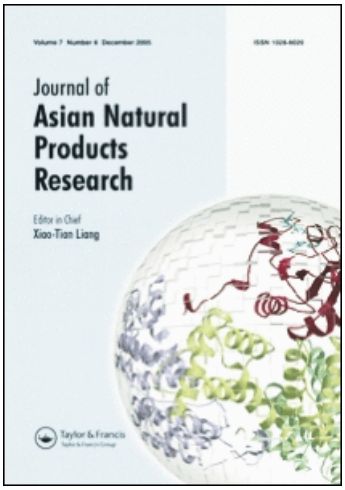

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A new cerebroside, Asperiamide A, from the marine fungus *Asperillus sp.*

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Note

A new cerebroside, Asperiamide A, from the marine fungus *Asperillus sp.*

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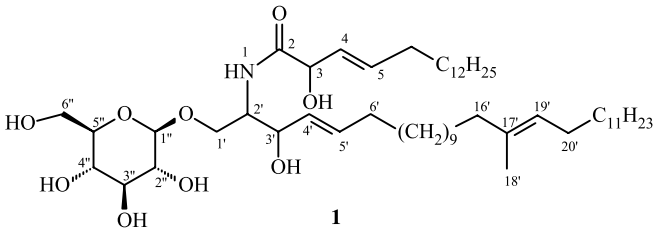
A new cerebroside, asperiamide A (**1**), and adenosine were produced from a marine fungus, identified as *Asperillus sp*., collected in the Mei Zhou Gulf, China. Two-dimensional NMR methods, FAB-MS, were used to established the structure of the new compound.

Keywords: Marine fungus; *Asperillus*; Cerebroside

1. Introduction

Cerebrosides and ceramides [1] have been isolated from a number of marine organisms such as sea stars, sea anemones, gorgonians, sponges, tunicates, dinoflagellates, and green algae. Some cerebroside and ceramides exhibited cytotoxic, antitumor [2,3], immunostimulatory [4], antifungal [5], antiviral [6]. In the search for bioactive components, two water soluble constituents, asperiamide A and adenosine [7], were isolated from the marine fungus *Asperillus sp.*, the current report describes the structural elucidation of a new compound, asperiamide A (**1**) and a known one, adenosine.



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2. Results and discussion

Asperiamide A (**1**) was obtained as a white amorphous powder. Its molecular formula was determined as $C_{54}H_{101}NO_9$ on the basis of HRFABMS. There were five degrees of unsaturation in the molecule according to the molecular formula. Compound **1** exhibited a molecular ion peak at *m/z* 906 $[M—H]^-$ in the negative FABMS. IR data of **1** indicated that it contains —OH, —NH (3368 cm^{-1}), C—H (—CH_2—, —CH_3, 2910, 2856 cm^{-1}), carbonyl (1649 cm^{-1}), double bonds (1636 cm^{-1}), methyl (1470, 1380 cm^{-1}), C—N and C—O (1078, 1036 cm^{-1}), tri-substitute vinyl (968 cm^{-1}). The ^1H and ^{13}C NMR spectra of **1** (table 1) were consistent with the presence of a secondary amide group (δ_H 7.83, 1H, d, $J = 9.2$ Hz, δ_C 52.9, δ_C 172.1) [8], and a glucopyranoyl group (table 1). Furthermore, the negative FABMS of **1** showed a quasimolecular ion at *m/z* 906 $[M—H]^-$; The 17′, 19′-double bond was assigned on the basis of the typical fragment ion at *m/z* 752 (100%) which was formed by elimination of undecene through McLafferty rearrangement (figure 1), and 4, 5-double bond was deduced by elimination of dodecene from the quasimolecular ion, and the fragment peak was at *m/z* 738 $[M—H—C_{12}H_{24}]^-$. In the ^1H NMR spectrum, the protons between δ_H 4.66–3.20 were assigned to protons attached to the carbons bearing a heteroatom. Two aliphatic chains were

Table 1. ^1H and ^{13}C NMR data of asperiamide A (**1**) in DMSO-d_6.

