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NOTE

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Asperaculin A, a Sesquiterpenoid from a Marine-Derived Fungus, *Aspergillus aculeatus*

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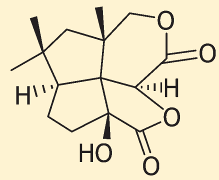
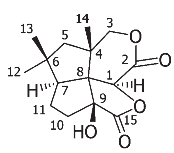
S Supporting Information

ABSTRACT: A novel sesquiterpenoid, asperaculin A (**1**), possessing a novel [5,5,5,6]fenestrane ring system, was isolated from the marine-derived fungus *Aspergillus aculeatus* CRI323-04. The structure of asperaculin A (**1**) was established by analysis of spectroscopic data. The name aspergillane is proposed for the sesquiterpene skeleton in asperaculin A (**1**).



Marine-derived fungi have become increasingly attractive to natural product chemists over the past decades partly because their secondary metabolites have diverse pharmaceutical activity. Fungal metabolites display various and potent biological activities,[1] and some have been used as drugs, for example, echinocandins (antifungal drugs), ergot alkaloids (for the treatment of migraine), cyclosporine (an immunosuppressive drug), and lovastatin (a cholesterol-lowering drug).[2−4] Fungi of the genus *Aspergillus* are sources of several bioactive compounds.[5,6] For example, *Aspergillus aculeatus* produces aculeacins A−G (antibiotics and antifungal agents)[7,8] and CJ-15,183 (squalene synthase inhibitor and antifungal agent).[9] Our previous investigation revealed that *A. aculeatus* CRI323-04 produced a new tyrosine-derived metabolite, aspergillusol A, an α-glucosidase inhibitor.[10] We further examined the minor constituents from the mycelia of the fungus CRI323-04 and isolated a novel sesquiterpenoid, namely, asperaculin A (**1**), together with a known compound, asperparaline A (aspergillimide),[11,12] from *A. aculeatus* CRI323-04. Herein, we report the isolation and structure elucidation of asperaculin A (**1**), whose skeleton is novel. Biosynthesis of **1** is also discussed in this paper.

The mycelial extract of *A. aculeatus* CRI323-04 was separated by size-exclusion chromatography and reversed-phase HPLC to yield asperaculin A (**1**) and a known oxindole alkaloid, asperparaline A[11] (also known as aspergillimide).[12] Spectral data of asperparaline A were identical in all respects to those reported.[11,12]



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Asperaculin A (**1**) was isolated as white needles. Analysis of NMR and APCITOF MS data determined the molecular formula of asperaculin A (**1**) as $C_{15}H_{20}O_5$. The IR spectrum exhibited absorptions at 3461 (hydroxyl group) and 1769 cm^{-1} (carbonyl group). The ^1H NMR spectrum ($CDCl_3$) of asperaculin A (**1**) displayed signals of oxygenated methine (δ_H 4.86) and methylene (δ_H 4.38 and 3.88), three singlet methyls (δ_H 1.00, 1.15, and 1.33), and four sp^3 methines and methylenes (δ_H 1.59−2.40) (Table 1). ^{13}C and DEPT spectra revealed that asperaculin A (**1**) contained 15 carbons, attributable to three methyls, four methylenes, two methines, and six nonprotonated carbons (two of which were identified as lactone groups). A partial structure H-7/H_2-11/H_2-10 was established from $^1H-^1H$ COSY correlations. Two singlet methyl groups, C-12 and C-13, were located on the same quaternary carbon, C-6, which was flanked by C-5 and C-7 due to HMBC correlations from H_3-12 to C-13; H_3-13 to C-12; and H_3-12 and H_3-13 to C-5, C-6, and C-7. There were HMBC correlations (acquired in DMSO-d_6) from OH-9 to both C-10 and C-15, placing C-9 between C-10 and a C-15 carbonyl ester. HMBC correlations of H-1 to two carbonyl ester carbons (C-15 and C-2) implied that C-1 was between the two carbonyl groups. Since oxygenated methylene H_2-3 appeared in a low-field region, it could link either with a carbonyl ester or with an ether functional group. The HMBC correlation from H_2-3 to a C-2 carbonyl ester group indicated that C-3 was linked to C-2 via an ester bond. The HMBC correlations from H_3-14 to C-3, C-4, C-5, and C-8 and from many protons located around the ring structure, including H-1, H_2-3α/β, H_2-5α/β, H-7, OH-9 (acquired in DMSO-d_6 at 600 MHz), H-10α, H_2-11α/β, and H_3-14, to a quaternary C-8 established the planar structure of asperaculin A (**1**), which contained a lactonized [5,5,5,6] fenestrane skeleton, having the characteristic of C-8 locating in the middle of the molecule. The relative configuration of asperaculin A (**1**) could be determined by NOESY correlations. The NOESY correlations between H-1 and H-7 and between H_3-12 and H-7 indicated that H-1, H-7, and H_3-12 were on the same plane,

