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Aspergilones A and B, two benzylazaphilones with an unprecedented carbon skeleton from the gorgonian-derived fungus *Aspergillus* sp.

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ABSTRACT

Two novel benzylazaphilone derivatives with an unprecedented carbon skeleton, aspergilone A (**1**), and its symmetrical dimer with a unique methylene bridge, aspergilone B (**2**), have been isolated from the culture broth of a marine-derived fungus *Aspergillus* sp. from a gorgonian *Dichotella gemmacea*. Their structures and relative stereochemistries of **1** and **2** were elucidated using a combination of NMR spectroscopy and X-ray crystallography. Compound **1** not only exhibited in vitro selective cytotoxicity but also showed potent antifouling activity.

Marine-derived fungi have proved to be a promising source of bioactive metabolites and a growing number of marine fungi have been reported to produce novel bioactive compounds.[1,2] As part of a program to discover new bioactive secondary metabolites from marine-derived fungi in the South China Sea,[3–5] the EtOAc extract of the culture broth of a marine-derived fungus *Aspergillus* sp.[6,7] isolated from a gorgonian *Dichotella gemmacea* exhibited significant cytotoxicity against A-549 human lung carcinoma cell line. Chemical investigation of the bioactive extract led to the discovery of two new azaphilone derivatives, aspergilones A and B (**1** and **2**) (Fig. 1), which possess an unprecedented carbon skeleton. Herein we report the isolation, structural determination and biological activity of these new compounds.

HPLC analysis of the EtOAc extract of the fungus *Aspergillus* sp. showed the presence of the evident UV absorption spectra (λ_{max} 199 and 323 nm). The extract was subjected to column chromatography on silica gel and Sephadex LH-20 successively. Further separation and purification using semi-preparative HPLC led to the isolation of compounds **1** and **2**.

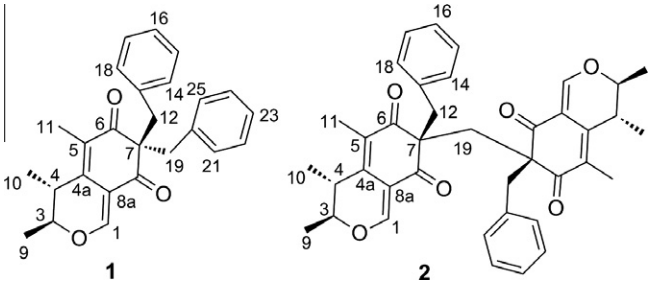

Figure 1. Structures of compounds **1** and **2**.

Aspergilone A (**1**)[8] was isolated as a colorless solid with the molecular formula of $C_{26}H_{26}O_3$ (14° of unsaturation) using HRESIMS (obsd $[M+H]^+$ at *m/z* 387.1974, calcd 387.1960). This molecular formula was also supported by both ^1H and ^{13}C NMR spectral data (Table 1). In its ^1H NMR spectrum, ten aromatic proton signals between δ_H 7.11 and 6.82 indicated that the presence of two mono-substituted benzene rings. An

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Table 1
NMR data (acetone-d_6) of **1**[a]

Position	δ_C, mult.	δ_H (J in Hz)	HMBC
1	155.1, –C	7.48, s	C-3, C-4a, C-8, C-8a
2	–	–	
3	79.3, CH	4.32, dq (6.6, 0.8)	C-1, C-4a, C-9
4	30.1, CH	2.58, dq (7.0, 0.8)	C-4a, C-5, C-8a, C-9, C-10
4a	145.2, –C	–	
5	126.5, –C	–	
6	200.3, –C	–	
7	66.5, –C	–	
8	199.3, –C	–	
8a	111.8, –C	–	
9	18.0, CH_3	0.71, d (6.6)	C-3, C-4,
10	16.9, CH_3	0.55, d (7.0)	C-3, C-4, C-4a
11	9.5, CH_3	1.59, s	C-4a, C-5, C-6, C-8a
12	47.0, CH_2	3.28, d (12.0) 3.14, d (12.0)	C-6, C-7, C-13, C-14, C-16, C-19
13	138.0, –C	–	
14/18	130.5, CH	6.89–6.87, m	C-13, C-15/17, C-19
15/17	127.2, CH	7.11–7.06, m	C-13, C-14/18
16	128.6, CH	7.11–7.06, m	C-14/18, C-15/17
19	45.6, CH_2	3.36, d (12.6) 3.22, d (12.6)	C-7, C-8, C-12, C-20, C-21, C-23
20	137.6, –C	–	
21/25	130.3, CH	6.84–6.82, m	C-19, C-20, C-22/24
22/24	127.1, CH	7.11–7.06, m	C-20, C-21/25
23	128.8, CH	7.11–7.06, m	C-21/25, C-22/24

[a] Measured at 600 MHz (^{1}H) and 150 MHz (^{13}C).

