Isolation and Structures of an Antifungal Antibiotic, Fusarielin A, and Related Compounds Produced by a *Fusarium* sp.

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(Received for publication August 22, 1994)

A new antifungal antibiotic, fusarielin A, and three related compounds, fusarielins B, C and D, were obtained from a culture of a *Fusarium* sp. The skeletal structure and the relative stereochemistry of fusarielin A were determined mainly on the basis of its NMR data, and the absolute structure was elucidated by using the exciton chirality method and the modified Mosher method. The structures of the other homologues were determined by comparison of their spectral data with those of fusarielin A.

In the course of our studies on antimitotic drugs, we have observed that low concentration of these compounds generally induce deformation of mycelia of *Pyricularia oryzae* germinated in 0.02% yeast extract solution. This biological feature was utilized as a screening assay for compounds that interfere with microtubule function. This paper reports on the isolation and structure elucidation of the compound designated as fusarielin A (**1**) that caused curling of mycelia of *P. oryzae*, and of its homologues, fusarielins B (**2**), C (**3**) and D (**4**) (Fig. 1).

Isolation and Purification

Among about 1,000 strains of fungi isolated from soils, the culture broth of *Fusarium* sp. K432 was found to induce "curling" of mycelia of *P. oryzae*. The fungal strain, was then cultured by standing for 20 days in a potato dextrose medium at 20°C. The acetone - benzene extract of a 2.9 liters culture was separated by column chromatographies as shown in Fig. 2, affording 900 mg of **1**, 26 mg of **2**, and a few mg each of **3** and **4**. Production of fusarielins by this fungus was temperature-dependent, and they were not produced at 27°C. The yields in shaken cultures were very low even at 20°C.

Physico-chemical Properties

Compounds **1**$\sim$**4** were obtained as white powders. They are all readily soluble in various organic solvents. The physico-chemical properties of these compounds are shown in Table 1. Their ^{1}H NMR and ^{13}C NMR data are summarized in Tables 2 and 3, respectively. Signal assignments for **1** were made based on chemical shifts, ^{1}H-^{1}H and ^{1}H-^{13}C COSY, heteronuclear multiple bond ^{1}H-^{13}C correlation spectroscopy (HMBC: Fig. 3) and NOESY (Fig. 4). Signal assignments for compounds **2**$\sim$**4** were made by comparison with the data of fusarielin A (**1**).

All the ^{1}H and ^{13}C NMR spectra were measured at 50°C, since measurements at room temperature gave poor resolution.

Skeletal Structures and Relative Stereochemistry

Fusarielin A (**1**), a white powder, has the formula $C_{25}H_{38}O_{4}$ based on the high resolution FAB mass

Fig. 1. Structures of fusarielins A, B, C and D.

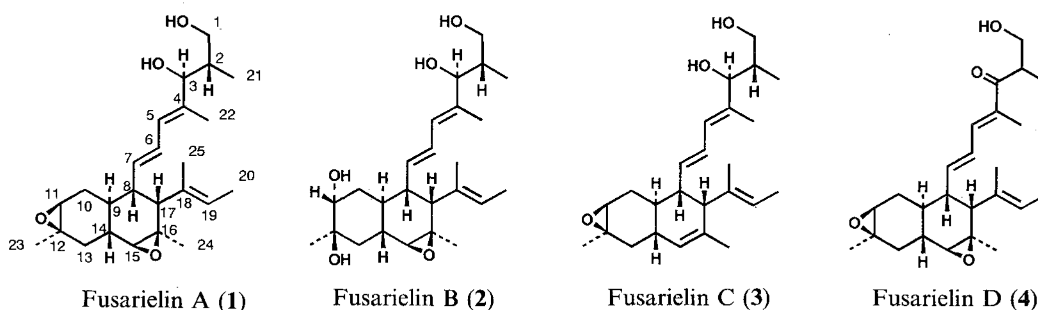


Fig. 2. Isolation procedure of fusarielins.

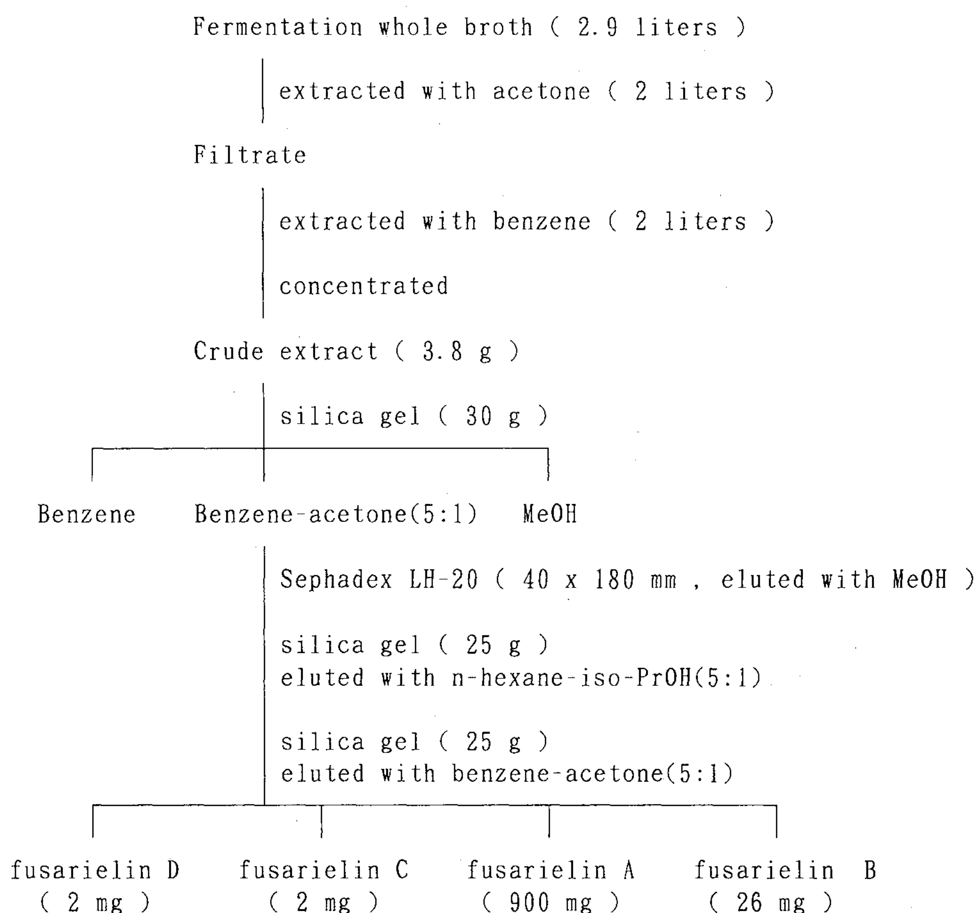


Table 1. Physico-chemical properties of fusarielins A, B, C and D.

