidente, A.; Berestetskiy, A.; Cimmino, A.; Tuzi, A.; Superchi, S.; Melck, D. Papyracillic acid, a phytotoxic 1,6-dioxaspiro[4,4]nonene produced by *Ascochyta agropyrina* var. *nana*, a potential mycoherbicide for *Elytrigia repens* bioncotrol. *J. Agric. Food Chem.* **2009**, *57*, 11168−11173.

(13) Andolfi, A.; Cimmino, A.; Vurro, M.; Berestetskiy, A.; Troise, C.; Zonno, M. C.; Motta, A.; Evidente, A. Agropyrenol and agropyrenal phytotoxins from *Ascochyta agropyrina* var. *nana*, a fungal pathogen of *Eltrigia repens*. *Phytochemistry* **2012**, *79*, 102−108.

(14) Evidente, A.; Capasso, R.; Vurro, M.; Bottalico, A. Ascosalitoxin, a phytotoxic trisubstituted salicylic aldehyde from *Ascochyta pisi*. *Phytochemistry* **1993**, *34*, 995−998.

(15) Strange, R. N. Phytotoxins associated with *Ascochyta* specie. In *Toxins in Plant Disease Development and Evolving Biotechnology*; Upadhyay, R. K., Mukerji, K. G., Eds.; Oxford and IBH Publishing: New Delhi, India, 1997; pp 167−182.

(16) Evidente, A.; Lanzetta, R.; Capasso, R.; Vurro, M.; Bottalico, A. Pinolidoxin, a phytotoxic nonenolide from *Ascochyta pinodes*. *Phytochemistry* **1993**, *34*, 999−100.

(17) Rubiales, D.; Fondevilla, S. Future prospects for Ascochyta blight resistance breeding in cool season food legumes. *Front. Plant Sci.* **2012**, *3*, 27 DOI: 10.3389/fpls.2012.00027.

(18) De Stefano, S.; Nicoletti, R.; Beato, O. Pachibasina e crisofanolo, due antrachinoni prodotti da *Trichoderma aureoviride*. *Tabacco* **1999**, *7*, 21−24.

(19) de Souza Borges, W.; Pupo, T.; Novel, M. anthraquinone derivatives produced by *Phoma sorghina*, an endophyte found in association with the medicinal plant *Tithonia diversifolia* (Asteraceae). *J. Braz. Chem. Soc.* **2006**, *17*, 929−934.

(20) Capasso, R.; Cristinzio, G.; Evidente, A.; Scognamiglio, F. Isolation, spectroscopy characterizzation and selective phytotoxic effects of main polyphenols from vegetable waste wate. *Phytochemistry* **1992**, *31*, 4125−4128.

(21) Wang, F.-Z.; Li, D.-H.; Zhu, T.-J.; Zhang, M.; Gu, Q.-Q. Pseurotin A1 and A2, two, new 1-oxa-7-azaspiro[4,4]non-2-ene-4,6-diones from the holothurian-derived fungus *Aspergillus fumigatus* WFZ-25. *Can. J. Chem.* **2011**, *89*, 72−76.

(22) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *Sir97*: A new tool for crystal structure determination and refinement. *J. Appl. Crystallogr.* **1999**, *32*, 115−119.

(23) Sheldrick, G. M. Foundations of crystallography. *Acta Crystallogr.* **2008**, *A64*, 112−122.

(24) Nakanishi, K.; Solomon, P. H. *Infrared Absorption Spectroscopy*, 2nd ed.; Holden Day: Oakland, CA, 1977; pp 17−44.

(25) Scott, A. I. *Intrepretation of the Ultraviolet Spectra of Natural Products*; Pergamon Press: Oxford, UK, 1964; pp 286−297.

(26) Berger, S.; Braun, S. *200 and More Basic NMR Experiments: a Practical Course*, 1st ed.; Wiley-VCH: Weinheim, Germany, 2004.

(27) Pretsch, E.; Bühlmann, P.; Affolter, C. Structure *Determination of Organic Compounds − Tables of Spectral Data*; Springer-Verlag: Berlin, Germany, 2000; pp 161−243.

(28) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH: Weinheim, Germany, 1987; pp 183−280.

(29) Sternhell, S. Correlation of interpretation of spin-spin coupling with structure. *Q. Rev.* **1969**, *23*, 236−269.

(30) Zhang, C.; Ondeyka, J. G.; Zink, D. L.; Basilio, A.; Vincente, F.; Collado, J.; Platas, G.; Huber, J.; Dorso, K.; Motyl, M.; Byrne, K.; Singh, S. B. Isolation, structure and antibacterial activity of pleosporone from a pleosporalean ascomycete discovered by using antisense strategy. *Bioorg. Med. Chem.* **2009**, *17*, 2162−2166.

(31) Xia, X. K.; Huang, H. R.; She, Z. G.; Shao, C. L.; Liu, F.; Cai, X. L.; Vrijmoed, L. L. P.; Lin, Y. C. ^{1}H and ^{13}C NMR assignments for five anthraquinones from the mangrove endophyte fungus *Halorosellinia* sp. (no. 1403). *Magn. Reson. Chem.* **2007**, *45*, 1006−1009.

