Isolation and Characterization of Bioactive Metabolites from Marine-Derived Filamentous Fungi Collected from Tropical and Sub-Tropical Coral Reefs

Michio NAMIKOSHI,*[,a] Hisayoshi KOBAYASHI,[b] Takeshi YOSHIMOTO,[a,1)] Shiori MEGURO,[a] and Kyoko AKANO[a]

Department of Ocean Sciences, Tokyo University of Fisheries,[a] Minato-ku, Tokyo 108–8477, Japan and Institute of Molecular and Cellular Biosciences, The University of Tokyo,[b] Bunkyo-ku, Tokyo 113–0032, Japan.
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Two new compounds, paecilospirone (1) and phomopsidin (2), and seven known compounds, chaetoglobosin A (3), griseofulvin (4), fusarielin A (5), fusapyrone (6), deoxyfusapyrone (7), and verrucarins J (8) and L acetate (9), have been isolated and characterized from marine-derived fungi collected in tropical and sub-tropical coral reef environments. The utility of marine-derived fungi as a source of bioactive secondary metabolites is discussed.

Key words bioactive metabolite; paecilospirone; marine fungi; isolation; phomopsidin; marine natural product

Marine-derived fungi have recently been utilized as a new source of novel bioactive secondary metabolites.[2)] We are currently involved in a research project to search for bioactive compounds from culture broths of marine-derived filamentous fungi.[3—5)] The fungi were isolated from marine organisms and submerged materials collected in tropical and sub-tropical coral reef environments of Okinawa, Yap, Pohnpei, and Palau. Secondary metabolites were isolated by the cytotoxicity assay against human (HL-60) and murine (L1210) leukemia cell lines and by the bioassay method we recently developed to search for antimitotic and antifungal compounds using conidia of *Pyricularia oryzae* P-2b.[3)] Two new compounds, paecilospirone (**1**)[4)] and phomopsidin (**2**),[5)] and seven known compounds, previously reported as terrestrial fungal metabolites, chaetoglobosin A (**3**), griseofulvin (**4**), fusarielin A (**5**), fusapyrone (**6**), deoxyfusapyrone (**7**), and verrucarins J (**8**) and L acetate (**9**), have been isolated and characterized from culture broths of nine marine-derived filamentous fungi. We describe here the detail of isolation and fermentation of the fungi and structures of the secondary metabolites, and discuss the utility of marine-derived fungi as a prolific source of biologically active natural products.

Isolation and Fermentation of Fungi Marine organisms (sponges, tunicates, soft corals, and algae) and submerged materials (leaves, branches, and dead algae) were collected by Scuba diving, and a few small pieces of substrates were homogenized with sterile seawater. The liquid portion was applied on an agar plate, and two to three pieces of substrate, if the structure remained after homogenization, were also applied on an agar plate. Mycelia grown on the plates were individually isolated and inoculated onto a slant.

Fungi were separately cultured on a plastic plate with a half nutrient potato-dextrose medium (1/2 PD), and the broth was added with MeOH and stored in a freezer.

Bioassay 1) Conidia Assay: The broth was evaporated to remove MeOH and adjusted to the original volume with water for bioassay. The conidia assay was performed as reported previously[3)] and as briefly mentioned in the Experimental section. Deformations of mycelia germinated from conidia of *P. oryzae* P-2b were observed.[3)]

2) Cytotoxicity: The broth was evaporated to dryness, and the residue was extracted with EtOH to remove the salt. The EtOH extract was used for bioassay. The human (HL-60) and murine (L1210) leukemia cell lines were each cultured in a 24-well assay plate with samples. The shape of cells was observed after 24, 48, and 72 h, and the inhibitory activity of proliferation was observed after 72 h by comparing the number of cells in sample wells with those in control wells.

Paecilospirone (1)[4)] The culture broth of *Paecilomyces* sp., isolated from the coral reef at Yap, showed a curing effect on mycelia in the conidia assay. Paecilospirone (**1**) was separated from the MeOH extract of mycelia. The molecular formula of **1**, $C_{32}H_{44}O_5$, was deduced from high-resolution (HR) FAB-MS and NMR data. ^{1}H- and ^{13}C-NMR data for **1** (Table 1) showed the presence of two benzene rings, one ketone and a secondary alcohol, and two hydrocarbon chains. The ^{1}H–^{1}H correlated spectroscopy (COSY) spectra of **1** revealed the connection of carbon bonds at 4–5–6, 1′–9–10–11, 15–16, 4′–5′, 9′–10′–11′, and 15′–16′ (Table 1). The connectivity of carbons 1 through 11 and 1′ through 11′ were elucidated by heteronuclear multiple-bond correlation (HMBC) experiments (Table 1).

The lengths of the two hydrocarbon chains were assigned from ^{13}C chemical shifts of C-10 to C-16 and C-10′ to C-16′ and confirmed by electrospray-ionization MS/collisionally induced dissociation (ESI-MS/CID)/mass spectra of **1** (Fig. 1).