	Fusarielin A	Fusarielin B	Fusarielin C	Fusarielin D
Appearance	White powder	White powder	White powder	White powder
Molecular formula	$C_{25}H_{38}O_4$	$C_{25}H_{40}O_5$	$C_{25}H_{38}O_3$	$C_{25}H_{36}O_4$
Elemental analysis				
Calcd:	C 73.60, H 9.54, O 16.86 (as $C_{25}H_{38}O_4 \cdot 0.3H_2O$)	C 71.39, H 9.58, O 19.02		
Found:	C 73.63, H 9.47, O 16.65	C 70.04, H 9.44, O 19.52		
HRFAB-MS (m/z)				
Calcd:	425.2668 (as $C_{25}H_{38}O_4Na$)	443.2773 (as $C_{25}H_{40}O_5Na$)	409.2719 (as $C_{25}H_{38}O_3Na$)	423.2511 (as $C_{25}H_{36}O_4Na$)
Found:	425.2656 $(M+Na)^+$	443.2763 $(M+Na)^+$	409.2698 $(M+Na)^+$	423.2480 $(M+Na)^+$
MP (°C)	68～72	138～140	66～70	56～58
$[\alpha]_D^{25}$	−132° (*c* 0.1125, MeOH)	−100° (*c* 0.135, MeOH)	−159° (*c* 0.14, MeOH)	−120° (*c* 0.08, MeOH)
UV λ_{max} nm (ε): MeOH	239 (28,400)	239 (24,200)	236 (22,200)	281 (13,200)
IR ν_{max} ($CHCl_3$) cm^{-1}	3620, 3480, 2970, 1382, 1090, 1060～1000, 975, 830	3440, 2970, 2850, 1380, 1090, 1040～1000	3620, 3480, 2970, 1382, 1430, 1030～1010, 978, 840	3625, 3520, 2970, 2925, 1650, 1630, 1450, 1430, 1380, 1350～1010, 980, 830

spectrum (HRFAB-MS) and elemental analysis. Alignments of vicinal protons and carbons were determined by ^1H-^1H and ^1H-^{13}C COSY experiments. Assignments of the signals through four quaternary carbons (at C-4, C-12, C-16 and C-18) were made by HMBC experiments. The presence of two hydroxy groups was suggested by the IR spectrum of **1** and was verified by acetylation to give a diacetate **5** (Fig. 5). The presence of two epoxy groups at the 11,12- and 15,16-positions was determined from the molecular formula, the chemical shifts of the

Table 2. ^{1}H NMR spectra of fusarielins A, B, C and D (δ_H (multiplicity), J in Hz, 500 MHz, DMSO-d_6, 50°C).

Position	A		B	
1	3.32 (1H, ddd)	$J_{gem}=10.4$, $J_{1,2}=6.0$, $J_{1,OH}=6.0$	3.32 (1H, ddd)	$J_{gem}=10.2$, $J_{1,2}=6.0$, $J_{1,OH}=5.0$
	3.53 (1H, ddd)	$J_{gem}=10.4$, $J_{1,2}=4.8$, $J_{1,OH}=4.8$	3.53 (1H, ddd)	$J_{gem}=10.2$, $J_{1,2}=4.0$, $J_{1,OH}=6.0$
2	1.62 (1H, m)		1.62 (1H, m)	
3	3.64 (1H, dd)	$J_{2,3}=8.2$, $J_{3,OH}=3.8$	3.65 (1H, dd)	$J_{2,3}=8.4$, $J_{3,OH}=3.8$
5	5.81 (1H, d)	$J_{5,6}=11.0$	5.80 (1H, dd)	$J_{5,6}=10.8$, $J_{5,19}=0.8$
6	6.19 (1H, dd)	$J_{5,6}=11.0$, $J_{6,7}=15.0$	6.13 (1H, dd)	$J_{5,6}=10.8$, $J_{6,7}=15.0$
7	5.13 (1H, dd)	$J_{6,7}=15.0$, $J_{7,8}=10.0$	5.14 (1H, dd)	$J_{6,7}=15.0$, $J_{7,8}=10.2$
8	2.10 (1H, ddd)	$J_{7,8}=10.0$, $J_{8,9}=11.0$, $J_{8,17}=5.0$	2.12 (1H, ddd)	$J_{7,8}=10.2$, $J_{8,9}=12.4$, $J_{8,17}=5.6$
9	1.21 (1H, m)		1.65 (1H, m)	
10_{ax}	0.99 (1H, dd)	$J_{gem}=15.0$, $J_{9,10}=12.0$, $J_{10,11}=0$	1.19 (1H, ddd)	$J_{gem}=13.8$, $J_{9,10}=13.2$, $J_{10,11}=2.4$
10_{eq}	1.73 (1H, ddd)	$J_{gem}=15.0$, $J_{9,10}=5.4$, $J_{10,11}=5.4$	1.29 (1H, ddd)	$J_{gem}=13.8$, $J_{9,10}=4.0$, $J_{10,11}=2.0$
11	2.91 (1H, d)	$J_{10_{ax},11}=0$, $J_{10_{eq},11}=5.4$	3.27 (1H, bs)	$J_{10,11}=2.4$, 2.0, $J_{11,OH}=3.8$, $J_{11,13}=0.3$
13_{ax}	1.61 (1H, dd)	$J_{gem}=13.8$, $J_{13,14}=10.8$	1.53 (1H, dd)	$J_{gem}=13.8$, $J_{13,14}=13.8$
13_{eq}	2.07 (1H, dd)	$J_{gem}=13.8$, $J_{13,14}=3.6$	1.42 (1H, ddd)	$J_{gem}=13.8$, $J_{13,14}=2.2$, $J_{11,13}=0.3$
14	1.50 (1H, ddd)	$J_{9,14}=13.0$, $J_{13,14}=10.8$, 3.6, $J_{14,15}=0$	1.78 (1H, ddd)	$J_{9,14}=13.2$, $J_{13,14}=13.8$, 2.2, $J_{14,15}=0$
15	2.70 (1H, s)	$J_{14,15}=0$	2.62 (1H, s)	$J_{14,15}=0$
17	2.46 (1H, d)	$J_{8,17}=5.0$	2.47 (1H, d)	$J_{8,17}=5.6$
19	5.18 (1H, q)	$J_{19,20}=6.8$	5.19 (1H, qd)	$J_{19,20}=6.6$, $J_{5,19}=0.8$
20	1.60 (3H, d)	$J_{19,20}=6.8$	1.60 (3H, d)	$J_{19,20}=6.6$
21	0.68 (3H, d)	$J_{2,21}=6.8$	0.68 (3H, d)	$J_{2,21}=6.8$
22	1.63 (3H, s)		1.60 (3H, s)	
23	1.26 (3H, s)		1.10 (3H, s)	
24	1.13 (3H, s)		1.12 (3H, s)	
25	1.60 (3H, s)		1.60 (3H, s)	
1-OH	4.22 (1H, dd)	$J=6.0$, 4.8	4.23 (1H, dd)	$J=6.0$, 5.0
3-OH	4.61 (1H, d)	$J=3.8$	4.60 (1H, d)	$J=3.8$
11-OH			4.23 (1H, d)	$J=3.8$
12-OH			4.04 (1H, s)	

