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Hippolachnin A, a New Antifungal Polyketide from the South China Sea Sponge *Hippospongia lachne*

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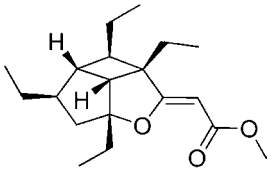
ABSTRACT



Hippolachnin A

Hippolachnin A (1), a polyketide possessing an unprecedented carbon skeleton with a four-membered ring, was isolated from the South China Sea sponge *Hippospongia lachne*. The structure was elucidated using MS and NMR spectroscopic analyses, and the absolute configuration was determined using a calculated ECD method. Hippolachnin A demonstrated potent antifungal activity against three pathogenic fungi, *Cryptococcus neoformans*, *Trichophyton rubrum*, and *Microsporum gypseum*, with a MIC value of 0.41 μM for each fungus.

For many years, diverse marine organisms have inspired researchers to identify novel marine natural products with pharmaceutical potential.[1] Marine sponges (Porifera) are one of the richest sources of bioactive secondary metabolites with chemical diversity. Sponges of the genus *Hippospongia* are well recognized as a rich source of terpenoids, such as sesquiterpenes,[2] sesterterpenes,[3] furanoterpenes,[4] triterpenoic acids,[5] and fatty acid derivatives.[6] These compounds exhibit antitumor,[7] anti-inflammatory,[8] and antifungal[9] activity. As part of our continuing efforts to discover bioactive marine natural products from South China Sea sponges,[10] bioassay-guided isolation of a portion of the EtOH extract of *H. lachne* with antifungal activity, collected off the Xisha Islands, resulted in the purification of an unprecedented polyketide, hippolachnin A (**1**), and

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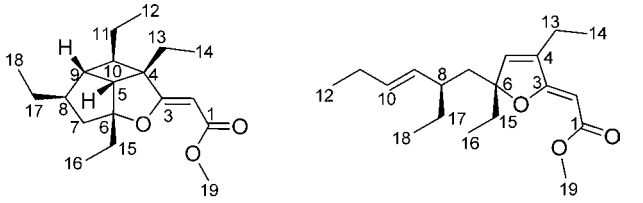
its proposed biogenic precursor, α,β-unsaturated methyl ester (**2**),[11] which was recently disclosed as a PPARγ antagonist.[11d] Hippolachnin A (**1**) exhibited potent antifungal activity against three pathogenic fungi (*Cryptococcus neoformans*, *Trichophyton rubrum*, and *Microsporum gypseum*) with an MIC value of 0.41 μM for all three species.



1 **2**

Hippolachnin A (**1**)[12] was isolated as a colorless oil. This compound's molecular formula was established as $C_{19}H_{30}O_3$ with five degrees of unsaturation with an HRESIMS ion peak at *m*/*z* 329.2092 $[M + Na]^+$. The IR absorption bonds at 2927, 1635, and 1217 cm^{-1} indicated the presence of an α,β-unsaturated ester subunit. The ^1H NMR spectrum showed the presence of four ethyl groups through four triplet methyl groups at δ_H 0.88 (6H, t, J = 7.5 Hz, 2 × CH_3 overlapped), 1.02 (3H, t, J = 7.5 Hz), and 0.80 (3H, t, J = 7.5 Hz), a methoxy signal at δ_H 3.66 (3H, s), and an olefinic proton at δ_H 4.56 (s) (Table 1). The ^{13}C and DEPT NMR spectra displayed 19 carbons, including four quaternary carbons (an ester carbonyl carbon at δ_C 167.3, an oxygenated sp^2 carbon at δ_C 181.1, an oxygenated sp^3 carbon at δ_C 104.2, and an aliphatic carbon at δ_C 56.6); five methine groups at δ_C 83.5, 49.4, 47.5, 46.3, and 52.9; five methylene peaks at δ_C 45.1, 30.8, 28.3, 24.6, and 23.3; and five methyl groups at δ_C 8.6, 9.6, 11.8, 13.0, and 50.4 (Table 1). The above-mentioned data indicated that **1** possessed one double bond and one carbonyl group, which accounted for two out of the five degrees of unsaturation, implying the presence of three rings in the structure.