The relative stereochemistry of **1** was determined from ^{1}H-NMR and rotating frame nuclear Overhauser and exchange spectroscopy (ROESY) data. The coupling constants between H-1′ and H-9, H-9 and H-10a, and H-9 and H-10b were 4.0, 4.0, and 12.0 Hz, respectively, which showed H-9 and 1′-OH group are pseudaxial and H-1′ is pseudequatorial orientation. The ROESY experiment revealed the nuclear Overhauser effect (NOE) correlations (Fig. 2) and confirmed the relative stereochemistry of **1**.

Paecilospirone (**1**) has a unique skeletal structure, spiro[chroman-2,1′(3′*H*)-isobenzofuran], which has only been reported as chemical reaction products. Two hemiacetal derivatives of spiro[chroman-2,1′(3′*H*)-isobenzofuran]-3,4-

* To whom correspondence should be addressed. e-mail: namikosh@tokyo-u-fish.ac.jp
Dedicated to the memory of Dr. Kyosuke Tsuda.

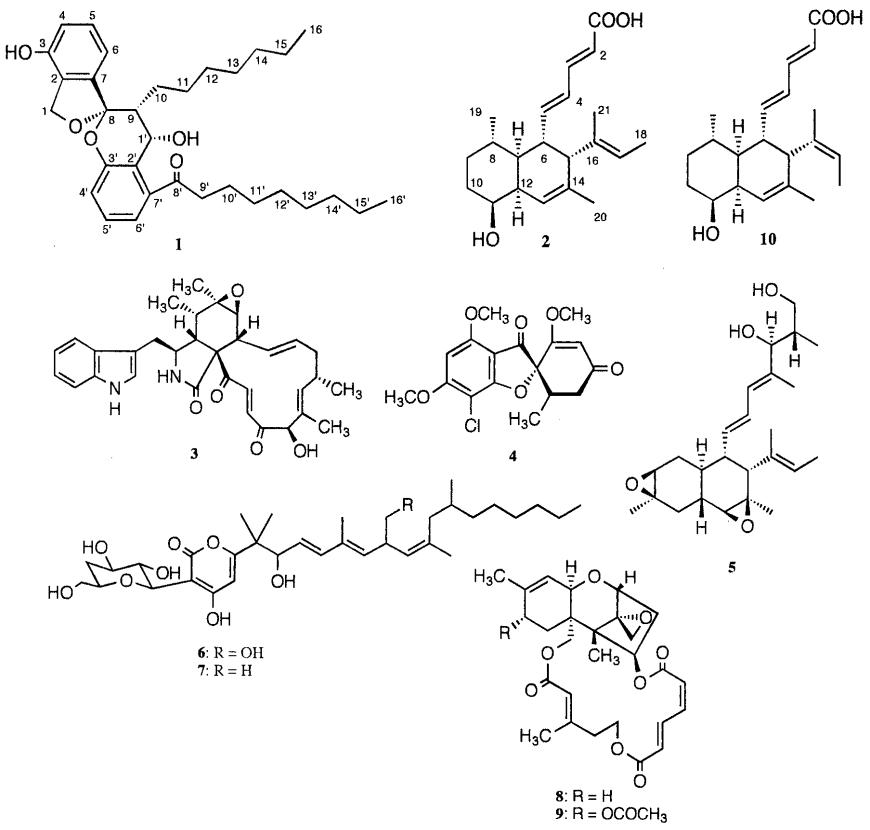

Chart 1

dione were synthesized by oxidation with periodic acid from 2′-hydroxymethylflavonol.[6] Two diastereomers of 4-hydroxy derivatives were prepared from phthalide with the mixture of metallic samarium and bromine (4 : 3).[7] Paecilospirone (**1**) is, therefore, the first example of this unique skeletal structure obtained as a biosynthetic product.

Paecilospirone (**1**) may be biosynthesized from two units of an octaketide, 2-hydroxy-6-(1-oxononyl)benzaldehyde *via* aldol condensation (Fig. 3). This would be a new route to dimeric derivatives biosynthesized from two polyketide units.[8]

The inhibitory activity of **1** to purified porcine brain microtubule proteins[9] was weak (20% inhibition at 50 μM), and accordingly, **1** was not antifungal at 25 μg/disc (disc diffusion assay) nor was it cytotoxic at 20 μg/ml to several tumor cell lines. The biosynthesis and biological activity of **1** are interesting subjects for future studies.

Phomopsidin (2)[5] *Phomopsis* sp. was isolated from a fallen mangrove branch on the bottom (−3 m) of a coral reef in Pohnpei. Phomopsidin (**2**), isolated from the broth filtrate, did not give an $(M+H)^+$ ion but showed the $(M+Na)^+$ ion at *m/z* 353 in the FAB and ESI mass spectra. The $(M-H)^-$ ion was detected at *m/z* 329 in the negative FAB-MS.

