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ANTIFUNGAL COMPOUNDS FROM *DIOSCOREA BATATAS* INOCULATED WITH *PSEUDOMONAS CICHORII*

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Key Word Index—*Dioscorea batatas*; Dioscoreaceae; Chinese yam; phytoalexins; antimicrobial activity; oxygenated bibenzyls; phenanthrenes.

Abstract—An induced and six preformed antifungal compounds were isolated from Chinese yam (*Dioscorea batatas*) inoculated with the bacterium *Pseudomonas cichorii*. The induced compound, a phytoalexin, was identified as dihydropinosylvin. The preformed compounds were characterized as oxygenated bibenzyls and phenanthrenes.

INTRODUCTION

We recently reported [1] the isolation of two sesquiterpenoid phytoalexins, costunolide and lettucenin A, from diseased lettuce leaves. In a continuation of our search for antimicrobial compounds of diseased vegetables, we have isolated several antifungal compounds from Chinese yam (*Dioscorea batatas* Decne = *D. opposita* Thunb.) inoculated with the bacterium *Pseudomonas cichorii*. Chinese yam occurs naturally in Japan where it is cultivated and its storage root is used as a food. Several phenanthrenes, dihydrophenanthrenes and bibenzyls have been isolated from *Dioscorea* species and related *Tamus* species [2–7]. Of these, batatasins I–V with dormancy-inducing activity have been isolated from dormant aerial bulbils of *D. batatas* [2]. The present paper describes the identification of a major antifungal compound, which qualifies as a phytoalexin, as dihydropinosylvin (3,5-dihydroxybibenzyl) [8] and the characterization of six preformed antifungal compounds, including two new phenols.

RESULTS AND DISCUSSION

Sliced tuber tissues of *D. batatas* were inoculated with a suspension of *P. cichorii* (*ca* 10^8 cells/ml) and incubated at 15° for 3 days. After being dried, the inoculated tissues were extracted with acetone and then with methanol. These extracts were submitted to sequential chromatography over silica gel and Sephadex LH-20, monitored by TLC bioassay, to give seven antifungal phenols: **1** (0.036% of the dry wt of the tissues), **2** (0.002%), **3** (0.013%, **4** (0.001%), **5** (0.003%), **6** (0.0004%), **7** (0.0004%).

The major phenol (**1**) had the molecular formula $C_{14}H_{14}O_2$ and gave a dimethyl ether (**1a**). The spectral data (^{1}H NMR, ^{13}C NMR, UV, IR and MS) indicated **1** to be 3,5-dihydroxybibenzyl (dihydropinosylvin), which was confirmed by direct comparison with an authentic synthetic sample [9]. This phenol was not detected in the extracts from intact healthy tissues of the plant, and hence qualified as a phytoalexin. As far as we know, this compound, dihydropinosylvin, is the first phytoalexin with a bibenzyl structure. It is also the first phytoalexin to be found in the Dioscoreaceae. The corresponding dehydro compound, pinosylvin, was reported as a phytoalexin of *Pinus* species [10].

Two groups of three phenols, **2–4** and **5–7**, exhibited spectroscopic characteristics of substituted bibenzyls and phenanthrenes (see Experimental), respectively [11–13]. Of these, phenol **2** had the molecular formula $C_{15}H_{16}O_2$ and formed a monomethyl ether (**2a**), which was identical to **1a**. Thus phenol **2**, showing the characteristic mass spectrum (*m/z* 137 $[C_8H_9O_2]^+$ and 91 $[C_7H_7]^+$), was formulated as 3-hydroxy-5-methoxybibenzyl. This compound has been reported, without spectral evidence, to occur in the heartwood of *Pinus albicaulis* Engelm [14]. Three phenols, **3**, **5** and **6**, were assigned molecular formulae $C_{15}H_{16}O_3$, $C_{17}H_{16}O_4$ and $C_{16}H_{14}O_4$, and were easily identified as 3,2′-dihydroxy-5-methoxybibenzyl (batatasin IV) [2], 6-hydroxy-2,4,7-trimethoxyphenanthrene (batatasin I) [15, 16] and 6,7-dihydroxy-2,4-dimethoxyphenanthrene [13], respectively, on the basis of their spectral data (^{1}H NMR, IR, UV and MS), which were virtually identical to the reported ones. This was supported by formation of the monoacetate (**5a**) of **5**, the diacetate (**6a**) of **6**, and the monomethyl ether (**5b**) [= dimethyl ether (**6b**) of **6**] of **5**.

