PERGAMON

Phytochemistry 60 (2002) 703–708

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Fungal melanin inhibitor and related compounds from *Penicillium decumbens*

Yuzo Fujii[a,*], Masahiro Asahara[b], Masakatsu Ichinoe[c], Hiromitsu Nakajima[d]

[a]*Department of Materials Science, Yonago National College of Technology, Yonago, Tottori 683-8502, Japan*
[b]*Department of Materials Science, Faculty of Engineering, Tottori University, Koyma, Tottori 680-8552, Japan*
[c]*Laboratory of Food Microbiology, Tokyo Kasei University, Itabashi, Tokyo 173-0003, Japan*
[d]*Department of Agricultural Chemistry, Faculty of Agriculture, Tottori University, Koyama, Tottori 680-8553, Japan*

Received 28 January 2002; received in revised form 7 May 2002

Abstract

Two polyketides, decumbenones A and B, and versiol were isolated from the culture filtrate of the fungus, *Penicillium decumbens*. Their respective structures were 1-(2,8-dihydroxy-1,2,6-trimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)-3-hydroxy-1-propanone and 1-(2,8-dihydroxy-1,2,6-trimethyl-1,2,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)-3-hydroxy-1-propanone based on NMR spectroscopic data, chemical conversion, and X-ray analysis. Decumbenone A inhibited melanization in *Magnaporthe grisea*, the rice blast pathogen, whereas decumbenone B like versiol did not.

Keywords: *Penicillium decumbens*; Melanin; Inhibitor; *Magnaporthe grisea*; Fungal metabolite; Decumbenone; Polyketide

1. Introduction

In many countries, rice blast disease caused by the phytopathogenic fungus, *Magnaporthe grisea*, seriously damages rice crops. This fungus infects host plants when a spore lands on a leaf surface, germinates, and produces an appressorium, a specialized cell that uses turgor pressure to penetrate the host cell (Howard et al., 1991). Studies of mutants confirm that melanization is critical for the development of the penetration pegs of appressoria. Nonfungitoxic antipenetrant compounds that interfere with melanin biosynthesis are called melanin biosynthesis inhibitors (Okuno et al., 1983, Woloshuk et al., 1980, 1983). Tricyclazole (5-methyl-1,2,4-triazolo[3,4-*b*]benzothiazole) is one such compound used in the field to control rice blast disease. Its antipenetrant activity is correlated with its ability to block the polyketide pathway leading to fungal melanin biosynthesis in the appressorial wall of *M. grisea*. Although tricyclazole very effectively controls rice blast disease, it has little or no toxicity to *M. grisea* in vitro (Sisler, 1986). Melanin biosynthesis inhibitors therefore would meet the requirements for high biochemical specificity with activity directed at none or only a few target sites in the pathogen and for low toxicity to non target organisms (Bell and Wheeler, 1986).

In our search for natural melanin formation inhibitors among metabolites produced by soil fungi, we found a fungus, identified as *Penicillium decumbens*, whose culture filtrate inhibited melanin production in *M. grisea*. *P. decumbens* has a worldwide distribution, most of the available data being from soils, swamps, minerals, or organic soil particles (Domsch et al., 1980). Its culture filtrate had three compounds, one identified as versiol, and two fungal metabolites named decumbenone A and B (Fig. 1) . Fukuyama et al. (1976, 1978) isolated versiol from *Aspergillus versicolor* and reported its structure. *P. decumbens* produced versiol and decumbenones A and B whose structures have hexa or octa hydronaphthalene skeletons bearing a side chain with carbonyl and hydroxyl groups. Of the three metabolites only decumbenone A inhibited melanin production in *M. grisea*. We report the isolation and structures

* Corresponding author. Tel.: +81-859-24-5152; fax: +81-859-24-5009.
E-mail address: fujii@yonago-k.ac.jp (Y. Fujii).

0031-9422/02/$ - see front matter
PII: S0031-9422(02)00196-6

of decumbenones A and B, as well as inhibition of melanin production in *M. grisea* by decumbenone A.

