JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 7417–7421 7417
DOI:10.1021/jf901052e

# Structure−Activity Relationship in the Interaction of Substituted Perinaphthenones with *Mycosphaerella fijiensis*

William Hidalgo,[†] Luisa Duque,[‡] Jairo Saez,[‡] Rafael Arango,[†] Jesús Gil,[†] Benjamín Rojano,[†] Bernd Schneider,[§] and Felipe Otálvaro*,[‡]

[†]Universidad Nacional de Colombia sede Medellín, Autopista Norte, Calle 64 Cra 65, AA 1027 Medellín, Colombia, [‡]Instituto de Química, Universidad de Antioquia, Calle 67 # 53-108, AA 1226 Medellín, Colombia, and [§]Max Planck Institut für Chemische Ökologie, Beutenberg Campus, Hans-Knöll-Strasse 8, 07745 Jena, Germany

The levels of native fungitoxic perinaphthenone phytoalexins in susceptible *Musa* varieties (banana), which are commercially grown in large plantations, are too low to provide plants with long-lasting protection against highly pathogenic fungi. Novel strategies for plant protection are necessary to reduce crop losses and to prevent the development of resistant fungal strains. The synthesis of novel fungicides based on the structures of perinaphthenone natural products is considered to be a promising strategy. Thirteen substituted perinaphthenones, among them two known natural products (**1**, **2**) and 11 synthetics (**3**−**13**), were evaluated for their activity against *Mycosphaerella fijiensis*, and their half-maximal inhibitory concentrations ($IC_{50}$) were calculated to establish structure−activity relationships (SAR). A SAR trend was hypothesized, leading to the design of a new compound, 4-methoxy-2-nitro-1*H*-phenalen-1-one (**14**); the new compound displayed significantly enhanced in vitro activity against *M. fijiensis* compared to other perinaphthenone derivatives. The activity of **14** was comparable to that of two commercial fungicides.

**KEYWORDS: *Mycosphaerella fijiensis*; banana; perinaphthenone; phenalenone; phototoxicity; reactive oxygen species**

## INTRODUCTION

Bananas are among the most important crops worldwide; they are a staple food in many developing countries and a crucial economic factor for the communities that produce them (*1*). Nearly all currently grown cultivars of *Musa* (banana) are derived from two species, *Musa acuminata* (A genome) and *Musa balbisiana* (B genome), and belong to the "Cavendish" subgroup. The commercial cultivation of genetically closely related clones of the Cavendish banana in monocultures has boosted outbreaks of invading insects and nematodes and resulted in devastating fungal infections. The appearance of phytopathogenic fungi such as *Fusarium oxysporum* and *Mycosphaerella fijiensis* and their global proliferation has caused the Panama disease and Black Sigatoka disease, among others. Detrimental crop losses have led to a debate about the future of banana production (*1*−*3*). Phenalenones (perinaphthenones) and their phenyl derivatives and oxidative transformation products have been identified as native defense compounds and are reported as phytoalexins of *Musa* (*4*−*6*). Resistant *Musa* varieties accumulate perinaphthenones to much higher levels in their tissue than susceptible varieties do (*7*). Hence, the enhancement of the biosynthetic potential for native perinaphthenones by either conventional breeding or genetic modification is considered to be a possible way to improve *Musa* plants; the susceptibility of such improved plants to fungal infection would be greatly reduced. Here, another promising approach, namely, designing synthetic fungitoxic agents based on the perinaphthenone structure, is reported.

Recently, some natural perinaphthenones, among them compounds **1** and **2** in *Musa acuminata* cv. 'Yangambi', were identified as the most potent class of compounds isolated from this plant; they are active against the ascomycetous fungal pathogen *Mycosphaerella fijiensis*. As a result, the perinaphthenone nucleus has come to be seen as an interesting structural motif for the development of a new class of fungicides (*7*). One reason for the interest in the perinaphthenone moiety is its plausible phototoxic mode of action (*8*−*10*): perinaphthenone, a well-known photosensitizer (*1*), is presumed to generate singlet molecular oxygen ($^1O_2$) and act as a catalyst for the production of other reactive oxygen species, which may in turn cause cell damage to the pathogen (*8*).

To produce singlet oxygen ($^3O_2 \rightarrow {}^1O_2$), the photon-excited perinaphthenone must undergo intersystem crossing to a triplet state from which deactivation can occur by different competing energy transfer processes such as phosphorescence, thermal decay, or radical reactions with other compounds (*12*). Therefore, suitable experimental conditions have to be designed for each particular plant pathosystem (*13*) to prove the involvement of $^1O_2$. Previous bioassays with natural perinaphthenones active against *M. fijiensis* were conducted in the dark (*7*). Because phototoxicity cannot be assessed under conditions of light exclusion, it was not clear if the tested compounds displayed

*Corresponding author (telephone 05742195653; fax 05742330120; e-mail pipelion@quimica.udea.edu.co).


