

# Phenalenone-Type Compounds from *Musa acuminata* var. “Yangambi km 5” (AAA) and Their Activity against *Mycosphaerella fijiensis*

Felipe Otálvaro,<sup>\*,†</sup> Juliana Nanclares,<sup>‡</sup> Luz Estella Vásquez,<sup>§</sup> Winston Quiñones,<sup>⊥</sup> Fernando Echeverri,<sup>⊥</sup> Rafael Arango,<sup>||</sup> and Bernd Schneider<sup>\*,▽</sup>

Departamento de Ingeniería, Universidad Nacional de Colombia sede Palmira, Carrera 32 Chapinero vía Candelaria, A.A 237, Palmira-Valle del Cauca, Colombia, Instituto de Química, Universidad de Antioquia, A.A 1226, Medellín, Colombia, Unidad de Biotecnología Vegetal, Corporación para Investigaciones Biológicas, Carrera 72 A # 78 B-141, Medellín, Colombia, Laboratorio de Química de Productos Naturales, Edificio SIU 234-235, Apartado Aéreo 1226, Medellín-Antioquia, Colombia, Escuela de Biociencias, Facultad de Ciencias, Universidad Nacional de Colombia sede Medellín, Autopista Norte, Carrera 64 Calle 65, A.A 3840, Medellín, Colombia, and Max-Planck Institut für Chemische Ökologie, Beutenberg Campus, Hans-Knöll-Strasse 8, 07745, Jena, Germany

Received March 2, 2007

Two perinaphthenone-type compounds (**1** and **2**) were isolated together with four known phenylphenalenones (**3–6**) from the rhizomes of *Musa acuminata* var. “Yangambi km 5”. The structures of the new phenalenones were assigned as 2-hydroxy-1*H*-phenalen-1-one (**1**) and 2-methoxy-1*H*-phenalen-1-one (**2**) on the basis of their spectroscopic data and were confirmed by synthesis. Compounds **1** and **2** displayed significantly enhanced activity against *Mycosphaerella fijiensis* in comparison with other phenylphenalenones.

Black leaf streak (also known as Black Sigatoka), caused by the ascomycetous fungal pathogen *Mycosphaerella fijiensis*, is currently one of the predominant diseases in *Musa* spp., attacking almost all cultivars of banana and plantains.<sup>1</sup> One of the reasons for the susceptibility of *Musa* species is the almost exclusive cultivation of seedless, sterile, and genetically uniform clones, especially the Cavendish banana. Efforts to breed resistant cultivars that can compete with Cavendish in terms of crop yield and market acceptance have had limited success. Hence, Cavendish continues to be the most consumed banana in industrial countries.<sup>1</sup> The frequent application of fungicides is the main control measure where a high incidence of pathogens exists. Since these cultivars develop resistance and because of environmental issues, new, environmentally friendly chemotherapeutic agents that can successfully protect the plant are being sought. Conventional breeding and genetic engineering may also offer ways to improve the resistance to fungal infection by *Musa* species.<sup>1</sup>

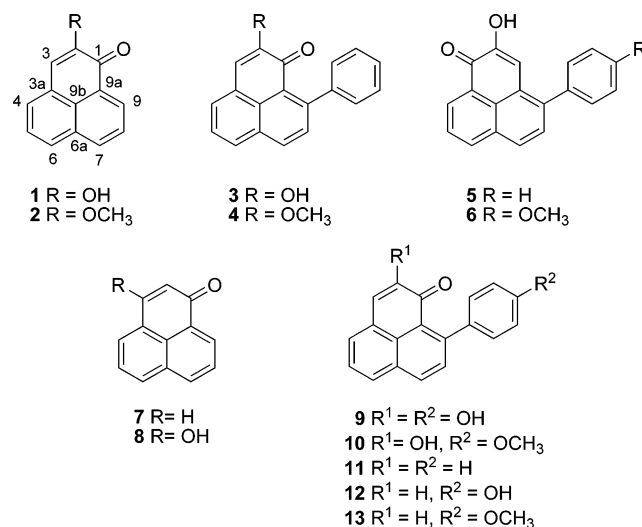
“Yangambi km 5”, a *Musa* dessert-type variety that produces small fruits, has shown resistance to several diseases and plagues including black leaf streak, Yellow Sigatoka disease (caused by *Mycosphaerella musicola*), and the borer nematode (*Radopholus similis*).<sup>2</sup> In the case of black leaf streak pathosystem, the incompatibility of *Musa* “Yangambi” and *Mycosphaerella fijiensis* activates fungal and plant metabolism. Incubating *M. fijiensis* with tricyclazole increased the concentration of 2,4,8-trihydroxytetralone, a fungal pentaketide metabolite. *Musa* plants, upon inoculation with *M. fijiensis*, respond with an increase of phenylalanine ammonia-lyase activity.<sup>3</sup> Activation of the phenylpropanoid pathway is in accordance with the well-documented occurrence of phenylphenalenone-type compounds in other Musaceae.<sup>4–7</sup>