2. Results and discussion

The EtOAc-soluble neutral fraction from the culture filtrate of *Penicillium decumbens* was subjected to a silica gel column chromatography, giving three fractions which respectively contained compounds **1**, **2** and **3**. These fractions, further purified by HPLC or recrystallization, yielded **1**, **2** or **3** in the pure state in respective yields of 4.3, 3.5 and 3.2 mg/l of medium. Compound **3** was identified as versiol based on a comparison of its spectroscopic data with data in the literature (Fukuyama et al., 1976, 1978). ^{13}C NMR data for versiol, not reported previously, are given in Table 1.

Compound **1** was obtained as a colorless oil. HREIMS and ^{13}C NMR spectroscopic data suggested the molecular formula $C_{16}H_{24}O_4$. Its IR absorption band at 1688 cm^{-1} and the ^{13}C resonance at δ 215.0 indicated a ketonic carbonyl in the molecule. Its IR spectrum showed an absorption band at 3358 cm^{-1} due to hydroxyl groups. Three protons that resonated at δ 3.20, 3.60, and 4.05 in the ^1H NMR spectrum of **1** were exchangeable with D_2O, indicative of three hydroxyls in the molecule. Its ^{13}C NMR spectrum showed three methyl, three methylene, six methines, and four quaternary carbons. Its ^1H NMR and ^1H–^1H COSY spectra indicated that the protons of the methylene (δ 3.80, C-1) were coupled with two protons (δ 2.71 and 3.06) of the methylene (C-2) and a proton (δ 3.60) of a hydroxyl group (1-OH). Moreover, as HMBC data for **1** showed a ketonic carbonyl carbon (C-3) adjacent to the methylene (C-2), **1** has a –CO–CH_2–CH_2–OH unit. The three methyl resonances in its ^1H NMR spectrum were two singlets and one doublet. Its ^1H–^1H COSY spectrum showed a coupling system in which methyl protons (H-16) resonating at δ 0.98 were coupled with the proton at δ 2.55 (H-8). Also in the ^1H–^1H COSY spectrum the methylene protons (H-7) resonating at δ 1.21 and 1.83 were coupled with two protons at δ 4.22 (H-6) and 2.55 (H-8), and the proton (H-6) resonating at δ 4.22 was coupled with the proton at δ 2.89 (H-5) and the hydroxy proton at δ 3.20 (6-OH). Four resonances assignable to olefinic carbons at δ 128.9 (C-11), 132.9 (C-10), 134.2 (C-9) and 134.6 (C-12) were present in the ^{13}C NMR spectrum of **1**. The olefinic protons resonating at δ 5.90 (H-11) and δ 5.36 (H-12) in the ^1H NMR spectrum were

Table 1
^1H (500 MHz) and ^{13}C (126 MHz) NMR spectroscopic data for compounds **1**, **2** and **3**