Published on Web 07/24/2009

photodynamic activity. However, when there is more than one mode of action, light exclusion is suitable for distinguishing phototoxicity from other activities. This paper reports the activity of two known natural (**1**, **2**) and 12 synthetic (**3**-**14**) perinaphthenones against *M. fijiensis* in the dark and under light-controlled conditions. Compounds **1**−**13** were chosen to gain information on structure−activity relationship (SAR) trends, which led to the design of a new compound, 4-methoxy-2-nitro-1*H*-phenalen-1-one (**14**).

## MATERIALS AND METHODS

**General Experimental Procedures.** $^1$H NMR, $^{13}$C NMR, DEPT 135, $^1$H−$^1$H COSY, HMBC, and HMQC spectra of synthetic compounds were recorded on a Bruker DRX 500 NMR spectrometer equipped with an ATM inverse probe (5 mm) or a Bruker AV 500 NMR spectrometer equipped with a TCI cryoprobe (5 mm) (Bruker-Biospin, Karlsruhe, Germany). The spectra were referenced to internal TMS in all cases. For routine measurements, a Bruker AMX III 300 spectrometer was employed. If not otherwise indicated, HREIMS was run on a Micromass MasSpec mass spectrometer (Micromass Ltd., Manchester, U.K.) at 70 eV with a direct insertion probe.

**Biological Material.** *M. 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. (*8*) Briefly, isolates of *M. fijiensis* were obtained from leaves infected with Black Sigatoka by discharging ascospores over a 2% aqueous potato dextrose agar (PDA) solution and were maintained on PDA in test tubes held at 25 ± 2 °C. Isolates of *M. fijiensis* were characterized by PCR amplification of the internally transcribed spacer region of rDNA to differentiate it from *M. musicola* (*14*). The following oligonucleotide sequences were employed: MF137 5′GGCGCCCC-CGGAGGCCGTCTA3′ (specific for *M. fijiensis*) and MM137 5′ GGC-GCCCCCGGAGGTCTCCTT3′ (specific for *M. musicola*) in conjunction with the nonspecific primer R635 5′GGTCCGTGTTTCAAGA-CGG3′ (*14*). Strains of *M. fijiensis* were classified according to the system of Cañas et al. (*15*) and are maintained in the Unidad de Biotecnología Vegetal UNALMED-CIB (Medellín, Colombia) under vouchers 060124 and 080105.

**Synthetic Methods.** 3-Hydroxyperinaphthenone (**3**) and perinaphthenone (**10**) were purchased from Aldrich (Milwaukee, WI) and, prior to being bioassayed, purified by preparative TLC using $Et_2O$/*n*-hexane (2:1) as an eluent ($R_f$ 0.21 and 0.68, respectively). 2-Hydroxyperinaphthenone (**1**) (*7*), 2-methoxyperinaphthenone (**2**) (*7*), 4-methoxyperinaphthenone (**6**) (*16*), 6-hydroxyperinaphthenone (**7**) (*17*), and 6-methoxyperinaphthenone (**8**) (*17*) were prepared and purified according to reported methods. Compounds **4** (*18*), **5** (*19*), **9** (*20*), **11** (*21*), **12** (*22*), and **13** (*20*) were previously reported, but their synthesis followed different procedures and/or their spectroscopic characterization is described here for the first time.

*3-Methoxy-1H-phenalen-1-one* (***4***). An ethanol-containing solution of diazomethane was prepared from Diazald using a standard procedure. This solution was added dropwise to 3-hydroxy-1*H*-phenalen-1-one (**3**) (5 mg, 0.026 mmol) until gas evolution had ceased. The reaction mixture was dried under a stream of nitrogen gas and the compound purified by preparative TLC (silica gel 60 $F_{254}$, 1 mm layer thickness, Merck, Darmstadt, Germany) using $CH_2Cl_2$-/MeOH (40:1, $R_f$ 0.70) as eluent to afford the desired compound (5 mg) as a yellow solid: $^1$H NMR ($C_3D_6O$, 500.13 MHz) $\delta$ 8.45 (dd, $J$ = 7.2, 1.3 Hz, H-9), 8.30 (dd, $J$ = 8.1, 1.3 Hz, H-7), 8.23 (dd, $J$ = 7.3, 1.2 Hz, H-4), 8.21 (dd, $J$ = 8.3, 1.2 Hz, H-6), 7.80 (dd, $J$ = 8.1, 7.2 Hz, H-8), 7.71 (dd, $J$ = 8.3, 7.3 Hz, H-5), 6.08 (s, H-2), 4.07 (s, −$OCH_3$); $^{13}$C NMR ($C_3D_6O$, 125.75 MHz) $\delta$ 184.9 (C-1), 166.8 (C-3), 134.9 (C-7), 133.1 (C-6), 133.1 (C-6a), 129.5 (C-9a), 129.4 (C-9), 128.0 (C-9b), 127.6 (C-8), 127.2 (C-5), 126.8 (C-4), 125.7 (C-3a), 104.3 (C-2), 56.7 (−$OCH_3$); HREIMS, $m/z$ 210.068131 (calcd for $C_{14}H_{10}O_2$, 210.068080).