The new phenol **4** had the molecular formula $C_{16}H_{18}O_3$ and formed a monomethyl ether (**4a**) which was identical to the dimethyl ether **3a** of **3**. Its mass spectrum (*m/z* 107 $[C_7H_7O]^+$ and 151 $[C_9H_{11}O_2]^+$) revealed that **4** was consisted of a hydroxybenzyl and a dimethoxybenzyl moiety. Hence the phenol was assigned the 2′-hydroxy-3,5-dimethoxybibenzyl structure.

The new phenol **7** had the same molecular formula ($C_{16}H_{14}O_4$) as **6** and gave a diacetate (**7a**) and a dimethyl ether (**7b**), the latter being identical to **5b** (= **6b**). Thus **7** possesses a 2,4,6,7-tetraoxygenated (OH or OMe) phenanthrene structure. In accordance with this, the ^{1}H NMR spectrum indicated the presence of two hydroxyl and two methoxyl groups [δ3.9 and 4.00 (each 3H, *s*)] as well as six

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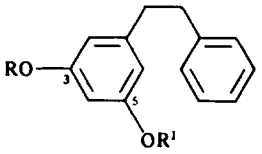
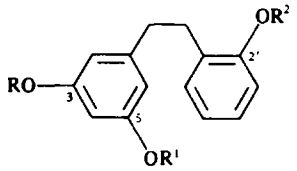
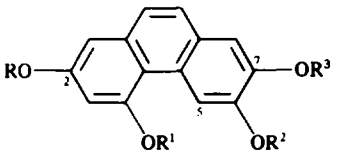


1 R = R¹ = H
1a (= **2a**) R = R¹ = Me
2 R = H, R¹ = Me



3 R = R² = H, R¹ = Me
3a (= **4a**) R = R¹ = R² = Me
4 R = R¹ = Me, R² = H



5 R² = H, R = R¹ = R³ = Me
5a R² = Ac, R = R¹ = R³ = Me
5b (= **6b** = **7b**) R = R¹ = R² = R³ = Me

6 R² = R³ = H, R = R¹ = Me
6a R² = R³ = Ac, R = R¹ = Me
7 R = R³ = H, R¹ = R² = Me
7a R = R³ = Ac, R¹ = R² = Me

aromatic protons [δ7.10 and 8.98 [12] (each 1H, *s*, H-8 and H-5),7.12 and 7.30 (each 1H, AB*q*, J = 8 Hz, H-9 and H-10 or vice versa) and 6.67 and 6.77 (each 1H, *d*, J = 2.5 Hz, H-1 and H-3 or vice versa)]. In the NOE difference spectra of **7**, NOEs were observed between the methoxy protons at δ4.00 and both the protons at δ6.67 (H-1 or H-3) and 8.98 (H-5), and also between the other methoxy protons at δ3.90 and the proton at δ8.98. This result clearly revealed that the methoxyl groups at δ4.00 and 3.90 were located at C-4 and C-6, respectively, and hence the two hydroxyl groups were placed at C-2 and C-7. This disposition of the substituents was consistent with the observations that in the ^{1}H NMR spectra, the signal due to H-5 was shifted slightly to lower field in passing from **7** to **7a**, while those due to H-1, H-3 and H-8 displayed large low-field shifts of 0.3–0.4 ppm, owing to the presence of adjacent acetoxyl groups [11, 17]. Thus phenol **7** is represented by the 2,7-dihydroxy-4,6-dimethoxyphenanthrene structure.

Coxon *et al.* [6] reported that antifungal compounds, found in the intact peel extracts of Brazilian yam, were either absent or present at much lower concentration in flesh extracts. In the present study, the six phenols **2**–**7** were detected by HPLC in extracts of intact outer tissues (peel) while only batatasin I (**5**) was found in the inner tissues (flesh). Contrary to our observation, Ireland *et al.* [18] reported that no defined batatasins could be detected in the extracts of peeled tubers (flesh) of *D. batatas*. These apparently different results may simply be a reflection of the use of different periods of incubation after peeling and slicing the tubers. Indeed, when the flesh was incubated for 6 days without inoculation, batatasin I (**5**) and some other compounds, pre-existing in the peel tissues, increased to a detectable amount.