The molecular formula, $C_{21}H_{30}O_3$, was assigned from the negative HR-FAB-MS and NMR data. The ^{1}H–^{1}H COSY spectra of **2** revealed the connectivity of carbons 2 to 6, 19–8–7–12–11–10, and 17–18. ^{1}H couplings between H-12 and 13, and H-6 and 15 were very small (J=*ca.* 0 Hz). The analysis of HMBC (Fig. 4) and nuclear Overhauser and exchange spectroscopy (NOESY) (Fig. 5) data finally elucidated the skeletal structure of **2**.

The relative stereochemistry of **2** was assigned from ^{1}H-NMR and NOESY data (Fig. 5). Two double bonds at the 2 and 4 positions were determined to have *E*-orientations from the ^{1}H coupling constants, 15.2 and 15.0 Hz, respectively, and *E*-orientation at Δ^{16} was assigned from the NOE observed between H-17 and 15. The signal at δ 3.57 (H-11) showed coupling constants of 11.2 Hz with H-10*a*, 4.6 Hz with H-10*e*, and 4.6 Hz with H-12, which revealed that the configurations of H-11 and 10*a* are axial and that H-10*e* and 12 are equatorial. Signals due to H-10*e* (δ 1.63) and H-12 (δ 2.65) showed a W-shaped long-range coupling. NOEs were observed between H-11/7, H-11/9*a*, H-7/9*a*, and H-7/H_3-19. These data showed that H-7, 9*a*, and 11 are axial, and the configuration of C-19 is equatorial, that is, H-8 is axial. The dihedral angle of H-12 and 13 was assumed to be *ca.* 90° ($J_{12,13}$=*ca.* 0 Hz), and H-13 showed an NOE with H-10*a* (δ 1.38) and a long-range coupling with H-15 (δ 2.76). An NOE was observed between H-15 and 8 (δ 1.42). The configuration of H-15 was, therefore, deduced to be pseudaxial. The dihedral angle of H-15 and H-6 was shown to be *ca.* 90°, since the coupling constant was small ($J_{6,15}$=*ca.* 0 Hz). NOEs observed between H-6/H-4 and H-6/H_3-19 revealed the stereochemistry at C-6. Consequently, the relative stereochemistry of phomopsidin **(2)** was assigned as shown.

The structure of **2** resembled that of MK8383 (**10**) isolated from terrestrial *Phoma* sp. as an antifungal component to several phytopathogens.[10] Although **10** could not be separated from **2** chromatographically, ^{1}H-NMR spectra of **2** and **12** measured by the same instrument (500 MHz) clearly distinguished the two compounds.[11] Reported ^{13}C-NMR data for **10**[10,11] were very similar to those of **2**, except for C-15 and 21, which are consistent with the different stereochemistry at the C-18 methyl group.

Table 1. ^{1}H- and ^{13}C-NMR Data for Paecilospirone (**1**)

C#	^{13}C	^{1}H mult. (J in Hz)	COSY (H#)	HMBC (C#)[a]	ROESY (H#)
1a	71.3	5.11 d (13.0)		2, 3, 4, 5, 6, 7, 9	1b
1b		5.21 d (13.0)		2, 3, 4, 5, 6, 7, 9	1a
2	126.4				
3	140.8				
4	115.9	6.80 d (8.0)	5	1, 2, 3, 6, 7	5
5	129.8	7.24 dd (7.5, 8.0)	4, 6	2, 3, 4, 6, 7, 8	4, 6
6	113.9	6.87 d (7.5)	5	2, 3, 4, 5, 7, 8	5, 9, 10a, 11a
7	150.5				
8	112.2				
9	42.4	2.49 ddd (12.0, 4.0, 4.0)	10b, 10a, 1′	8, 10, 11	6, 10a, 11a, 1′
10a	25.2	1.07 m	9, 10b, 11a, 11b	8, 9, 11, (12), 1′	6, 9, 10b, 11b
10b		1.80 m	9, 10a, 11a, 11b	8, 9, 11, (12), 1′	10a, 11a, 11b, 1′, 1′-OH
11a	26.7	1.20 m	10a, 10b, 11b	9	6, 9, 10b, 11b, 1′
11b		1.57 m	10a, 10b, 11a	9, (12), (13)	10a, 10b, 11a, 1′
12	29.3[b]	1.25—1.35 m			
13	29.2[b]	1.25—1.35 m			
14	31.8	1.2—1.3 m			
15	22.6	1.2—1.3 m	16		
16	14.1	0.84 t (7.0)[c]	15	14, 15	
1′	60.7	5.11 dd (7.0, 4.0)	9, 1′-OH	8, 9, 2′, 3′, 7′	9, 10b, 11a, 11b, 1′-OH
2′	123.5				
3′	139.7				
4′	121.4	6.97 dd (7.0, 2.0)	5′	2′, 3′, 6′, 7′	5′
5′	128.9	7.28 m	4′	2′, 3′, 4′, 7′	4′
6′	121.5	7.27 m		2′, 3′, 4′, 7′, 8′	9′
7′	152.2				
8′	206.8				
9′	42.3	2.97 m	10′	8′, 10′, (11′)	6′, 10′
10′	24.4	1.74 m	9′, 11′	8′, 9′, (11′), (12′)	9′
11′	29.6[b]	1.25—1.35 m	10′		
12′	29.4[b]	1.25—1.35 m			
13′	29.2[b]	1.25—1.35 m			
14′	31.8	1.2—1.3 m			
15′	22.6	1.2—1.3 m	16′		
16′	14.1	0.88 t (7.0)[c]	15′	14′, 15′	
1′-OH		4.36 d (7.0)	1′	9, 10, 1′	10b, 1′

C#: carbon number. H#: proton number. *a*) Carbon number in the parenthesis for HMBC data indicates the most probable signal giving the correlation among the five overlapped signals at δ_C 29.2—29.6. *b*, *c*) These signals may be exchanged within the same mark.