NO.	*^1H*	*^{13}C*	
1	7.83 (1H, d, $J = 9.2$ Hz)		
2		172.1	C
3	4.79 (1H, d, $J = 7.2$ Hz)	72.0	CH
4	5.74 (1H, dd, $J = 15.6, 7.2$ Hz)	129.0	CH
5	6.05 (1H, dt, $J = 15.6, 6.2$ Hz)	130.6	CH
6	1.94 (2H, overlap)	31.8	CH_2
7–15	1.20–1.46	28.9–29.3	—CH_2—
16	1.25 (2H, m)	31.3	CH_2
17	1.25 (2H, m)	22.4	CH_2
18	0.84 (3H, t, $J = 7.1$ Hz)	13.8	CH_3
1′	3.95 (1H, dd, $J = 10.4, 3.2$ Hz)	68.8	CH_2
	4.35 (1H, dd, $J = 10.4, 6.0$ Hz)		
2′	4.19 (1H, m)	52.9	CH
3′	4.32 (1H, d, $J = 7.2$ Hz)	70.6	CH
4′	5.67 (1H, dd, $J = 16.0, 7.2$ Hz)	130.9	CH
5′	5.92 (1H, dt, $J = 16.0, 7.2$ Hz)	131.1	CH
6′	1.95 (2H, m)	32.2	CH_2
7′ −15′	1.20–1.46	28.9–29.3	—CH_2—
16′	1.88 (2H, overlap)	39.0	CH_2
17′		134.7	C
18′	1.52 (3H, s)	15.7	CH_3
19′	5.34 (1H, t, $J = 6.0$ Hz)	123.6	CH
20′	1.93 (1H, m)	31.8	CH_2
21′–28′	1.20–1.46	28.9–29.3	—CH_2—
29′	1.25 (2H, m)	31.3	CH_2
30′	1.25 (2H, m)	22.4	CH_2
31′	0.84 (3H, t, $J = 7.1$ Hz)	13.8	CH_3
1″	4.66 (1H, d, $J = 7.9$ Hz)	103.5	CH
2″	3.42 (1H, t, $J = 7.9$ Hz)	73.4	CH
3″	3.50 (1H, t, $J = 9.0$ Hz)	76.9	CH
4″	3.54 (1H, m)	70.1	CH
5″	3.59 (1H, m)	76.6	CH
6″	3.89 (1H, dd, $J = 11.6, 3.2$ Hz)	61.1	CH_2
	4.06 (1H, dd, $J = 11.6, 4.0$ Hz)		

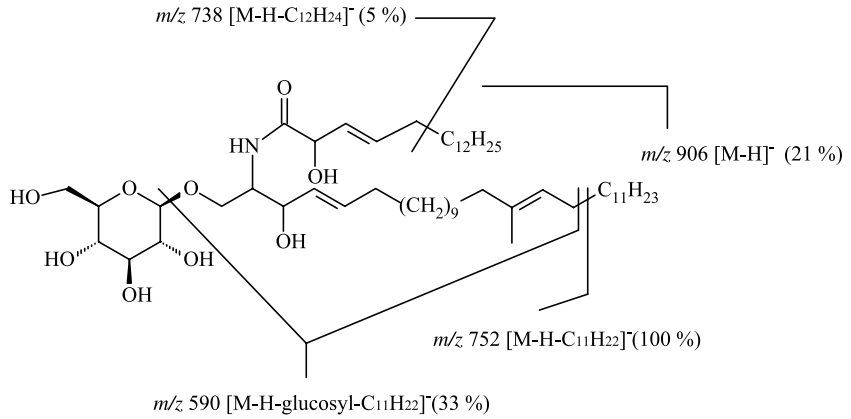

Figure 1. The key negative FAB mass fragments of **1**.

suggested by the presence of signals for methylene groups at δ_H 1.20–1.46, and three methyl groups at δ_H 1.52 (s, 3H), and 0.84 (t, 6H). In the ^{13}C NMR spectrum, an intense signal at δ_C 29–30 indicated methylene groups of aliphatic side-chains. Three double bond signals, including two disubstituted and one trisubstituted, were exhibited at δ_C 134.7 (C), 131.1 (CH), 130.9 (CH), 130.6 (CH), 129.0 (CH), and 123.6 (CH), and δ_H 6.05 (1H, dt, $J = 15.6$, 7.2 Hz, H-5), 5.92 (1H, dt, $J = 16.0$, 7.2 Hz, H-5′), 5.74 (1H, dd, $J = 15.6$, 6.2 Hz, H-4), 5.67 (1H, dd, $J = 16.0$, 7.2 Hz, H-4′), 5.34 (1H, t, $J = 6.0$ Hz), these coupling constants ($J = 15.6$ Hz and $J = 16.0$ Hz) suggested that two disubstituted double bonds were *trans*. Compound **1** showed an anomeric proton δ_H 4.66 (1H, d, $J = 7.9$ Hz) and anomeric carbon δ_C 103.5 suggesting β-configuration for the sugar which was detected as glucose by acid hydrolysis. The position of the sugar was confirmed by HMBC spectrum (figure 2).

The structural elucidation and assignments of complete proton and carbon signals were achieved by 2D NMR techniques and chemical methods. The carbonyl group position was assigned by the long-range HMBC correlations between H-3 at δ 4.79 and C-2 at δ 172.1, and between H-1 at δ 7.83 and C-2, between H-4 at δ 5.74 and C-2; the positions of three double bonds were also confirmed by the correlations of HMBC spectrum (see figure 2): between H-3 at δ 4.79 and C-4 at δ 129.0, C-5 at δ 130.6, between H-3′ at δ 3.93 and C4′ at δ 130.9, C-5′ at δ 131.1, and