Position	C		D	
1	3.32 (1H, ddd)	$J_{gem}=10.4$, $J_{1,2}=6.0$, $J_{1,OH}=6.0$	3.31 (1H, ddd)	$J_{gem}=10.6$, $J_{1,2}=6.0$, $J_{1,OH}=5.0$
	3.54 (1H, ddd)	$J_{gem}=10.4$, $J_{1,2}=4.8$, $J_{1,OH}=4.8$	3.55 (1H, ddd)	$J_{gem}=10.6$, $J_{1,2}=7.0$, $J_{1,OH}=5.0$
2	1.62 (1H, m)		3.42 (1H, ddq)	$J_{1,2}=6.0$, 7.0, $J_{2,21}=6.8$
3	3.65 (1H, dd)	$J_{2,3}=8.0$, $J_{3,OH}=3.8$		
5	5.83 (1H, dd)	$J_{5,6}=11.0$, $J_{5,19}=0.8$	7.10 (1H, d)	$J_{5,6}=11.0$
6	6.23 (1H, dd)	$J_{5,6}=11.0$, $J_{6,7}=15.0$	6.46 (1H, dd)	$J_{5,6}=11.0$, $J_{6,7}=15.0$
7	5.22 (1H, dd)	$J_{6,7}=15.0$, $J_{7,8}=10.0$	5.77 (1H, dd)	$J_{6,7}=15.0$, $J_{7,8}=10.0$
8	2.06 (1H, ddd)	$J_{7,8}=10.0$, $J_{8,9}=11.0$, $J_{8,17}=5.6$	2.55 (1H, ddd)	$J_{7,8}=10.0$, $J_{8,9}=11.0$, $J_{8,17}=5.0$
9	1.22 (1H, m)		1.30 (1H, m)	
10_{ax}	1.10 (1H, dd)	$J_{gem}=15.0$, $J_{9,10}=12.0$, $J_{10,11}=0$	1.03 (1H, dd)	$J_{gem}=15.0$, $J_{9,10}=12.0$, $J_{10,11}=0$
10_{eq}	1.82 (1H, ddd)	$J_{gem}=15.0$, $J_{9,10}=5.4$, $J_{10,11}=5.4$	1.75 (1H, ddd)	$J_{gem}=15.0$, $J_{9,10}=5.4$, $J_{10,11}=5.4$
11	2.92 (1H, d)	$J_{10_{ax},11}=0$, $J_{10_{eq},11}=5.4$	2.91 (1H, d)	$J_{10_{ax},11}=0$, $J_{10_{eq},11}=5.4$
13_{ax}	1.35 (1H, dd)	$J_{gem}=14.0$, $J_{13,14}=12.0$	1.63 (1H, dd)	$J_{gem}=13.8$, $J_{13,14}=12.0$
13_{eq}	2.00 (1H, dd)	$J_{gem}=14.0$, $J_{13,14}=3.6$	2.08 (1H, dd)	$J_{gem}=13.8$, $J_{13,14}=3.0$
14	1.77 (1H, m)	$J_{9,14}=13.2$, $J_{13,14}=12.0$, 3.6, $J_{14,15}=0.4$	1.53 (1H, ddd)	$J_{9,14}=13.0$, $J_{13,14}=12.0$, 3.0, $J_{14,15}=0$
15	5.23 (1H, br s)	$J_{14,15}=0.4$	2.74 (1H, s)	$J_{14,15}=0$
17	2.53 (1H, br d)	$J_{8,17}=5.6$	2.52 (1H, d)	$J_{8,17}=5.0$
19	5.13 (1H, qd)	$J_{19,20}=6.8$, $J_{5,19}=0.8$	5.23 (1H, br q)	$J_{19,20}=6.8$
20	1.56 (3H, d)	$J_{19,20}=6.8$	1.62 (3H, d)	$J_{19,20}=6.8$
21	0.68 (3H, d)	$J_{2,21}=6.8$	0.93 (3H, d)	$J_{2,21}=6.8$
22	1.63 (3H, s)		1.78 (3H, s)	
23	1.23 (3H, s)		1.27 (3H, s)	
24	1.47 (3H, s)		1.14 (3H, s)	
25	1.52 (3H, s)		1.64 (3H, s)	
1-OH	4.23 (1H, dd)	$J=6.0$, 4.8	4.46 (1H, t)	$J=5.0$
3-OH	4.62 (1H, d)	$J=3.8$		

Table 3. ^{13}C NMR spectra of fusarielins A, B, C and D (δ, ppm, 125 MHz, DMSO-d_6, 50°C).

Carbon	A ($^1J_{C-H}$=)	B	C	D	Carbon	A ($^1J_{C-H}$=)	B	C	D
1	63.8 t (140)	63.8 t	63.8 t	64.3 t	14	33.5 d (130)	36.8 d	33.9 d	33.6 d
2	38.4 d (130)	38.4 d	38.4 d	41.5 d	15	61.8 d (178)	63.6 d	126.0 d	61.9 d
3	78.9 d (144)	79.0 d	78.9 d	204.2 s	16	60.2 s	60.4 s	134.4 s	60.3 s
4	137.6 s	137.8 s	137.3 s	133.7 s	17	52.9 d (128)	53.6 d	53.9 d	52.8 d
5	125.2 d (150)	125.6 d	125.3 d	144.7 d	18	133.1 s	133.8 s	134.0 s	133.0 s
6	126.5 d (150)	126.4 d	126.4 d	127.4 d	19	124.0 d (157)	124.0 d	122.0 d	124.6 d
7	134.3 d (150)	135.8 d	134.9 d	138.1 d	20	13.2 q (126)	13.5 q	13.2 q	13.4 q
8	42.8 d (133)	43.5 d	47.5 d	43.5 d	21	13.7 q (126)	14.0 q	13.7 q	13.7 q
9	32.3 d (128)	29.8 d	33.2 d	32.6 d	22	11.5 q (126)	11.8 q	11.4 q	11.6 q
10	30.0 t (128)	34.4 t	29.7 t	30.1 t	23	22.4 q (126)	27.8 q	22.5 q	22.6 q
11	58.5 d (179)	72.8 d	58.3 d	58.6 d	24	21.5 q (127)	22.0 q	21.6 q	21.6 q
12	57.7 s	70.5 s	57.5 s	57.9 s	25	17.8 q (125)	18.0 q	17.8 q	17.8 q
13	35.3 t (126)	39.0 t	37.4 t	35.4 t					

Fig. 3. ^1H-^{13}C correlations of **1** by HMBC experiment.

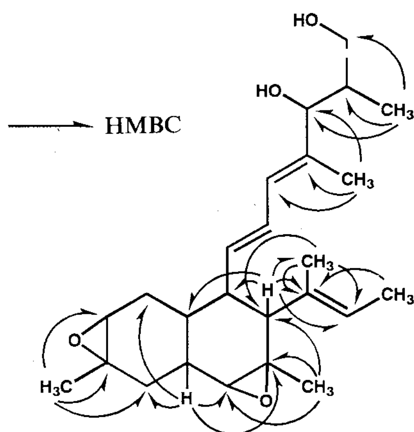


Fig. 4. ^1H-^1H correlations of **1** by NOE experiment.

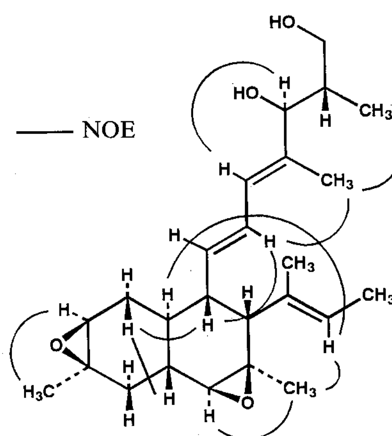
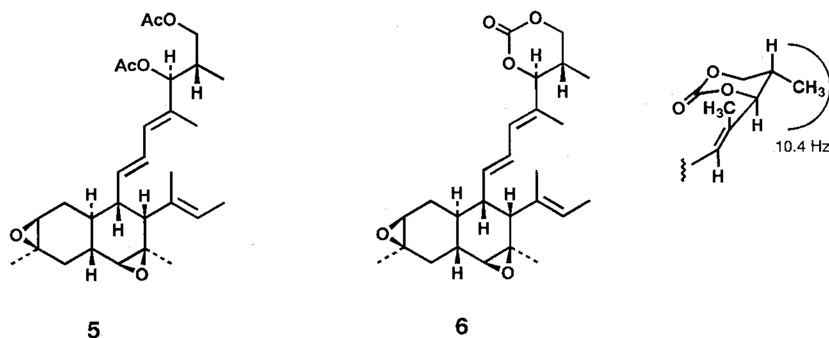


Fig. 5. Structures of **5** and **6**.



^1H and ^{13}C NMR signals of these positions and the relatively large $^1J_{CH}$ values for C-11 ($^1J_{C\text{-}11,H\text{-}11}$ = 179 Hz) and C-15 ($^1J_{C\text{-}15,H\text{-}15}$ = 178 Hz) (see Table 2).

