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Structure−Activity Relationships of Derivatives of Fusapyrone, an Antifungal Metabolite of *Fusarium semitectum*

CLAUDIO ALTOMARE,*,† RAFFAELE PENGUE,‡ MARA FAVILLA,† ANTONIO EVIDENTE,‡ AND ANGELO VISCONTI†

Istituto di Scienze delle Produzioni Alimentari, CNR, 70125 Bari, Italy, and Dipartimento di Scienze del Suolo, della Pianta e dell'Ambiente, Università di Napoli Federico II, 80055 Portici, Italy

Fusapyrone (**1**) and deoxyfusapyrone (**2**) are two 3-substituted-4-hydroxy-6-alkyl-2-pyrones isolated from *Fusarium semitectum* that have considerable antifungal activity against molds. Because of their low zootoxicity and selective action they are potentially utilizable along with biocontrol yeasts for control of postharvest crop diseases. Seven derivatives of **1** (**3** and **5**−**10**) and one derivative of **2** (**4**) were obtained by chemical modifications of the glycosyl residue, the 2-pyrone ring, the aliphatic chain, or a combination thereof, and a structure−activity correlation study was carried out with regard to their zootoxicity and antifungal activity. Derivatives **7**−**10**, as well as **1**, were slightly zootoxic in *Artemia salina* (brine shrimp) bioassays, whereas pentaacetylation of **1** into **3**, **5**, and **6** resulted in a strong increase in toxicity. Compound **4**, the tetraacetyl derivative of **2**, was as toxic as **2**. Because the structural changes of **1** that resulted in an increase of biological activity in *A. salina* bioassay were those that affected mainly the water solubility of the molecule, it appears that toxicity is related to hydrophobicity. Compounds **1** and **2** showed strong antifungal activity toward *Botrytis cinerea*, *Aspergillus parasiticus*, and *Penicilliun brevi-compactum* (minimum inhibitory concentration at 24 h = 0.78−6.25 μg/mL). Among derivatives **3**−**10**, only compounds **7**, **9**, and **10** retained some activity, limited to *B. cinerea* and at high concentration (25−50 μg/mL). None of the compounds **1**−**10** inhibited the growth of the biocontrol yeasts *Pichia guilliermondii* and *Rhodotorula glutinis* at the highest concentration tested (50 μg/mL).

KEYWORDS: **α-Pyrone; antifungal compound; biological control; *Botrytis cinerea*; *Aspergillus parasiticus*; *Penicilliun brevi-compactum***

INTRODUCTION

Fungi are a well-known source of bioactive compounds, and the research for isolation of novel fungal antibiotics and agrochemicals that blossomed more than 40 years ago is still very active today. In addition to their potential use as agrochemicals or chemotherapeutics, bioactive compounds are also useful as lead molecules, by serving as structural models for the design of new synthetic compounds with higher activity or stability. In particular, these compounds may disclose novel mechanisms of action capable of overcoming the acquired resistance of plant pathogens to known antibiotics. Moreover, the combined use of bioactive natural compounds with biocontrol agents, for example, antagonistic yeasts, has been proposed as a profitable approach to achieve additive and/or synergistic effects and provide greater consistency and efficacy of disease control (*1*).

Evidente et al. (*2*) described the chemical structures of two antifungal compounds, named fusapyrone (**1**) and deoxyfusapyrone (**2**) (**Figure 1**), isolated from rice cultures of the soil fungus *Fusarium semitectum* Berk. & Rav. Compounds **1** and **2** are 3-substituted 4-hydroxy-6-alkyl-2-pyrones that consist of a highly functionalized aliphatic chain and a 4-deoxy-β-*xylo*-hexopyranosyl *C*-glycosyl moiety bound to the C-6 and C-3 of the 2-pyrone ring, respectively. Other secondary metabolites containing the pyran-2-one moiety (α-pyrones) have been reported to be produced by fungi belonging to several genera, including *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, and *Trichoderma*, and they exhibit a wide range of biological activities, for example, antibiotic, antifungal, cytotoxic, neurotoxic, and phytotoxic (*3*). Members of this class of compounds have also been investigated for their potent antitumor (*4, 5*) and HIV protease inhibiting (*6*−*8*) properties and for their plant growth regulating activity (*9*−*11*).