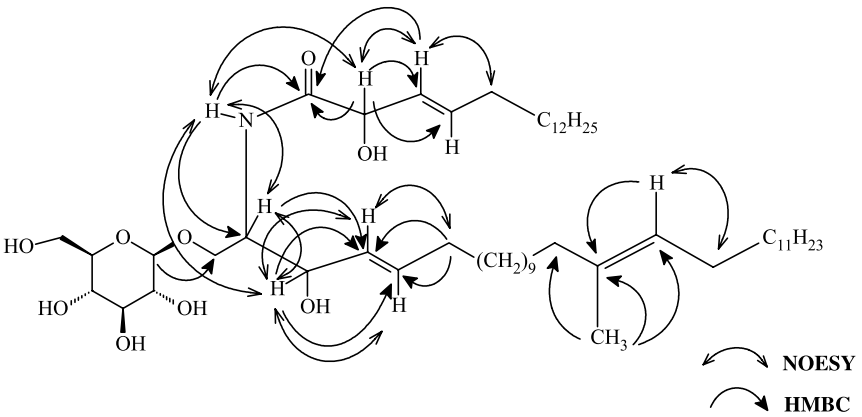

Figure 2. The Nosey and HMBC correations of **1**.

between the protons of methyl (H-18′) at δ 1.52 and C-17′ at δ 134.7, C-19′ at δ 123.6. Based on the NOESY experiment (figure 2), the structure for **1** was further illustrated, the NOESY correlations were from H-1 to H-3, H-2′, H-3′, from H-4 to H-3, H-6, and from H-4′ to H-3′, H-6′. Thus the structure of asperiamide A was concluded to be **1**.

3. Experimental

3.1 General experimental procedures

FAB-MS were taken on a VG Autospec 3000 system spectrometer. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AM-400 spectrometer and the solvent is DMSO-d_6. Optical rotations were taken on a JASCO-20C digital polarimeter, and the IR spectrum was recorded with a Perkin-Elmer 1750 FTIR spectrometer. Chromatographic stationary phase used RP-18 (40–60 μm, Merck), silica gel (160–200 mesh), and Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co., Ltd.). The following solvent systems were used: $CHCl_3$—MeOH—H_2O (80:20:2, and 70:30: 5) and MeOH—H_2O (0–100%) for the alkaloids. Spot of TLC was detected by spraying with 5% H_2SO_4 followed by heating.

3.2 Fungal isolation and culture

The fungal strain (culture MF-34#) was isolated from the sea water collected in Mei-Zhou Gulf, Fujian Province, China, in 2000 and identified as an *Asperillus sp.* by Prof. Chen Bi-E. The fungus was cultured (20 L) 25 days at 24°C in the medium: soluble starch (1%), yeast extract (0.2%), $MgSO_47H_2O$ (0.05%), KH_2PO_4 (0.1%), sea water (100%).

3.3 Extraction and isolation

The mycelium and broth were separated by filtration. The mycelial mat was extracted with MeOH. The combined extract (5.1 g) was subjected to Dianon gel column and eluted with water and methanol. Evaporation of the methanol eluate yielded 1.3 g of a brown fraction. The fraction was chromatographed on silica gel to give four fractions. Each fraction was chromatographed on RP-18 gel column (solvent: MeOH—H_2O, 10%–70%) and Si gel column using $CHCl_3$—MeOH—H_2O (80:20:2–70:30:5) as solvent to yield **1** (24 mg), **2** (87 mg).

3.4 Acid hydrolysis of 1

Compound **1** (10 mg) was dissolved in 10 ml of a solution (MeOH: 1 M H_2SO_4—1:1) and refluxed for 6 h. The reaction mixture was neutralized with 2 N NaOH and extracted with $CHCl_3$, the aqueous layer was concentrated to dryness for identification. D-glucose was detected by HPTLC by comparison with authentic sample (solvent system: $CHCl_3$—MeOH—H_2O/7:3:0.5 (9 ml) + HOAc (1 ml).

Asperiamide A (**1**) White amorphous powder, $C_{54}H_{101}NO_9$, $[\alpha]_D^{21}$ -16 (c 0.46, MeOH); Negative FAB-MS *m/z* 906 $[M—H]^-$ (21) 889 (3), 752 (100), 738 (5),, and 590 (33); HRFABMS *m/z* 906.7403 (calcd for $C_{54}H_{101}NO_9$) $[M—H]^-$; IR ν_{max}(liquid): 3368,

2910, 2856, 1649, 1636, 1540, 1470, 1380, 1078, 1036, 968 cm^{-1}; ^1H NMR and ^{13}C NMR see table 1.

Adenosine Brown amorphous powder, $C_{10}H_{13}N_5O_4$, Negative FAB-MS *m/z* 266 $[M-H]^-$; ^1H NMR (DMSO) δ_H: 8.34 (1H, s, Ad-H-8), 8.12 (1H, s, Ad-H-2), 7.34 (2H, br. s, NH_2), 5.86 (1H, d, $J = 4.9$ Hz), 4.59 (1H, t, $J = 4.2$ Hz), 4.13 (1H, br s), 3.95 (1H, br. d, $J = 2.0$ Hz), 3.65 (1H, m), 3.52 (1H, m); and ^{13}C NMR δ_C: 156.2 (C), 152.4 (CH), 149.1 (C), 139.9 (CH), 119.4 (C), 88.0 (CH), 85.9 (CH), 73.5 (C), 70.7 (CH), 61.7 (CH_2).

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