	1[a]		**2**[a]		**3**[b]	
Position	δ_C	δ_H[c]	δ_C	δ_H[c]	δ_C	δ_H[c]
1	58.9	3.80 (2H, *m*)	57.6	3.75 (2H, *q*, 6.0)	60.0	3.97 (1H, *ddd*, 12.1, 11.8, 3.4) 4.11 (1H, *dd*, 11.8, 8.9)
2	44.6	2.71 (1H, *dt*, 17.3, 5.8) 3.06 (1H, *ddd*, 17.3, 7.0, 5.8)	44.5	2.70 (1H, *dt*, 18.0, 6.0) 3.07 (1H, *dt*, 18.0, 6.0)	38.6	2.36 (1H, *dd*, 15.1, 3.4) 2.87 (1H, *ddd*, 15.1, 12.1, 8.9)
3	215.0		215.3		212.8	
4	58.3		57.0		57.0	
5	43.0	2.89 (1H, *dt*, 3.0, 3.5)	46.4	1.83 (1H, *dd*, 18.0, 1.0)	41.7	3.32 (1H, *q*, 3.0)
6	66.3	4.22 (1H, *m*)	66.3	4.11 (1H, *m*)	67.0	3.96 (1H, *m*)
7	39.8	1.21 (1H, *ddd*, 13.3, 11.4, 4.8) 1.83 (1H, *ddt*, 13.3, 1.5, 4.0)	44.1	1.12 (*ddd*, 13.0, 12.5, 2.5) 1.68 (1H, *ddt*, 13.0, 2.0, 3.5)	39.2	1.29 (1H, *m*) 1.95 (1H, *dt*, 4.6, 13.3)
8	26.2	2.55 (1H, *m*)	26.3	1.97 (1H, *m*)	25.3	2.62 (1H, *m*)
9	134.2	5.56 (1H, *s*)	42.1	0.72 (1H, *t*, 12.5) 1.78 (1H, *ddt*, 12.5, 2.0, 3.5)	135.5	5.72 (1H, *brs*)
10	132.9		31.3	2.44 (1H, *dddt*, 12.5, 11.0, 2.5, 2.0)	129.9	
11	128.9	5.90 (1H, *d*, 9.7)	130.2	5.35 (1H, *dd*, 10.0, 2.0)	132.2	6.21 (1H, *d*, 9.5)
12	134.6	5.36 (1H, *d*, 9.7)	134.0	5.31 (1H, *dd*, 10.0, 2.0)	128.2	5.44 (1H, *d*, 9.5)
13	75.0		73.8		78.6	
14	26.8	1.13 (3H, *s*)	27.4	1.05 (3H, *s*)	20.8	1.28 (3H, *s*)
15	14.7	1.48 (3H, *s*)	13.2	1.54 (3H, *s*)	13.1	1.12 (3H, *s*)
16	21.5	0.98 (3H, *d*, 7.3)	21.8	0.85 (3H, *d*, 7.0)	21.0	1.04 (3H, *d*, 7.1)
1-OH		3.60		3.42		
6-OH		3.20		3.25		1.77
13-OH		4.05		3.86		

[a] Taken in acetone-d_6.
[b] Taken in $CDCl_3$.
[c] Intensities, multiplicities and *J* values (Hz) shown in parentheses.

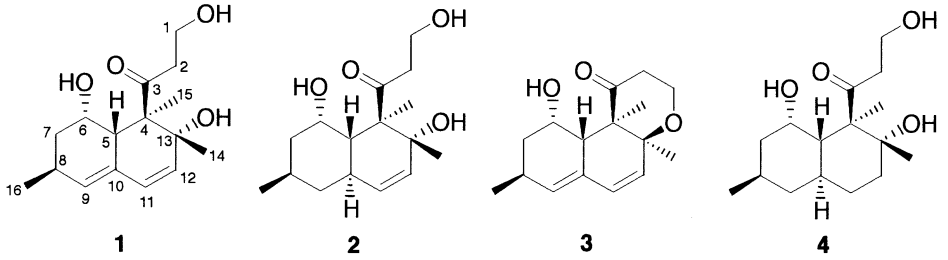

Fig. 1. Structures of compounds **1**–**4**.

coupled to each other at the coupling constant of 9.7 Hz. Another double bond is present between the methine (C-9) and quaternary (C-10) carbons. HMBC data for **1** indicated that the methyl (H-16) and methylene (H-7) protons had three-bond correlations to the olefinic methine carbon at C-9 and that the methine proton (H-6) had a three-bond correlation to the olefinic quaternary carbon at C-10. Therefore **1** has a cyclohexene ring consisting of C-5, 6, 7, 8, 9 and 10. HMBC correlations were found from the olefinic proton, H-11, to the five carbons C-5, 9, 10, 12 and 13. The olefinic methine, C-11, therefore is adjacent to the olefinic quaternary carbon, C-10, of the double bond in the cyclohexene ring. Two sp^3 quaternary carbons (δ 75.0 and 58.3) were confirmed from ^{13}C NMR and DEPT data for **1**. HMBC correlations exist from the methyl protons, H-15, to four carbons, C-3, 4, 5 and 13, and from the methyl protons, H-14, to three carbons, C-4, 12 and 13. HMBC data for **1** indicate that the quaternary carbon (C-13) is attached to the hydroxyl group, the methyl carbon (C-14), the quaternary carbon (C-4), and the olefinic methine carbon (C-12). These findings indicate the presence of a decalin skeleton with a conjugated diene. The structure of decumbenone A (**1**) therefore is 1-(2,8-dihydroxy-1,2,6-trimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)-3-hydroxy-1-propanone.