Hence, it was anticipated that the “Yangambi” variety, as a member of the *Musa* genus, would also produce phytoalexins belonging to the phenylphenalenone structural class. Furthermore, the correlation of different accumulation profiles of phenylphe-

nalenes with different resistance traits of *M. fijiensis*<sup>8</sup> suggested that these phytoalexins could also be involved in the resistance of “Yangambi”.

This paper reports the occurrence of two new and four known phenalenone-type compounds in “Yangambi” and their activity in response to in vitro cultures of *M. fijiensis*.

Ethyl acetate extracts from the rhizomes of *Musa acuminata* cv. “Yangambi” (group AAA) were analyzed for the occurrence of phenylphenalenone-type compounds. Apart from compounds **1** and **2**, the known phenylphenalenones 2-hydroxy-9-phenyl-1*H*-phenalen-1-one (**3**, anigorufone),<sup>9</sup> 2-methoxy-9-phenyl-1*H*-phenalen-1-one (**4**, methoxyanigorufone),<sup>10</sup> 2-hydroxy-4-phenyl-1*H*-phenalen-1-one (**5**, 4'-dehydroxyirenone, isoanigorufone),<sup>11</sup> and 2-hydroxy-4-(4-hydroxyphenyl)-1*H*-phenalen-1-one (**6**, 4'-methoxyirenone)<sup>12</sup> were isolated and identified by comparison with authentic standards. Compound **4** was obtained as the major compound (5 mg kg<sup>-1</sup> fresh material) followed by compound **1** (1 mg kg<sup>-1</sup>). The concentration levels of compounds **2**, **3**, **5**, and **6** varied in the range 0.2–0.5 mg kg<sup>-1</sup>.



\* Corresponding authors. (F.O.) Tel: 05722717000, ext 35710. Fax: 05722717008. E-mail: ifotalvarot@palmira.unal.edu.co. (B.S.) Tel: 049 3641 571600. Fax: 049 3641 571600. E-mail: schneider@ice.mpg.de.

<sup>†</sup> Universidad Nacional de Colombia sede Palmira.

<sup>‡</sup> Universidad de Antioquia.

<sup>§</sup> Corporación para Investigaciones Biológicas.

<sup>⊥</sup> Sede de Investigación Universitaria-Universidad de Antioquia.

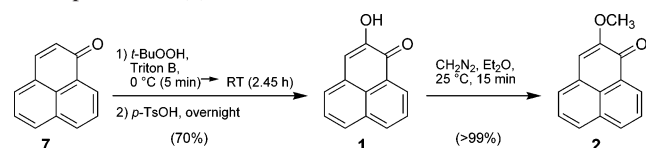
<sup>||</sup> Universidad Nacional de Colombia sede Medellín.

<sup>▽</sup> Max-Planck Institut für Chemische Ökologie.