The antimicrobial activity of all the phenols (**1**–**7**) was examined against 24 species of fungi and 6 genera of bacteria (41 strains), some of the results being listed in Table 1. It was found that: (1) fungi were affected more effectively than bacteria; (2) bibenzyls **1**–**4** appeared to be more active than phenanthrenes **5**–**7**; and (3) dihydropinosylvin (**1**) completely inhibited the growth of *Pyricularia oryzae* at 75 ppm, while it did not show any significant antibacterial activity even at 400 ppm against some strains of the genera *Erwinia* and *Pseudomonas*.

EXPERIMENTAL

Mps: uncorr.; ^{1}H (100 or 400 MHz) and ^{13}C (25 MHz) NMR: $CDCl_3$, unless otherwise stated. MS: direct inlet, 70 eV.

Induction and isolation of dihydropinosylvin (**1**). Chinese yam tubers (*Dioscorea batatas*) of an unknown variety were purchased locally. The tubers were cut into 4 mm thick slices, which were kept in a moist chamber at 15° for 1 day and then inoculated with *Pseudomonas cichorii* (*ca* 10^8 cells/ml). After being incubated at 15° for 3 days, the browned slices were air-dried at 60°. The slices (111 g) were extracted with Me_2CO and then with MeOH, and the respective solns were evaporated under red. pres. below 35° to give an Me_2CO (0.3 g) and a MeOH extract (1.6 g), respectively. Both extracts were submitted separately to 2D-TLC analysis; a set of plates was prepared and developed with Et_2O and CH_2Cl_2–MeOH (49:1). One plate was examined under UV light and then sprayed with a ceric sulphate–H_2SO_4 soln and heated, while the other plate was used for bioassay with *Bipolaris leersiae* as the test fungus [19]. The Me_2CO extract showed several spots with weak antifungal activity. Most of these spots were also detected in the Me_2CO extract of intact peel tissues, which were obtained by removing a 1 mm (approx.) thick layer from the outer surface of the tubers. The purification procedure of these pre-existing components will be described below (see *Large-scale isolation*). By contrast, the MeOH extracts revealed one major

Table 1. Inhibition of microbial growth by compounds 1–7*

Fungus or bacterium	1		2		3		4		5		6		7	
	100†	25	100	25	100	25	100	25	100	25	100	25	100	25
Alternaria japonica	+	+	+	+	+	±	+	+	–	–	–	–	±	–
	+		+				+							
Botrytis alli	+	+	+	±	+	±	+	+	±	–	±	–	+	–
	+	+	+				+							
Caloneeteria graminicola	+	+	+	+	+	±	+	+	±	–	+	+	+	+
Pyricularia oryzae	+	+	+	+	+	+	+	+	+	+	+	–	±	–
					+		+							
Fusarium solani f. sp. *pici*	+	+	+	±	+	±	+	+	+	–	–	–	+	–
Agrobacterium tumefaciens	+	+	+	–	+	–	+	+	+	–	+	–	+	–
					+		+						+	
Bacillus subtilis	+	+	+	–	+	–	+		±	–	+	–	+	–
					+						+		+	
Pseudomonas cichorii		–	–	–	–	–			–	–	–	–	–	–
P. solanacearum	–	–	–	–	–	–	–		–	–	–	–	–	–
Xanthomonas campestris pv. *oryzae*	±	–	+	±	–	–	–		–	–	+	+	±	–

*Fungal and bacterial growth were observed 2 days after incubation at 25° in potato dextrose (fungi) and potato sucrose (bacteria) broth media. Intensity of antifungal activity was scored and classified into four grades: —, normal growth; ±, about half of the normal growth; +, a little growth; + +, no growth.

†Concentration, ppm.

spot showing strong antifungal activity, which was not detected in extracts of either the intact peel or the flesh. The MeOH extract was then treated with EtOAc, and the EtOAc-soluble fraction was separated by chromatography over silica gel with CH_2Cl_2–MeOH (19:1), each fraction being checked by the TLC bioassay. Combined active fractions (67 mg) were purified by chromatography over silica gel with Et_2O to give more active fractions (34 mg), from which **1** (28 mg) was isolated after chromatography on Sephadex LH-20 with MeOH.

