Golmaenone, A New Diketopiperazine Alkaloid from the Marine-Derived Fungus Aspergillus sp.

Yong Li,¹ Xifeng Li,¹ Se-Kwon Kim,¹ Jung Sook Kang,¹ Hong Dae Choi,² Jung Rae Rho,² and Byeng Wha Son*¹,a

¹ Department of Chemistry, Pukyong National University; Busan 608–737, Korea; ² College of Dentistry, Pusan National University; Busan 609–739, Korea; and ³ Department of Chemistry, Dongeui University; Busan 614–714, Korea. © 2004 Pharmaceutical Society of Japan

Received October 29, 2003; accepted January 21, 2004

A new diketopiperazine alkaloid, golmaenone (1) and related alkaloids, neoechinulin A (2) and L-alanyl-L-tryptophan anhydride (3), have been isolated from the culture broth of the marine-derived fungus Aspergillus sp. The structure and absolute stereochemistry of the new compound (1) was assigned by spectroscopic methods and the advanced Marfey’s method. Compounds 1 and 2 exhibited a significant radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC₅₀ values of 20 and 24 μM, respectively, which are similar to the positive control, ascorbic acid (IC₅₀ 20 μM). Compounds 1 and 2 also showed an ultraviolet-A (UV-A) (320—390 nm) protecting activity with ED₅₀ values of 90 and 170 μM, respectively, which are more active than oxybenzone (ED₅₀, 350 μM) currently being used as sunscreen.

Key words diketopiperazine alkaloid; golmaenone; neoechinulin A; L-alanyl-L-tryptophan anhydride; marine-derived fungus; Aspergillus sp.

Diketopiperazines are widespread microbial products commonly found in nutrient rich cultures of both terrestrial¹ and marine fungi.²,³ Diketopiperazines are of interest because of their activity in various pharmacological assay systems.⁴

As part of a program to explore the bioactive metabolites produced by the fungi isolated from marine habitats,⁵ we investigated the bioactive constituents of the marine algicloous fungus and isolated a new golmaenone (1) in addition to neoechinulin A (2) and L-alanyl-L-tryptophan anhydride (3).

A fungal strain (culture # MFA 212) was isolated from the surface of the marine red alga Lomentaria catenata collected at Golmae Village, Ulsan City, Korea in 2002, and it was identified by fatty acid methyl ester analysis (FAME) as a Aspergillus sp.⁶ The fungus was cultured (10 l) in a seawater-based medium.⁷

The culture broth and mycelium were separated, and the broth was extracted with ethyl acetate to provide a crude extract (1.5 g), which was subjected to a combination of column chromatography on silica gel (n-hexane/EtOAc) and octadesyl silica (ODS) gel (H₂O/MeOH) to furnish three fractions containing diketopiperazines 1 (20 mg), 2 (120 mg), and 3 (35 mg). Further purifications of each fraction by HPLC (YMC ODS-A, MeOH) yielded a new golmaenone (1) (12 mg), as well as neoechinulin A (2) (95 mg) and L-alanyl-L-tryptophan anhydride (3) (7 mg).

Golmaenone (1)⁸ was isolated as a yellow solid which was thought to have a molecular composition of C₁₉H₂₁N₃O₄ from the high resolution (HR)-FAB-MS and ¹³C-NMR data.

Since 1 showed eleven unsaturations in HR-FAB-MS, it implied that 1 contained four carbonyl, five double bonds, and two rings. The IR spectrum of 1 showed absorptions for free amide (3433, 1697 cm⁻¹) and hydrogen-bonded amide (3242, 1629 cm⁻¹) functionality. The UV spectrum of 1 showed the presence of conjugated amide [222 nm (log ε 1.8), 327 (1.9), 368 (1.7)] chromophores.

In the ¹H-NMR spectrum, three protons were exchanged by D₂O, suggesting that 1 has three amide protons [δ 6.61 (1H, s, H-11), 11.57 (1H, s, H-14), 11.44 (1H, s, H-15)]. Detailed analyses of the ¹H- and ¹³C-NMR spectra of 1, including the results from distortionless enhancement by polarization transfer (DEPT), ¹H-detected heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC) experiments, revealed signals ascribable to a methyl substituted diketopiperazine [δ 6.61 (1H, br s, H-11), 4.40 (1H, qd, J=7.0, 1.8 Hz, H-12), 11.57 (1H, s, H-14), 1.66 (3H, d, J=7.0 Hz, H₂-22), 140.0 (C-9), 1.573 (C-10), 51.8 (C-12), 166.4 (C-13), 21.1 (C-22)], 1,2-disubstituted benzene [δ 7.96 (1H, dd, J=8.0, 1.5 Hz, H-3), 7.14 (1H, dd, J=8.2, 8.0, 1.0 Hz, H-4), 7.57 (1H, dd, J=8.6, 8.2, 1.5 Hz, H-5), 8.74 (1H, dd, J=8.6, 1.0 Hz, H-6), 141.4 (C-1), 123.6 (C-2), 130.5 (C-3), 122.6 (C-4), 135.4 (C-5), 121.3 (C-6)], 2,2-dimethyl-3-butenamide [δ 11.44 (1H, s, H-15), 6.12 (1H, dd, J=17.5, 10.5 Hz, H-18), 5.31, 5.37 (each 1H, d, J=10.5, 17.5 Hz, respectively, H₂-19), 1.43 (6H, s, CH₃-20/21), 175.9 (C-16), 46.8 (C-17), 142.4 (C-18), 114.9 (C-19), 24.8 (C-20/21), 1,3,3-trisubstituted propenone [δ 7.22 (1H, s, H-8), 195.1 (C-7), 102.3 (C-8), 140.0 (C-9)] (Table 1).