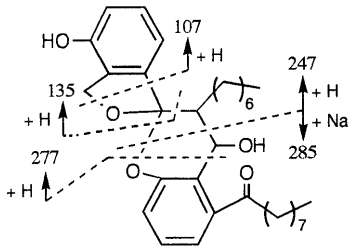

Fig. 1. Fragment Ions Detected in the ESI-MS/CID/Mass Spectra of Paecilospirone (**1**)

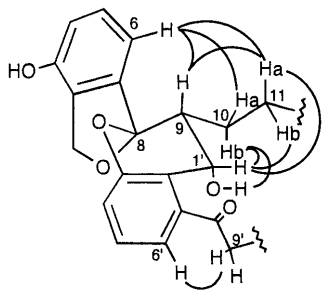

Fig. 2. NOE Correlations Assigned from the ROESY Spectrum of Paecilospirone (**1**)

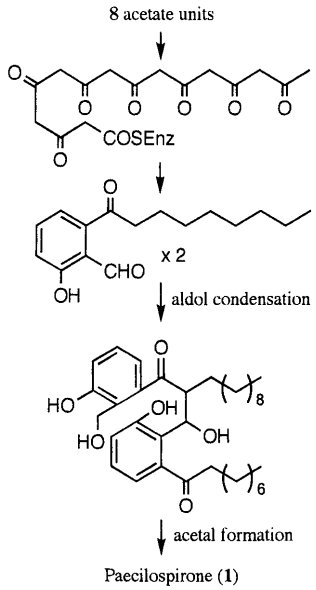

Fig. 3. A Probable Biosynthetic Pathway to Paecilospirone (**1**)

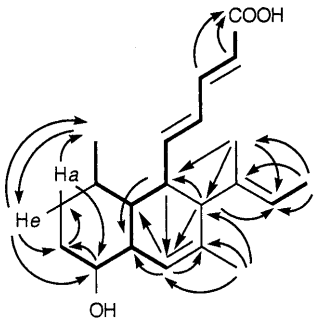

Fig. 4. COSY (—) and Selected HMBC Date for Phomopsidin (**2**)

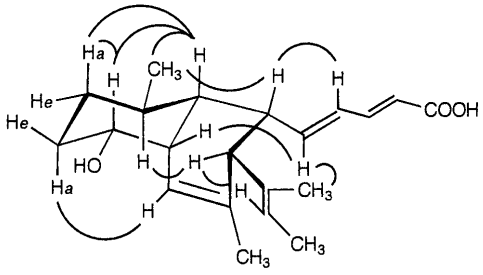

Fig. 5. NOE Correlations Assigned from the NOESY Spectrum of Phomopsidin (**2**)

The microtubule assembly assay using purified porcine brain microtubule proteins was performed as reported previously,[9] and **2** showed strong inhibitory activity (IC_{50}, 5.7 μM). Colchicine, rhizoxin, and compound **10** showed IC_{50} of 10, 4, and 8 μM, respectively, in the same experiment. Structurally similar compounds, fusarielins, possessing a *trans*-decaline moiety, did not show any inhibitory activity.[9] The *cis*-decaline structure may, therefore, be important to the activity.

Phomopsidin (**2**) would biogenetically be synthesized *via* a biological Diels–Alder reaction similar to the biosynthesis of solanapyrones and other decaline derivatives.[12]

Characterization of Known Compounds The culture broth of *Chaetomium* sp., isolated from a green alga at Yap, showed bead shape deformation on mycelia of *P. oryzae* in the conidia assay.[3] Bioassay guided separation of the broth filtrate as mentioned in the Experimental section gave chaetoglobosin A (**3**).[13] The structure of **3** was elucidated based on the spectral data (^{1}H- and ^{13}C-NMR, 2-dimensional (2D) NMR, mass, IR, and UV spectra) and confirmed by direct comparison with the authentic sample.

The characteristic curling effect on mycelia of *P. oryzae* in the conidia assay was observed by broths of *Penicillium* sp. and *Fusarium* sp., both isolated at Pohnpei. TLC analysis of the EtOAc extracts from the broths of *Penicillium* sp. and *Fusarium* sp. suggested the presence of griseofulvin (**4**)[14] and fusarielin A (**5**),[9] respectively. The compounds were separated by TLC and compared directly with the authentic samples.