The <sup>1</sup>H NMR and <sup>1</sup>H–<sup>1</sup>H COSY spectra of compound **1** displayed eight signals corresponding to two ABX spin systems attributable to H-4–H-5–H-6 and H-7–H-8–H-9 and two singlets

**Table 1.**  $^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) Spectroscopic Data of Compounds **1** and **2** Measured in Acetone- $d_6$ 

position	<b>1</b>		<b>2</b>	
	$\delta_{\text{H}}$ (mult.; $J_{\text{HH}}$ in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult.; $J_{\text{HH}}$ in Hz)	$\delta_{\text{C}}$
1		180.9		180.1
2		150.0		154.8
3	7.20 s	114.2	7.20 s	113.8
3a		128.9		129.9
4	7.84 d (7.0)	130.8	7.82 d (7.1)	130.5
5	7.66 dd (8.2, 7.0)	127.6	7.64 dd (8.2, 7.1)	128.5
6	8.06 d (8.2)	130.2	8.03 d (8.2)	130.2
6a		132.4		133.5
7	8.43 d (8.0)	136.9	8.37 d (8.0)	136.5
8	7.89 dd (7.5, 8.0)	127.2	7.85 dd (7.5, 8.0)	128.2
9	8.64 d (7.5)	131.5	8.56 d (7.5)	130.9
9a		127.9		129.9
9b		124.9		125.8
OCH <sub>3</sub>			3.89 s	56.2

**Scheme 1.** Synthesis of Compounds **1** and **2** from Perinaphthenone (**7**)

of H-3 and  $-\text{OH}$ ; the latter disappeared after addition of  $\text{D}_2\text{O}$ . The signal at low field ( $\delta$  8.64; Table 1) suggested a proton in the *peri* position to an electron-withdrawing group. This proton signal was attributed to H-9, and HMBC and HMQC correlations were used to assign the other  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals as shown in Table 1. For example, the doublet of H-9 and the singlet at  $\delta$  7.20 (H-3) exhibited HMBC correlations through three bonds with the C-1 carbonyl ( $\delta$  180.9). HMBC cross signals of H-3, H-4, H-6, H-7, and H-9 with a carbon atom at  $\delta$  124.9 were used to assign the latter to C-9b. This pattern suggested a phenalenone molecule without a phenyl ring. The structure of compound **1** was confirmed as 2-hydroxy-1H-phenalen-1-one by EIMS and HREIMS. The synthesis of compound **1** was achieved by epoxidation of perinaphthenone, followed by acid treatment of the intermediary epoxide (Scheme 1).

The  $^1\text{H}$  NMR spectrum of **2** was very similar to that of **1**, but a singlet integrating for three protons appeared at  $\delta$  3.89 instead of the exchangeable singlet of the hydroxyl group, suggesting that **2** is a methoxy derivative of **1**. All  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals were assigned unambiguously from the HMBC and HMQC spectra of **2** (Table 1). HREIMS was used to confirm the structure of compound **2** as 2-methoxy-1H-phenalen-1-one. Methylation of compound **1** by diazomethane resulted in a quantitative yield of compound **2** (Scheme 1).

Quiñones et al. have reported the biological activity against *M. fijiensis* of four natural phenylphenalenones and four 9-phenylphenalenone-related compounds lacking an OH group at the C-2 position.<sup>13</sup> Commercial perinaphthenone (**7**) was also assayed and displayed significant mycelial growth and spore germination inhibition; its effects on spore germination were even more effective than those of some commercial fungicides.<sup>13</sup> The activity of compounds **1** and **2** (synthetic samples) against *M. fijiensis* was examined, under the conditions reported by Quiñones et al. (see bioassay methods 1 and 3 in the Experimental Section), along with synthetic compound **3** and a sample of isolated compound **4**. In addition, 3-hydroxy-1H-phenalen-1-one (**8**, 3-hydroxyperinaphthenone), 2-hydroxy-9-(4-methoxyphenyl)-1H-phenalen-1-one (**10**), and 9-(4-hydroxyphenyl)-1H-phenalen-1-one (**12**) were assayed. In order to evaluate reproducibility of the bioassay, some phenalenones that had been tested previously were also subjected to the assay.<sup>13</sup>

Those compounds were 1H-phenalen-1-one (**7**, perinaphthenone), 2-hydroxy-9-(4-hydroxyphenyl)-1H-phenalen-1-one (**9**, hydroxy-anigorufone), 9-phenyl-1H-phenalen-1-one (**11**), and 9-(4-methoxyphenyl)-1H-phenalen-1-one (**13**). Due to insufficient amounts being obtained, compounds **5** and **6** were not assayed.