The connection of the functional groups in 1, which led to the planar structure, was achieved on the basis of HMQC and HMBC correlations. Key HMBC correlations between H-15 and C-2, C-6 and C-16; between H-8 and C-7 and C-10; between H₂-20/21 and C-16 and C-18; and between H-14 and C-8, C-10 and C-12, clearly estab-
lished the planar structure of 1.

The geometry of C-8/C-9 double bond in compound 1 was determined to be (Z) configuration on the basis of the chemical shifts of H-8 (δ 7.22 (1H, s) and H-14 (δ 11.57 (1H, s)), which were shifted to the low field by the deshielding effect of the carbonyl group on β-vinyl proton and by the hydrogen-bonding with 7-carbonyl group, respectively.

The stereochemistry of the alanine residue was determined by the advanced Marfey’s method. For this analysis two enantiomeric alanine isomers were derivatized with 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (L-FDAA), and analyzed by reversed-phase HPLC. The retention times of the corresponding enantiomers (2S and 2R) were observed with 9.6 and 10.6 min, respectively. Analogous derivatization of the acid hydrolyzate of compound 1 followed by HPLC analysis and comparison with the standard derivatives enabled us to deduce 12S configuration.

Compounds 2 and 3 have been isolated from the more polar fractions and were identified as neoechinulin A, which was previously isolated as an antioxidative substance from the fungal genera Aspergillus and L-alanyl-L-tryptophan anhydride, respectively.

Compounds 1 and 2 exhibited a significant radical scavenging activity against DPPH with IC₅₀ values of 20 and 24 µM, respectively, which are similar to the positive control, ascorbic acid (IC₅₀, 20 µM). Compounds 1 and 2 also showed a UV-A protecting activity with ED₅₀ values of 90 and 170 µM, respectively, which are more active than oxybenzone (ED₅₀, 350 µM) currently being used as sunscreen. The further biological evaluation of 1 is in progress.

Acknowledgements CD and Mass spectral data were kindly provided by the Korea Basic Science Institute. This work was supported by the Brain Korea 21 Project in 2003 (F020).

References and Notes
6) The fungal strain was identified as a Aspergillus sp. based on fatty acid methyl ester analysis and growth characteristics (Korean Culture Center of Microorganisms, Seoul, Korea). Their analysis showed a similarity index of 0.62.
7) The fungus was cultured (20 l) for 30 d (static) at 29 °C in SWS medium: soytonye (0.1%), soluble starch (1%), and seawater (100%).
8) Golmaenone (1) was isolated as a yellow solid which showed: mp 160—161 °C (from CHCl₃). [α]D₅max (CHCl₃) nm (log ε): 222 (1.8), 247 (2.0), 327 (1.9), 368 (1.7). CD [α]D₅max (CHCl₃) nm (Ar): 230 (+0.5), 239 (−0.2), 253 (−0.4), 305 (+0.1), 346 (−0.1). LR-FAB-MS m/z: 378 [M+Na]⁺, 356 [M+H]⁺; HR-FAB-MS m/z: 378.1428 (Calcd for C₉H₁₄NO₄Na: 378.1430). See Table 1 for NMR spectral data.
11) Samples (0.5 mg) of each compounds 1—3 were subjected to acid hydrolysis with 6 N HCl (1 ml) at 110 °C for 12 h. The hydrolyzates were dried, resuspended in H₂O (100 µl), and derivatized with L-FDAA. The l-FDAA derivatives, from the hydrolyzates, were compared with similar derivatized standard amino acids (L-alanine and D-alanine) by TLC detection at 340 nm using an isocratic elution of MeCN–0.1% (v/v) aqueous TFA (1:1).
12) Neoechinulin A (2) was isolated as a colorless solid which showed spectral data virtually identical to that reported in the literature. The NMR data was reassigned as follows: H-NMR (CDCl₃): δ 8.32 (1H, s, H-1), 7.27 (1H, dd, J = 7.8 Hz, H-4), 7.18 (1H, dd, J = 7.8, 7.5 Hz, H-5), 7.16 (1H, dd, J = 7.5, 7.5 Hz, H-6), 7.36 (1H, d, J = 7.3 Hz, H-7), 7.21 (1H, s, H-8), 7.45 (1H, brs, H-11), 4.30 (1H, qd, J = 7.0, 1.7 Hz, H-12), 6.40 (1H, s, H-14), 6.07 (1H, dd, J = 17.5, 10.5 Hz, H-16), 5.23 (1H, d, J = 10.5 Hz, H-17), 5.19 (1H, d, J = 17.5 Hz, H-18), 1.53 (6H, s, H-3/19), 1.60 (3H, d, J = 7.0 Hz, H-20). lL-NMR (CDCl₃): δ: 143.8 (C-2), 102.9 (C-3), 126.9 (C-3a), 118.9 (C-4), 121.0 (C-5), 122.3 (C-6), 111.2 (C-7), 134.3 (C-7a), 111.9 (C-8), 124.5 (C-9/10), 159.8 (C-10), 51.7 (C-12, 165.7 (C-13), 39.2 (C-15), 144.3 (C-16), 113.3 (C-17), 27.3 (C-18), 27.4 (C-19), 20.9 (C-20).