*4-Hydroxy-1H-phenalen-1-one* (***5***). 4-Methoxyperinaphthenone (**6**) (200 mg, 0.95 mmol) was treated with 47% HBr (2.8 mL) in acetic acid (20 mL) and refluxed under nitrogen for 9 h. Acetic acid was removed under vacuum, and the crude mixture was extracted with $CH_2Cl_2$/$H_2O$. The organic layer was dried ($Na_2SO_4$), concentrated, and purified by preparative TLC using $CH_2Cl_2$/MeOH (40:1, $R_f$ 0.20) as eluent to afford an orange powder (102 mg, 55%): $^1$H NMR ($C_3D_6O$, 500.13 MHz) $\delta$ 8.51 (dd, $J$ = 7.9, 1.2 Hz, H-9), 8.38 (d, $J$ = 9.0 Hz, H-3), 7.91 (dd, $J$ = 7.4, 1.2 Hz, H-7), 7.80 (d, $J$ = 9.4 Hz, H-6), 7.48 (dd, $J$ = 7.9, 7.4 Hz, H-8), 6.84 (d, $J$ = 9.4 Hz, H-5), 6.82 (d, $J$ = 9.0 Hz, H-2); $^{13}$C NMR ($C_3D_6O$, 125.75 MHz) $\delta$ 176.6 (C-4), 175.2 (C-1), 138.2 (C-6), 135.6 (C-3), 132.9 (C-7), 131.0 (C-9b), 128.8 (C-9), 128.1 (C-6a), 127.7 (C-5), 127.3 (C-9a), 123.7 (C-8), 118.0 (C-2), 116.6 (C-3a); HREIMS, $m/z$ 196.053394 (calcd for $C_{13}H_8O_2$, 196.052430).

*2-Amino-1H-phenalen-1-one* (***9***). A balloon filled with hydrogen was fitted to a 10 mL round-bottom flask charged with the catalyst (10% Pd/C, 2.5 mg) and a solution of **13** (5 mg) in MeOH (5 mL). The mixture was stirred at 25 °C for 10 min, after which the catalyst was filtered and the solvent evaporated. Preparative TLC (*n*-hexane/ethyl acetate 2:1, $R_f$ 0.62) afforded the desired compound (4 mg, 98%) as a deep red solid: $^1$H NMR ($C_3D_6O$, 500.13 MHz) $\delta$ 8.61 (dd, $J$ = 7.3, 1.2 Hz, H-9), 8.33 (dd, $J$ = 8.0, 1.2 Hz, H-7), 7.88 (dd, $J$ = 7.9, 1.5 Hz, H-6), 7.83 (dd, $J$ = 7.3, 8.0 Hz, H-8), 7.59 (dd, $J$ = 7.3, 1.5 Hz, H-4), 7.56 (dd, $J$ = 7.3, 7.9 Hz, H-5), 6.89 (s, H-3); $^{13}$C NMR ($C_3D_6O$, 125.75 MHz) $\delta$ 180.9 (C-1), 143.0 (C-2), 136.3 (C-7), 133.1 (C-6a), 131.1 (C-3a), 130.5 (C-9), 129.2 (C-9a), 128.2 (C-5), 127.9 (C-4), 127.8 (C-6), 127.5 (C-8), 124.7 (C-9b), 110.0 (C-3); LC-HRESIMS (Micromass Quattro II tandem quadrupole mass spectrometer, Micromass Ltd., Manchester, U.K.), $m/z$ 194.06097 (calcd for $C_{13}H_8NO$, 194.06059).