Acremonium sp. from Yap and *Colletotrichum* sp. from Pohnpei showed a strange deformation (lumpy mycelia) in the conidia assay. The MeOH extract of mycelia of *Acremonium* sp. gave fusapyrone (**6**)[15,16] and a very small amount of deoxyfusapyrone (**7**).[16] The mycelia extract of *Colletotrichum* sp. afforded deoxyfusapyrone (**7**) as the only bioactive component. The structures of **6** and **7** were assigned by mass, ^{1}H- and ^{13}C-NMR, and 2D NMR spectra.

The EtOAc extract of whole broth of an unidentified strain (97F95), isolated from a sponge body collected at Aka Island, Okinawa, showed strong cytotoxicity to HL-60 and L1210. Verrucarin J (**8**)[17,18] was obtained as the only cytotoxic component of this strain. The structures of these compounds were elucidated based on ^{1}H-, ^{13}C-NMR, and 2D NMR spectral data. The fungus is not identified, since no conidium has been generated on several agar plates. The IC_{50} values of **8** to HL-60 and L1210 were both 2.5 ng/ml.

An unidentified strain (95F137), isolated from a sponge body at Pohnpei, showed cytotoxicity to HL-60. The bioactive compound was isolated by similar separation procedures as above and the structure characterized by ^{1}H-, ^{13}C-NMR, and 2D NMR spectral data as verrucarin L acetate (**9**).[19] The IC_{50} value of **9** to HL-60 was 1.0 ng/ml.

Discussion

Fungi living in marine environments are expected to have different biosynthetic pathways than their terrestrial counterparts. The differences are thought to result from the adaptation to the higher pressure and especially to the presence of salt. Therefore, the marine-derived fungi will be important sources of unique components. Although most of the secondary metabolites obtained from marine-derived fungi are parallel, if not identical, to those from terrestrial fungi, a few marine-derived fungi produced compounds having unique carbon skeletons.[2] Paecilospirone (**1**) is a remarkable example of a novel structure isolated for the first time from a natural source and also of a new biosynthetic pathway. Dimerization of two octaketide units, probably *via* aldol condensation (Fig. 3), has not been observed in terrestrial fungi. Another example of marine-derived fungus produced a chlorinated polyketide derivative which required seawater for the biosynthesis, while other metabolites were produced in freshwater culture medium by the same fungus.[20] We observed a similar phenomenon of salt being required in the culture of two fungi for the production of secondary metabolites (unpublished data).

Marine-derived fungi also show different ratios of biosynthetic products as observed in the present study. *Colletotrichum* sp. and unidentified strains 97F95 and 95F137 produced, respectively, deoxyfusapyrone (**7**), verrucarin J (**8**), and verrucarin L acetate (**9**) as the main components, while these compounds were isolated as minor products from terrestrial fungi *Fusarium semitectum*,[16] *Myrothecium* spp.,[18] and *Myrithecium verrucaria*,[18] respectively.

This study also showed that the same compounds were isolated from different genera of marine-derived fungi to those of terrestrial fungi. The marine-derived *Acremonium* sp. and *Colletotrichum* sp. produced fusapyrone (**6**) and deoxyfusapyrone (**7**), but these compounds were first isolated from the terrestrial *Fusarium* spp.[15,16]

The results obtained in this study suggest that marine-derived fungi are useful not only for a source of novel bioactive substances but also for new producers of known compounds which were isolated from terrestrial fungi as minor components with insufficient amounts

Experimental

NMR spectra were measured either on a JEOL JNM A-500 NMR spectrometer or a Varian UNITY INOVA-500 spectrometer. Mass spectra were obtained by either a JEOL HX-110 mass spectrometer (FAB mode) or a

Finnigan TSQ 700 triple quadrupole mass spectrometer (ESI mode). UV and IR spectra were recorded on a Shimadzu UV-300 and on a JASCO A-102, respectively.

Isolation of Fungi Marine organisms (sponges, tunicates, soft corals, and algae) and submerged material (leaves, branches, and dead algae) were collected by Scuba diving in coral reefs at Yap (1994), Pohnpei (1995), Palau (1995, 1998), and Aka Island, Okinawa (1996—1998). The substrates were sealed in sterile plastic bags in the water and stored in a cooler box with coolant. Treatment of the substrates was done within 3 h in a laboratory of the research vessel Sohgen-maru (Yap, Pohnpei, Palau 1995), the training vessel Umitaka-maru (Palau 1998), or in the Akajima Marine Science Laboratory (Aka Island). Three to five small pieces of each substrate were placed in a sterile mortar with 1—2 ml of sterile seawater and homogenized with a pestle. One- to two-hundred microliters of the liquid portion was placed on an agar plate (0.02% yeast extract, 0.1% soluble starch, 2% agar, and 200 ppm chloramphenicol in 90% natural seawater). The substrate remaining in the mortar was pressed by the pestle to remove liquid and further dried with sterile paper towels, and two to three pieces were applied on an agar plate. The plates were placed in the research room (25—26 °C) of the ship and then incubated at 20 °C in incubators after returning to the university.