Dihydropinosylvin (**1**). Viscous oil; TLC (silica gel), R_f 0.42 (Et_2O) and 0.10 (CH_2Cl_2–MeOH, 49:1); Gibbs reagent, purple; high-resolution MS *m/z* 214.0989 $[M]^+$ (calc. for $C_{14}H_{14}O_2$: 214.0994); EIMS *m/z* (rel. int.): 214 $[M]^+$ (49), 123 $[C_7H_7O_2]^+$ (66), 91 $[C_7H_7]^+$ (100); UV λ_{max}^{EtOH} nm (log ε): 210 (4.31), 274 (3.19), 282 (3.18); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3330, 1602, 1147; ^1H NMR: δ2.78 (4H, *s*, $ArC\underline{H_2}C\underline{H_2}Ar$), 4.84 (2H, *br*, 2OH, exchangeable with D_2O), 6.2 (3H, *m*, H-2, H-4 and H-6), 7.2 (5H, *m*, H-2′–H-6′); ^{13}C NMR: δ37.28 and 37.57 (each *t*, $Ar\underline{C}H_2\underline{C}H_2Ar$), 100.54 (*d*, C-4), 108.20 (*d*, C-2 and C-6), 125.90 (*d*, C-4′), 128.34 (*d*, C-2′, C-3′, C-5′ and C-6′), 141.49 (*s*, C-1′), 144.95 (*s*, C-1), 156.37 (*s*, C-3 and C-5). Compound **1** was prepared from 3,5-dimethoxybenzaldehyde and benzyl chloride by a reported procedure [9]. The synthetic sample was identical to the natural one in all respects.

Large-scale isolation. The inoculated, dried tuber slices (4.6 kg), prepared in the same manner as described above, were extracted successively with Me_2CO and MeOH to give an Me_2CO (13 g) and a MeOH extract (85 g). The Me_2CO extract was treated with EtOAc (60 ml) to yield an EtOAc-soluble fraction (12 g), which was purified by chromatography over silica gel with EtOAc. The eluate was evaporated to leave a brown oil (10 g), which was separated into 5 fractions by chromatography on Sephadex LH-20 (50ϕ × 350 mm) with EtOAc–MeOH (1:9). The third (260 ml) and fourth (480 ml) fractions were evaporated to leave oily residues, R_1 (968 mg) and R_2 (294 mg), which showed a single and three spots by the TLC bioassay, respectively. Residue R_1, showing several spots on TLC under UV (254 nm) and with ceric sulphate reagent, was separated further into six fractions (190, 220, 80, 220, 420 and 500 ml) by chromatography over silica gel (100 g) with CH_2Cl_2–MeOH (49:1). The second fraction, showing two spots on TLC (bioassay), was evaporated and fractionated by chromatography over silica gel with CH_2Cl_2–MeOH (99.5:0.5) to give **4** (10 mg) and **2** (29 mg) in pure state. The fourth and fifth fractions, each showing a single spot on TLC (bioassay, UV and ceric sulphate), were purified in the same manner as the second fraction to yield **3** (111 mg) and **1** (303 mg), respectively. Residue R_2, showing three fluorescent spots under UV (366 nm), was separated into eight fractions (60, 20, 40, 10, 20, 40, 500 and 1000 ml) by chromatography over silica gel (30 g) with CH_2Cl_2–MeOH (49:1). The fourth and fifth fractions, showing the same single spot on TLC [bioassay and UV (366 nm)], were combined and evaporated to leave a crystalline residue (40 mg), which was purified by chromatography over silica gel (5 g) with CH_2Cl_2–MeOH (99.5:0.5) to give **7** (18 mg) in pure state. The second and sixth fractions, each showing a single spot on TLC, were concentrated to leave crystalline **5** (118 mg) and **6** (20 mg).

The MeOH extracts, showing no UV (366 nm) fluorescent spot on TLC, were fractionated practically in the same manner as the Me_2CO extracts to give **1** (1.35 g), **2** (67 mg), **3** (493 mg) and **4** (41 mg). The total amounts of antifungal compounds from the diseased tubers were as follows: **1** (1.65 g), **2** (96 mg), **3** (604 mg), **4** (51 mg), **5** (118 mg), **6** (20 mg) and **7** (18 mg).