Despite their close structural similarity, **1** and **2** exhibit distinct biological activities. In particular, **1** has been shown to be a potent antifungal compound with low zootoxicity in *Artemia salina* L. larvae mortality bioassays, whereas **2** had higher zootoxicity (LC_{50} to *A. salina* = 37.1 μM) (*12*). Therefore, in addition to the well-known bioactive properties of the α-pyrone ring, the functionalities of the aliphatic chain also seem to have

* Author to whom correspondence should be addressed (telephone +39 080 5929318; fax +39 080 5929371; e-mail claudio.altomare@ispa.cnr.it).
† Istituto di Scienze delle Produzioni Alimentari, CNR.
‡ Università di Napoli Federico II.

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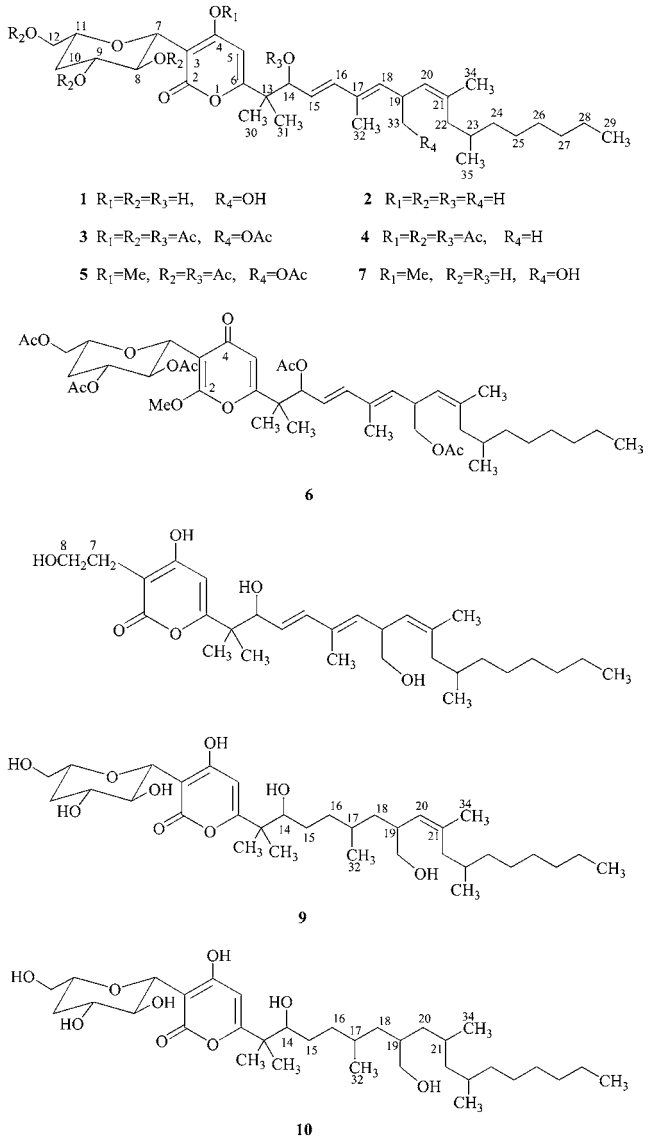

Figure 1. Structures of fusapyrone (**1**), 3-(4-deoxy-β-*xylo*-hexopyranosyl)-4-hydroxy-6-(2-hydroxy-7-hydroxymethyl-1,1,5,9,11-pentamethyl-3,5,8-heptadecatrienyl)-2*H*-pyran-2-one; deoxyfusapyrone (**2**), 3-(4-deoxy-β-*xylo*-hexopyranosyl)-4-hydroxy-6-(2-hydroxy-1,1,5,7,9,11-hexamethyl-3,5,8-heptadecatrienyl)-2*H*-pyran-2-one; and synthetic derivatives **3**–**10**.

a role in affecting, both quantitatively and qualitatively, the biological activity of these natural compounds.

In this study we investigated structure−activity relationships, with respect to both antifungal and zootoxic activity, of these compounds and seven different chemical derivatives of **1** and one derivative of **2**. The aims of this study were to (1) verify if new molecules with increased antifungal activity could be generated and (2) evaluate the toxicity of the derivatives in the perspective of the potential use of these molecules as agrochemicals or chemotherapeutics. Derivatives were obtained by chemical modifications of the glycosyl residue, the 2-pyrone ring, the aliphatic chain, or a combination thereof. Antifungal activity was tested on both phytopathogenic molds and biocontrol yeasts.

