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, 12R,13S-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, 12R,13S-

*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 collection (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 H2O to furnish 12R,13S-dihydroxyfumitremorgin C (1, 5.0 mg), fumitremorgin C (2, 80 mg), and brevianamide F (3, 60 mg).

12R,13S-Dihydroxyfumitremorgin C (1): a pale yellow solid; [α]D +18.1° (c 0.3, CHCl3); IR (neat) νmax 3420, 3380, 3280, 1670, 1665 cm−1; 1H NMR (400 MHz, DMSO-d6) δ 7.90 (1H, s, H-1), 5.95 (1H, br d, J = 9.5 Hz, H-3), 4.15 (1H, br d, J = 9.0, 7.0 Hz, H-6), 2.25 and 2.40 (each 1H, m, Hα- and Hβ-24), 3.50 (1H, dd, J = 15.5, 5.5 Hz, Hβ-13), 7.45 (1H, d, J = 9.0 Hz, H-16), 6.84 (1H, d, J = 9.0, 2.0 Hz, H-17), 6.90 (1H, d, J = 9.0 Hz, H-19), 4.90 (1H, d, J = 9.5 Hz, H-21), 1.65 (3H, s, H-23), 2.00 (3H, s, H-24), 3.85 (3H, s, 18-OCH3); 13C NMR (100 MHz, DMSO-d6) 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), 15.62 (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-OCH3); 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; [α]D −101° (c 0.5, AcOH); IR (KBr) νmax 3280, 1670, 1650 cm−1; 1H NMR (DMSO-d6, 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, Hα-8), 3.23 (1H, m, Hb-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, Hα- and Hβ-15), 1.67 (1H, m, H-16), 1.95 (1H, dt, J = 7.0, 3.2 Hz, H-16), 1.37 and 1.62 (each 1H, m, Hα- and Hβ-17), 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-OCH3); EIMS m/z (rel. int.) 285 [M]+ (55), 595 (22), 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 multidrug-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

12R,13S-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, 3380 cm−1) and amide (1680, 1665 cm−1) groups. In the UV spectrum, I 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, I showed signals due to an N-H proton (δ 10.73 (1H, s, H-1)), a 1,2,3-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-CH3)], and two methyl [δ 1.92 (3H, s, H-23), 1.58 (3H, s, H-24)] groups along with signals due to several methine and methylene groups (Experimental). The 13C NMR spectrum of 1, analyzed by the DEPT method, indicated the presence of two amide-carbonyls [δ 170.5 (s, C-5) and 170.0 ppm] and a methoxy [δ 34.2 ppm].