The mycelia grown on an agar plate or on a substrate were inoculated on a slant in a culture tube. The cultures are maintained in the Tokyo University of Fisheries as TUF strain numbers designated in the individual description.

Fermentation of Fungi Each fungus was cultured on a plastic plate for screening bioassays with 15 ml of 1/2 PD (hot water (500 ml) extract of potato (100 g), 10 g dextrose, 500 ml natural seawater) for three weeks at 20 °C. MeOH (8 ml) was added and the broth was stored in a freezer (−30 °C).

This sample was filtered, and 1.0 ml of filtrate was evaporated to about half the volume, and then the volume adjusted to 1.0 ml with water. This solution was used for the conidia assay.

For cytotoxicity assays, 1.0 ml of the above filtrate was evaporated to dryness, dissolved in EtOH, and filtered. The EtOH extract was evaporated and used for bioassays.

Bioassay 1) Conidia Assay: The assay was performed as described previously.[3] Briefly, a suspension (50 μl) of conidia of *P. oryzae* P-2b in sterile water containing 0.2% yeast extract was placed in each well of a 96-well assay plate, and the sample solution (50 μl) was added to the first well. The suspension was mixed and 50 μl moved to the second well. The procedure was repeated to the last well of the column. The assay plates were incubated for 16 h at 27 °C, and the shape of mycelia germinated from the conidia was observed and compared with controls (negative: water, positive: rhizoxin) under an inverted microscope.

2) Cytotoxicity: The human (HL-60) and murine (L1210) leukemia cell lines were incubated in Roswell Park Memorial Institute 1640 and minimal essential medium (modified Eagle's medium), respectively, using 24-well assay plates. Ten microliters of the EtOH solution of each sample in the required concentration was placed in a well and the solvent evaporated. The suspension (1 ml, 1×10^4 cells/ml) of L1210 or HL-60 was added to each well and incubated at 37 °C for 72 h in a CO_2 incubator (5% CO_2). The shape of cells was observed after 24, 48, and 72 h under an inverted microscope. The number of vital cells after 72 h was compared between sample and control wells using WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disufophenyl)-2*H*-tetrazolium, monosodium salt][21] (Cell Counting Kit-8[36]).

Paecilospirone (1) *Paecilomyces* sp. 94F79 was cultured in ten 500-ml flasks (each 150 ml, 1/2 PD, 50% natural seawater) for three weeks at 20 °C and filtered. The MeOH extract (1.5 g) of mycelia was subjected to solid phase extraction with octadecylsilanated silica gel (ODS), and the bioactive fraction (100% MeOH eluate) was separated by silica gel column chromatography (benzene, benzene–acetone=11 : 1 and 4 : 1, and then MeOH). The fraction eluted with benzene–acetone (11 : 1) was purified with a silica gel column (benzene : acetone=98 : 2) to give 8.5 mg of **1**, $[\alpha]_D^{25}$ +202.5° (c=0.37, MeOH); HR-FAB-MS: $[M+Na]^+$ m/z 531.3087, Calcd for $C_{32}H_{44}O_5Na$, 531.3086; UV λ_{max} nm (MeOH): 206, 218, 251, 278, 299; IR (KBr): 3340, 2930, 2860, 1666, 1607, 1582, 1452, 1096, 1060, 1000, 840 cm^{-1}. ^{1}H- and ^{13}C-NMR data are listed in Table 1.

Phomopsidin (2) *Phomopsis* sp. 95F47 was cultured in ten 500-ml flasks (each 100 ml of 1/2 PD, 50% natural seawater) for three weeks at 20 °C. Acetone was added to the cultured broth and filtered. The filtrate was extracted with benzene, and the extract was separated by a silica gel column (benzene, benzene : acetone=2 : 1, and then MeOH). The bioactive fraction (benzene–acetone eluate) was subjected to silica gel column chromatography (benzene : acetone=4 : 1) followed by HPLC (ODS, $CH_3CN : H_2O$= 1 : 1) to afford 12.0 mg of **2**, $[\alpha]_D^{27}$ +31° (c=0.1, MeOH); HR-FAB-MS $[(M-H)^-$, m/z 329.2121, Calcd for $C_{21}H_{29}O_3$, 329.2125]; UV (MeOH) λ_{max} nm (ε): 260 (23000); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.77 (d, 15.2 Hz, H-2), 7.18 (dd, 15.2, 10.2 Hz, H-3), 6.15 (dd, 15.0, 10.2 Hz, H-4), 6.26 (dd, 15.0, 9.0 Hz, H-5), 2.78 (m, H-6), 1.25 (m, H-7), 1.42 (m, H-8), 1.00 (m, H-9*a*), 1.65 (m, H-9*e*), 1.38 (m, H-10*a*), 1.63 (m, H-10*e*), 3.57 (m, 11.2, 4.6, 4.6 Hz, H-11), 2.65 (m, H-12), 5.73 (s, $J_{12,13}$=*ca.* 0 Hz, H-13), 2.76 (s, $J_{6,15}$=*ca.* 0 Hz, H-15), 5.22 (q, 6.5 Hz, H-17), 1.55 (dd, 6.5, 0.8 Hz, H-18), 0.94 (d, 6.5 Hz, H-19), 1.60 (s, H-20), 1.48 (s, H-21); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.9 (C-1), 120.6 (C-2), 146.9 (C-3), 129.0 (C-4), 148.8 (C-5), 45.3 (C-6), 49.3 (C-7), 29.9 (C-8), 34.0 (C-9), 31.1 (C-10), 73.3 (C-11), 39.1 (C-12), 124.3 (C-13), 136.1 (C-14), 51.3 (C-15), 136.4 (C-16), 123.5 (C-17), 13.5 (C-18), 19.4 (C-19), 22.3 (C-20), 16.8 (C-21).