(32) Prakash, A. Refinement of the crystal structure of anthraquinone. *Acta Crystallogr.* **1967**, *22*, 439−440.

(33) Zhu, L. C.; Zhao, Z. G.; Yu, S. J. 2-Hydroxy-1,6,7,8-tetramethoxy-3-methylanthraquinone. *Acta Crystallogr.* **2008**, *E64*, o371.

(34) Andolfi, A.; Maddau, L.; Cimmino, A.; Linaldeddu, B. T.; Franceschini, A.; Serra, S.; Basso, S.; Melck, D.; Evidente, A. Cyclobotryoxide, a phytotoxic metabolite produced by the plurivorous pathogen *Neofusicoccum australe*. *J. Nat. Prod.* **2012**, *75*, 1785−1791.

(35) Blotch, P.; Tamm, C. Pseurotin, a new metabolite of *Pseudeurotium ovalis* Stolk having an unusual hetero-spirocyclic system. *Helv. Chim. Acta* **1976**, *59*, 133−137.

(36) Hayashi, A.; Fujioka, S.; Nukina, M.; Kawano, T.; Shimada, A.; Kimura, Y. Fumoquinones A and B, nematocidal quinones produced by *Aspergillus fumigatus*. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 1607−1702.

(37) Li, H.-Q.; Li, X.-J.; Wang, Y.-L.; Zhang, Q.; Zhang, A.-L.; Gao, J.-M.; Zhang, X.-C. Antifungal metabolites from *Chaetomium globosum*, an endophytic fungus in *Ginkgo biloba*. *Biochem. Syst. Ecol.* **2011**, *39*, 876−879.

(38) Li, X.-J.; Zhang, Q.; Zhang, A.-L.; Gao, J.-M. Metabolites from *Aspergillus fumigatus*, an endophytic fungus associated with *Melia azedarach*, and their antifungal, antifeedant, and toxic acrivities. *J. Agric. Food Chem.* **2012**, *60*, 3424−3431.

(39) Chen, H.; Fujita, M.; Feng, Q. H.; Clardy, J.; Fink, G. R. Tyrosol is a quorum-sensing molecule in *Candida albicans*. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5048−5052.

(40) Roberts, M. R.; Paul, N. D. Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defence against pests and pathogens. *New Phytol.* **2006**, *170*, 677−699.

(41) Sarpeleh, A.; Tate, M. E.; Wallwork, H.; Catcheside, D.; Able, A. J. Characterisation of low molecular weight phytotoxins isolated from *Pyrenophora teres*. *Physiol. Mol. Plant Pathol.* **2008**, *73*, 154−162.
(42) Liao, H. L.; Chung, K. R. Cellular toxicity of elsinochrome phytotoxins produced by the pathogenic fungus, *Elsinoe fawcettii* causing citrus scab. *New Phytol.* **2008**, *177*, 239−250.
(43) Heiser, I.; Hess, M.; Schmidtke, K. U.; Vogler, U.; Miethbauer, S.; Liebermann, B. Fatty acid peroxidation by rubellin B, C and D, phytotoxins produced by *Ramularia collo-cygni* (Sutton et Waller). *Physiol. Mol. Plant Pathol.* **2004**, *64*, 135−143.
(44) Triantaphylides, C.; Havaux, M. Singlet oxygen in plants: production, detoxification and signaling. *Trends Plant Sci.* **2009**, *14*, 219−228.
(45) Peever, T. L. Role of host specificity in the speciation of *Ascochyta* pathogens of cool season food legumes. *Eur. J. Plant Pathol.* **2007**, *119*, 119−126.
(46) Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Academic Press: London, UK, 1983; pp 140−147.
(47) Dewick, P. M. *Medicinal Natural Products − A Biosyntheic Approach*; Wiley: Chichester, UK, 2009; pp 103−109.
(48) Osbourn, A. E.; Lanzotti, V. *Plant-Derived Natural Products − Synthesis, Function, and Application*; Springer: Dordrecht, The Netherlands, 2009; pp 27−28.
(49) Evidente, A.; Rodeva, R.; Andolfi, A.; Stoyanova, Z.; Perrone, C.; Motta, A. Phytotoxic polyketides produced by *Phomopsis foeniculi*, a strain isolated from diseased Bulgaria fennel. *Eur. J. Plant Pathol.* **2011**, *130*, 173−182.
(50) Zhang, J. Y.; Tao, L. Y.; Liang, Y. J.; Chen, L. M.; Mi, Y. J.; Zheng, L. S.; Wang, F.; She, Z. G.; Lin, Y. C.; Wah To, K. K.; Fu, L. W. Anthracenedione derivatives as anticancer agents isolated from secondary metabolites of mangrove endophytic fungi. *Mar. Drugs* **2010**, *8*, 1469−1481.