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# 12,13-Dihydroxyfumitremorgin C, Fumitremorgin C, and Brevianamide F, Antibacterial Diketopiperazine Alkaloids from the Marine-Derived Fungus *Pseudallescheria* sp.

**Dahai Zhang, Dedi Noviendri, Muhammad Nursid, Xiudong Yang, and Byeng Wha Son***

*Department of Chemistry, Pukyong National University, Busan 608-737, Korea*

**Abstract** – Dioxopiperazine alkaloids, 12*R*,13*S*-dihydroxyfumitremorgin C (**1**), fumitremorgin C (**2**), and brevianamide F (**3**), were isolated from the marine-derived fungus *Pseudallescheria*, and the absolute stereostructures of compounds **1** - **3** were elucidated on the basis of chemical and physicochemical evidence. Compounds **1** - **3** showed an antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. The MIC (minimum inhibitory concentration) values of compounds **1** - **3** were 125 μg/mL for all strains.
**Keywords** – Marine-derived fungus, *Pseudallescheria* sp., 12,13-dihydroxyfumitremorgin C, fumitremorgin C, brevianamide F, antibacterial activity

## Introduction

The exploitation of the marine environment has been intriguingly successful in recent years in the search for structurally unusual and biologically active natural products (Blunt *et al*., 2007). To avoid depletion of marine resources and to enable access to large quantities of interest in compounds, there is a particular interest in those marine organisms that are culturable. Thus, we are studying fungi isolated from marine sources for their potential of providing new natural products. In a combined approach of biological and chemical screening we are gaining a thorough understanding of the secondary metabolite pattern of this fungus. *Pseudallescheria* sp. was selected from our screening program for further studies, because of a rich metabolite pattern as detected by TLC on Si gel with various staining reagents, and antimicrobial activity of its extract against *Staphylococcus aureus* (SA), methicillin-resistant *S. aureus* (MRSA), and multidrug-resistant *S. aureus* (MDRSA) strains.

As part of an effort to discover biologically active natural products from marine microorganisms (Zhang *et al*., 2007), we have investigated bioactive constituents of the marine-derived fungus *Pseudallescheria* sp., and isolated mild antibacterial diketopiperazines, 12*R*,13*S*-dihydroxyfumitremorgin C (demethoxycyclotryprostatin C, **1**) (Abraham and Arfmann, 1990; Cui *et al*., 1996), fumitremorgin C (**2**) (Buckingham *et al*., 1994; Cui *et al*., 1996), and brevianamide F (**3**) (Birch and Russell, 1972).

This paper briefly describes the isolation and structural elucidation of **1-3**, and evaluation of their antibacterial activities.

## Experimental

**Fungal isolation and culture** – The fungal strain, *Pseudallescheria* sp., was isolated from the surface of the drift wood collected at Namhae Island, Gyeongnam Province, Korea, in 2003 and identified based on the morphological evaluation and fatty acid methyl ester analysis (Korean Culture Center of Microorganism, Seoul, Korea, a similarity index of 0.65). A voucher specimen is deposited at Pukyong National University with the code MFB262.

The fungus was cultured (10 L) for 30 days (static) at 29 °C in SWS medium consisting of soytone (0.1%), soluble starch (1.0%), and seawater (100%).

**Extraction and isolation** – The culture broth and mycelium were separated, and the broth (10 L) was extracted with ethyl acetate to provide crude extract (800 mg), which was subjected to silica gel flash chromatography and eluted with *n*-hexane-EtOAc (5 : 1),

*Author for correspondence
Fax: +82-51-628-8147; E-mail: sonbw@pknu.ac.kr

*n*-hexane-EtOAc (1 : 1), *n*-hexane-EtOAc (1 : 5), *n*-hexane-EtOAc (1 : 10), and finally EtOAc. Each collections (30 mL each) were combined on the basis of their TLC profiles to yield five major fractions. Medium pressure liquid chromatography (MPLC) of fractions 3 and 4 on ODS by elution with MeOH afforded compounds **1**, **2**, and **3**, respectively. The isolated compounds were further purified by HPLC (YMC ODS-A, MeOH) utilizing a 30 min gradient program of 50% to 100% MeOH in $H_2O$ to furnish 12*R*,13*S*-dihydroxyfumitremorgin C (**1**, 5.0 mg), fumitremorgin C (**2**, 8.0 mg), and brevianamide F (**3**, 6.0 mg).

