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

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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 IC_{50} values of 20 and 24 μM, respectively, which are similar to the positive control, ascorbic acid (IC_{50}, 20 μM). Compounds 1 and 2 also showed an ultraviolet-A (UV-A) (320—390 nm) protecting activity with ED_{50} values of 90 and 170 μM, respectively, which are more active than oxybenzone (ED_{50}, 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 terrestrial[1] 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 algicolous 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 anlaysis (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 (H_2O/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 $C_{19}H_{21}N_3O_4$ from the high resolution (HR)-FAB-MS and ^{13}C-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 ^{1}H-NMR spectrum, three protons were exchanged by D_2O, 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 ^{1}H- and ^{13}C-NMR spectra of **1**, including the results from distortionless enhancement by polarization transfer (DEPT), ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multple-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, H_3-22), 140.0 (C-9), 157.3 (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, ddd, J=8.2, 8.0, 1.0 Hz, H-4), 7.57 (1H, ddd, 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, H_2-19), 1.43 (6H, s, CH_3-20/21), 175.9 (C-16), 46.8 (C-17), 142.4 (C-18), 114.9 (C-19), 24.8 (C-20/21)], and 1,3,3-trisubstitited 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-3 and C-7; between H-8 and C-7 and C-10; between H_3-20/21 and C-16 and C-18; and between H-14 and C-8, C-10 and C-12, clearly estab-

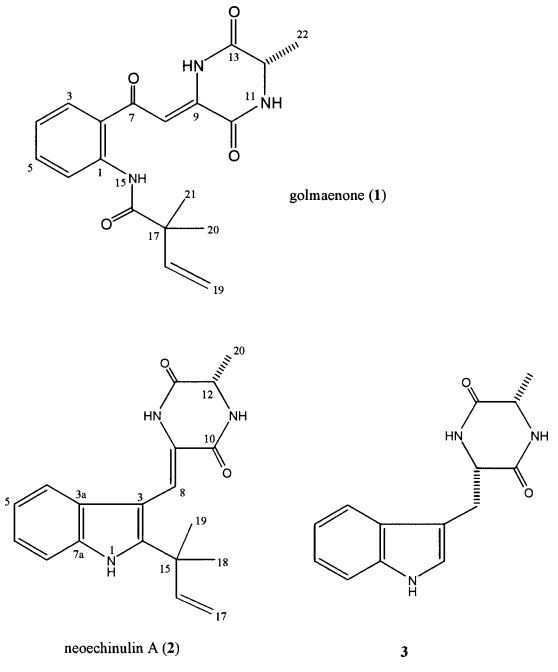

Fig. 1

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Table 1. NMR Spectral Data for Golmaenone (**1**)[a,b]

C#	δ_H (mult., J)	δ_C (mult.)	HMBC (H to C)
1		141.4	
2		123.6	
3	7.96 (dd, 8.0, 1.5)	130.5	1, 4, 5, 7
4	7.14 (ddd, 8.2, 8.0, 1.0)	122.6	2, 3, 5, 6
5	7.57 (ddd, 8.6, 8.2, 1.5)	135.4	1, 3, 4
6	8.74 (dd, 8.6, 1.0)	121.3	1, 2, 4
7		195.1	
8	7.22 (s)	102.3	7, 10
9		140.0	
10		157.3	
11	6.61 (br s)		9, 13
12	4.40 (qd, 7.0, 1.8)	51.8	10, 13, 22
13		166.4	
14	11.57 (s)		10, 12
15	11.44 (s)		2, 6, 16
16		175.9	
17		46.8	
18	6.12 (dd, 17.5, 10.5)	142.4	16, 17, 20/21
19	5.37 (d, 17.5)	114.9	17, 18
	5.31 (d, 10.5)		
20/21	1.43 (s)	24.8	16, 17, 18
22	1.66 (d, 7.0)	21.1	12, 13

a) Recorded in $CDCl_3$ at 400 MHz (^1H) and 100 MHz (^{13}C). *b*) Assignments aided by DEPT, HMQC, and HMBC.

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[9] and by the hydrogen-bonding with 7-carbonyl group, respectively.

The stereochemistry of the alanine residue was determined by the advanced Marfey's method.[10] 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 (2*S* and 2*R*) 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 12*S* configuration.[11]

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*,[9,12,13] and L-alanyl-L-tryptophan anhydride,[14] respectively.

Compounds **1** and **2** exhibited a significant radical scavenging activity against DPPH with IC_{50} values of 20 and 24 μM, respectively, which are similar to the positive control, ascorbic acid (IC_{50}, 20 μM). Compounds **1** and **2** also showed a UV-A protecting activity with ED_{50} values of 90 and 170 μM, respectively, which are more active than oxybenzone (ED_{50}, 350 μM) currently being used as sunscreen.

The further biological evaluation of **1** is in progress.

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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: soytone (0.1%), soluble starch (1.0%), and seawater (100%).
8) Golmaenone (**1**) was isolated as a yellow solid which showed: mp 160—161 °C (from $CHCl_3$). $[\alpha]_D$ +7.1° (c=0.4, $CHCl_3$). IR (neat) cm^{-1}: 3433, 3242, 1697, 1629, 1578, 1510, 1446, 1378, 1310, 1218, 1164, 1021, 916, 752. UV λ_{max} ($CHCl_3$) nm (log ε): 222 (1.8), 247 (2.0), 327 (1.9), 368 (1.7). CD λ_{max} ($CHCl_3$) nm ($\Delta\varepsilon$): 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 $C_{19}H_{21}N_3O_4Na$: 378.1430). See Table 1 for NMR spectral data.
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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 H_2O (100 μl), and derivatized with L-FDAA. The L-FDAA derivatives, from the hydrolyzates, were compared with similarly derivatized standard amino acids (L-alanine and D-alanine) by HPLC [YMC ODS-A (10×250 mm), 10 μm, flow rate 1 ml/min, UV detection at 340 nm] using an isocratic elution of MeCN–0.1% (v/v) aqueous TFA (1 : 1). The retention times of L-FDAA derivatives of standard amino acids, L-alanine and D-alanine, were 9.6 and 10.6 min, respectively, and the retention times of L-FDAA derivatives of hydrolyzates were 9.6 min, respectively.
12) Neoechinulin A (**2**) was isolated a colorless solid which showed spectral data virtually identical to that reported in the literature.[9,13] The NMR data was reassigned as follow: ^1H-NMR ($CDCl_3$) δ: 8.32 (1H, s, H-1), 7.27 (1H, d, 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, 7.3 Hz, H-6), 7.36 (1H, d, J=7.3 Hz, H-7), 7.21 (1H, s, H-8), 7.45 (1H, br s, 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_a-17), 5.19 (1H, d, J=17.5 Hz, H_b-17), 1.53 (6H, s, H_3-18/19), 1.60 (3H, d, J=7.0 Hz, H_3-20). ^{13}C-NMR ($CDCl_3$) δ: 143.8 (C-2), 102.9 (C-3), 126.0 (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), 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).
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