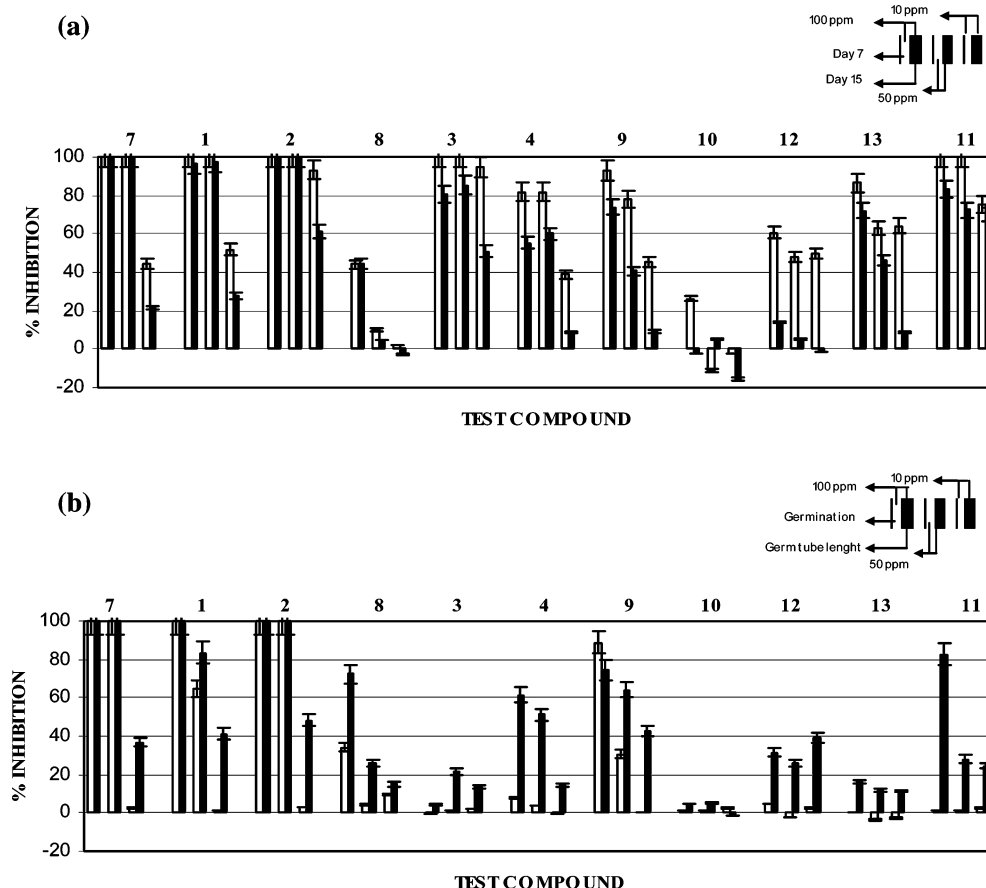
Compounds **1** and **2**, together with perinaphthenone (**7**), were the most active antifungal compounds assayed. The complete inhibition of mycelial growth was observed at 50–100 ppm for the three compounds, and this remained in effect for 15 days (bioassay method 1; Figure 1a). 3-Hydroxyperinaphthenone (**8**) displayed very low activity. Phenylphenalenones with an unsubstituted phenyl ring, such as anigorufone (**3**), methoxyanigorufone (**4**), and 9-phenylphenalenone (**11**), showed a moderate activity especially on the mycelia. The activity was significantly reduced for compounds **9** and **13** and was even lower for compounds **10** and **12**, all of which are substituted by OH or OCH<sub>3</sub> in the *p*-position of the phenyl ring. With regard to its effects on spore germination (bioassay method 3; Figure 1b), compound **2** was as active as perinaphthenone (**7**) at 100–50 ppm and even slightly more active than **7** at 10 ppm. Compound **1** was also active at 100 ppm. The remaining compounds assayed had very little effect on spores, except for hydroxyanigorufone (**9**) and 9-phenylphenalenone (**11**) at 100 ppm. Compounds **3**, **7**, and **11** displayed levels of bioactivity that are similar to those reported earlier,<sup>13</sup> but compound **9** was more active in the present bioassays (Figure 1a,b), and compound **13** was more active on mycelia growth (Figure 1a). In order to calculate the half-maximal inhibitory concentration (IC<sub>50</sub>) values of compounds **1** and **2** for *M. fijiensis*, a 96-well microtiter plate photometric bioassay was conducted<sup>14</sup> (see bioassay method 2 in the Experimental Section), revealing values of 23.4 ppm for 2-hydroxy-1H-phenalen-1-one (**1**) and 19.3 ppm for 2-methoxy-1H-phenalen-1-one (**2**). All compounds were tested by bioassay methods 1 and 2, and similar results were obtained; however, bioassay method 2 required less of the test compounds.