**12*R*,13*S*-Dihydroxyfumitremorgin C (1):** a pale yellow solid; $[\alpha]_D$ + 18.1° (*c* 0.3, $CHCl_3$); IR (neat) $\nu_{max}$ 3420, 3280, 1680, 1665, 1475, 1450, 1375, 745 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (ε) 224 (19500), 270 (6500) nm; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 10.73 (1H, s, H-1), 5.75 (1H, d, *J* = 9.4 Hz, H-3), 4.39 (1H, dd, *J* = 9.6, 7.0 Hz, H-6), 1.86 and 2.28 (each 1H, m, $H_a$- and $H_b$-7), 1.86 (1H, m, $H_2$-8), 3.42 and 3.47 (each 1H, m, $H_a$- and $H_b$-9), 5.51 (1H, d, *J* = 3.2 Hz, H-13), 7.61 (1H, d, *J* = 8.6 Hz, H-16), 6.62 (1H, dd, *J* = 8.6, 2.2 Hz, H-17), 6.82 (1H, d, *J* = 2.2 Hz, H-19), 4.76 (1H, d, *J* = 9.4 Hz, H-21), 1.92 (3H, s, $H_3$-23), 1.58 (3H, s, $H_3$-24), 6.34 (1H, s, 12-OH), 5.22 (1H, d, *J* = 3.2 Hz, 13-OH), 3.74 (3H, s, 18-$OCH_3$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 130.0 (s, C-2), 49.2 (d, C-3), 170.5 (s, C-5), 58.3 (d, C-6), 28.8 (t, C-7), 22.1 (t, C-8), 45.0 (t, C-9), 166.1 (s, C-11), 83.1 (s, C-12), 67.9 (d, C-13), 105.9 (s, C-14), 120.6 (s, C-15), 120.6 (d, C-16), 108.4 (d, C-17), 155.2 (s, C-18), 94.7 (d, C-19), 137.4 (s, C-20), 124.4 (d, C-21), 132.7 (s, C-22), 25.4 (q, C-23), 18.0 (q, C-24), 55.1 (q, 18-OMe); EIMS *m/z* (rel. int.) 411 $[M]^+$ (38), 394 (5), 280 (3), 243 (80), 228 (8), 214 (13), 200 (100), 187 (55), 159 (18), 70 (22).

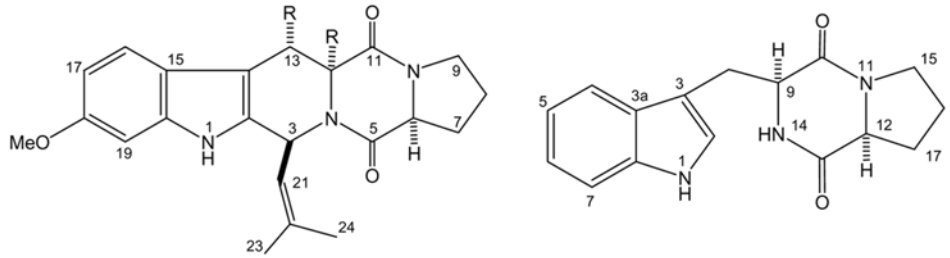
**Fumitremorgin C (2):** a pale yellow solid; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 7.90 (1H, s, H-1), 5.95 (1H, br d, *J* = 9.5 Hz, H-3), 4.15 (1H, br dd, *J* = 9.0, 7.0 Hz, H-6), 2.25 and 2.40 (each 1H, m, $H_a$- and $H_b$-7), 2.00 (2H, m, $H_2$-8), 3.62 (2H, m, $H_2$-9), 4.20 (1H, br dd, *J* = 11.0, 5.5 Hz, H-12), 3.10 (1H, dd, *J* = 15.5,11.0 Hz, $H_a$-13), 3.50 (1H, dd, *J* = 15.5, 5.5 Hz, $H_b$-13), 7.45 (1H, d, *J* = 9.0 Hz, H-16), 6.84 (1H, dd, *J* = 9.0, 2.0 Hz, H-17), 6.90 (1H, d, *J* = 2.0 Hz, H-19), 4.90 (1H, d, *J* = 9.5 Hz, H-21), 1.65 (3H, s, $H_3$-23), 2.00 (3H, s, $H_3$-24), 3.85 (3H, s, 18-$OCH_3$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 132.0 (s, C-2), 51.2 (d, C-3), 169.5 (s, C-5), 59.3 (d, C-6), 28.8 (t, C-7), 23.1 (t, C-8), 45.0 (t, C-9), 165.4 (s, C-11), 56.5 (d, C-12), 22.0 (t, C-13), 106.0 (s, C-14), 120.6 (s, C-15), 119.0 (d, C-16), 109.4 (d, C-17), 156.2 (s, C-18), 95.2 (d, C-19), 137.0 (s, C-20), 124.3 (d, C-21), 133.7 (s, C-22), 25.4 (q, C-23), 18.0 (q, C-24), 55.6 (q, 18-OMe); EIMS *m/z* (rel. int.) 379 $[M]^+$ (83), 364 (15), 336 (12), 324 (35), 281 (100), 267 (6), 253 (10), 239 (18), 227 (30), 212 (66), 199 (27), 176 (16), 70 (19).