*2-Bromo-1H-phenalen-1-one* (***11***). The method of Imanzadeh et al. (*23*) for bromination was adapted in this case. Flame-dried neutral alumina (1.4 g), *N*-bromosuccinimide (303 mg, 1.7 mmol), and perinaphthenone (186 mg, 1 mmol) were ground in a mortar at room temperature until a uniform color was perceived. The mixture was then transferred to a test tube and heated at 45 °C for 3 h. Extraction with $CH_2Cl_2$ (20 mL) and preparative TLC (*n*-hexane/dichloromethane 1:1, $R_f$ 0.28) afforded a bright yellow solid (150 mg, 58%, 98% based on recovered perinaphthenone): $^1$H NMR ($C_3D_6O$, 500.13 MHz) $\delta$ 8.63 (d, $J$ = 7.4 Hz, H-9), 8.48 (s, H-3), 8.46 (d, $J$ = 8.2 Hz, H-7), 8.27 (d, $J$ = 8.2 Hz, H-6), 8.02 (d, $J$ = 7.1 Hz, H-4), 7.92 (dd, $J$ = 8.2, 7.4 Hz, H-8), 7.74 (dd, $J$ = 8.2, 7.1 Hz, H-5); $^{13}$C NMR ($C_3D_6O$, 125.75 MHz) $\delta$ 178.6 (C-1), 144.2 (C-3), 136.7 (C-7), 133.5 (C-6), 133.3 (C-6a), 133.0 (C-4), 132.3 (C-9), 129.4 (C-9a), 128.7 (C-3a), 128.5 (C-8), 128.2 (C-5), 127.4 (C-9b), 126.0 (C-2); HREIMS, $m/z$ 257.967261 (calcd for $C_{13}H_7{}^{79}BrO$, 257.968026).

*2-Chloro-1H-phenalen-1-one* (***12***). Concentrated (65%) $HNO_3$ (186 $\mu$L) was added to perinaphthenone (**10**) (100 mg, 0.56 mmol) dissolved in concentrated (36%) HCl (5 mL), and the mixture was stirred for 3 h at room temperature. Liquid−liquid partition between $H_2O$ and $CH_2Cl_2$ (3 × 15 mL) followed by preparative TLC (*n*-hexane/dichloromethane 2:3, $R_f$ 0.65) afforded the desired compound in 55% yield as a yellow solid: $^1$H NMR ($C_3D_6O$, 500.13 MHz) $\delta$ 8.66 (dd, $J$ = 7.4, 1.2 Hz, H-9), 8.49 (dd, $J$ = 8.1, 1.2 Hz, H-7), 8.28 (dd, $J$ = 8.3, 1.1 Hz, H-6), 8.27 (d, $J$ = 0.5 Hz, H-3), 8.05 (ddd, $J$ = 7.1, 1.1, 0.5 Hz, H-4), 7.95 (dd, $J$ = 8.1, 7.4 Hz, H-8), 7.77 (dd, $J$ = 8.3, 7.1 Hz, H-5); $^{13}$C NMR ($C_3D_6O$, 125.75 MHz) $\delta$ 178.7 (C-1), 140.1 (C-3), 136.8 (C-7), 133.9 (C-2), 133.4 (C-6), 133.3 (C-6a), 133.1 (C-4), 132.1 (C-9), 129.9 (C-9a), 128.5 (C-8), 128.2 (C-5), 128.1 (C-3a), 127.1 (C-9b); HREIMS, $m/z$ 214.018414 (calcd for $C_{13}H_7{}^{35}ClO$, 214.018543).

*2-Nitro-1H-phenalen-1-one* (***13***). The method reported by Dokunikhin et al. (*20*) was employed in which a mixture of concentrated (65%) nitric acid (42 $\mu$L) and concentrated (98%) sulfuric acid (121 $\mu$L) was added dropwise to a cooled solution (0 °C) of perinaphthenone (**10**) (100 mg, 0.6 mmol) dissolved in concentrated sulfuric acid (2 mL). The reaction mixture was stirred for 1 h at room temperature, $H_2O$ (10 mL) was added, and the mixture was extracted with $CH_2Cl_2$ (3 × 15 mL). The organic layer was dried and concentrated in vacuo. Preparative TLC (*n*-hexane/ethyl acetate 1:1, $R_f$ 0.65) afforded the desired compound (yellow solid) in 30% yield (55% based on recovered perinaphthenone): $^1$H NMR ($C_3D_6O$, 500.13 MHz) $\delta$ 8.77 (s, H-2), 8.71 (dd, $J$ = 7.3, 1.2 Hz, H-9), 8.59 (dd, $J$ = 8.1, 1.2 Hz, H-7), 8.03 (dd, $J$ = 7.3, 8.1 Hz, H-8), 7.91 (dd, $J$ = 7.2, 8.3 Hz, H-5), 7.50 (dd, $J$ = 7.3, 1.1 Hz, H-6), 7.40 (dd, $J$ = 7.2, 1.1 Hz, H-4); $^{13}$C NMR ($C_3D_6O$, 125.75 MHz) $\delta$ 175.2 (C-1), 147.9 (C-2), 138.8 (C-3), 137.7 (C-7), 137.6 (C-4), 136.6 (C-6), 133.3 (C-6a), 132.4 (C-9), 130.5 (C-9a), 129.0 (C-8), 128.6 (C-5), 127.8 (C-9b), 125.2 (C-3a); HREIMS, $m/z$ 225.043327 (calcd for $C_{13}H_7NO_3$, 225.042593).