Compounds **1** and **2** constitute the first phenalenones lacking a phenyl group that have been isolated from the genus *Musa*. As far as we know, only one related compound of that type has been reported for plants, namely, 4-hydroxy-2-methoxyphenalen-1-one, which was previously isolated from *Strelitzia reginae*.<sup>15</sup> The activity of the two compounds **1** and **2** against *M. fijiensis* is the most potent reported for a secondary metabolite isolated from *Musa* and emphasizes their key role in the Yangambi cultivar's defense against *M. fijiensis*. However, further experiments are required to clarify if these compounds are constitutive metabolites or if they are produced *de novo* after plants have been infected with the pathogen.

## Experimental Section

**General Experimental Procedures.** The melting point of compound **1** was measured without correction on a Büchi apparatus. UV spectra were obtained using a Shimadzu UV-160A spectrophotometer.  $^1\text{H}$  NMR,  $^1\text{H}$ – $^1\text{H}$  COSY, HMBC, and HMQC spectra were recorded on a Bruker Avance DRX 500 NMR spectrometer equipped with an inverse detection microprobe head (2.5 mm) or a 5 mm TXI CryoProbe. A broadband-decoupled microprobe head (2.5 mm) was used for measuring  $^{13}\text{C}$  NMR spectra at 125.75 MHz. The spectra were referenced to internal TMS in all cases. For routine measurements, Bruker Avance 400 or Bruker AMXIII 300 spectrometers were employed. EIMS and HREIMS were run on a Micromass MassSpec mass spectrometer at 70 eV with a direct insertion probe.

**Plant Material and Fungi.** Healthy rhizomes of *Musa acuminata* cv. “Yangambi” (group AAA) were supplied by Instituto Colombiano Agropecuario (ICA), Colombia, in March 2003. *M. acuminata* cv. “Yangambi” is continuously maintained at the same institute. *Mycosphaerella fijiensis* was isolated from naturally infected banana leaves supplied by the Asociación de Bananeros de Colombia (AUGURA) from Apartadó-Colombia according to the method reported by Quiñones et al.<sup>13</sup> Briefly, isolates of *M. fijiensis* were obtained from ascospores after discharge from leaves infected with Black Sigatoka and were maintained on potato dextrose agar (PDA) in test tubes held at  $28 \pm 2$  °C.



**Figure 1.** Antifungal activity of compounds 1–4 and 7–13 on *Mycosphaerella fijiensis*. (a) Effect on the mycelia weight measured after 7 and 15 days of incubation as determined by bioassay method 1. (b) Effect on spores. Germination and length of the germ tubes were established microscopically for 50–150 ascospores after an incubation period of 24 h (determined by bioassay method 3).