3-Hydroxy-5-methoxybibenzyl (**2**). Mp 45–49°, lit. 49–50° [14]; TLC, R_f 0.42 (CH_2Cl_2–MeOH, 49:1); high-resolution MS *m/z*: 228.1139 $[M]^+$ (calc. for $C_{15}H_{16}O_2$: 228.1149); EIMS *m/z* (rel. int.): 228 $[M]^+$ (49), 137 (100), 91 (61); UV λ_{max}^{EtOH} nm (log ε): 205

(3.61), 228 (sh, 2.96), 273 (2.06), 281 (2.06); IR ν_{max}^{neat} cm^{-1}: 3490–3250, 1610, 1140; ^1H NMR: δ2.87 (4H, *s*, ArC$\underline{H_2}$C$\underline{H_2}$Ar), 3.75 (3H, *s*, OMe), 5.05 (1H, *s*, OH, exchangeable with D_2O), 6.27 (3H, *m*, H-3, H-4 and H-5), 7.25 (5H, *m*, H-2′–H-6′).

Compound **2** (3 mg) in Me_2CO (1 ml) was heated with Me_2SO_4 (8 drops) and K_2CO_3 (10 mg) under reflux for 30 hr with stirring. The ppts. were removed by filtration while hot and washed with Me_2CO. The filtrate and washings were combined and evaporated to leave a residue, which was dissolved in Et_2O. After being washed with 25% NH_4OH and dried, the Et_2O soln was evaporated to give 3,5-dimethoxydihydropinosylvin (**2a**). This compound was also prepared by methylation of **1** and was identical to a synthetic specimen obtained by a reported procedure [9].

3,2′-Dihydroxy-5-methoxybibenzyl (*batatasin IV*) (**3**). Mp 98–99° (needles from $CHCl_3$–CCl_4), lit. 99.5–100.5° [3]; TLC, R_f 0.13 (CH_2Cl_2–MeOH, 49:1); high-resolution MS *m/z*: 244.1001 $[M]^+$ (calc. for $C_{15}H_{16}O_3$: 244.1099); EIMS *m/z* (rel. int.): 244 $[M]^+$ (19), 138 (14), 137 (16), 107 (100), 77 (16); UV λ_{max}^{EtOH} nm (log ε): 209 (3.60), 222 (sh, 3.31), 274 (2.78), 280 (sh, 2.74); IR ν_{max}^{KBr} cm^{-1}: 3510–3255, 1610, 1590, 1490, 1450, 1300, 1260, 1195, 1150, 1060, 750; ^1H NMR: 2.85 (4H, *s*, ArC$\underline{H_2}$C$\underline{H_2}$Ar), 3.73 (3H, *s*, OMe), 4.86 and 5.08 (each 1H, *s* (*br*) 2OH, exchangeable with D_2O), 6.30 (3H, *m*, H-2, H-4 and H-6), 6.80 and 7.08 (each 2H, *m*, H-3′–H-6′).

2′-Hydroxy-3,5-dimethoxybibenzyl (**4**). Viscous oil; TLC, R_f 0.50 (CH_2Cl_2–MeOH, 49:1); high-resolution MS *m/z*: 258.1269 $[M]^+$ ($C_{16}H_{18}O_3$ requires: 258.1256); EIMS *m/z* (rel. int.): 258 $[M]^+$ (63), 151 (71), 107 (100); UV λ_{max}^{EtOH} nm (log ε): 205 (3.65), 223 (sh, 3.16), 273 (2.63), 280 (2.59); IR ν_{max}^{neat} cm^{-1}: 3560–3280, 1595, 1465, 1205, 1155, 1065, 750; ^1H NMR: δ2.88 (4H, *s*, ArC$\underline{H_2}$C$\underline{H_2}$Ar), 3.75 (6H, *s*, 2OMe), 4.67 (1H, *s*, OH, exchangeable with D_2O), 6.34 (3H, *s*, H-3, H-4 and H-5), 6.81 and 7.10 (each 2H, *m*, H-2′–H-4′).