Chaetoglobosin A (3) *Chaetomium* sp. 94F49 was cultured in 10 plates of 1/2 PD (50% seawater, each 25 ml) at 20 °C for three weeks and filtered. The filtrate was extracted with HP-20 (MeOH eluate) and then with the mixture of water, acetone, and benzene. The organic layer was concentrated, and the residue was chromatographed on silica gel with benzene, benzene–acetone and then methanol to afford 7 mg of **3**, UV λ_{max} nm (MeOH): 222; IR (KBr): 3490, 3440, 2990, 1695, 1618, 1460, 1300, 1160, 980, 910 cm^{-1}. The structure was confirmed by direct comparison with the authentic sample using HPLC (ODS, $CH_3CN : H_2O$=55 : 45).

Griseofulvin (4) *Penicillium* sp. 95F629 was cultured in three plates (each 15 ml of 1/2 PD). The broth was added with acetone and extracted with EtOAc. The EtOAc extract was separated by TLC (benzene : acetone=3 : 2) to give **4**, which was compared with the authentic sample of griseofulvin by TLC (Rf=0.71).

Fusarielin A (5) The broth of *Fusarium* sp. 95F858 cultured in plates (1/2 PD) was filtered, and mycelia were extracted with MeOH. The solid phase extraction of mycelia extract by ODS (80% MeOH eluate) and of broth filtrate by HP-20 (100% MeOH eluate) showed bioactivity. These fractions gave similar spots on TLC (benzene : acetone=3 : 2), and the bioactive component (**5**) was separated by TLC. TLC analysis (Rf=0.65, red spot by sulfuric acid followed by heating) of **5** with the authentic sample revealed that **5** was identical to fusarielin A.

Fusapyrone (6) and Deoxyfusapyrone (7) *Acremonium* sp. 94F74 was cultured in ten plates (1/2 PD, each 25 ml). The broth was filtered, and the mycelia were extracted with MeOH, then subjected to solid phase extraction with ODS. The 85% MeOH eluate was separated by HPLC (ODS, 80% MeOH) to give **6** and **7**. Fusapyrone (**6**), HR-FAB-MS: $[M+H]^+$ m/z=607.3827, Calcd for $C_{34}H_{55}O_9$, 607.3846; IR (KBr): 3420, 2940, 1651, 1502, 1451, 970 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.88 (H-35), 0.90 (H-29), 1.17 (H-30), 1.24 (H-31), 1.24 (10H, H-24, 25, 26, 27, 28), 1.61 (H-23), 1.66 (H-34), 1.84 (H-32), 1.89 (H-10), 1.96, 2.06 (H-22), 3.44 (H-33a), 3.5 (3H, H-12, 33b), 3.6 (2H, H-11, 19), 3.7 (H-9), 4.21 (H-8), 4.49 (H-14), 4.67 (H-7), 5.09 (H-20), 5.39 (H-18), 5.69 (H-15), 6.00 (H-5), 6.37 (H-16); ^{13}C-NMR (125 MHz, $CD_3OD : C_6D_6$=2 : 1): δ 169.4 (C-2), 97.7 (C-3), 180.2 (C-4), 106.8 (C-5), 169.2 (C-6), 76.3 (C-7), 73.2 (C-8), 74.3 (C-9), 35.7 (C-10), 77.4 (C-11), 65.0 (C-12), 44.8 (C-13), 77.9 (C-14), 126.5 (C-15), 138.4 (C-16), 134.2 (C-17), 134.6 (C-18), 41.8 (C-19), 126.7 (C-20), 136.7 (C-21), 41.0 (C-22), 32.0 (C-23), 38.0 (C-24), 28.2 (C-25), 30.5 (C-26), 32.8 (C-27), 23.5 (C-28), 14.5 (C-29), 22.6 (C-30), 20.8 (C-31), 13.4 (C-32), 66.7 (C-33), 24.0 (C-34), 20.1 (C-35). Deoxyfusapyrone (**7**), HR-FAB-MS: $[M+H]^+$ m/z=591.3884, Calcd for $C_{34}H_{55}O_8$, 591.3897.