**Extraction and Isolation.** Freshly collected rhizomes of *M. acuminata* cv. “Yangambi” (20 kg) were washed, chopped, and pressed to eliminate water, which was discarded. The remaining plant material (approximately 10 kg) was immediately extracted with ethanol (5 L) in a percolator system. This crude extract was evaporated to 25% of its initial volume to remove the organic solvent and was extracted with EtOAc. The EtOAc extract was evaporated (~3 g of dried extract obtained) and subjected to column chromatography (Sephadex LH-20) using *n*-hexane–CH<sub>2</sub>Cl<sub>2</sub>–MeOH (5:3:1) as eluent. Fourteen fractions were separated and subjected to TLC to detect phenylphenalenones using diethyl ether–*n*-hexane (7:1). TLC analysis afforded fractions four to nine as being the most interesting, due to the appearance of colored spots after spraying with a mixture of sulfuric acid in acetic acid (1:9) and heating (90–100 °C). Pure compounds were obtained by preparative reversed-phase HPLC of these fractions. A LiChrospher 100 RP-18 column (10  $\mu$ m; 250  $\times$  10 mm; flow rate 3.5 mL min<sup>-1</sup>; UV 254 nm) was used employing a MeCN (0.1% TFA)–H<sub>2</sub>O (0.1% TFA) gradient of 30:70%  $\rightarrow$  90:10% in 50 min  $\rightarrow$  30:70% in 5 min (total run time 55 min); 20, 4, 10, 100, 3, and 3 mg of compounds 1–6 were obtained respectively from this extract.

**2-Hydroxy-1*H*-phenalen-1-one (1,2-hydroxyperinaphthenone):** orange powder, mp 176–178 °C; *t*<sub>R</sub> 18.4 min; UV–vis (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 230 (4.42), 260 (4.29), 330 (3.96), 360 (3.89), 430 (3.87) nm; fluorescence emission not detected in the range 400–700 nm; <sup>1</sup>H NMR and <sup>13</sup>C NMR (see Table 1); EIMS *m/z* 196 [M]<sup>+</sup> (100), 168 (59), 139 (46); HREIMS *m/z* 196.0528 (calcd for C<sub>15</sub>H<sub>8</sub>O<sub>2</sub>, 196.0524).

**2-Methoxy-1*H*-phenalen-1-one (2,2-methoxyperinaphthenone):** yellow oil; *t*<sub>R</sub> 19.7 min; UV–vis (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 230 (4.20), 260 (4.16), 330 (3.74), 360 (3.82), 420 (3.80) nm; fluorescence emission  $\lambda_{\text{max}}$  520 nm; <sup>1</sup>H NMR and <sup>13</sup>C NMR (see Table 1); HREIMS *m/z* 210.0686 (calcd for C<sub>14</sub>H<sub>10</sub>O<sub>2</sub>, 210.0681).

**Synthetic Methods. 2-Hydroxy-1*H*-phenalen-1-one (1,2-hydroxyperinaphthenone).** To a cooled solution (ice–water bath) containing 200 mg of perinaphthenone (97 wt %) in benzene were added 100  $\mu$ L of aqueous *tert*-butylhydroperoxide (70 wt %) and 100  $\mu$ L of TritonB

(40 wt % in MeOH). After 5 min cooling, the solution was allowed to warm at ambient temperature and then stirred for 45 min before a second equal portion of *tert*-butylhydroperoxide and TritonB was added. Formation of the epoxide was observed by TLC (CH<sub>2</sub>Cl<sub>2</sub>) during a period of 2 h. *p*-Toluenesulfonic acid monohydrate (200 mg) was then added, and the resulting mixture was stirred overnight. The product was purified by column chromatography on silica gel 60 (particle size 0.040–0.063 mm) (Merck, Darmstadt, Germany), using CH<sub>2</sub>Cl<sub>2</sub> as an eluent to obtain 145 mg of 2-hydroxy-1*H*-phenalen-1-one (**1**) as an orange powder (70% yield). All reagents were purchased from Aldrich and used as supplied.

**2-Methoxy-1*H*-phenalen-1-one (2).** An ethanol-containing solution of diazomethane was prepared from Diazald using a standard procedure. This solution was added dropwise to 2-hydroxy-1*H*-phenalen-1-one (**1**) (50 mg, 0.25 mmol) until gas evolution had ceased. The reaction mixture was dried under a stream of nitrogen gas to afford 51 mg of the desired compound as a yellow oil. Analytical data of compounds **1** and **2** matched those of compounds isolated from the plant material.