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Asperaculin A (1)

position	δ_C[a]	δ_H[a] (mult, *J* in Hz)	δ_C[b]	δ_H[b] (mult, *J* in Hz)
1	76.5	4.86, s	76.3	5.31, s
2	167.6		168.5	
3	75.7	4.38, d (11.7, H_α); 3.88, d (11.7, H_β)	74.4	4.45, d (11.6, H_α); 3.85, d (11.6, H_β)
4	44.9		44.7	
5	52.5	1.86, d (14.3, H_α); 1.59, d (14.3, H_β)	51.9	1.79, d (14.0, H_α); 1.56, d (14.0, H_β)
6	40.2		39.0	
7	60.7	2.24, dd (7.2, 9.2)	59.3	2.03, dd (9.7, 7.7)
8	65.5		63.8	
9 (−OH)	84.3		83.1	6.43, s
10	35.9	2.40, m (H_α); 1.98, m (H_β)	33.0	2.17, m (H_α); 1.87, m (H_β)
11	24.0	1.74, m (H_α); 1.86, m (H_β)	23.4	1.85, m (H_α); 1.57, m (H_β)
12	32.2	1.15, s	31.1	1.15, s
13	27.0	1.00, s	26.8	0.94, s
14	23.1	1.33, s	23.6	1.25, s
15	177.1		177.7	

[a] Acquired in $CDCl_3$ (600 MHz). [b] Acquired in DMSO-d_6 (600 MHz).

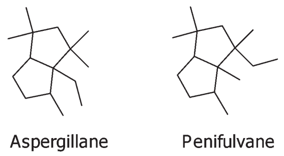
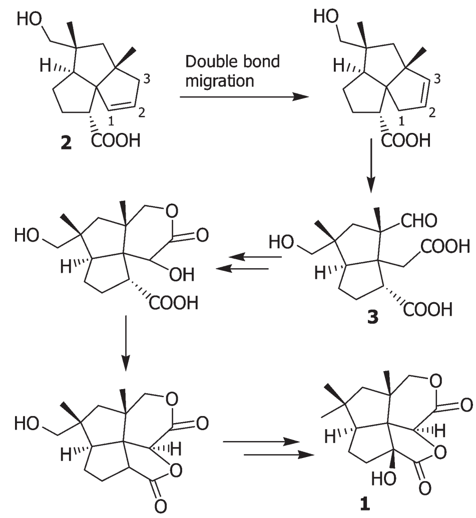


Figure 1. Two novel skeletons of fungal sesquiterpenes.

while the NOESY correlation between OH-9 and H_3-14 established a coplanarity of these protons. Although available NMR data could not establish the configuration of a quaternary C-8, the C-8 stereochemistry in **1** should, upon biosynthetic analogy, be similar to that of penifulvins (A−E),[13,14] closely related sesquiterpenoids derived from silphinenes.

