

COMMUNICATIONS TO THE EDITOR

**Phomopsidin, a New Inhibitor of Microtubule
Assembly Produced by *Phomopsis* sp.
Isolated from Coral Reef in Pohnpei**

Sir:

Antimitotic natural products, such as taxol, ansamitocin P-3, vinblastine, and griseofulvin, are useful antitumor and antifungal agents. Recently, curacin A, which is a potential antitumor agent, was isolated from marine cyanobacterium (blue-green alga) *Lyngbya majuscula* as an antimitotic cytotoxic component.¹⁾ We have reported an effective assay method for antimitotic substances, which observes deformations of mycelia germinated from conidia of *Pyricularia oryzae* P-2b, and certain antimitotic compounds can be detected by the characteristic curling effect of the mycelia.²⁾ Curacin A, which inhibits the assembly of microtubule proteins, showed the curling effect.

This bioassay method has been applied to fermentation broths of filamentous fungi isolated from marine environments (invertebrates, algae, submerged materials and so on) in Pohnpei and Palau. A new antimitotic component **1** has been isolated from a fungal strain TUF 95F47. We describe here the isolation and fermentation of the fungus and isolation, structure, and biological activity of the compound.

The filamentous fungus, TUF 95F47 was isolated from a fallen mangrove branch on the bottom (−3 m) of a coral reef in Pohnpei during the Pohnpei/Palau expedition by the research vessel *Sohgen-maru* operated by Marine Biotechnology Institute in 1995. The fungus was isolated from a colony formed from the substrate inoculated on an agar plate (1/2 nutrient Potato Dextrose Agar (1/2 PDA) medium, 80% sea water containing 100 ppm of chloramphenicol) in a clean room on the ship. It was maintained on a slant (1/2 PDA, 50% sea water) as a stock culture. Identification of the fungus was done in the usual manner using PDA and Oat Meal Agar as media, and TUF 95F47 was identified as a *Phomopsis* sp. The fungus was cultured for a screening assay with 1/2 nutrient Potato Dextrose (1/2 PD) medium (50% sea water) in a plate (15 ml) for 3 weeks at 20°C.

Phomopsis sp. strain TUF 95F47 was cultured in ten 500 ml Erlenmeyer flasks (each 100 ml of 1/2 PD, 50% sea water) for 3 weeks at 20°C. The curling effect on the mycelia in the bioassay performed as reported²⁾ and was

observed with the broth filtrate. Acetone was added to the cultured broth and filtered. The filtrate was extracted with benzene, and the active component was separated by two consecutive silica gel column chromatographies (benzene - acetone, 2 : 1 and 4 : 1, respectively) and HPLC (ODS, CH₃CN - H₂O = 1 : 1) to afford 12.0 mg of **1**, [α]_D²⁷ + 31° (*c* 0.1, MeOH), UV (MeOH) λ_{\max} nm (ϵ): 260 (23,000).

¹H and ¹³C NMR data for **1**, assigned by ¹H-¹H COSY, HMQC, and HMBC spectra, are listed in Table 1. Compound **1** did not give an (M+H)⁺ ion but showed the (M+Na)⁺ ion at *m/z* 356 in the FAB and ESI mass spectra, but the (M-H)[−] ion was detected at *m/z* 329 in the negative FAB-MS spectrum. The molecular formula, C₂₁H₃₀O₃, was assigned from the negative HR-FAB-MS [(M-H)[−], *m/z* 329.2121 (Δ −0.4)] and NMR data. The ¹H-¹H COSY spectra of **1** revealed the connectivity of carbons 2 to 6, 19-8-7-12-11-10, and 17-18. ¹H couplings between H-12 and 13, and H-6 and 15 were very small (*J* = ~0 Hz). The skeletal structure of **1** was finally elucidated by the analysis of HMBC and NOESY spectral data as listed in Table 1.

The relative stereochemistry of **1** was assigned from ¹H NMR and NOESY data (Table 1). Two double bonds at the 2 and 4 positions were determined to have *E*-orientations from the ¹H 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*.

Fig. 1. Structures of phomopsidin (**1**) isolated from *Phomopsis* sp. strain TUF 95F47 and MK8383 (**2**).

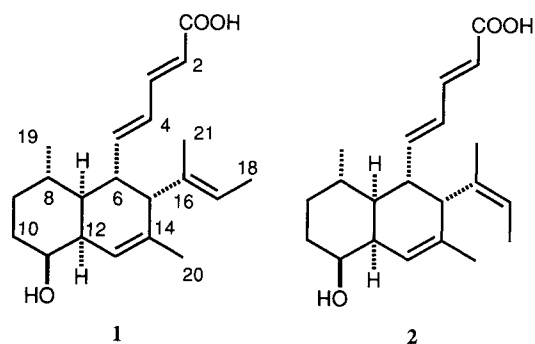


Table 1. ^1H (500 MHz) and ^{13}C NMR (125 MHz) data for **1** and **2** in methanol- d_4 .