Anigorufone (**3**), hydroxyanigorufone (**9**), 2-hydroxy-9-(4-methoxyphenyl)-1*H*-phenalen-1-one (**10**), 9-phenyl-1*H*-phenalen-1-one (**11**), 9-(4-hydroxyphenyl)-1*H*-phenalen-1-one (**12**), and 9-(4-methoxyphenyl)-1*H*-phenalen-1-one (**13**) were synthesized according to a reported method<sup>16</sup> and purified by column chromatography (silica gel 60, particle size 0.040–0.063 mm) (Merck) using CH<sub>2</sub>Cl<sub>2</sub> as eluent and preparative TLC (silica gel 60 F<sub>254</sub>, 1 mm layer thickness, Merck, Darmstadt, Germany) using Et<sub>2</sub>O–*n*-hexane (7:1) as eluent before being used in bioassays. Purity was checked by analytical HPLC using conditions described by Kamo et al.<sup>17</sup>

Perinaphthenone (**7**) and 3-hydroxyperinaphthenone (**8**) were purchased from Aldrich (Milwaukee, WI) and, prior to bioassays, purified by preparative TLC using Et<sub>2</sub>O–*n*-hexane (2:1) as an eluent (*R*<sub>f</sub> 0.68 and 0.21, respectively).

**Bioassays. Inhibition of Mycelial Growth (Method 1).** *M. fijiensis* was cultivated on potato dextrose agar (PDA; Becton Dickinson) at 27 °C. Aqueous suspensions of conidia were obtained by washing the

surface of isolated colonies with sterile distilled water. These conidia were dispersed in PDA medium in Petri dishes and incubated for 4 days. Isolated small colonies weighing  $2 \pm 1$  mg were placed in Petri dishes (20 colonies each) with PDA medium containing different concentrations (10, 50, 100 ppm) of the compounds dissolved in EtOH (3.7% max. in the medium). Colony weight was determined after 7 and 15 days of incubation (10 colonies per day) and compared to the weight of colonies grown on a blank medium containing the same concentration of EtOH but not the compound to be assayed. Experiments were performed in quadruplicate and analyzed by one-way analysis of variance (ANOVA), with excellent reproducibility.

**Microtiter Well Method (Method 2).** The method reported by Peláez et al.<sup>14</sup> was used for this bioassay in which fresh monospore subcultures of different *M. fijiensis* strains grown for 15–20 days at 27 °C in PDA were used as the source to obtain the inoculum. Mycelia from these subcultures were resuspended in sterile distilled water. The suspension was then fragmented by vortexing with glass beads of 6 mm diameter (Schott, Wertheim, Germany) during 1–2 min. Filtration with sterile “etamine” cloth (100  $\mu$ m pores) afforded uniform mycelial fragments. The concentration of mycelial fragments was measured in a Neubauer counting chamber (1/10 mm deep, bright line; Boeco, Boeckel & Co, Hamburg, Germany) and adjusted to  $2 \times 10^4$ ,  $2 \times 10^5$ , and  $2 \times 10^6$  mycelial fragments/mL with water, to test the best working inoculum dilution. Growth inhibition was determined in sterile, flat-bottomed 96-well microtiter plates with low-evaporation lids (Falcon-Becton-Dickinson). Each well was filled with 50  $\mu$ L of Sabouraud broth (BBL Becton-Dickinson), 50  $\mu$ L of the fungal inoculum, and 50  $\mu$ L of the different compound solutions in order to obtain final concentrations of 0, 5, 10, 20, 30, 40, and 50 ppm. Wells filled with 50  $\mu$ L of Sabouraud medium, 50  $\mu$ L of H<sub>2</sub>O, and 50  $\mu$ L of inoculum were used as the positive control. Blanks consisted of 50  $\mu$ L of Sabouraud medium and 100  $\mu$ L of H<sub>2</sub>O. The microtiter plates were incubated at 27 °C for several days. Mycelial growth was measured using a spectrophotometer (Biorad model 550). Readings of the OD<sub>595 nm</sub> for the suspensions in each well were taken daily and up to 17 days. IC<sub>50</sub> is defined as the concentration that is able to inhibit 50% of growth.