MATERIALS AND METHODS

General Experimental Procedures. IR and UV spectra were determined neat and in methanol solution, respectively, on an IR FT-1720X spectrometer (Perkin-Elmer, Norwalk, CT) and a Perkin-Elmer Lamda 3B spectrophotometer; ^{1}H and ^{13}C NMR spectra were recorded at 400 or 250 MHz and at 100 or 62.5 MHz, respectively, in CD_3OD, on Bruker spectrometers (Bruker, Karlsruhe, Germany). The same solvent was used as internal standard. Carbon multiplicities were determined by DEPT spectra (*13*) using Bruker microprograms. ES and FABMS were taken at 70 eV and in glycerol/thioglycerol, respectively, on Perkin-Elmer API 100 LC-MS and Fisons ProSpec (VG Instruments, Manchester, U.K.) spectrometers. Analytical and preparative TLC were performed on Kieselgel 60 F_{254}, 0.25 and 0.50 mm, respectively, silica gel (Merck, Darmstadt, Germany) or on Stratochrom KC-18, F_{254}, 0.25 mm, reverse phase (Whatman, Clifton, NJ) plates; the spots were visualized by exposure to UV radiation and/or by spraying with 10% H_2SO_4 in methanol and then with 5% phosphomolybdic acid in methanol, followed by heating at 110 °C for 10 min. Medium-pressure column chromatography was performed on Kieselgel 60, 230−400 mesh silica gel (Merck), using a model 681 chromatography pump (Büchi, Flawil, Switzerland) fixed at 20 bar.

Production and Purification of Fusapyrone and Deoxyfusapyrone. *Fusarium semitectum* (strain ITEM 1623) was grown on autoclaved wheat kernels under conditions previously reported (*1*). Fusapyrone (**1**) and deoxyfusapyrone (**2**) were purified according to the chromatographic method described by Evidente et al. (*14*).

Acetyl Derivative of Deoxyfusapyrone and Fusapyrone and Its Methylated Derivatives. 8,9,12,14,33-Pentaacetylfusapyrone and its 4-*O*-methyl derivative, 8,9,12,14-tetraacetyldeoxyfusapyrone, and the 2-*O*-methyl derivative of the isomeric γ-pyrone of **3** (**3**, **5**, **4**, and **6**, respectively), were obtained as previously described (*2*).

4-*O*-Methylfusapyrone (7). To a solution of **1** (30.2 mg) in methanol (2 mL) was added an ethereal solution of CH_2N_2. The reaction was carried out at room temperature under stirring and stopped after 4 h by evaporation under a N_2 stream. The crude residue was purified by preparative silica gel TLC, using EtOAc/H_2O/MeOH (8.5:2:1, v/v/v) as eluent, to give 1 mg of 4-*O*-methylfusapyrone (**7**). The reaction was repeated several times to obtain 15 mg of **7** as an amorphous solid: UV λ_{max} (log ϵ) 235 (3.94), 283 (3.55); ^{1}H NMR, differed from that of **1** only in the singlet (OMe) at δ 3.97; ESMS (−), *m*/*z* 619 $[M - H]^-$; HREIMS, *m*/*z* 620.3914 $[M]^+$; calcd for $C_{35}H_{56}O_9$, 620.3924.

3-(2-Hydroxyethyl)aglycon Derivative of Fusapyrone (8). Fusapyrone (**1**) (115.2 mg) dissolved in methanol (2.5 mL) was oxidized with $NaIO_4$ (85 mg/mL). After 1 h, the reaction was stopped with ethylene glycol (300 mL), and the residue obtained by workup was reduced with methyl morpholine borane (MMB, 400 mg/2 mL) at 50 °C under stirring. The reaction was stopped after 3 h with methanol, and the crude residue obtained by the workup was purified by preparative silica gel TLC, using EtOAc/H_2O/MeOH (8.5:2:1, v/v/v) as eluent, to give 27.3 mg of **8** as an amorphous solid resistant to crystallization: UV λ_{max} (log ϵ) 238 (4.19), 285 (3.67); ^{1}H NMR, differed from that of **1** in the absence of the glycosidic protons and the presence of the signals δ 3.65 (1H, t, J = 6.5 Hz, H_2C-8) and 2.66 (1H, t, J = 6.5 Hz, H_2C-7); ESMS (−) and (+), *m*/*z* 503 $[M - H]^-$ and 527 $[M + Na]^+$, respectively; HREIMS, *m*/*z* 504.3451 $[M]^+$; calcd for $C_{30}H_{48}O_6$, 504.3440.