The structure of **2** was found by comparison of its NMR spectral data with those of **1**. Based on HREIMS and ^{13}C NMR findings, **2** has the molecular formula $C_{16}H_{26}O_4$. Its degree of unsaturation therefore is four, whereas that of **1** is five. Only two resonances were assignable to the olefinic carbon (δ 130.2 and 134.0) in the ^{13}C NMR spectrum of **2**, as opposed to four to the olefinic carbon in **1**. The ^{13}C NMR spectrum of **2** had two new signals for methine (δ 31.3) and methylene (δ 42.1) carbons, instead of two olefinic carbon signals as in **1**. This was supported by the absence of UV absorption by **2**. In the ^1H–^1H COSY of **2** the olefinic proton resonating at δ 5.35 (H-11) was coupled with two protons at δ 5.31 (H-12) and 2.44 (H-10). The methine proton (δ 2.44, H-10) was coupled with the other methine (δ 1.83, H-5) and methylene (δ 0.72 and 1.78, H-9) protons. Two protons (H-9) of the methylene were coupled with the proton (δ 1.97, H-8) of the methine-bearing exomethyl group. In the HMBC spectrum of **2**

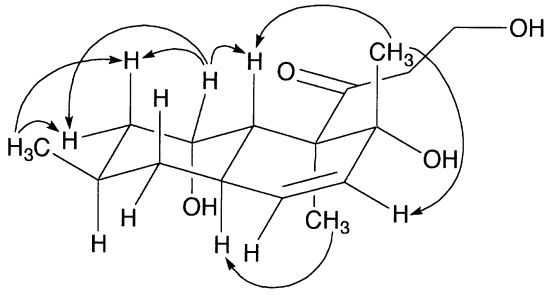

Fig. 2. Key NOEs of decumbenone B (**2**).

the methyl protons (H-14) have three-bond correlations to the olefinic methine carbon (C-12). HMBC correlations were found from the olefinic proton, H-12, to the four carbons, C-4, 10, 11 and 13, and from the olefinic proton, H-11, to the three carbons, C-5, 10 and 13. These results indicate that a double bond exists between C-11 and 12. HMBC data for **2** indicate that the methine carbon (C-10) is attached to the methine carbon (C-5), the methylene carbon (C-9), and the olefinic methine carbon (C-11). HMBC correlations were found from the methine proton, H-8, to the two methylene carbons, C-7 and 9, and from the methylene protons, H-9, to the five carbons, C-5, 8, 10, 11 and 16. In the HMBC spectrum the methylene protons (H-7) have three-bond correlations to the methylene carbon (C-9). The presence of a –CO–CH_2–CH_2–OH unit as **1** was confirmed by the NMR data. These NMR findings indicate that **2** has a structure in which the double bond between C-9 and 10, present in **1,** is hydrogenated to a single bond. The structure of **2** therefore is 1-(2,8-dihydroxy-1,2,6-trimethyl-1,2,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)-3-hydroxy-1-propanone.