The relative stereochemistry at positions 2 and 3, 4 through 7 and 8 through 17 was elucidated as follows. Treatment of **1** with *N,N*-carbonyl diimidazole gave a cyclic carbonate **6** bridged between C-1 and C-3 (Fig. 5). The coupling constant between H-2 and H-3 of **6** was shown to be 10.4 Hz, indicating a *trans*-diaxial relationship of these two protons, and, accordingly, a *threo* relationship at positions 2 and 3. *E*-Orientations of the 4,5 and 6,7-enes were elucidated from the observed NOE between H-6 and H_3-22 (Fig. 4), and a large coupling constant between H-6 and H-7 (15.0 Hz).

Regarding the relative stereochemistry of the decalin moiety (on C-8 through C-17), *trans*-diaxial relationships between H-8 and H-9, H-9 and one of H-10 (appearing at δ 0.99), and H-9 and H-14 were indicated by their coupling constants (11.0, 12.0 and 10.8 Hz, respectively). The H-11 signal (δ 2.91) appeared as a doublet (J = 5.4 Hz). ^1H-^1H COSY showed no coupling between this proton and H-10_{axial} (appearing at δ 0.99), indicat-

ing the relative stereochemistry at the 11,12-position to be as shown in Fig. 1. In this structure, the dihedral angle between H-10_{axial} and H-11 is *ca.* 90°. Similarly, the H-15 signal was seen as a singlet, showing no coupling between H-14 and H-15. This suggested that the dihedral angle between H-14 and H-15 should be *ca.* 90°. The stereochemistry at the 17-position was determined from the coupling constant between H-8 and H-17 (5.0 Hz), which indicated a *syn* relationship of these two protons. These data established the relative stereochemistry of the decalin moiety (position 8 through 17).

The stereochemistry of the 18,19-ene was elucidated based on the NOE observed between H-19 and H-9. Only the *E*-orientation of this double bond allows to locate H-17 to be located close to H-9.

Fusarielin B (**2**), a white powder, gave a HRFAB-MS spectrum and an elemental analysis consistent with the formula $C_{25}H_{40}O_5$ which formally coincide with monohydration of fusarielin A (**1**). Some differences were seen in the ^{1}H and ^{13}C signals of **1** and **2** around the positions 11, 12, suggesting that the 11,12-epoxide present in **1** had been hydrolytically opened to generate a 11,12-diol in **2**. This was supported by lower shifts of the H-11, C-11 and C-12 signals of **2** relative to those of **1** as well as coupling between H-11 and an OH proton. The relative stereochemistry of the diol moiety was elucidated from the lower shifts of the H-9 and H-14 signals in the ^{1}H NMR spectrum of **2**, caused by the 1,3-diaxial relationships between these hydrogens and OH-11 and OH-12, respectively, as well as a W-type coupling observed between H-11 and H-13. The rest of the structure including the stereochemistry was determined by correlation of **1** with **2** through acid-catalyzed epoxide opening.

Fusarielin C (**3**), a white powder, has the formula $C_{25}H_{38}O_3$, which is one oxygen atom less than that of **1**, based on the HRFAB-MS. The fact that the signals of two olefinic carbons appeared at δ 126.0 and 134.4 ppm in the ^{13}C NMR spectrum of **3** at the expense of the two epoxide carbon signals (δ 61.8 and δ 60.2) observed in the spectrum of **1** suggested that one of the epoxy groups present in **1** is replaced by an olefin group in **3**. The ^{1}H NMR spectrum of **3** showed an additional olefinic proton signal at δ 5.23 ppm as a doublet (J=0.4 Hz), establishing the location of the double bond at the 15, 16 position.

Fusarielin D (**4**), a white powder, gave a HRFAB-MS molecular ion corresponding to the formula $C_{25}H_{36}O_4$, which is two hydrogen atoms less than that of **1**. Its IR spectrum showing strong bands at 1650 cm^{-1} and 1630 cm^{-1} and the UV spectrum, showing a maximum at 281 nm (ε=13,200), indicated the presence of a conjugated dienone group. This was proved by its NMR spectra (Tables 2, 3). In the ^{13}C NMR spectrum of **4**, a carbonyl carbon signal appeared at δ 204 ppm at the expense of a hydroxylated tertiary carbon (δ 78.9) observed in the spectrum of **1**, and in the ^{1}H NMR spectrum of **4**, a proton doublet due to H-3 had disappeared and the signals assigned to H-5, H-6 and H-7 were shifted to lower fields. These spectral data established the structure of **4**.

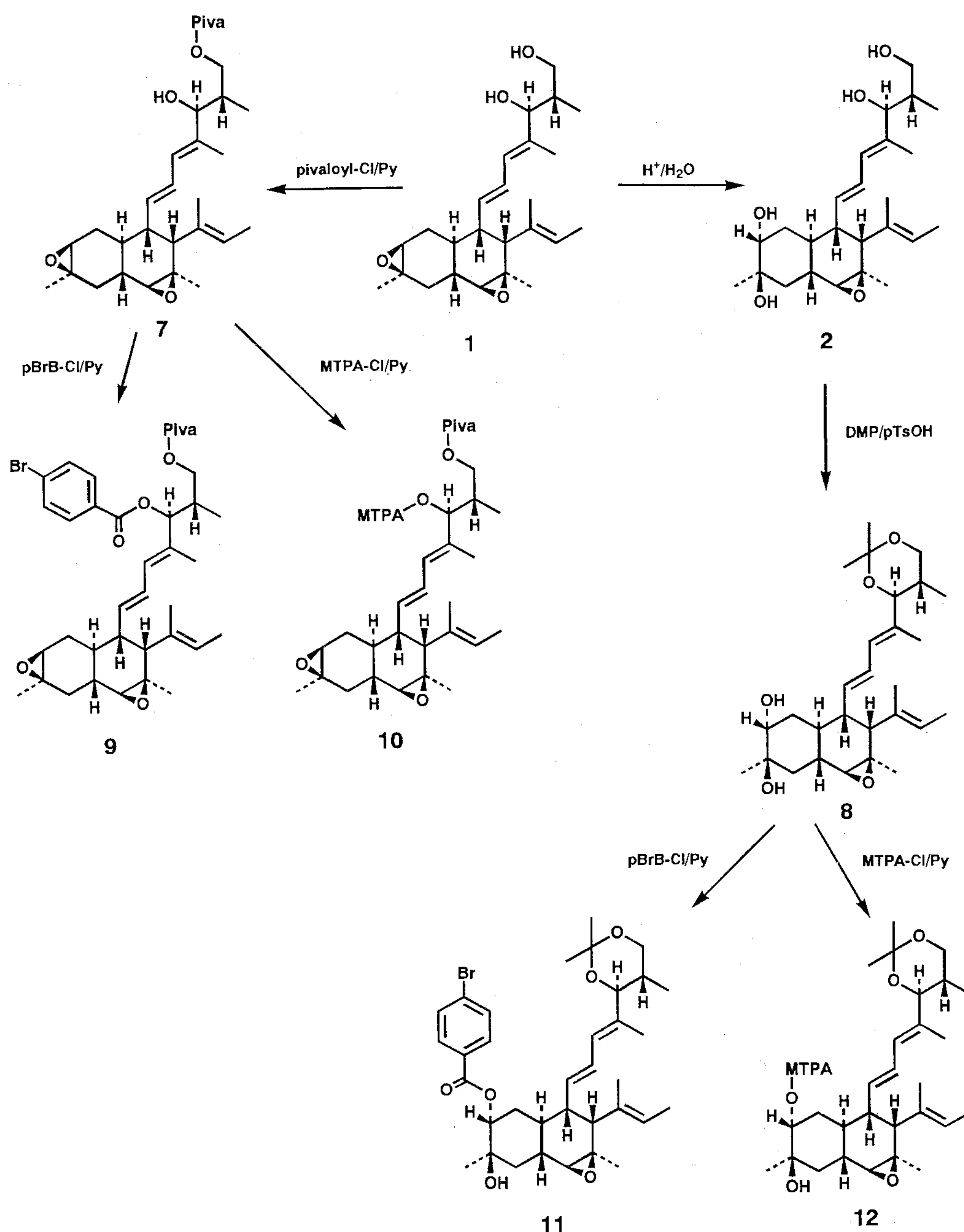
Compounds **1**~**4** are structurally related to compactin (ML-236B)[1], betaenones[2], nargenicin A_1[3] and some other polyketide compounds which possess a functionalized decalin moiety in their structures.