Catalytic Hydrogenation of Fusapyrone (1). Fusapyrone (**1**) (65.8 mg) dissolved in methanol (4 mL) was hydrogenated using PtO_2 as catalyst. After 18 h at room temperature in the dark and under atmospheric pressure with continuous stirring, the reaction was stopped by filtration, and the residue, obtained by evaporation, was purified by preparative reverse phase TLC using MeCN/H_2O (7:3, v/v) as eluent affording the 15,16,17,18,20,21-hexahydro- (**10**) and the 15,16,17,18-tetrahydro- (**9**) derivative of **1**, both as amorphous solids resistant to crystallization (27.2 and 18.0 mg, R_f values of 0.42 and 0.52, respectively). Derivative **9** had UV λ_{max} (log ϵ) 281 (3.76) and its ^{1}H NMR differed from that of **1** in the absence of the olefinic protons (CH-15-CH-18) and the upfield shift of Me-32 (d, J = 6.5 Hz) at δ 0.91 and H-14 (m) at δ 3.8−3.2. Moreover, H-20 (d, J = 9.3 Hz) and Me-34 (d, J = 1.5 Hz) appeared at δ 5.09 and 1.70 respectively, whereas the methylene and methyne protons CH_2-15, CH_2-16, CH_2-18, and CH-17 gave a complex system at δ 1.8−1.0. The ^{13}C NMR differed from that of **1** in the presence of the only two olefinic signals of C-21 (s) and C-20 (d) at δ 136.6 and 130.1, respectively, whereas the C-18, C-16, C-15 (all t), and C-17 (d) appeared at δ 41.2, 41.1, 30.7, and

32.3, respectively. ESMS (−) and (+) *m*/*z* were 609 $[M - H]^-$ and 611 $[M + H]^+$, respectively. HREIMS *m*/*z* was 610.4069 $[M]^+$; calcd for $C_{34}H_{58}O_9$, 610.4081. Derivative **10** showed UV λ_{max} (log ϵ) 280 (3.86) and its ^{1}H NMR differed from that of **1** in the absence of all the olefinic protons and the upfield shift of both Me-32 (d, $J = 6.5$ Hz) and Me-34 (d, $J = 6.5$ Hz) centered at δ 0.91 and H-14 (m) at δ 3.8−3.2. ESMS (−) and (+) *m*/*z* were 611 $[M - H]^-$ and 635 $[M + Na]^+$ and 613 $[M + H]^+$, respectively. HREIMS *m*/*z* was 612.4226 $[M]^+$; calcd for $C_{34}H_{60}O_9$, 612.4237.

***Artemia salina* Bioassay.** The brine shrimp (*A. salina* L.) assay was performed as described by Eppley (*15*) with minor modifications. Brine shrimp eggs were obtained from a local pet shop. Eggs were hatched in artificial seawater [3.3% (w/v) marine salts in distilled water] by incubation in Petri dishes at 26 °C for 36 h, in the dark. Larvae were then transferred into 24-well culture plates (Corning, NY). Each well contained 30−40 brine shrimp larvae in 500 μL of marine water. The compounds were dissolved in MeOH and serially diluted to obtain a range of concentrations (200−0.8 μM). The methanolic solutions were transferred to the wells to a final solvent concentration of 1% (v/v). Each assay included negative control (1% MeOH in seawater). Tests were performed in quadruplicate. The percentage of larvae mortality was determined after exposure to the α-pyrones for 24 h at 27 °C and transformed in probits. The LC_{50} of each of the active compounds was calculated with data from three independent experiments by using the standard procedure of probit analysis (*16*) performed with the computer program POLO−PC (*17*).