Decumbenone A (**1**) has five chiral centers in its structure, whereas decumbenone B (**2**) has six. In the NOE experiments for **2**, irradiation of H-6 caused NOE enhancement of the signals at H-5 and H-7, and irradiation of H-14 caused NOE enhancement of the signals at H-5 and H-12 (Fig. 2). NOE enhancement of the signal of H-10 occurred on irradiation of H-15 and of the signals of methylene protons, H-7, on irradiation of H-16, indicative that the relative configuration of **2** is 4*R**, 5*S**, 6*S**, 8*R**, 10*R**, 13*R**. This was confirmed by

X-ray analysis (Fig. 3). In the NOESY experiments for **1**, the methine proton (H-5) showed NOE correlations to the methyl protons (H-15) and the methine proton (H-6). This result indicates that these relative configurations at C-5, 6 and 15 of **1** are similar to those of **2**. Catalytic hydrogenations of **1** and **2** gave the same product (**4**). Consequently, the relative stereochemistry of **1** is 4*R**, 5*S**, 6*S**, 8*S**, 13*R**. Three compounds produced by the same microorganism, *Penicillium decumbenone*, have the same relative stereochemistry except C-13 position. The relative configuration at C-13 in **3** is *S**, and that in **1** and **2** is *R**. This indicates that not dehydration but substitution reaction occurs in versiol (**3**) formation from **1**, viz. tetrahydropyranone ring formation of **3**.

In the bioassay done with paper discs and *Magnaporthe grisea* (*Pyricularia oryzae* IFO 31177), **1** at 10 μg/disc caused pigment accumulation that turned the mycelia reddish-brown, whereas the water control caused melanization that turned the color black. Tricyclazole at 0.1 μg/disc caused a reddish-brown change in mycelial color. This inhibition of melanization by **1**, which disappeared within a few days, suggests that **1** is a competitive inhibitor of the melanization enzyme in *M. grisea*. **1** at 1.0 mg/disc was not toxic to *M. grisea*, whereas tricyclazole at 10 μg/disc produced a white mycelium with antibiotic activity against *M. grisea*. At 100 μg/disc the effects of **2** and **3** on the mycelia were similar to the effect of the water control. The diene of decumbenone A (**1**) is an essential structural unit for its inhibition on *M. grisea* melanization, compared with decumbenone B (**2**). The structural difference in compounds **1** and **3** also suggested the importance of the $COCH_2CH_2OH$ unit of **1** for its inhibition. Further experiments are being planned to clarify the precise mode of action of decumbenone A.

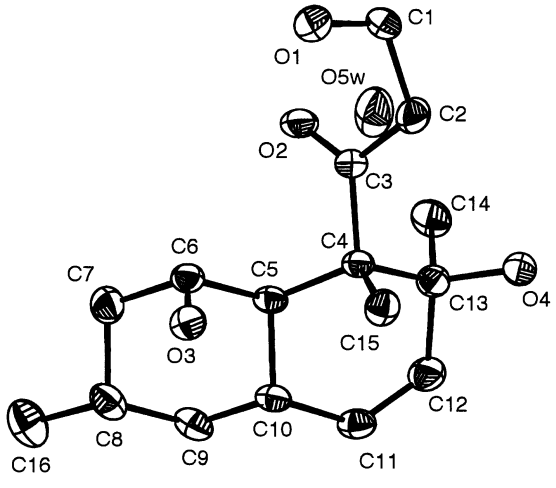

Fig. 3. ORTEP diagram of the decumbenone B (**2**) molecule.

3. Experimental

3.1. General

Melting points, determined in a Yanagimoto micro melting point apparatus, are uncorrected. IR spectra were recorded using a JASCO FT-IR-5300 spectrometer. Optical rotation was measured with a Horiba SEPA-200 high sensitive polarimeter, and the CD spectrum with a JASCO J-720 spectropolarimeter. EIMS were obtained with a Jeol A505HA spectrometer (direct probe, 70 eV), and NMR spectra with a Jeol JNM EC-500 NMR spectrometer. Chemical shifts were referenced against acetone-d_6 (δ_H 2.00, δ_C 30.3 and 206.0) and $CDCl_3$ (δ_H 7.26, δ_C 77.0). Preparative HPLC was done with a Cosmosil $5C_{18}$-AR-II column (Nacalai Tesque, 10 × 250 mm) and monitored at 220 nm.