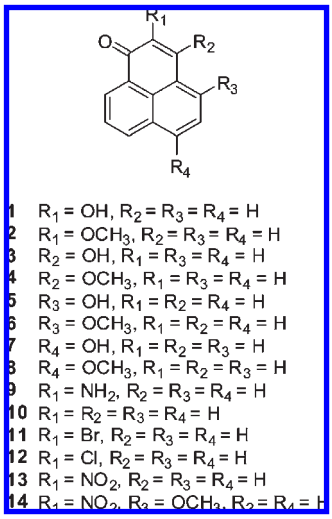


**Figure 1.** Synthetic perinaphthenones (**1**−**14**) assayed in this work. Compounds **1** and **2** occur in *M. acuminata* var. Yangambi (*7*).

*4-Methoxy-2-nitro-1H-phenalen-1-one* (***14***). Concentrated $HNO_3$ (30 $\mu$L) was added to 4-methoxyperinaphthenone (**6**) (70 mg, 0.3 mmol) dissolved in acetic acid (7 mL), and the mixture was stirred for 3 h at room temperature. Evaporation of the solvent was followed by liquid−liquid partition between $H_2O$ and $CH_2Cl_2$. Preparative TLC purification (*n*-hexane/ethyl acetate 2:1, $R_f$ 0.14) afforded **14** (yellow solid, 28 mg, 33%, 65% based on recovered **6**): $^1$H NMR ($C_3D_6O$, 500.13 MHz) $\delta$ 8.94 (s, H-2), 8.70 (dd, $J$ = 7.6, 1.2 Hz, H-9), 8.54 (d, $J$ = 9.3 Hz, H-6), 8.47 (dd, $J$ = 7.9, 1.2 Hz, H-7), 7.85 (dd, $J$ = 7.6, 7.9 Hz, H-8), 7.74 (d, $J$ = 9.3 Hz, H-5), 4.30 (s, $-OCH_3$); $^{13}$C NMR ($C_3D_6O$, 125.75 MHz) $\delta$ 174.4 (C-1), 164.5 (C-4), 146.1 (C-2), 140.7 (C-6), 137.5 (C-7), 133.2 (C-9), 132.8 (C-3), 130.7 (C-9a), 129.1 (C-9b), 128.7 (C-6a), 126.7 (C-8), 115.3 (C-5), 110.2 (C-3a), 57.9 ($-OCH_3$); HREIMS, $m/z$ 255.053327 (calcd for $C_{14}H_9NO_4$, 255.053158).

**Bioassays.** A microtiter well method (*7*) was applied according to which measures of the mycelial growth were taken after 8 days of incubation (logarithmic growth phase of the fungi) in the dark or under light-controlled conditions. For the light-controlled experiments, the plates were placed at a distance of 1 m from 4 × 2 white light tubes (Sylvania F32T8, 20 W, Daylight 154) in an incubation chamber with a 12 h photoperiod. In all cases, the working inoculum concentration was adjusted to $2 \times 10^5$ mycelial fragments/mL. Statistical analysis was performed according to the method of Lazzaro et al. (*9*). The $IC_{50}$ value was obtained by interpolation in the corresponding myceliar growth versus phenalenone concentration plots. All statistical analyses were done using the SAS software version 9.1.