Antifungal Activity Tests. Antifungal activity was tested against three filamentous fungi that are agents of pre- and postharvest plant diseases (*Botrytis cinerea* Pers. *ex* Fr., *Aspergillus parasiticus* Speare, *Penicilliun brevi-compactum* Dierckx) and against two yeasts [*Pichia guilliermondii* Wickerham and *Rhodotorula glutinis* (Fres.) Harrison]. The filamentous fungi were from the collection of the Istituto di Scienze delle Produzioni Alimentari (ISPA), Bari, Italy; the yeasts were kindly provided by Dr. G. Arras, of the ISPA, Section of Sassari, Italy. The minimal inhibitory concentration (MIC) of compounds **1**−**10** against both filamentous fungi and yeasts was determined by a broth dilution method in 96-well microtitration plates. Sterile Czapek Dox broth (Difco, Detroit, MI), at pH 7 (35 g of lyophilized medium dissolved in 1 L of phosphate buffer pH 7 and sterilized in the autoclave for 20 min at 110 °C), was used for the preparation of serial dilutions of the test compounds. Ten 2-fold dilutions (50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, and 0.09 μg/mL) were prepared with each compound; 180 μL of each dilution was then transferred into eight replicated microwells. Medium controls (medium without test compounds and with solvent) were also included. As reference standards for comparison of antifungal activity, the fungicides benomyl and dicloran in pure form (Dr. Ehrenstorfer GmbH, Augsburg, Germany) were used. Because of their low solubility in water, the chemical fungicides were tested in the range of concentrations from 25 to 0.09 μg/mL. Inoculum of the fungi was obtained from fresh cultures on potato dextrose agar. Each microwell was inoculated with 20 μL of fungal suspension containing either 10^5 conidia/mL (filamentous fungi) or 5×10^6 cells/mL (yeasts) in sterile Czapek Dox broth (pH 7). Plates were incubated at 27 °C for a maximum of 72 h, and growth was observed every 24 h under a reverse microscope. The MIC was identified as the highest dilution that resulted in the complete absence of growth of the test fungus. Antifungal tests were performed three times.

RESULTS AND DISCUSSION

Chemical transformations were conducted to modify the three chemical moieties present in fusapyrone (**1**) and to prepare several key derivatives to investigate structure−activity relationships in comparison to **1** and **2**. By reaction with diazomethane, **1** was converted into the 4-*O*-methyl derivative (**7**), which showed a modification of the pyrone moiety. The oxidation with sodium periodate of the sugar residue at C-3 followed by the reduction of the oxidized intermediate gave the 3-(2-hydroxyethyl)- derivative of fusapyrone (**8**), showing the degradation of the glycosyl moiety. Finally, the catalytic hydrogenation of **1** allowed the preparation of two derivatives modified on the alkyl side chain attached at C-6. Derivatives **9** and **10** were obtained from this reaction and showed the partial and total saturation of the three olefinic bonds of polysubstituted heptadecatrienyl residue at C-6, respectively. The latter was also modified in deoxyfusapyrone (**2**), which presents a methyl group at C-19 with respect to the hydroxymethyl group of **1**. The three structural components present in fusapyrone (**1**) were all modified, although in a reversible form, by acetylation of all the hydroxy groups carried out under standard conditions, thereby converting **1** into the pentaacetylderivative (**3**). The same chemical transformation was performed on deoxyfusapyrone (**2**) to obtain the corresponding tetraacetyl derivative (**4**), which is also modified on the same three moieties. Finally, by reaction with diazomethane the pentaacetyl derivative of **1** was converted in part into the corresponding 4-*O*-methyl derivative (**5**) and in part to the 2-*O*-methyl derivative of the isomeric γ-pyrone (**6**), which allowed us to test also a derivative of a 2-hydroxy-4-pyrone.

Table 1. Toxicity of Fusapyrone (**1**), Deoxyfusapyrone (**2**), and Their Derivatives (**3**–**6**) to *Artemia salina* Larvae after 24 h of Exposure

compd[a]	MW	LC_{50} (μM)	95% confidence limit (μM)	slope	LC_{50} (μg/mL)
1	606	>200.0			
2	590	37.1[b]			21.9
3	816	85.6	71.7–105.9	1.721	69.8
4	758	39.7	31.4–49.9	1.791	30.1
5	830	46.1	35.1–65.4	1.167	38.3
6	830	51.9	40.6–70.5	1.459	43.1

[a] Compounds not listed were not toxic at the highest concentration tested (200 μM). [b] Altomare et al. (*11*).