Absolute Structures

Fusarielin A (**1**) gave fusarielin B (**2**), through epoxide opening, on treatment with *p*-toluenesulfonic acid. This indicates that the structures of **1** and **2** are identical to each other, except for the 11 and the 12 positions, including the absolute stereochemistry. Their absolute stereochemistry was then determined in the following manner. The *p*-bromobenzoates **9** and **11**, and the 2-methoxy-2-trifluoromethylphenylacetates (MTPA) **10** and **12** were prepared from fusarielins A and B as shown in Scheme 1 to determine the absolute configurations by the exciton chirality method[4] and the modified Mosher method[5], respectively.

The ^{1}H NMR spectrum of **9** showed NOE between H-3 and H-5, indicating that the two hydrogens are oriented in a *syn* relationship in solution. The CD spectrum (MeOH) of **9** showed well-split intense Cotton effects, $\Delta\varepsilon_{249}$ +47.6 and $\Delta\varepsilon_{231}$ −40.6 (Fig. 6), indicating that the projection of the two chromophores, the *p*-bromobenzoyl group at C-3 and the diene (C-4 through C-7), should be clockwise. These two results allowed us to assign the *S* configuration for C-3. Similarly, the CD spectrum (MeOH) of **11** showed well-split intense Cotton effects, $\Delta\varepsilon_{248}$ +66.6 and $\Delta\varepsilon_{230}$ −24.1 (Fig. 7), indicating the projection of the two chromophores, the *p*-bromobenzoyl group at C-11 and the diene moiety, to be clockwise, and hence the *S* configuration at C-8 was assigned. These conclusions were supported by a modified Mosher method analysis using the ^{1}H-chemical shift differences between the (*R*) and (*S*)-MTPA esters of each of **10** and **12** ($\Delta\delta=\delta(S)-\delta(R)$), and the results are illustrated in Fig. 8. The absolute structures of **1** and **2** have thus been established. The stereochemistry of fusarielins C and D was tentatively assigned by analogy

Scheme 1. Synthetic pathways of **9**~**12**.



with that of **1** and **2**.

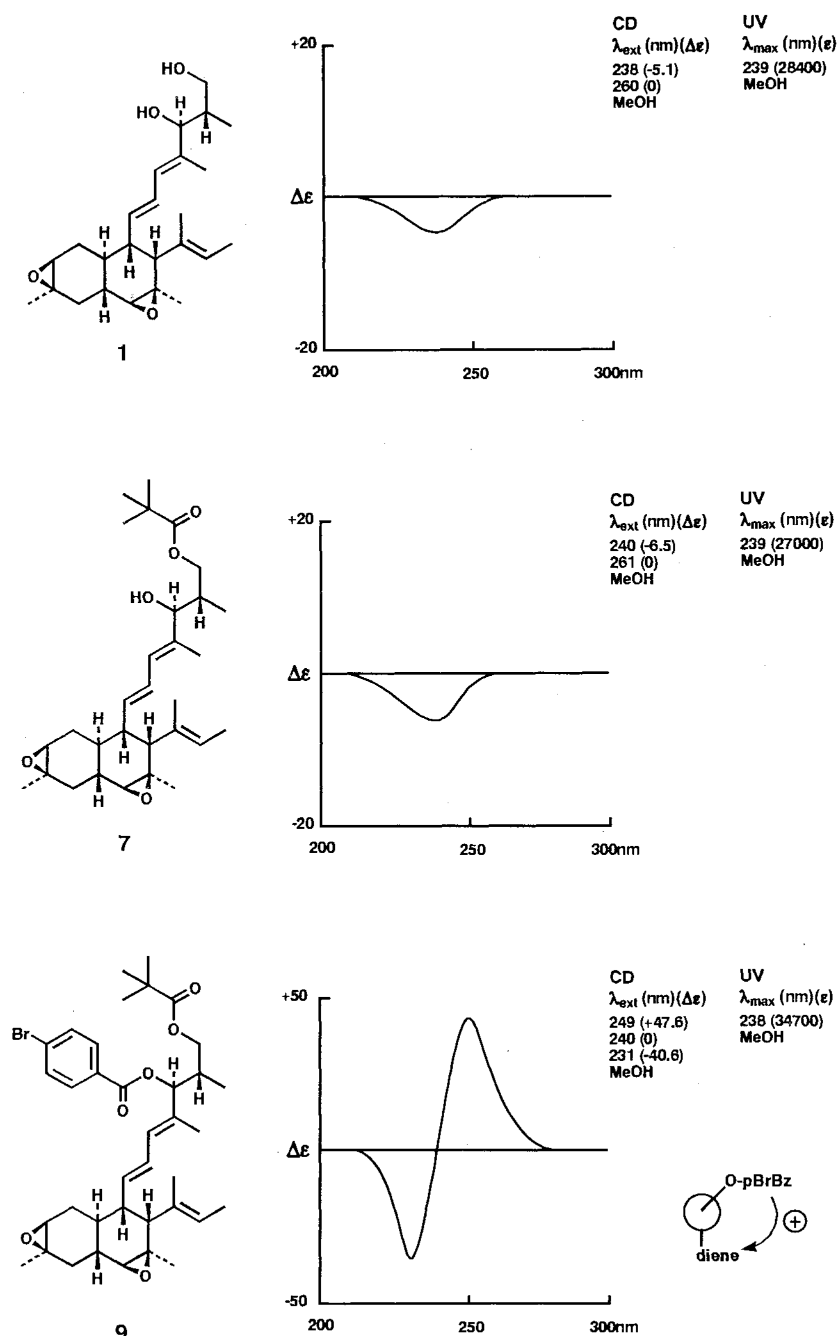
Biosynthesis of Fusarielin A

Inspection of the structure of fusarielin A led us to consider a biosynthetic route through the acetate-malonate pathway. In order to establish the origin of the carbon atoms in **1**, incorporation experiments with [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]acetate, [3-^{13}C]propionate, and L-[*methyl*-^{13}C]methionine were carried out with cultures of the K432 strain. The ^{13}C-labeling patterns after incorporation of these precursors were determined from the respective ^{13}C NMR spectra and are shown in Fig. 9. The incorporation pattern of [1,2-$^{13}C_2$]acetate was confirmed by matching of $^1J_{CC}$ values. Propionate was not incorporated and the five methyl carbons, C-21, C-22, C-23, C-24 and C-25, all originated from methionine. It was concluded that fusarielin A (**1**) is biogenetically a penta-methylated decaketide.

Biological Activity

Fusarielin A (**1**) showed a narrow antifungal spectrum (Table 4), and its cytotoxicity against HeLa S3 and NCI-H69 cells was rather weak. The IC_{50} values were 54.6 μM and 48.6 μM, respectively. This compound did not inhibit the assembly of microtubule protein prepared from porcine brain even at 200 μM concentration. Fusarielin B (**2**) had no inhibitory activity on the growth

Fig. 6. CD spectra of **1** and its derivatives **7** and **9**.



of bacteria, fungi or tumor cells.