3.2. Fungal material

The fungus (M-189), isolated from a soil sample collected in Yonago, Tottori Prefecture, Japan, was identified as *Penicillium decumbens*, based on its morphological features (Domsch et al., 1980). It has been maintained on potato-dextrose agar.

3.3. Extraction and isolation

The fungus was grown without shaking at 24 °C for 14 days in the dark in 500 ml conical flasks containing 200 ml of medium consisting of glucose (30 g/l), peptone (3 g/l) the extract from 50 g/l of malt, and water. Each culture (10 l) was filtered, and the filtrate was extracted with EtOAc (3 × 5 l) at pH 2.0. The EtOAc solution was washed with 1 M $NaHCO_3$ aq. then dried over Na_2SO_4, and concentrated to give the EtOAc-soluble neutral fraction (2.8 g). The latter residue was subjected to Si gel column chromatography (70 g of Wakogel C-200, 30 × 200 mm), then developed with 500 ml (100 ml × 5) each of 20, 30 and 40% acetone in *n*-hexane. The second fraction, eluted with 20% acetone in *n*-hexane (235 mg), was recrystallized, yielding a colorless solid **3** (65 mg). The fifth fraction (450 mg), eluted with 30% acetone in *n*-hexane, was purified by Si gel flash cc (25 g of Wakogel FC-40, 20 × 160 mm) with 250 ml (10 ml × 25) each of 60, 70 and 80% EtOAc in benzene. Fractions 16–21, eluted with 70% EtOAc in benzene, were combined and dried by evaporation. The residue (123 mg), purified by HPLC (60% MeOH, 0.8 ml/min), gave **1** as a colorless oil (86 mg). Fractions 3–18, eluted with 80% EtOAc in benzene, were combined and dried by evaporation. The residue (100 mg), recrystallized from acetone in benzene, gave colorless needles of **2** (70 mg).

3.4. Decumbenone A (**1**)

Colorless oil. $[\alpha]_D^{24}$ +54° (*c* EtOH, 0.50). UV λ_{max}^{EtOH} nm (log ε): 240 (3.93). IR ν_{max}^{KBr} cm^{-1}: 3358, 2960, 2930, 1688, 1458, 1379, 1214, 1162, 1112, 1073, 1044. ^1H NMR and ^{13}C NMR data: see Table 1. ^1H–^{13}C HMBC correlations: see Table 2. EIMS *m*/*z*: 280 $[M]^+$ (9), 262 (42), 244 (22), 219 (19), 189 (100), 171 (31), 161 (42), 147 (34), 73 (26). HREIMS *m*/*z*: 280.1677 (calc. for $C_{16}H_{24}O_4$, 280.1675).

3.5. Decumbenone B (**2**)

Colorless needles (acetone-benzene). Mp 113–114 °C. $[\alpha]_D^{24}$ +8° (*c* EtOH, 0.50). IR ν_{max}^{KBr} cm^{-1}: 3434, 2998, 2918, 1688, 1665, 1636, 1460, 1394, 1379, 1367, 1255, 1226, 1166, 1125, 1079, 1052, 1028. ^1H NMR and ^{13}C NMR data: see Table 1. ^1H–^{13}C HMBC correlations: H1/C2, C3; H2/C1, C3; H5/C4, C6, C10, C11, C13, C15; H7/C6, C8, C9, C16; H8/C7, C9; H9/C5, C8, C10, C11, C16; H11/C5, C10, C13; H12/C4, C10, C11, C13; H14/C4, C12, C13; H15/ C3, C4, C5, C13; H16/C8. EIMS *m*/*z*: 282 $[M]^+$ (8), 264 (94), 246 (49), 234 (19), 233 (19), 203 (71), 193 (40), 191 (79), 173 (69), 163 (70), 149 (100), 139 (55), 124 (60), 109 (75). HREIMS *m*/*z*: 282.1831 (calc. for $C_{16}H_{26}O_4$, 282.1831).