olefinic proton signal at δ_H 7.48, two methine signals at δ_H 4.32 (dq, J = 6.6, 0.8 Hz), 2.58 (dq, J = 7.0, 0.8 Hz) and three methyl groups at δ_H 1.59 (s), 0.71 (d, J = 6.6 Hz) and 0.55 (d, J = 7.0 Hz) were observed. Moreover, two methylenes were characterized by two AB doublets at δ_H 3.28 (d, J = 12.0 Hz) and 3.14 (d, J = 12.0 Hz), and at δ_H 3.36 (d, J = 12.6 Hz) and 3.22 (d, J = 12.6 Hz). The ^{13}C NMR spectral data of **1** showed two ketonic carbonyl carbons (δ_C 200.3 and 199.3), four olefinic carbons, and 12 aromatic carbon signals assignable to two mono-substituted benzene rings, three methyl groups including one olefinic methyl, two methylenes, two methine, and one quaternary carbon. The 14° of unsaturation inherent in the molecular formula of **1**, coupled with data showing the presence of two carbonyls, four olefinic carbons, and 12 aromatic carbons assigned to two benzene rings, indicated that aspergilone A (**1**) possesses another two rings. A further analysis of its NMR data (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY and HMBC spectra) of **1** revealed the presence of an azaphilone structure.[9] The correlations between H-3 and H-4, H_3-9; H-4 and H_3-10 in the ^{1}H–^{1}H COSY spectrum revealed the CH_3–CH–CH–CH_3 subunit in **1**. In the HMBC spectrum, two benzyl groups attached to C-7 were confirmed by the correlations from H_2-12 to C-6, C-7 and from H_2-19 to C-7, C-8, respectively. The structure of **1** as shown in Figure 1 was established. Considering the exceptionally high field shifts of H_3-9 (0.71) and H_3-10 (0.55) which were strongly shielded by the ring current of benzene, the sites of the protons of the two methyl groups (9-CH_3 and 10-CH_3) should lie above the two benzene ring plane, respectively. Finally, the above-mentioned deduction and the relative stereochemistry of aspergilone A (**1**) were unambiguously confirmed by single-crystal X-ray analysis as shown in Figure 2. The molecular structure of **1** showed that the methyl groups of 9-CH_3 and 10-CH_3 lie exactly above the plane of the corresponding benzene rings. Then the relative configurations of two chiral centers were (3S^*, 4R^*).

Aspergilone B (**2**)[10] was isolated as a colorless solid. HREIMS analysis of **2** gave an $[M]^+$ ion at m/z 604.2820 which is consistent with a molecular formula of $C_{39}H_{40}O_6$ (calcd for $C_{39}H_{40}O_6$, 604.2819) with 20° of unsaturation. The ^{1}H NMR and ^{13}C NMR data

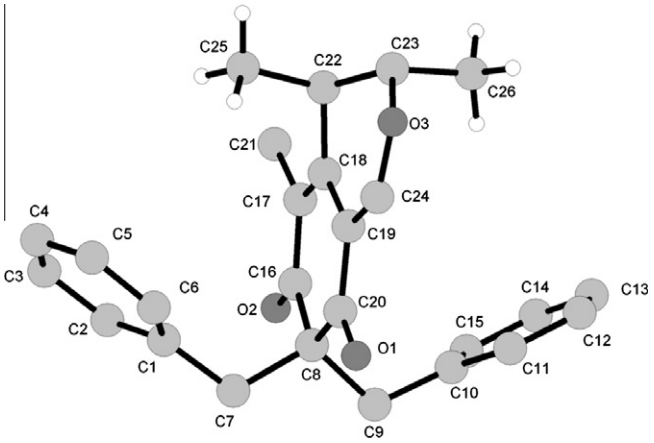

Figure 2. Molecular structure of aspergilone A (**1**).

(Table 2) of **2** were very similar to those of **1**. The most noticeable difference in the ^{1}H NMR spectrum of **2** was that all ^{1}H NMR signals of benzylazaphilone showed double integrating intensity except for the additional methylene group, suggesting that **2** was a symmetrical dimer of **1**, connected at C-7 via a methylene bridge (C-19). The HMBC correlations from H_2-12 to C-19 and from H_2-19 to C-7, C-8 confirmed that the position of the methylene group was at C-7. Accordingly, the planar structure of aspergilone B (**2**) was determined by comparison of its spectral data with those of **1**. Additional evidence that confirmed the structure of **2** came from the EIMS experiment that illustrated prominent fragment ions in support of the structure (Fig. 3). The relative configurations of C-3 and C-4 in compound **2** were assigned as identical to **1** on the basis of the ^{1}H–^{1}H coupling constants and interpretation of 1D NOE experiment. However, the relative configuration of C-7 could not be confirmed because of lacking NOE correlations for H_2-12 and H_2-19. A more detailed comparison of the ^{1}H NMR data of compound **2** with that of **1** revealed that the methyl signal (H_3-10) signal shifted downfield to δ_H 1.07 (0.52 ppm difference), suggesting that there was no effect of the ring current of benzene over it. While the other methyl signal (H_3-9) in **2** shifted much more upfield (δ_H 0.48) compared to that of **1** indicated that this methyl was further influenced by the aromatic anisotropic shielding effect of the only one benzene. The above data together with the structure of **1** strongly suggested that the protons of 9-CH_3 with the conspicuously high field shifts should locate at the same side with the benzene ring plane, so that the relative configurations of **2** could be determined as 3S^*, 4R^* and 7S^*.

Aspergilones A and B (**1** and **2**) were the latest examples of azaphilone derivatives with the benzyl group and aspergilone B (**2**) is also the first azaphilone dimer with a unique methylene bridge. Although numerous natural dimers, including flavonoids,[11] macrolides,[12] alkaloids,[13] anthraquinones,[14] xanthones[15] and naphthopyranones[16], have been reported to date, the methylene bridge is highly unusual among natural dimers. The co-isolation of aspergilones A and B (**1** and **2**) suggests that **2** could be biosynthesized by two molecules of debenzylaspergilone A and one molecule of formaldehyde in the fungus. The discovery of aspergilone B (**2**) also provides additional evidence that there may be a formaldehyde biosynthetic system in nature.[17–19]

Compound **1** was found to exhibit in vitro selective cytotoxicity toward HL-60 human promyelocytic leukemia, MCF-7 human breast adenocarcinoma and A-549 human lung carcinoma cell lines with IC_{50} values of 3.2, 25.0 and 37.0 μg/mL, respectively.[20] However, compound **2** was found to be relatively noncytotoxic (IC_{50} >50 μg/mL) against these three tumor cell lines.