Experimental

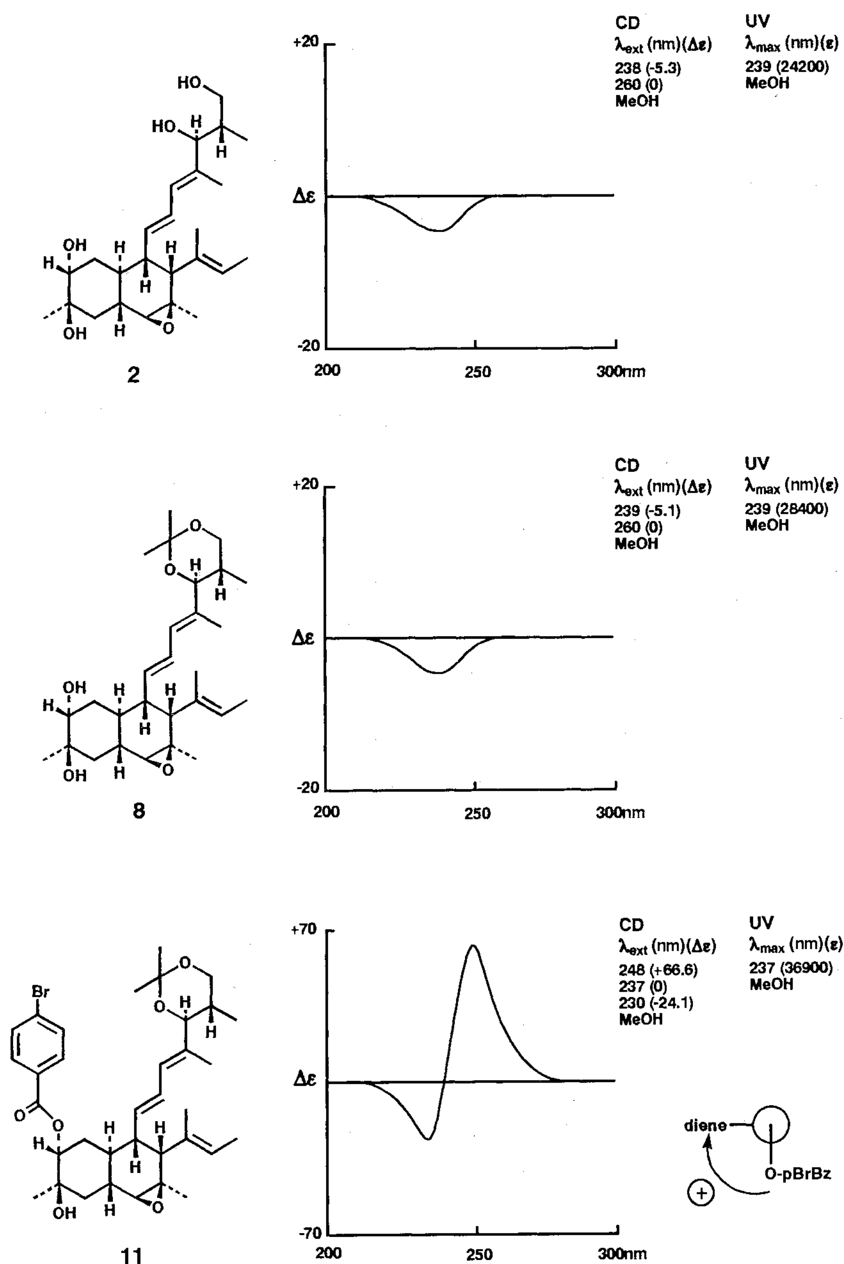
General

UV spectra were measured on a Shimadzu apparatus, model UV-300, and the maxima were given in nm (extinction ε). IR spectra were measured on a JASCO A-102 apparatus and are recorded in cm^{-1}. ^{1}H and ^{13}C NMR spectra were measured on a JEOL JNM A-500 NMR spectrometer; chemical shifts are given in ppm (δ) relative to TMS as internal standard; s = singlet, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublet, t = triplet, q = quartet, br = broad, coupling constants J were given in Hz. Mass spectra were measured on a JEOL HX-110 apparatus. Optical rotations and CD spectra were taken in methanol solutions on a JASCO DIP-140 polarimeter and on a JASCO J-20A recording spectropolarimeter, respectively. Thin layer chromatography were carried out on Merck Kieselgel 60F-254 plates.

Isolation of Fusarielins A (**1**), B (**2**), C (**3**) and D (**4**)

Fusarium sp. K432 strain was cultured in flasks containing 100 ml each of potato dextrose medium (potato 200 g, dextrose 20 g per liter of water), by standing at 20°C for 20 days. The acetone - benzene extract of total 2.9 liters culture was separated by a silica gel column chromatography (30 g). The column was

Fig. 7. CD spectra of **2** and its derivatives **8** and **11**.



eluted with benzene, benzene-acetone (5 : 1) and methanol, successively. The benzene-acetone eluent was further separated by column chromatographies (see Fig. 2) to give white powders of **1** (900 mg), **2** (26 mg), **3** (2 mg) and **4** (2 mg). Their physico-chemical properties are listed in Tables 1, 2 and 3.

Fusarielin A Diacetate (**5**)

Compound **1** was treated with pyridine-acetic anhydride to give a diacetate **5**. $C_{29}H_{42}O_6$; EI-MS (M^+); ^1H NMR ($CDCl_3$) 0.88 (3H, d, J=6.8 Hz), 1.22 (5H, m), 1.37 (3H, s), 1.61 ~ 1.77 (12H, m), 2.05 (3H, s, OAc), 2.07 (3H, s, OAc), 2.16 ~ 2.10 (3H, m), 2.54 (1H, br s), 2.72 (1H, s), 2.95 (1H, d, J=5.4 Hz), 4.25 (1H, dd, J=11.0, 5.8 Hz), 5.03 (1H, d, J=9.4 Hz), 5.12 (1H, dd, J=15.0, 10.0 Hz), 5.21 (1H), 5.85 (1H, d, J=11.0 Hz), 6.16 (1H, br t).

Fusarielin A Cyclic Carbonate (**6**)

Compound **1** (20 mg; 50 μM) and *N*,*N*-carbonyl diimidazole (10.5 mg; 65 μM) in dry benzene (1 ml) was kept stirred overnight at room temperature. The reaction solution was shaken with water to remove excess reagent and the generated imidazole. The organic layer was dried and evaporated to give **6** (17 mg). $C_{26}H_{36}O_4$; EI-MS 428 (M^+); ^1H NMR (DMSO-d_6, 50°C) 0.77 (3H, d, J=6.8 Hz, H-21), 1.02 (1H, dd, J=15.0, 12.0, 0 Hz, H-10_{ax}), 1.13 (3H, s, H-24), 1.23 (1H, m, H-9), 1.27 (3H, s, H-23), 1.51 (1H, ddd, J=13.0, 10.8, 3.6, 0 Hz, H-14), 1.60 (6H, s, H-20, 25), 1.64 (1H, dd, J=13.8, 10.8 Hz, H-13_{ax}), 1.68 (3H, s, H-22), 1.75 (1H, ddd, J=15.0, 5.4, 5.4 Hz, H-10_{eq}), 2.08 (1H, dd, J=13.8, 3.6 Hz, H-13_{eq}), 2.15 (1H, ddd, J=10.0, 11.0, 5.8 Hz, H-8), 2.22 (1H, m, H-2), 2.48 (1H, d, J=5.0 Hz, H-17), 2.72 (1H, s, H-15), 2.91 (1H, d, J=5.4, 0 Hz, H-11), 4.06 (1H, dd, J=10.8,

Fig. 8. $\Delta\delta$ values in MTPA esters **10** and **12**.