Asperaculin A (**1**) is structurally related to penifulvins (A−E).[13,14] Penifulvin A, a novel sesquiterpeniod with activity against the fall armyworm, *Spodoptera frugiperda*, was initially separated as a major metabolite from *Penicillium griseofulvum*,[13] while its oxidized analogues, penifulvin B−E, were discovered later in the same year.[14] Herein, the skeleton of asperaculin A (**1**) was named "aspergillane", while that of penifulvins was proposed as "penifulvane" (Figure 1). The structure of asperaculin A (**1**) is very similar to that of penifulvin D, except for the transposition of a carbonyl (C-2) and an oxygen in an ester group. Asperaculin A (**1**) is possibly biosynthesized from silphinenes,[14] which are metabolites of plants and fungi.[15,16] As shown in Scheme 1, the proposed biosynthesis of asperaculin A (**1**) starts from a double-bond migration (C1/C2 to C2/C3) of the silphinene intermediate **2**. The C2/C3 double bond then undergoes oxidative cleavage to yield the intermediate **3**, which in turn undergoes sequences of oxidation and lactonizations to finally give asperaculin A (**1**). The double-bond migration is considered as a key step leading to the difference in aspergillane and penifulvane skeletons. Asperaculin A (**1**) did not exhibit cytotoxic activity (at 50 μg/mL) against HepG2, MOLT-3, A549, and HuCCA-1 cancer cell lines.

Scheme 1. Proposed Biosynthesis of Asperaculin A (1)



■ EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on a digital Electrothermal 9100 melting point apparatus and reported without correction. Optical rotations were measured at the sodium D line (590 nm) on a JASCO DIP-370 digital polarimeter. UV−vis spectra were obtained using a Shimadzu UV-1700 PharmaSpec spectrophotometer. FTIR data were obtained using a universal attenuated total reflectance attachment on a Perkin-Elmer Spectrum One spectrometer. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Bruker AVANCE 600 NMR spectrometer (operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C). APCITOF MS spectra were obtained on a Bruker MicroTOF$_{LC}$ spectrometer.

Fungal Material, Extraction, and Isolation. *A. aculeatus* CRI323-04 was isolated from a sponge, *Xesto-spongia testudinaria*, collected from Phi Phi Island, Krabi Province, Thailand. The strain was cultured in 10 L of potato dextrose broth, which was prepared using seawater. Mycelia were separated by filtration and were macerated in MeOH and CH_2Cl_2, respectively. Crude MeOH extract was partitioned with hexane to remove lipid and extracted with EtOAc to yield 3.10 g of a crude extract. The extract was first fractionated by Sephadex LH-20, eluted with MeOH, producing nine fractions. Fractions 2 and 3, containing sesquiterpenoid, were further isolated by preparative reversed-phase HPLC to yield asperaculin A (**1**)

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(70.8 mg) (45% MeOH in water; t_R = 22.3 min) and asperparaline A (29.3 mg) (55% MeOH in water; t_R = 12.0 min).

Asperaculin (1): white needles; mp 190−192 °C; $[\alpha]^{28}_{D}$ −10.2 (c 0.23, in MeOH); UV ($CDCl_3$) λ_{max} (log ε) 228.50 (2.30) nm; IR ($CHCl_3$) ν_{max} 3461, 2924, 1769, 1660, 1469 cm^{-1}; ^1H NMR, ^{13}C NMR data, see Table 1; APCITOF MS observed m/z 281.1388 ($C_{15}H_{20}O_5$ + H)$^+$ and 303.1225 ($C_{15}H_{20}O_5$ + Na)$^+$, calcd 281.1389 ($C_{15}H_{20}O_5$ + H)$^+$ and 303.1208 ($C_{15}H_{20}O_5$ + Na)$^+$, respectively.

Cytotoxicity Assay. Cytotoxic activity for HepG2, HuCCA-1, and A549 cancer cell lines was evaluated with the MTT assay,[17] while that for the MOLT-3 cell line was assessed using the XTT assay.[18] Doxorubicin and etoposide were the reference drugs.

ASSOCIATED CONTENT

Supporting Information. ^1H, ^{13}C NMR and 2D spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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