3.6. Versiol (**3**)

$[\alpha]_D^{24}$ −64° (*c* 1.5, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε) 242 (4.06). IR ν_{max}^{KBr} cm^{-1}: 3470, 2956, 2928, 2870, 1686, 1386, 1309, 1249, 1174, 1118, 1104, 1077, 1060, 1040. ^1H NMR and ^{13}C NMR data: see Table 1. EIMS *m*/*z*: 262 $[M]^+$ (2), 244 (42), 22 9 (21), 201 (11), 188 (12), 172 (100), 157 (39), 149 (11), 146 (12), 131 (10), 119 (8), 91 (10).

3.7. Tetrahydrodecumbenone A and dihydrodecumbenone B (**4**)

Compound **1** (9.6 mg) in EtOAc (3 ml) containing PtO_2 (4 mg) was stirred under H_2 for 8 h at room temp. The reaction mixture was filtered then dried by evaporation. Purification of the residue by HPLC (70% MeOH, 0.3 ml/min) gave compound **4** (0.4 mg, t_R 78.1 min). By the same procedure, compound **2** (9.8 mg) also was allowed to react for 2 h with PtO_2 (2.0 mg). The products were separated by HPLC (60% MeOH, 1.2 ml/min) to give compound **4** (8.2 mg, t_R 39.8 min). CD: $[\theta]_{296}$-78100 (EtOH; *c* 4.0). ^1H NMR ($CDCl_3$, 500 MHz): δ 3.86 (3H, *m*), 3.14 (1H, *ddd*, *J* = 18.1, 7.5, 3.6 Hz), 2.60 (1H, *ddd*, *J* = 18.1, 6.6, 3.4 Hz), 1.86–1.71 (4H, *m*), 1.67–1.53 (3H, *m*), 1.51 (3H, *s*), 1.34 (1H, *ddd*, *J* = 12.8, 4.2, 2.9 Hz), 1.19 (1H, *m*), 1.11 (3H, *s*), 1.05 (1H, *m*), 0.86 (3H, *d*, *J* = 6.4 Hz), 0.69 (1H, *m*).

Table 2
HMBC spectral data of **1**

H No.	Correlation with carbon atoms
H2	C1, C3
H5	C4, C9, C10, 15
H6	C5, C8, C10
H7	C5, C6, C8, C9, C16
H8	C16
H9	C5, C8
H11	C5, C9, C10, C12, C13
H12	C4, C10, C11
H14	C4, C12, C13
H15	C3, C4, C5, C13
H16	C7, C8, C9

3.8. Paper disc method

Magnaporthe grisea (*Pyricularia oryzae* IFO 31177) was grown at 28 °C in a Petri dish (90 × 20 mm) containing 20 ml of malt extract medium composed of glucose (30 g/l), peptone (3 g/l) and the extract from 50 g/l of malt with 2% agar until colony diameter was 3 or 4 cm. Petri dishes were kept for a few days in a refrigerator at 2–10 °C. A fungal sample in 50 μl of Me_2CO was applied to a paper disc which then was dried to remove the Me_2CO. A disc (8 mm) containing the sample was placed beside the colony on the agar in the Petri dish, and the dish incubated at 28 °C for 24–48 h under near-ultraviolet (352 nm) light. Inhibition of melanin formation was checked from the reverse side of the plate.

3.9. X-ray crystal structure analysis of **2**

Single crystals of **2**·H_2O suitable for X-ray crystal structure analysis were obtained by recrystallization from acetone in benzene. Crystal data for **2**·H_2O: colorless, block, $C_{16}H_{26}O_4 \cdot H_2O$, $M_r = 300.39$, crystal dimension 0.5 × 0.1 × 0.1 mm, monoclinic, space group $P2_1$ (No. 4), $a = 6.3086$ (5) Å, $b = 9.2786(9)$ Å, $c = 14.406(1)$ Å, $\beta = 95.372(2)°$, $V = 839.6(1)$ $Å^3$, $\rho_{calc.} = 1.188$ g/cm^3, $Z = 2$, $\mu(MoK_\alpha) = 0.87$ cm^{-1}. Intensity data were collected at 296 ± 1 K up to the maximum 2θ value of 54.8° on a Rigaku RAXIS Rapid-S imaging plate area detector with graphite-monochromated MoK_α ($\lambda = 0.71070$ Å) radiation. A total of 44 oscillation images, each oscillating at 5° and exposed for 25 min, were collected for **2**·H_2O. Data were corrected for Lorentz and polarization effects. Correction was made for secondary extinction (coefficient = 2.546800). The structure was determined by direct methods (SIR92) (Altomare et al., 1994) and expanded by Fourier techniques (DIRDIF99) (Beurskens et al., 1999). Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included at the calculated positions but not refined. Total reflections corrected 6070, of which 2005 were unique ($R_{int} = 0.044$). The final $R_1 = 0.057$,