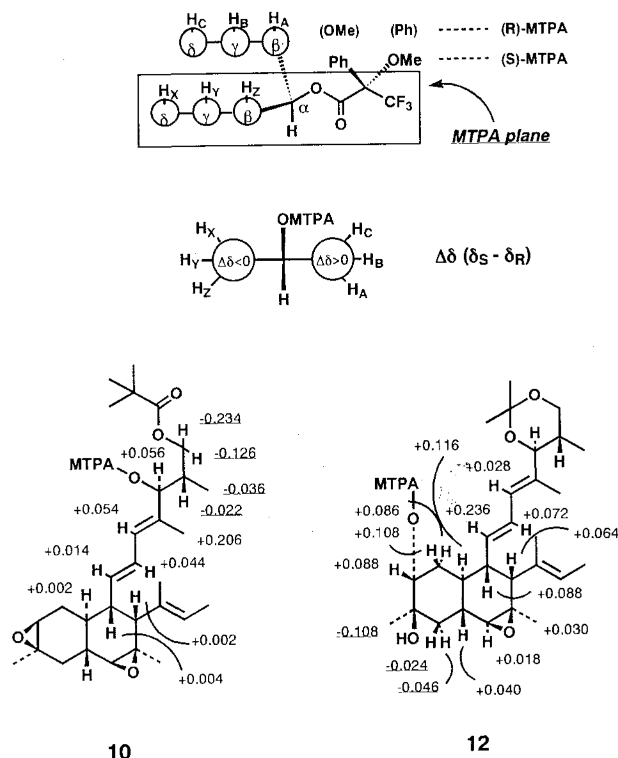
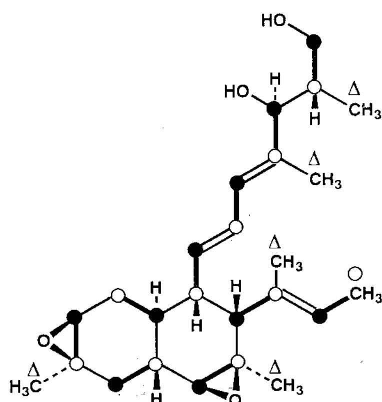


Fig. 9. Schematic representation of incorporation of biosynthetic precursors into fusarielin A.



10.8 Hz, H-1), 4.28 (1H, dd, J=10.8, 4.8 Hz, H-1), 4.60 (1H, d, J=10.4 Hz, H-3), 5.20 (1H, q, J=6.8 Hz, H-19), 5.32 (1H, dd, J=15.0, 10.0 Hz, H-7), 6.03 (1H, d, J=11.0 Hz, H-5), 6.26 (1H, dd, J=11.0, 15.0 Hz, H-6).

Fusarielin A Pivaloate (7)

To compound **1** (40.2 mg; 10 μM) in pyridine (40 μl) was added pivaloyl chrolide (25 μl) and the whole was stirred at room temperature for 20 minutes. To the reaction mixture was added water. The mixture was extracted with benzene. After evaporation of the solvent, the crude pivaloate **7** was purified by a silica gel column chromatography and HPLC (Nakarai 5C_{18}, eluted with 80% acetonitrile) to give **7**. $C_{30}H_{46}O_5$; FAB-MS 509 (M+Na$^+$).

Table 4. Antifungal activity fusarielin A.

Organism	MIC (μg/ml)
Aspergillus fumigatus 11268	3.1
Alternaria kikuchiana	50
Colletotricum lindemuthianum	25
Fusarium nivale	12.5
Fusarium oxysporum f. sp. *lycopersici*	>100
Fusarium solani f. sp. *phaseoli*	>100
Helminthosporium oryzae	25
Pyricularia oryzae	50
Rhizoctonia solani	>100
Rhizopus chinensis	>100

Fusarielin B Isopropylidene Acetal (8)

To compound **2** (21 mg; 50 μM) in dry acetone (1 ml) was added 2,2-dimethoxypropane (200 μl) and p-toluenesulfonic acid (1 mg) and the whole was stirred at room temperature for a few minutes. After neutralization of the reaction mixture with aq. $NaHCO_3$, water was added to the mixture which was extracted with benzene. After evaporation of solvent, the residue was chromatographed on a silica gel column to give **8** (21 mg). $C_{28}H_{44}O_5$; FAB-MS 483 (M+Na$^+$).

p-Bromobenzoyl Ester of Fusarielin A Pivaloate (9)

To pivaloate **7** (3.5 mg; 7.2 μM) in pyridine was added p-bromobenzoyl chrolide (3.5 mg; 16 μM) and dimethylamino pyridine (0.1 mg). The whole was stirred at room temperature overnight. After neutralization of the reaction mixture with aq. $NaHCO_3$, water was added to the mixture which was then extracted with benzene. After evaporation of the solvent, the residue was chromatographed on a silica gel column to give **9**. $C_{37}H_{49}O_6Br$; FAB-MS 693 (M+Na$^+$); ^{1}H NMR (DMSO-d_6, 50°C) 0.90 (3H, d, H-21), 0.97 (1H, dd, H-10), 1.12 (12H, s, H-24, CH_3-piva), 1.22 (1H, m, H-9), 1.25 (3H, s, H-23), 1.48 (1H, ddd, H-14), 1.57 (6H, s, H-20, 22), 1.62 (1H, dd, H-13), 1.72 (1H, ddd, H-10), 1.74 (3H, d, H-25), 2.06 (1H, dd, H-13), 2.12 (1H, ddd, H-8), 2.35 (1H, m, H-2), 2.45 (1H, d, H-17), 2.71 (1H, s, H-15), 2.88 (1H, d, H-11), 4.03 (2H, d, H-1), 5.16 (1H, q, H-19), 5.22 (1H, dd, H-3), 5.25 (1H, dd, H-7), 6.01 (1H, d, H-5), 6.22 (1H, dd, H-6), 7.74 (2H, d, H-Ph), 7.88 (2H, d, H-Ph).

MTPA Esters of Fusarielin A Pivaloate (10)

To pivaloate **7** (3.5 mg; 7.2 μM) in pyridine (40 μl) was added (R)-MTPA chrolide or (S)-MTPA chrolide (5 μl; 20 μM). The whole was stirred at room temperature overnight. After neutralization of the reaction mixture with aq. $NaHCO_3$, water was added to the mixture which was then extracted with benzene. After evaporation of the solvent, the residue was chromatographed on a silica gel column and HPLC (Sensyu aquasil SS, eluted with 3.5% isopropanol-n-hexane) to give MTPA esters **10**.

(*R*)-MTPA ester ((***R***)-**10**): ^{1}H NMR (DMSO-d_6, 50°C) 0.85 (3H, d, H-21), 1.03 (1H, dd, H-10), 1.13 (3H, s, H-24), 1.25 (9H, s, CH_3-piva), 1.24 (1H, m, H-9), 1.27 (3H, s, H-23), 1.50 (3H, s, H-22), 1.52 (1H, ddd, H-14), 1.59 (3H, d, H-20), 1.60 (3H, s, H-25), 1.65 (1H, dd, H-13), 1.75 (1H, ddd, H-10), 2.08 (1H, dd, H-13), 2.14 (1H, ddd, H-8), 2.24 (1H, m, H-2), 2.46 (1H, d, H-17), 2.72 (1H, s, H-15), 2.91 (1H, d, H-11), 3.93 (1H, dd, H-1), 4.06 (1H, dd, H-1), 5.19 (1H, q, H-19), 5.23 (1H, dd, H-3), 5.30 (1H, d, H-7), 6.02 (1H, d, H-5), 6.21 (1H, dd, H-6).