**Brevianamide F (3):** a colorless solid: $[\alpha]_D$ −101° (*c* 0.5, AcOH); IR (KBr) $\nu_{max}$ 3280, 1670, 1650 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 400 MHz) δ 10.86 (1H, s, 1-NH), 7.17 (1H, s, H-2), 7.56 (1H, d, *J* = 7.5 Hz, H-4), 6.96 (1H, dd, *J* = 7.0, 7.5, H-5), 7.05 (1H, dd, *J* = 7.0, 8.0 Hz, H-6), 7.32 (1H, d, *J* = 8.0, H-7), 3.06 (1H, dd, *J* = 14.5, 5.5 Hz, $H_a$-8), 3.23 (1H, m, $H_b$-8), 4.30 (1H, t, *J* = 5.5 Hz, H-9), 4.06 (1H, dd, *J* = 8.6, 8.0 Hz, H-12), 7.74 (1H, s, 14-NH), 3.26 and 3.36 (each 1H, m, $H_a$- and $H_b$-15), 1.67 (1H, m, $H_a$-16), 1.95 (1H, dt, *J* = 7.0, 3.2 Hz, $H_b$-16), 1.37 and 1.62 (each 1H, m, $H_a$- and $H_b$-17); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) δ 124.3 (d, C-2), 109.2 (s, C-3), 127.3 (s, C-3a), 118.6 (d, C-4), 118.1 (d, C-5), 120.8 (d, C-6), 111.2 (d, C-7), 135.9 (s, C-7a), 25.7 (t, C-8), 55.2 (d, C-9), 165.4 (s, C-10), 58.3 (d, C-12), 168.9 (s, C-13), 44.5 (t, C-15), 27.6 (t, C-16), 21.8 (t, C-17); EIMS *m/z* (rel. int.) 283 $[M]^+$ (54), 154 (42), 130 (100), 103 (21), 102 (11), 77 (29), 70 (44).

**Antibacterial assay** – The *in vitro* antibacterial activity of the fermentation broth and purified samples were evaluated by a conventional 2-fold serial dilution method using *S. aureus*, methicillin-resistant *S. aureus*, and multidurg-resistant *S. aureus* as indicator strains. A 5 mL suspension containing 105 cells per mL was used as inoculum of the test organism. The MIC values were determined after the inoculation for 18 hours at 37 °C (Li *et al*., 2003).

## Results and Discussion

12*R*,13*S*-Dihydroxyfumitremorgin C (**1**) was obtained in the form of a pale yellow solid. The IR spectrum of **1** suggested the presence of hydroxyl or amine (3420, 3280 $cm^{-1}$) and amide (1680, 1665 $cm^{-1}$) groups. In the UV spectrum, **1** showed characteristic absorption curve suggestive of a 6-*O*-methylindole chromophore with absorption maxima at 224 (ε 19500) and 270 (6500) nm (Cui *et al*., 1996). In the $^1H$ NMR spectrum, **1** showed signals due to an N-H proton [δ 10.73 (1H, s, H-1)], a 1,2,4-trisubstituted benzene ring [δ 7.61 (1H, d, *J* = 8.6 Hz, H-16), 6.62 (1H, dd, *J* = 8.6, 2.2 Hz, H-17), 6.82 (1H, d, *J* = 2.2 Hz, H-19)], a methoxy [δ 3.74 (3H, s, 18-$OCH_3$)], and two methyl [δ 1.92 (3H, s, $H_3$-23), 1.58 (3H, s, $H_3$-24)] groups along with signals due to several methine and methylene groups (Experimental). The $^{13}C$ NMR spectrum of **1**, analyzed by the DEPT method, indicated the presence of two amide-carbonyls [δ 170.5 (s, C-5) and



12*R*,13*S*-dihydroxyfumitremorgin C: R = OH

fumitremorgin C (**2**): R = H

brevianamide F (**3**)