## RESULTS AND DISCUSSION

To check for photodynamic activity, 2-hydroxyperinaphthenone (**1**) and 2-methoxyperinaphthenone (**2**) (**Figure 1**), both of which occur in *M. acuminata* var. 'Yangambi' (*7*), were assayed for their activity against *M. fijiensis* (mycelial growth inhibition) under light-controlled conditions using a slight modification of a reported protocol (*7*) (**Figure 2a**). The activity of natural compounds **1** and **2** did not significantly change under the influence of light. This made us ask whether hydroxyl and methoxyl groups generally prevent the photodynamic activities of perinaphthenones or if substituents attached to the ortho position relative to the carbonyl group were responsible. To address this question, compounds **3**−**8** (**Figure 1**) were synthesized and assayed under the same conditions (**Figure 2a**). With the exception of compounds **1** and **2**, replacing a hydroxyl group with a methoxyl group increases the activity by >3-fold. Because no significant effect of light was observed in this series (Tukey variance analysis, 95% confidence), an increase in lipophilicity may explain the enhanced activity of the methoxylated compounds. That compound **1** and its *O*-methyl analogue **2** (**Figure 2a**) show nearly equal activities in the bioassay can be rationalized in terms of a strong intramolecular hydrogen bond present in **1**, which gives it an even lower polarity than its *O*-methyl counterpart **2** as revealed by the $R_f$ values (0.77 for **2** and 0.88 for **1**) in normal phase silica gel ($CH_2Cl_2$/MeOH 40:1). It is worth noting that compounds **3**, **5**, and **7** offer the possibility of strong intermolecular hydrogen bonding (*24*), which seems to diminish the activity. That light had no effect on the activities of compounds **1**−**8** against *M. fijiensis* prompted us to explore other SAR trends in the perinaphthenone series.

Polycyclic aromatic $\alpha,\beta$-unsaturated ketones are well recognized for their Michael acceptor ability (*25*); therefore, perinaphthenones seemed to be a good candidate for this mode of action. Accordingly, electron-withdrawing groups attached at the C-2 position of perinaphthenone should increase the activity, and, conversely, the opposite effect should be observed for electron-donating groups. To explore this possibility, perinaphthenone derivatives **9**−**13** (**Figure 1**) were chosen with substituents that affect the electron density of the double bond by either inductive or conjugation effects, and these were assayed in the dark. Electron-withdrawing groups attached to the C-2 position of the perinaphthenone nucleus dramatically increase the activity of perinaphthenones (**Figure 2b**). 2-Nitroperinaphthenone (**13**) was the most active compound in this series (**Figure 2b**), as expected for a conjugated nitroalkene under a Michael acceptor mode of action (*26*). Compounds **10**−**13** were the only ones in this series for which activity was enhanced under light (Tukey variance analysis, 95% confidence). Specifically, compounds **11**−**13** with electron-withdrawing groups at C-2 displayed activity enhancement with both strains of *M. fijiensis*. This confirms previous evidence indicating that electron-withdrawing groups in position C-2 of perinaphthenone result in molecules with high quantum yields of $^1O_2$ production (*27*). In the case of perinaphthenone (**10**), significant differences between activities in the light and the dark were observed only for strain 060124 (**Figure 2b**).

Comparison of the *O*-methyl derivatives **4**, **6**, and **8** with the unsubstituted perinaphthenone **10** revealed that the *O*-methyl group always increased activity, compound **6** being the most active. This finding demonstrated the relevance of a methoxyl substituent at C-3, C-4, or C-6 in this nucleus and offered us an opportunity to explore whether an additional electron-withdrawing group at C-2 of *O*-methyl derivatives **4**, **6**, or **8** could have a synergistic effect. Therefore, 2-nitro-4-methoxyperinaphthenone (**14**) (**Figure 1**) was synthesized, and its activity was measured under light-controlled experiments (**Figure 2b**).

The $IC_{50}$ value of compound **14** was the lowest among all tested perinaphthenone compounds, and its in vitro activity was comparable to that of the commercial fungicides benomyl and propiconazole (**Figure 2b**). Moreover, the activity of 2-nitro-4-methoxyperinaphthenone (**14**) was significantly enhanced in the presence of light. Comparison of the $IC_{50}$ value of the 2-nitro-4-methoxy-substituted perinaphthenone **14** with the values of the 4-methoxy derivative **6** and the 2-nitro derivative **13** (**Figure 2**) suggests that the two functional groups affect the Michael acceptor capacity of the perinaphthenone structure without disturbing the photodynamic properties of the core nucleus. In summary, these findings demonstrate that the activity of perinaphthenones against *M. fijiensis* can be enhanced by introducing electron-withdrawing groups at C-2 and a methoxyl substituent at C-4. The effects of other types of substituents at C-4 on the activity against *M. fijiensis* deserve more attention.