(*S*)-MTPA ester ((***S***)-**10**): ^{1}H NMR (DMSO-d_6, 50°C) 0.81 (3H, s, H-21), 1.02 (1H, dd, H-10), 1.13 (3H, s, H-24), 1.16 (9H, s, CH_3-piva), 1.23 (1H, m, H-9), 1.27 (3H, s, H-23), 1.51 (1H, ddd, H-14), 1.58 (3H, d, H-20), 1.59 (3H, s, H-25), 1.64 (1H, dd, H-13), 1.71 (3H, s, H-22), 1.73 (1H, ddd, H-10), 2.08 (1H, ddd, H-13), 2.15 (1H, ddd, H-8), 2.22 (1H, m, H-2), 2.47 (1H, d, H-17), 2.72 (1H, s, H-15), 2.89 (1H, d, H-11), 3.70 (1H, dd, H-1), 3.92 (1H, dd, H-1), 5.18 (1H, q, H-19), 5.28 (1H, dd, H-3), 5.31 (1H, dd, H-7), 6.07 (1H, d, H-5), 6.25 (1H, ddd, H-6).

p-Bromobenzoyl Ester of Fusarielin B Isopropyliden Acetal (11)

To acetal **8** (4.6 μg; 10 μM) in pyridine (40 μl) was added *p*-bromobenzoyl chrolide (3.5 mg; 16 μM) and dimethylamino pyridine (0.1 mg). The whole was stirred at room temperature overnight. Water was added to the reaction mixture which was then extracted with benzene. After evaporation of the solvent, the residue was chromatographed on a silica gel column and on HPLC (Sensyu aquasil SS, eluted with 3.5% isopropanol - *n*-hexane) to give **11**. $C_{35}H_{47}O_6Br$; FAB-MS 644 (M^+); ^{1}H NMR (DMSO-d_6, 50°C) 0.53 (3H, d, H-21), 1.11 (3H, s, H-24), 1.16 (3H, s, H-23), 1.24 (3H, s, isopro-CH_3), 1.35 (3H, s, isopro-CH_3), 1.37 (1H, ddd, H-13), 1.45 ~ 1.72 (5H, m, H-2, 9, 10, 12), 1.52 (3H, d, H-20), 1.55 (3H, s, H-25), 1.60 (3H, s, H-22), 1.92 (1H, ddd, H-14), 2.22 (1H, ddd, H-8), 2.50 (1H, d, H-17), 2.73 (1H, s, H-15), 3.44 (1H, dd, H-1), 3.62 (1H, dd, H-1), 3.81 (1H, d, H-3), 4.76 (1H, s, OH-12), 4.82 (1H, d, H-11), 5.07 (1H, dd, H-7), 5.25 (1H, q, H-19), 5.87 (1H, d, H-5), 6.16 (1H, dd, H-6), 7.77 ~ 7.84 (4H, m, Ph).

MTPA Esters of Fusarielin B Isopropyliden Acetal (12)

To acetal **8** (5.5 mg; 12 μM) in pyridine (60 μl) was added (*R*)-MTPA chrolide or (*S*)-MTPA chrolide (8 μl; 32 μM). The whole was stirred at room temperature overnight. Water was added to the reaction mixture which was then extracted with benzene. After evaporation of the solvent, the residue was chromatographed on a silica gel column and on HPLC (Sensyu aquasil SS, eluted with 3.5% isopropanol - *n*-hexane) to give **12**.

(*R*)-MTPA ester ((***R***)-**12**): ^{1}H NMR (DMSO-d_6, 50°C) 0.59 (3H, d, H-21), 1.09 (3H, s, H-24), 1.12 (3H, s, H-23), 1.27 (3H, s, isopro-CH_3), 1.34 (1H, m, H-9), 1.37 (3H, s, H-25), 1.41 (3H, s, isopro-CH_3), 1.41 (1H, dd, H-10), 1.41 (isopro-CH_3), 1.42 (1H, dd, H-13), 1.48 (3H, d, H-20), 1.50 (1H, ddd, H-10), 1.64 (3H, s, H-22), 1.67 (1H, dd, H-13), 1.73 (1H, m, H-2), 1.89 (1H, ddd, H-14), 2.14 (1H, ddd, H-8), 2.45 (1H, d, H-17), 2.66 (1H, s, H-15), 3.50 (1H, dd, H-1), 3.65 (1H, dd, H-1), 3.90 (1H, d, H-3), 4.83 (1H, d, H-11), 4.87 (1H, dd, H-7), 5.10 (1H, q, H-19), 5.89 (1H, d, H-5), 6.18 (1H, dd, H-6).

(*S*)-MTPA ester ((***S***)-**12**): ^{1}H NMR (DMSO-d_6, 50°C) 0.59 (3H, d, H-21), 1.01 (3H, s, H-23), 1.13 (3H, s, H-24), 1.25 (3H, s, isopro-CH_3), 1.36 (1H, dd, H-13), 1.38 (3H, s, isopro-CH_3), 1.39 (3H, d, H-20), 1.45 (1H, dd, H-10), 1.50 (3H, ddd, H-10), 1.51 (3H, s, H-25), 1.52 (1H, m, H-9), 1.64 (1H, dd, H-13), 1.66 (3H, s, H-22), 1.73 (1H, m, H-2), 1.90 (1H, m, H-14), 2.23 (1H, ddd, H-8), 2.51 (1H, d, H-17), 2.68 (1H, s, H-15), 3.49 (1H, dd, H-1), 3.66 (1H, dd, H-1), 3.88 (1H, d, H-3), 4.92 (1H, d, H-11), 5.10 (1H, dd, H-7), 5.15 (1H, q, H-19), 5.92 (1H, d, H-5), 6.25 (1H, dd, H-6).

Preparation of Microtubule Protein

Microtubule protein was prepared from porcine brains as described previously[6]. The protein concentrations were determined by the method of LOWRY *et al.*[7] using bovine serum albumin as a standard. Microtubule assembly assay were carried out in MES buffer, containing 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), 1 mM EGTA, 0.5 mM $MgCl_2$, 1 mM 2-mercaptoethanol and 1 mM GTP (pH 6.5).

Microtubule Assembly Assay

Microtubule assembly was monitored spectroscopically by using Shimadzu UV-300 apparatus equipped with a thermostatically regurated liquid circurator. The temperature was held at 37°C, and changes in turbidity were monitored at 400 nm. For the drug-protein study, drug dissolved in DMSO was added to 1 ml buffer solution containing 2 mg microtubule protein. The final DMSO concentrations were less than 1%.

Acknowledgment

The authors are indebted to the Institute of Science and Technology, Inc. for the evaluation of cytotoxicity and antimicrobial activity. Gratitude is due to Prof. N. HARADA of Tohoku University, Sendai for helpful discussion. The authors thank also to Prof. Y. SATO of Kyoritsu College of Pharmacy for the measurement of CD spectra. The work was supported in part by Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture of Japan.

References

1) ENDO, A.; M. KURODA & Y. TSUJITA: ML-236A, ML-236B and ML-236C, new inhibitors of cholesterogenesis produced by *Penicillium citrinum*. J. Antibiotics 29: 1346 ~ 1348, 1976
2) ICHIHARA, A.; H. OIKAWA, K. HAYASHI & S. SAKAMURA: Structures of betaenones A and B, novel phytotoxins from *Phoma betae* Fr. J. Am. Chem. Soc. 105: 2907 ~ 2908, 1983

3) CELMER, W. D.; G. N. CHUMURNY, C. E. MOPPETLE, R. S. WARE, P. C. WATTS & E. B. WHIPPLE: Structure of natural antibiotic CP-47. 444: J. Am. Chem. Soc. 102: 4203, 1980
4) HARADA, N. & K. NAKANISHI: Circular Dichroic Spectroscopy-exciton Coupling in Organic Stereochemistry. University Science Book and Oxford University Press, Oxford, 1983
5) OHTANI, I.; T. KUSUMI, Y. KASHMAN & H. KAKISAWA: High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. J. Am. Chem. Soc. 113: 4092~4096, 1991
6) TAKAHASHI, M.; S. IWASAKI, H. KOBAYASHI, S. OKUDA, T. MURAI, Y. SATO, T. HARAGUCHI-HIRAOKA & H. NAGANO: Studies on macrocyclic lactone antibiotics XI. Antimitotic and anti-tubulin activity of new antitumor antibiotics, rhizoxins and its homologues. J. Antibiotics 40: 66~72, 1987