**Inhibition of Spore Germination (Method 3).** Ascospores discharged from infected banana leaves over Petri dishes containing different concentrations (10, 50, 100 ppm) of the compounds dispersed in PDA were used. Germination and length of the germ tubes were established microscopically for 50–150 ascospores after an incubation period of 24 h at 27 °C. Experiments were done in quadruplicate, with excellent reproducibility.

**Acknowledgment.** We thank Dr. A. Svatos for mass spectroscopic analyses, A. Gallego for collaboration with bioassays, and E. Wheeler for editorial assistance. This research was financially supported by Universidad de Antioquia (Programa de Sostenibilidad), Instituto Colombiano para el Desarrollo de la Ciencia y Tecnología “Francisco José de Caldas” (COLCIENCIAS, grant 2213-12-17825), Corporación para Investigaciones Biológicas (CIB), Universidad Nacional de Colombia (grants 2010100516 and 20101006122), and the Max-Planck-Institut für Chemische Ökologie. F.O. thanks COLCIENCIAS and Max-Planck-Institut für Chemische Ökologie for fellowships.

## References and Notes

- (1) Marín, D. H.; Romero, R. A.; Guzman, M.; Sutton, T. B. *Plant Dis.* **2003**, *87*, 208–222.
- (2) Vargas, A.; Sandoval, J. *InfoMusa* **2005**, *14*, 6–10.
- (3) Hoss, R.; Helbig, J.; Bochow, H. *J. Phytopathology* **2000**, *148*, 387–394.
- (4) Hirai, N.; Ishida, H.; Koshimizu, K. *Phytochemistry* **1994**, *37*, 383–385.
- (5) Luis, J. G.; Quiñones, W.; Echeverri, F.; Grillo, T.; Kishi, M.; Garcia, F.; Torres, F.; Cardona, G. *Phytochemistry* **1996**, *41*, 753–757.
- (6) Binks, H.; Greenham, R.; Luis, J. G.; Gowen, S. R. *Phytochemistry* **1997**, *45*, 47–49.
- (7) Kamo, T.; Hirai, N.; Iwami, K.; Fujioka, D.; Ohigashi, H. *Tetrahedron* **2001**, *57*, 7649–7656.
- (8) Otálvaro, F.; Echeverri, F.; Quiñones, W.; Torres, F.; Schneider, B. *Molecules* **2002**, *7*, 331–340.
- (9) Cooke, R. G.; Thomas, R. L. *Aust. J. Chem.* **1975**, *28*, 1053–1057.
- (10) Luis, J. G.; Fletcher, W. Q.; Echeverri, F.; Abad, T.; Kishi, M. P.; Perales, A. *Nat. Prod. Lett.* **1995**, *6*, 23–30.
- (11) Luis, J. G.; Lahlou, E. H.; Andrés, L. S. *Nat. Prod. Lett.* **1999**, *13*, 299–304.
- (12) Luis, J. G.; Fletcher, W. Q.; Echeverri, F.; Grillo, T. *Tetrahedron* **1994**, *50*, 10963–10970.
- (13) Quiñones, W.; Escobar, G.; Echeverri, F.; Torres, F.; Rosero, Y.; Arango, V.; Cardona, G.; Gallego, A. *Molecules* **2000**, *5*, 974–980.
- (14) Peláez, J.; Vázquez, L. E.; Díaz, T. J.; Castañeda, D. A.; Rodríguez, E.; Arango, R. E. *Rev. Fac. Nal. Agr. Medellín* **2006**, *59*, 3425–3433.
- (15) Hölscher, D.; Schneider, B. *J. Nat. Prod.* **2000**, *63*, 1027–1028.
- (16) Otálvaro, F.; Quiñones, W.; Echeverri, F.; Schneider, B. *J. Label. Compd. Radiopharm.* **2004**, *47*, 147–159.
- (17) Kamo, T.; Hirai, N.; Tsuda, M.; Fujioka, D.; Ohigashi, H. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2089–2098.

NP070091E