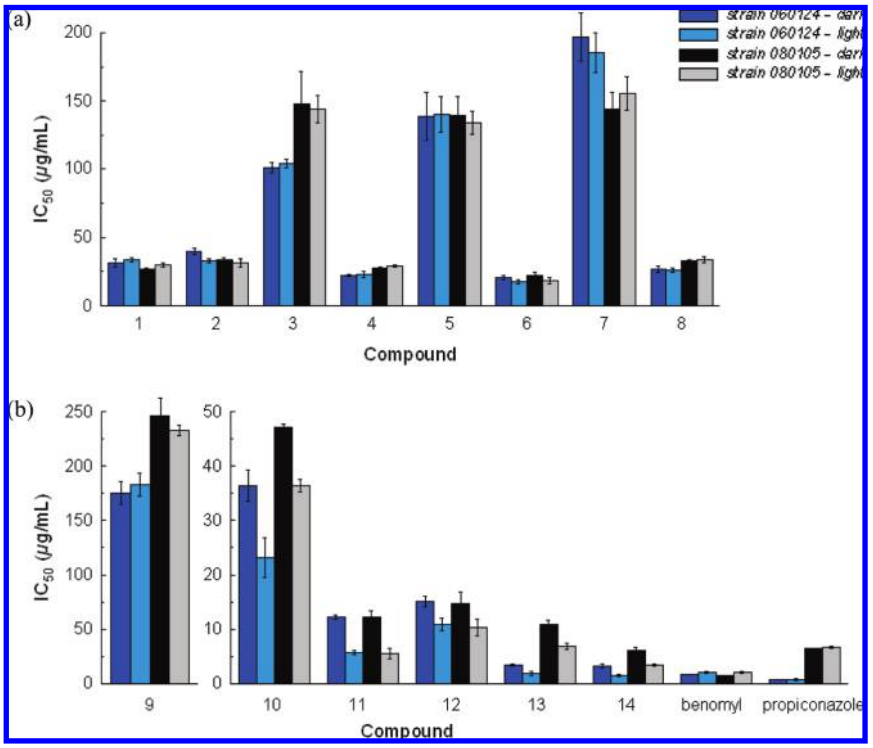


**Figure 2.** Antifungal activity of compounds **1**−**14** on *Mycosphaerella fijiensis*. The effect on mycelial growth was measured in the dark and under photoperiods of 12 h during 8 days of incubation. Commercial fungicides (benomyl and propiconazole) were tested for reference purposes.

## ACKNOWLEDGMENT

We thank Sybille Lorenz for mass spectrometric analyses and Emily Wheeler for editorial assistance.