To compare the biological activities of **1**, **2**, and their derivatives, we used the 50% lethal concentration (LC_{50}) to *A. salina* larvae and MIC to several filamentous fungi and yeasts. Toxicity data of compounds **3**−**6**, compared to **1** and **2**, are summarized in **Table 1**. Among the fusapyrone (**1**) derivatives, compounds **7**−**10** were only slightly toxic to *A. salina*, similarly to **1** ($LC_{50} > 200$ μM). Pentaacetylation of **1** into **3**, **5**, and **6** resulted in an increase of toxicity, to a higher extent in the two latter derivatives containing methylation in the α- and γ-position, respectively. Compound **4**, the tetraacetyl derivative of deoxyfusapyrone (**2**), did not show a significant difference in toxicity from the lead molecule.

The only structural changes of **1** that resulted in an increase of biological activity in the *A. salina* bioassay were those that affected mainly the water solubility of the molecule. In fact, the conversion of the three hydroxy groups of the glycosyl residue, as well as those of the side chain, resulting in the corresponding acetyl derivative **3**, lowered the polarity of the molecule. Moreover, the derivatization of the hydroxy pyrone group into the corresponding methyl ether, resulting in derivatives **5** and **6**, further lowered the polarity and, consequently, the water solubility of these compounds. Hence, it appears that toxicity is correlated to hydrophobicity.

The brine shrimp (*A. salina*) lethality assay has been reported to be useful as a preliminary test for the identification of cytotoxic substances (*18*, *19*). According to McLaughlin (*19*), compounds with $LC_{50} < 1000$ ppm are considered to be potentially toxic against tumor cell lines and worthy of being evaluated in more specific antitumor systems. On the basis of the results of the present study, compounds **2**−**6** could be evaluated for this use.

The antifungal bioassays showed the same results in the three independent experiments performed. The antifungal activity tests

Table 2. Antifungal Activity of Fusapyrone (**1**), Deoxyfusapyrone (**2**), and Some Derivatives, in Comparison with Chemical Fungicides Benomyl and Dicloran, toward Filamentous Fungi

species and strain	compd[b]	MIC[a] (μg/mL) 24 h	48 h	72 h
Aspergillus parasiticus ITEM-11	**1**	6.25	6.25	12.50
	2	0.78	0.78	0.78
	benomyl	>25.00	>25.00	>25.00
	dicloran	>25.00	>25.00	>25.00
Botrytis cinerea ITEM-966	**1**	1.56	3.12	6.25
	2	0.78	1.56	1.56
	7	25.00	50.00	50.00
	9	50.00	50.00	50.00
	10	50.00	50.00	50.00
	benomyl	0.19	0.19	0.19
	dicloran	3.12	6.25	6.25
Penicillium brevi-compactum ITEM-449	**1**	0.78	3.12	6.25
	2	0.78	1.56	1.56
	benomyl	>25.00	>25.00	>25.00
	dicloran	12.50	25.00	>25.00

[a] Minimum inhibitory concentration. [b] Compounds not listed did not inhibit fungal growth at the highest concentration tested (50 μg/mL). The highest concentration of chemical fungicides tested was 25 μg/mL.

showed that all of the modifications made to **1** and **2** resulted in a loss of toxicity toward filamentous fungi. Among derivatives **3**−**10**, only compounds **7**, **9**, and **10** retained some activity, limited to *B. cinerea* and only at high concentration (**Table 2**), whereas all of the others were inactive. The antifungal activities of **1** and **2** were shown to be considerably higher than that of either benomyl or dicloran on *A. parasiticus* and *P. brevi-compactum*, where only benomyl was able to reduce the germ tube elongation but not to inhibit the germination of conidia. Compounds **1** and **2** were also more active than dicloran, but not benomyl, toward *B. cinerea*. None of the compounds **1**−**10** inhibited the growth of yeasts at the highest concentration tested (50 μg/mL).

Structurally **1** and **2** consist of a hydrophilic sugar residue opposed to a long hydrophobic chain and, therefore, possess an amphiphilic nature that in other cases has been linked to antifungal and antibiotic properties (*20*, *21*). These molecules, known as biosurfactants, interact with plasma membranes and cause loss of membrane integrity and cell lysis. A similar mechanism may be involved in the antifungal activities of **1**, **2**, and, to a lesser extent, **7**, **9**, and **10**. The different sensitivities exhibited by filamentous fungi and yeasts is likely due to diversity in cell wall structure and permeability to these molecules or to a variable fatty acid composition of plasma membranes (*22*). Should this be the mechanism of action of α-pyrones, the probability of developing insensitive strains would be low, because development of resistance would require a major structural change in the chemical makeup of the plasma membrane. This is particularly important for those phytopathogenic fungi, such as *B. cinerea*, that easily develop resistance to chemical pesticides. Fusapyrone (**1**), applied as a 100 μg/mL solution, was found to be very active in inhibiting the development of gray mold (*B. cinerea*) on wounded grapes in vitro (*23*).