Compounds **3** and **4** were treated separately with Me_2SO_4 in the same manner as **2** to give the same methyl ether (**3a** = **4a**), 3,5-2′-trimethoxybibenzyl; ^1H NMR: δ2.86 (4H, *s*, ArC$\underline{H_2}$C$\underline{H_2}$Ar), 3.77 (6H, *s*, 2OMe), 3.83 (3H, *s*, OMe), 6.35 (3H, *m*, H-2, H-4 and H-6), 6.91 and 7.14 (each 2H, *m*, H-3′–H-6′).

6-Hydroxy-2,4,7-trimethoxyphenanthrene (*batatasin I*) (**5**). Mp 146–147° (needles from MeOH), lit. 145–147° [17]; TLC, R_f 0.60 (CH_2Cl_2–MeOH, 49:1); high-resolution MS *m/z*: 284.1045 $[M]^+$ (calc. for $C_{17}H_{16}O_4$: 284.1042); EIMS *m/z* (rel. int.): 284 $[M]^+$ (100), 269 (28), 241 (13), 142 (19); UV λ_{max}^{EtOH} nm (log ε): 220 (sh, 4.33), 259 (5.04), 282 (4.27), 294 (sh, 4.12), 304 (sh, 3.96), 327 (3.49), 343 (3.85), 360 (4.00); IR ν_{max}^{KBr} cm^{-1}: 3600–3550, 1610, 1575, 1505, 1305, 1195, 1115, 880, 855, 825, 815; ^1H NMR: δ3.93, 4.02 and 4.07 (each 3H, *s*, 3OMe), 5.80 (1H, *br*, OH, exchangeable with D_2O), 6.74 and 6.87 (each 1H, *d*, *J* = 2.5 Hz, H-1 and H-3 or vice versa), 7.19 (1H, *s*, H-8), 7.50 and 7.62 (each 1H, *d*, *J* = 8 Hz, H-9 and H-10 or vice versa), 9.10 (1H, *s*, H-5).

6,7-Dihydroxy-2,4-dimethoxyphenanthrene (**6**). Mp 210–213° (plates from MeOH), lit. 213–214° [13]; TLC, R_f 0.13 (CH_2Cl_2–MeOH, 49:1); high-resolution MS *m/z*: 270.0869 $[M]^+$ (calc. for $C_{16}H_{14}O_4$: 270.0846); EIMS *m/z* (rel. int.): 270 $[M]^+$ (100), 255 (29), 277 (27), 212 (24), 184 (19), 135 (18); UV λ_{max}^{EtOH} nm (log ε): 260 (4.93), 283 (4.18), 294 (sh, 4.02), 305 (3.88), 329 (3.41), 344 (3.75), 362 (3.88); IR ν_{max}^{KBr} cm^{-1}: 3500–3230, 1615, 1585, 1490, 860, 840, 810; ^1H NMR (Me_2CO-d_6): δ3.90 and 4.08 (each 3H, *s*, 2OMe), 6.77 and 6.96 (each 1H, *d*, *J* = 2 Hz, H-1 and H-3 or vice versa), 7.28 (1H, *s*, H-8), 7.48 and 7.60 (each 1H, *d*, *J* = 8 Hz, H-9 and H-10 or vice versa), 8.27 (2H, *br*, 2OH, exchangeable with D_2O), 9.10 (1H, *s*, H-5).

Compound **6** (2 mg) was treated with Ac_2O (1 ml) and C_5H_5N (1 ml) at room temp. for 3 hr and worked up as usual to give 6,7-diacetoxy-2,4-dimethoxyphenanthrene, mp 144–147°, lit. 146–147° [12]; EIMS *m/z*: 354 $[M]^+$; ^1H NMR (Me_2CO-d_6): δ2.20 and 2.22 (each 3H, *s*, 2OAc), 3.83 and 4.00 (each 3H, *s*, 2OMe), 6.76 and 6.96 (each 1H, *d*, *J* = 2.5 Hz, H-1 and H-3 or vice versa), 7.63 (3H, *s* (*br*), H-8, H-9 and H-10), 9.24 (1H, *s*, H-5).