## LITERATURE CITED

(1) Marin, D. H.; Romero, R. A.; Guzmán, M.; Sutton, T. B. Black Sigatoka: an increasing threat to banana cultivation. *Plant Dis*. **2003**, *87*, 208–222.
(2) Knight, J. Crop improvement: a dying breed. *Nature* **2003**, *421*, 568–570.
(3) Jones, D. Novel and conventional path to a better banana. *Nature* **2003**, *421*, 889–889.
(4) Luis, J. G.; Echeverri, F.; Quiñones, W.; Brito, I.; López, M.; Torres, F.; Cardona, G.; Aguiar, Z.; Pelaez, C.; Rojas, M. Irenolone and emenolone: two new types of phytoalexins from *Musa paradisiaca*. *J. Org. Chem*. **1993**, *58*, 4306–4308.
(5) Hirai, N.; Ishida, H.; Koshimizu, K. A phenalenone-type phytoalexin from *Musa acuminata*. *Phytochemistry* **1994**, *37*, 383–385.
(6) Binks, R. H.; Greenham, J. R.; Luis, J. G.; Gowen, S. R. A phytoalexin from roots of *Musa acuminata* var. Pisang sipulu. *Phytochemistry* **1997**, *45*, 47–49.
(7) Otálvaro, F.; Nanclares, J.; Vásquez, L. E.; Quiñones, W.; Echeverri, F.; Arango, R.; Schneider, B. Phenalenone-type compounds from *Musa acuminata* var. "Yangambi km 5" (AAA) and their activity against *Mycosphaerella fijiensis*. *J. Nat. Prod*. **2007**, *70*, 887–890.
(8) Quiñones, W.; Escobar, G.; Echeverri, F.; Torres, F.; Rosero, Y.; Arango, V.; Cardona, G.; Gallego, A. Synthesis and antifungal activity of *Musa* phytoalexins and structural analogs. *Molecules* **2000**, *5*, 974–980.
(9) Lazzaro, A.; Corominas, M.; Martí, C.; Flors, C.; Izquierdo, L. R.; Grillo, T. A.; Luis, J. G.; Nonell, S. Light- and singlet oxygen-mediated antifungal activity of phenylphenalenone phytoalexins. *Photochem. Photobiol. Sci*. **2004**, *3*, 706–710.
(10) Flors, C.; Nonell, S. Light and singlet oxygen in plant defense against pathogens: phototoxic phenalenone phytoalexins. *Acc. Chem. Res*. **2006**, *39*, 293–300.
(11) Schmidt, R.; Tanielian, C.; Dunsbach, R.; Wolff, C. Phenalenone, a universal reference compound for the determination of quantum yields of singlet oxygen $O_2(^1\Delta_g)$ sensitization. *J. Photochem. Photobiol. A: Chem*. **1994**, *79*, 11–17.
(12) Flors, C.; Ogilby, P. R.; Luis, J. G.; Grillo, T. A.; Izquierdo, L. R.; Gentili, P.; Bussotti, L.; Nonell, S. Phototoxic phytoalexins. Processes that compete with the photosensitized production of singlet oxygen by 9-phenylphenalenones. *Photochem. Photobiol*. **2006**, *82*, 95–103.
(13) Berenbaum, M. R.; Larson, R. A. Flux of singlet oxygen from leaves of phototoxic plants. *Experientia* **1988**, *44*, 1030–1032.
(14) Johanson, A.; Crowhurst, R. N.; Rikkerink, E. H. A.; Fullerton, R. A.; Templeton, M. D. The use of species-specific DNA probes for the identification of *Mycosphaerella fijiensis* and *M. musicola*, the causal agents of sigatoka disease of banana. *Plant Pathol*. **1994**, *43*, 701–707.
(15) Cañas, G. P.; Angarita, M. J.; Restrepo, J. M.; Rodríguez, P.; Moreno, C. X.; Arango, R. Analysis of the CYP51 gene and encoded protein in propiconazole-resistant isolates of *Mycosphaerella fijiensis*. *Pest Manag. Sci*. **2009**, *65*, 892−899.
(16) Nanclares, J.; Gil, J.; Rojano, B.; Saez, J.; Schneider, B.; Otálvaro, F. Synthesis of 4-methoxy-1*H*-phenalen-1-one: a subunit related to natural phenalenone-type compounds. *Tetrahedron Lett*. **2008**, *49*, 3844–3847.
(17) Otálvaro, F.; Quiñones, W.; Echeverri, F.; Schneider, B. Synthesis of [*phenyl*-$^{13}C_6$]lachnanthocarpone and other $^{13}$C-labelled phenylphenalenones. *J. Labelled Compd. Radiopharm*. **2004**, *47*, 147–159.
(18) Goldman, M. The anomalous reduction of 7,9-diketoperinaphthane and its methyl ether. *J. Am. Chem. Soc*. **1954**, *76*, 4032–4033.
(19) Loudon, J. D.; Razdan, R. K. Rearrangements of chromanones and dihydrocoumarins by aluminium chloride. *J. Chem. Soc*. **1954**, 4299–4303.
(20) Dokunikhin, N. S.; Solodar, S. L.; Vinogradov, L. M. Transformations of polycyclic ketones. XIII. * Nitration of phenalenone. *Zh. Org. Khim*. **1979**, 2360–2366.
(21) Fieser, L. F.; Newton, L. W. Reactions of perinaphthane derivatives. *J. Am. Chem. Soc*. **1942**, *64*, 917–921.
(22) Dokunikhin, N. S.; Solodar, S. L.; Stavinchuk, V. G. Reactions of polycyclic ketones. IX. Synthesis and properties of some phenalenone and benzanthrone salts. *Zh. Org. Khim*. **1976**, *12*, 186–191.
(23) Imanzadeh, G.; Zamanloo, M.; Eskandari, H.; Shayesteh, K. A new ring bromination method for aromatic compounds under

solvent-free conditions with NBS/$Al_2O_3$. *J. Chem. Res.* **2006**, 151–153.
(24) Haddon, R. C. Symmetrical hydrogen bonding: molecular orbital theory of the $\pi$-electron component. *J. Am. Chem. Soc.* **1980**, *102*, 1807–1811.
(25) Ahn, B. Z.; Sok, D. E. Michael acceptors as a tool for anticancer drug design. *Curr. Pharm. Des.* **1996**, *2*, 247–262.
(26) Rai, V.; Namboothiri, I. N. A theoretical evaluation of the Michael-acceptor ability of conjugated nitroalkenes. *Eur. J. Org. Chem.* **2006**, 4693–4703.
(27) Nonell, S.; Gonzáles, M.; Trull, F. R. 1*H*-Phenalen-1-one-2-sulfonic acid: an extremely efficient singlet molecular oxygen sensitizer for aqueous media. *Afinidad* **1993**, *50*, 445–450.

**Received March 30, 2009. Revised manuscript received June 10, 2009. Accepted July 14, 2009. This research was financially supported by Universidad de Antioquia, Universidad Nacional de Colombia sede Medellín, and the Max Planck Institut für Chemische Ökologie.**