The two yeasts (*P. guilliermondii* and *R. glutinis*) that were utilized in our bioassays are effective biological control agents that have been successfully used for the control of postharvest diseases of citrus fruits (*24*). These particular strains were chosen as test organisms in order to assess their tolerance to the α-pyrones for a possible use of α-pyrones in combination with biocontrol agents for plant disease control. Therefore, the combined use of **1** and **2** with antagonistic yeasts appears to be a feasible approach for the reduction of synthetic pesticides. Compound **1** appears to be particularly interesting because of its lower zootoxicity.

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LITERATURE CITED

(1) El-Ghaouth, A.; Smilanick, J. L.; Brown, G. E.; Ippolito, A.; Wisniewski, M.; Wilson, C. L. Application of *Candida saitoana* and glycolchitosan for the control of postharvest diseases of apple and citrus fruit under semi-commercial conditions. *Plant Dis.* **2000**, *84*, 243−248.

(2) Evidente, A.; Conti, L.; Altomare, C.; Bottalico, A.; Sindona, G.; Segre, A. L.; Logrieco, A. Fusapyrone and deoxyfusapyrone, two antifungal α-pyrones from *Fusarium semitectum*. *Nat. Toxins* **1994**, *2*, 4−13.

(3) Dickinson, J. M. Microbial pyran-2-ones and dihydropyran-2-ones. *Nat. Prod. Rep.* **1993**, *10*, 71−98.

(4) Suzuki, K.; Kuwahara, A.; Yoshida, H.; Fujita, S.; Nishikiori, T.; Nakagawa, T. NF00659A1, A2, A3, B1 and B2, novel antitumor antibiotics produced by *Aspergillus* sp. NF00659. I. Taxonomy, fermentation, isolation and biological activities. *J. Antibiot.* **1997**, *50*, 314−317.

(5) Kondoh, M.; Usui, T.; Kobayashi, S.; Tsuchiya, K.; Nishikawa, K.; Nishikiori, T.; Mayumi, T.; Osada, H. Cell cycle arrest and antitumor activity of pironetin and its derivatives. *Cancer Lett.* **1998**, *126*, 29−32.

(6) Thaisrivongs, S.; Romero, D. L.; Tommasi, R. A.; Janakiraman, M. N.; Strohbach, J. W.; Turner, S. R.; Biles, C.; Morge, R. R.; Johnson, P. D.; Aristoff, P. A.; Tomich, P. K.; Lynn, J. C.; Horng, M. M.; Chong, K. T.; Hinshaw, R. R.; Howe, W. J.; Finzel, B. C.; Watenpaugh, K. D. Structure-based design of HIV protease inhibitors: 5,6-dihydro-4-hydroxy-2-pyrones as effective, nonpeptidic inhibitors. *J. Med. Chem.* **1996**, *39*, 4630−4642.

(7) Poppe, S. M.; Slade, D. E.; Chong, K. T.; Hinshaw, R. R.; Pagano, P. J.; Markowitz, M.; Ho, D. D.; Mo, H.; Gorman, R. R., 3rd; Dueweke, T. J.; Thaisrivongs, S.; Tarpley, W. G. Antiviral activity of the dehydropyrone PNU-140690, a new nonpeptidic human immunodeficiency virus protease inhibitor. *Antimicrob. Agents Chemother.* **1997**, *41*, 1058−1063.

(8) Turner, S. R.; Strohbach, J. W.; Tommasi, R. A.; Aristoff, P. A.; Johnson, P. D.; Skulnick, H. I.; Dolak, L. A.; Seest, E. P.; Tomich, P. K.; Bohanon, M. J.; Horng, M. M.; Lynn, J. C.; Chong, K. T.; Hinshaw, R. R.; Watenpaugh, K. D.; Janakiraman, M. N.; Thaisrivongs, S. Tipranavir (PNU-140690): A potent, orally bioavailable nonpeptidic HIV protease inhibitor of the 5,6-dihydro-4-hydroxy-2-pyrone sulfonamide class. *J. Med. Chem.* **1998**, *41*, 3467−3476.