**Fig. 1.** Structures of the antibacterial compounds (**1-3**).

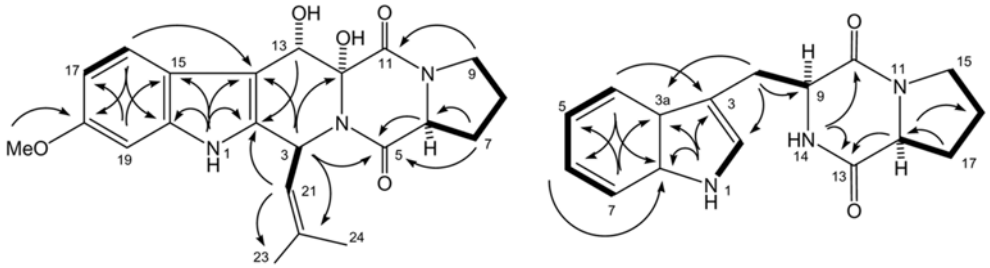


**Fig. 2.** Structures of **1** and **3** elucidated by $^{1}$H-$^{1}$H COSY (▬) and HMBC ( → ).

166.1 (s, C-11) ], an oxygen bearing sp$^2$ carbon [δ 155.2 (s, C-18)], a methoxy [δ 55.1 (q, 18-OMe)], and two methyl [δ 25.4 (q, C-23), 18.0 (q, C-24)] groups together with four sp$^2$ and three sp$^3$ methines, five sp$^2$ and a sp$^3$ quaternary carbons, and three methylene groups (Experimental). Detailed analyses of the $^{1}$H and $^{13}$C NMR spectra of **1**, including the results from DEPT, COSY, HMQC, HMBC, and NOESY experiments, suggested that the structure of **1** was composed of a 6-methoxyindole moiety, a diketopiperazine forming a pentacyclic ring skeleton, and an isoprenyl residue (Fig. 1, 2). The connection and position of the functional groups in **1** were achieved on the basis of COSY and HMBC correlations. Key COSY and HMBC correlations showed the connections of C2-C3-N4, C12-C13-C14, and C6-C7 bonds as well as the positions of 12,13-dihydroxyl, 18-methoxyl, and isoprenyl groups (Fig. 2). These spectroscopic features revealed that compound **1** had the same structural features of 12,13-dihydroxyfumitremorgin C (**1**) (Abraham and Arfmann, 1990; Cui *et al*., 1996). Thus, compound **1** was characterized as a derivative of fumitremorgins (Buckingham *et al*., 1994), and direct comparison of NMR data of **1** with those of 12,13-dihydroxyfumitremorgin C (Abraham and Arfmann, 1990; Cui *et al*., 1996) provided additional support in justifying the gross structure shown for **1** (Fig. 1).

Fumitrmorgin C (**2**) was obtained as a pale yellow solid. The general features of the LREIMS and NMR spectra of **2** closely resembled those of 12*R*,13*S*-dihydroxyfumitremorgin C (**1**), except for the NMR signals at C-12 and C-13 that were changed from sp$^3$-quaternary and methine carbons [δ 83.1 (s, C-12); 5.51 (1H, d, *J* = 3.2 Hz, H-13), 67.9 (d, C-13)] in compound **1** to sp$^3$-methine and methylene carbons [δ 4.20 (1H, br dd, *J* = 11.0, 5.5 Hz, H-12), 56.5 (d, C-12); 3.10 (1H, dd, *J* = 15.5, 11.0 Hz, H$_a$-13), 3.50 (1H, dd, *J* = 15.5, 5.5 Hz, H$_b$-13), 22.0 (t, C-13) ] in **2**. Detailed analyses of the $^{1}$H and $^{13}$C NMR spectra of **2**, including the results from DEPT, COSY, HMQC, and HMBC experiments, suggested that metabolite **2** is fumitremorgin C (Buckingham *et al*., 1994; Cui *et al*., 1996).

Brevianamide F (**3**) was a colorless solid obtained from the broth extract. The $^{1}$H and $^{13}$C NMR spectra, including DEPT, showed 1,2-disubstituted benzene, two amide-carbonyls, two sp$^3$ methines, and four sp$^3$ methylenes. Detailed analyses of the $^{1}$H and $^{13}$C NMR spectra of **3**, including the results from DEPT, HMQC, and HMBC experiments, suggested the presence of monosubstituted indole, a diketopiperazine forming a pentacyclic ring skeleton, and an sp$^3$ methyene residue. The connection of the partial groups in **3** were achieved on the basis of COSY and HMBC correlations. Key COSY and HMBC correlations showed the connections of C3-C8-C9, C12-C17, and N11-C15 bonds (Fig. 2). On the basis of the

foregoing evidences, compound **3** was assigned to be brevianamide F (Fig. 1)( Birch and Russell, 1972).

Diketopiperazines **1-3** exhibited a mild antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. The MIC values of compounds **1-3** were 125 μg/mL for all strains.

Compounds **1** and **2,** isolated from *Aspergillus fumigatus,* were reported as a new group of G2/M-phase inhibitors of the mammalian cell cycle (Cui *et al*., 1996), and compound **3** was isolated from *Penicillium brevicompactum* (Birch and Russell, 1972). But, isolation of compounds **1-3** from the genus of *Pseudallescheria* is the first report. Diketopiperazines are widespread microbial products commonly found in nutrient rich cultures of both terrestrial and marine fungi, and they are also of interest because of their activity in various pharmacological assay systems (Li *et al*., 2004).

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