2,7-Dihydroxy-4,6-dimethoxyphenanthrene (**7**). Mp 193–194° (rhombics from MeOH); TLC, R_f 0.25 (CH_2Cl_2–MeOH, 49:1); high-resolution MS *m/z*: 270.0887 $[M]^+$ ($C_{16}H_{14}O_4$ requires: 270.0846); EIMS *m/z* (rel. int.): 270 $[M]^+$ (100), 255 (29), 227 (15), 135 (12); UV λ_{max}^{EtOH} (log ε): 261 (4.92), 282 (4.15), 295 (4.05), 347 (3.64), 364 (3.75); IR ν_{max}^{KBr} cm^{-1}: 3550–3150, 1610, 1590, 1490, 870, 855, 800; ^1H NMR (Me_2CO-d_6): δ3.90 and 4.00 (each 3H, *s*, 2OMe), 6.67 and 6.77 (each 1H, *d*, *J* = 2.5 Hz, H-3 and H-1), 7.10 (1H, *s*, H-8), 7.12 and 7.30 (each 1H, *d*, *J* = 8 Hz, H-9 and H-10 or vice versa), 7.66 and 8.40 (each 1H, *s*, 2OH), 8.98 (1H, *s*, H-5).

Compound **7** was acetylated in the same manner as **6** to give 2,7-diacetoxy-4,6-dimethoxyphenthrene, mp 226–229° (rhombics from MeOH); ^1H NMR (Me_2CO-d_6): δ2.19 and 2.20 (each 3H, *s*, 2OAc), 4.07 and 4.08 (each 3H, 2OMe), 6.97 and 7.20 (each 1H, *d*, *J* = 2.5 Hz, H-3 and H-1), 7.51 (1H, *s*, H-8), 7.54 and 7.63 (each 1H, *d*, *J* = 8 Hz, H-9 and H-10 or vice versa), 9.19 (1H, *s*, H-5).

Compounds **5**, **6** and **7** were methylated separately with Me_2SO_4 in the same way as **2** to give the same methyl ether (**5b** = **6b** = **7b**), 2,4,6,7-tetramethoxyphenanthrene, mp 138–140°, lit. 139–140° [11]; EIMS *m/z*: 298 $[M]^+$; ^1H NMR: δ3.95, 4.03, 4.09 and 4.10 (each 3H, *s*, 4OMe), 6.75 and 6.89 (each 1H, *d*, *J* = 2.5 Hz, H-1 and H-3 or vice versa), 7.21 (1H, *s*, H-8), 7.52 and 7.64 (each 1H, *d*, *J* = 8 Hz, H-9 and H-10 or vice versa), 9.11 (1H, *s*, H-5).

HPLC analysis. Peel tissues were obtained by removing a 1 mm (approx.) thick layer from the outer surface of intact tubers. The remaining inner tissues were sliced and used as flesh tissues. After being kept in a moist chamber at 20° for 1 day, the peel and flesh tissues were treated in the same manner as described in the Isolation procedure to give peel and flesh tissue extracts. Moreover, the extracts from the diseased flesh tissues were also prepared by inoculation of the tissues and subsequent incubation and extraction. Each of the resulting three extracts was submitted separately to HPLC analysis on a μ Porasil column (Waters Radial-PAK) [solvent, CH_2Cl_2–hexane–HOAc–MeOH (47:47:5:1); flow rate, 1 ml/min; UV detector (260 nm)]. The R_ts of authentic samples were checked, prior to the analysis of the extracts: **1**, 15.0 min; **2**, 5.5 min, **3**, 10.7 min; **4**, 5.0 min; **5**, 6.0 min; **6**, 11.0 min; **7**, 8.0 min. The results of the analysis are described in the text.

Antimicrobial testing. Plastic microtitre plates with 96 holes (7ϕ × 10 mm) were used for assays. To each of the holes were added 20 μl 5% Me_2CO soln of the test compound and 80 μl of the medium (potato dextrose and potato sucrose broth for fungi and bacteria, respectively), and then microquantities of a bacterial soln (10^9 cells/ml) or a microblock (0.5 × 0.5 × 0.5 mm) of the mycelium. After incubation at 20–25° for 2 days, inhibition was determined by measurement of the areas of mycelical growth of the fungi and of the degree of cell propagation, judged from the observed turbidity, of the bacteria.

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