(9) Nakajima, H.; Nishimura, K.; Hamasaki, T.; Kimura, Y.; Yokota, T. Structure of neovasinone, a new α-pyrone plant growth regulator produced by the fungus *Neocosmopora vasinfecta*. *Agric. Biol. Chem.* **1987**, *51*, 1221−1224.

(10) Kobayashi, S.; Tsuchiya, K.; Kurokawa, T.; Nakagawa, T.; Shimada, N.; Iitaka, Y. Pironetin, a novel plant growth regulator produced by *Streptomyces* sp. NK10958. II. Structural elucidation. *J. Antibiot.* **1994**, *47*, 703−707.

(11) Tsuchiya, K.; Kobayashi, S.; Nishikiori, T.; Nakagawa, T.; Tatsuta, K. NK10958P, a novel plant growth regulator produced by *Streptomyces* sp. *J. Antibiot.* **1997**, *50*, 259−260.

(12) Altomare, C.; Perrone, G.; Zonno, M. C.; Evidente, A.; Pengue, R.; Fanti, F.; Polonelli, L. Biological characterization of fusapyrone and deoxyfusapyrone, two bioactive secondary metabolites of *Fusarium semitectum*. *J. Nat. Prod.* **2000**, *63*, 1131−1135.

(13) Breitmaier, E.; Voelter, W. *Carbon 13 NMR Spectroscopy*; VCH: Weinheim, Germany, 1987; pp 73−106.
(14) Evidente, A.; Amalfitano, C.; Pengue, R.; Altomare, C. High-performance liquid chromatography for the analysis of fusapyrone and deoxyfusapyrone, two antifungal α-pyrones from *Fusarium semitectum*. *Nat. Toxins* **1999**, *7*, 133−137.
(15) Eppley, R. M. Sensitivity of brine shrimp (*Artemia salina*) to trichothecenes. *J. Assoc. Off. Anal. Chem.* **1974**, *57*, 618−620.
(16) Finney, D. J. *Probit Analysis: Statistical Treatment of the Sigmoid Response Curve*; Cambridge University Press: London, U.K., 1964.
(17) LeOra Software. *POLO−PC a User's Guide to Probit or Logit Analysis*; LeOra Software, Berkeley, CA, 1987.
(18) Logrieco, A.; Moretti, A.; Fornelli, F.; Fogliano, V.; Ritieni, A.; Caiaffa, M. F.; Randazzo, G.; Bottalico, A.; Macchia, L. Fusaproliferin production by *Fusarium subglutinans* and its toxicity to *Artemia salina*, SF-9 insect cells, and IARC/LCL 171 human B lymphocytes. *Appl. Environ. Microbiol.* **1996**, *62*, 3378−3384.
(19) McLaughlin, J. M. Crown gall tumours on potato discs and brine shrimp lethality: Two simple bioassays for higher plant screening and fractionation. In *Methods in Plant Biochemistry*; Hostettmann, K., Ed.; Academic Press: New York, 1991; Vol. 6, Chapter 1, pp 1−32.
(20) Stanghellini, M. E.; Miller, R. M. Biosurfactants: Their identity and potential efficacy in the biological control of zoosporic plant pathogens. *Plant Dis.* **1997**, *81*, 4−12.
(21) Vollenbroich, D.; Pauli, G.; Özel, M.; Vater, J. Antimycoplasma properties and application in cell culture of surfactin, a lipopeptide antibiotic from *Bacillus subtilis*. *Appl. Environ. Microbiol.* **1997**, *63*, 44−49.
(22) Gruiz, K. Fungitoxic activity of saponins: Practical use and fundamental principles. *Adv. Exp. Med. Biol.* **1996**, *404*, 527−534.
(23) Altomare, C.; Perrone, G.; Stornelli, C.; Bottalico, A. Prove di lotta contro la muffa grigia dell'uva con fusapirone, un metabolita di *Fusarium. Quaderni della Scuola di specializzazione in Scienze viticole ed enologiche.* **1998**, *22*, 59−66.
(24) Arras, G.; Scherm, B.; Migheli, Q. Improving biocontrol activity of *Pichia guilliermondii* against post-harvest decay of oranges in commercial packing-houses by reduced concentrations of fungicide. *Biocontrol Sci. Technol.* **2002**, *12*, 547−553.

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