C#	1				2	
	^{13}C	^1H	HMBC (C#)	NOESY (H#)	$^{13}\text{C}^a$	^1H
1	170.9 s	—			170.8 s	—
2	120.6 d	5.77 d (15.2)	1, 3		120.8 d	5.78 d
3	146.9 d	7.18 dd (15.2, 10.2)	1, 2, 4, 5		146.8 d	7.18 dd
4	129.0 d	6.15 dd (15.0, 10.2)	2, 3, 5, 6	6	129.5 d	6.16 dd
5	148.8 d	6.26 dd (15.0, 9.0)	3, 4, 6	12, 21	148.3 d	6.34 dd
6	45.3 d	2.78 m	4, 5, 12, 13, 15	4, 19	44.7 d	
7	49.3 d ^b	1.25 m	12, 13	9a, 11, 19	solv. ^c	
8	29.9 d	1.42 m	7, 19	15	30.1 d	
9	34.0 t	(a) 1.00 m (e) 1.65 m	11, 19 8, 10, 11, 19	7, 11	34.0 t	1.02
10	31.1 t	(a) 1.38 m (e) 1.63 m	9, 11, 12 9, 11, 12	13	31.0 t	
11	73.3 d	3.57 m (11.2, 4.6, 4.6)	10, 12	7, 9a	73.2 d	3.61 m
12	39.1 d	2.65 m		5	39.0 d	2.71 br. s
13	124.3 d	5.73 s ($J_{12,13} = \sim 0$)	7, 12, 14, 20	10a	123.5 d	5.72 br. s
14	136.1 s	—			136.4 s	—
15	51.3 d	2.76 s ($J_{6,15} = \sim 0$)	6, 13 17, 21	8, 17	43.1 d	
16	136.4 s	—			136.3 s	—
17	123.5 d	5.22 q (6.5)	6, 15, 16, 18, 21	15	123.9 d	5.37 m
18	13.5 q	1.55 dd (6.5, 0.8)	6, 15, 16, 17, 21		13.5 q	1.62 d
19	19.4 q	0.94 d (6.5)	7, 8, 9	6, 7	19.6 q	0.97 d
20	22.3 q	1.60 s	13, 14, 15		22.3 q	1.61 s
21	16.8 q	1.48 s	6, 15, 16, 17, 18	5	22.5 q	1.54 s

^a Reported value (100 MHz)³⁾.^b Calibrated from field gradient 2D NMR spectra.^c Overlapped with solvent.

Signals due to H-10e (δ 1.63) and H-12 (δ 2.65) showed a W-shaped long-range coupling. NOEs were observed between H-11/7, H-11/9a, H-7/9a, and H-7/H₃-19. These data showed that H-7, 9a, 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° ($J_{12,13} = \sim 0$ Hz), and H-13 showed an NOE with H-10a (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 *pseudo-axial*. The dihedral angle of H-15 and H-6 was shown to be *ca.* 90°, since the coupling constant was small ($J_{6,15} = \sim 0$ Hz). NOEs observed between H-6/H-4 and H-6/H₃-19 revealed the stereochemistry at C-6. Consequently, the relative stereochemistry of phomopsidin (**1**) was assigned as shown in Fig. 1.

The structure of **1** resembled that of MK8383 (**2**) isolated from terrestrial *Phoma* sp. as an antifungal component to several phytopathogens³⁾. Although **2** could not be separated from **1** chromatographically, ^1H NMR spectra of **1** and **2** measured by the same instru-

ment (500 MHz) clearly distinguished the two compounds.[†] Reported ^{13}C NMR data for **2**^{3,†)} listed in Table 1 were very similar to those of **1**, except for C-15 and 21, which are consistent with the different stereochemistry at the C-18 methyl group. Compounds **1** and **2** would biogenetically be synthesized *via* a biological Diels-Alder reaction similar to the biosynthesis of solanapyrones and other decaline derivatives.^{4,5)}

The microtubule assembly assay using purified porcine brain microtubule proteins was performed as reported previously,⁶⁾ and **1** showed strong inhibitory activity (IC_{50} , 5.7 μM). Colchicine and rhizoxin showed IC_{50} of 10 and 4 μM , respectively, in the same experiment. Structurally similar compounds, fusarielins, possessing a *trans*-decaline moiety, did not show any inhibitory activity.⁶⁾ The *cis*-decaline structure may, therefore, be important to the activity. It is suggested that MK8383 (**2**) shows similar activity to **1**.

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[†] The authentic sample of MK8383 (**2**) and the ^{13}C NMR, ^1H - ^1H and ^1H - ^{13}C COSY and HMBC spectra were provided by Meiji Seika Kaisha, LTD. The ^1H NMR spectrum of **2** was measured to compare directly with that of **1**.

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