Colletotrichum sp. 95F7 was cultured in 1/2 PD as above. The MeOH extract of mycelia showed a similar deformation on mycelia of *P. oryzae* in the conidia assay to that of the extract from *Acremonium* sp. The bioactive component was separated by ODS (85% MeOH eluate) followed by TLC ($CH_2Cl_2 : MeOH : H_2O$=80 : 19 : 1) and identified as deoxyfusapyrone (**7**) by TLC and HPLC (ODS, 80% MeOH) with compounds **6** and **7** obtained from *Acremonium* sp.

Verrucarin J (8) An unidentified strain 97F95, isolated at Aka Island, was cultured in 1/2 PD (each 150 ml, six 500-ml flasks), and the broth was added with acetone (450 ml) and extracted with EtOAc. The extract was separated by a silica gel column (benzene–EtOAc, benzene–acetone, and then MeOH) followed by HPLC (ODS, 70% MeOH) to afford **8** (0.4 mg), UV λ_{max} nm (MeOH): 263; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.88 (d, 5.0, H-2), 2.16 (m, H-3a), 2.54 (m, H-3b), 6.00 (m, H-4), 1.84 (m, H-7a), 1.98 (m, H-7b), 2.00 (H-8), 5.28 (br d, 5.0 Hz, H-10), 3.68 (d, 5.5 Hz, H-11), 2.83 (d, 4.0 Hz, H-13a), 3.12 (d, 4.0 Hz, H-13b) 0.83 (s, H-14), 3.98 (d, 12.5 Hz, H-15a), 4.42 (d, 12.5 Hz, H-15b), 1.72 (s, H-16), 5.85 (s, H-2′), 2.53 (H-4′), 4.15 (m, H-5′a), 4.44 (m, H-5′b), 6.02 (d, 15.5 Hz, H-2″), 8.09 (dd, 15.5, 11.2 Hz, H-3″), 6.62 (dd, 11.2, 11.0 Hz, H-4″), 6.10 (d, 11.0 Hz, H-5″); ^{13}C-NMR (125 Hz, $CDCl_3$): δ 79.0 (C-2), 35.1 (C-3), 75.3 (C-4), 48.8 (C-5),

43.0 (C-6), 20.8 (C-7), 27.7 (C-8), 140.4 (C-9), 118.6 (C-10), 67.3 (C-11), 65.5 (C-12), 48.1 (C-13), 7.00 (C-14), 63.3 (C-15), 23.3 (C-16), 166.1 (C-1′), 118.1 (C-2′), 156.6 (C-3′), 40.2 (C-4′), 60.4 (C-5′), 17.2 (C-6′), 165.5 (C-1″), 127.4 (C-2″), 139.1 (C-3″), 139.5 (C-4″), 125.5 (C-5″), 165.8 (C-6″).

Verrucarin L Acetate (9) An unidentified strain 95F137, isolated at Palau, was cultured in 1/2 PD (six 500-ml flasks), and the broth was added with acetone (450 ml) and extracted with EtOAc. The EtOAc extract was separated by a silica gel column (benzene, benzene–acetone, and then MeOH) followed by TLC (benzene : acetone=4 : 1) to afford **9**, UV λ_{max} nm (MeOH): 262; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.84 (d, 5.0 Hz, H-2), 2.21 (m, H-3a), 2.49 (m, H-3b), 5.93 (dd, 8.0, 4.0 Hz, H-4), 2.19 (m, H-7), 5.19 (m, H-8), 5.70 (br d, 5.5 Hz, H-10), 3.80 (d, 5.5 Hz, H-11), 2.84 (d, 4.0 Hz, H-13a), 3.11 (d, 4.0 Hz, H-13b), 0.81 (s, H-14), 4.21 (d, 12.5 Hz, H-15a), 4.54 (d, 12.5 Hz, H-15b), 1.76 (s, H-16), 1.95 (s, H-18), 5.77 (br s, H-2′), 2.54 (m, H-4′), 4.13 (ddd, 11.0, 11.0, 3.5 Hz, H-5′a), 4.49 (ddd, 11.0, 4.0, 4.0 Hz, H-5′b), 2.28 (d, 1.0 Hz, H-6′), 6.01 (d, 15.5 Hz, H-2″), 8.00 (dd, 15.5, 11.5 Hz, H-3″), 6.62 (dd, 11.5, 10.5 Hz, H-4″), 6.09 (d, 10.5 Hz, H-5″); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 78.9 (C-2), 34.9 (C-3), 74.8 (C-4), 49.0 (C-5), 42.2 (C-6), 26.5 (C-7), 68.8 (C-8), 136.5 (C-9), 123.8 (C-10), 67.0 (C-11), 65.3 (C-12), 47.9 (C-13), 7.0 (C-14), 64.4 (C-15), 20.5 (C-16), 170.9 (C-17), 21.0 (C-18), 165.6 (C-1′), 117.7 (C-2′), 157.0 (C-3′), 40.2 (C-4′), 60.4 (C-5′), 17.1 (C-6′), 165.5 (C-1″), 127.8 (C-2″), 138.8 (C-3″), 139.9 (C-4″), 125.1 (C-5″), 165.8 (C-6″).

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