$wR_2 = 0.192$ for 3309 ($I > -10\sigma(I)$), 219 parameters; max./min. residual electron density 0.41/−0.40 $e^-/Å^3$. All calculations were done with a CrystalStructure crystallographic software package. Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, No. CCDC-176735. Copies of this information may be obtained free of charge from: The Director CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax. (int code) + 44(1223)336–033 or Email: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Acknowledgements

We thank Miss Hiroko Tani for her assistance with the X-ray measurements, Miss Wakako Yanagida for her help with the NMR measurements and Mr. Naoya Ueda, Miss Miki Nagao, Mr. Hiromichi Nakagawa, Miss Ai Adachi, Miss Ritsuko Kobayashi and Miss Momoko Hamasaki for their technical assistance.

References

Altomare, A., Cascarano, G., Giacovazzo, C., Guagliardi, A., Burla, M., Polidori, G., Camalli, M., 1994. SIR92—a program for automatic solution of crystal structures by direct methods. Journal of Applied Crystallography 27, 435.

Bell, A.A., Wheeler, M.H., 1986. Biosynthesis and functions of fungal melanins. Annual Review of Phytopathology 24, 411–451.

Beurskens, P. T., Admiraal, G., Beurskens, G., Bosman, W. P., de Gelder, R., Israel, R., Smits, J. M. M., 1999. The DIRDIF-99 Program System. Technical Report of the Crystallography Laboratory, University of Nijimegen, The Netherlands.

Domsch, K. H., Gams, W., Anderson, T.-H., 1980. Compendium of Soil Fungi, Vol. 1. Academic Press, London, pp. 561.

Fukuyama, K., Tsukihara, T., Katsube, Y., Hamasaki, T., Hatsuda, Y., 1976. Structure of versiol, a new metabolite from *Aspergillus versicolor*. Tetrahedron Letters, 189–190.

Fukuyama, K., Katsube, Y., Hamasaki, T., Hatsuda, Y., 1978. Structure and absolute configuration of versiol, a metabolite from *Aspergillus versicolor*. Journal of the Chemical Society, Perkin Transactions II, 683–686.

Howard, R.J., Ferrari, M.A., Roach, D.H., Money, N.P., 1991. Penetration of hard substrates by a fungus employing enormous turgor pressures. Proceedings of the National Academy of Sciences USA 88, 11281–11284.

Okuno, T., Matsuura, K., Furusawa, I., 1983. Recovery of appressorial penetration by some melanin precursors in *Pyricularia oryzae* treated with tricyclazole and in a melanin deficient mutant. Journal of Pesticide Science 8, 357–360.

Sisler, H.D., 1986. Control of fungal diseases by compounds acting as antipenetrants. Crop Protection 5, 306–313.

Woloshuk, C.P., Sisler, H.D., Tokousbalides, M.C., Dutky, S.R., 1980. Melanin biosynthesis in *Pyricularia oryzae*: site of tricyclazole inhibition and pathogenicity of melanin-deficient mutants. Pesticide Biochemistry and Physiology 14, 256–264.

Woloshuk, C.P., Sisler, H.D., Vigil, E.L., 1983. Action of the antipenetrant, tricyclazole, on appressoria of *Pyricularia oryzae*. Physiological Plant Pathology 22, 245–259.