The structure of **1** was further established after interpreting 2D NMR experiments (Figure 1). The $^1H-^1H$ COSY correlations of H_2-13/H_3-14, H_2-11/H_3-12, H_2-15/H_3-16, and H_2-17/H_3-18 indicated the presence of four ethyl groups, meanwhile the correlations of H-5/H-9, H-8/H-7b, and H_2-17/H-8 revealed the linkage of CH-5/CH-9 and CH_3-18/CH_2-17/CH-8/CH_2-7, respectively (Figure 1). Accordingly, the connectivity of the above subunits with the remaining atoms enabled assembly into the planar structure of **1** by interpreting the HMBC spectrum in detail. HMBC correlations from both H_3-14 and H_2-13 to C-4, and H_2-13 to C-3, C-5, and C-10 placed the ethyl CH_2-13/CH_3-14 on C-4 as well as linked three carbon bonds, C-3/C-4, C-4/C-10, and C-4/C-5. This assignment was confirmed by HMBC correlations of H-5/C-10, C-13 and H-10/C-4, C-13. Due to the overlap of H-10/H-15a, and H-9/H-13a in the ^1H NMR spectrum, the HMBC cross peaks of H-9 with C-4, C-11, and C-10 could not be clearly distinguished. Fortunately, the HMBC correlations from H-8 to C-10, from H_2-17 to C-7, C-8, and C-9, and from both H-10 and H_2-11 to C-9 could be clearly observed, which not only placed the ethyl CH_2-17/CH_3-18 at C-8 and linked the carbon bond C-8/C-9 but also revealed the presence of a cyclobutane moiety.

Furthermore, H_2-15 showed HMBC correlations with C-5, C-6, and C-7, which connected a cyclopentane moiety and located the ethyl CH_2-15/CH_3-16 at C-6. The remaining ethyl was placed at C-10 on account of the long-ranged correlations of H-11b with C-4, C-9, and C-10. Consequently, a 4/5-fused bicyclic system was unambiguously established.

Moreover, a pair of ^4J correlations from both H-15a and H-15b to C-3 was observed in the HMBC spectrum (Figure 1 and Figure S11 in Supporting Information (SI)), which indicated the presence of an ether between C-3 and C-6 and formed a tetrahydrofuran ring, accounting for their downfield chemical shifts of C-6 and C-3 and the remaining one degree of unsaturation.

In addition, HMBC correlations from the olefinic proton H-2 to C-1, C-3, and C-4 and from the methoxy H_3-19 to C-1 suggested the α,β-unsaturated ester was located at C-4, which was confirmed by the ^3J correlation between H_2-13 and C-3. Thus, the planar structure of **1** was finally determined as shown in Figure 1.

The geometry of double bond $\Delta^{2,3}$ was determined as *Z* based on the NOESY correlation between H-2 and H-13b and was confirmed by comparison of the chemical shift of proton H-2 (δ_H 4.56) with data in the literature (δ 4.85 for *Z*, δ 5.23 for *E*).[13]

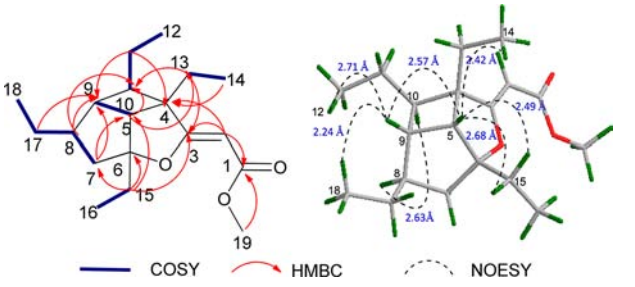


Figure 1. Key COSY, HMBC, and NOESY correlations of **1**.

The relative configuration of **1** was determined using a NOESY spectrum (Figure 1). The observed key NOESY correlations of H-5 with H-9, H_3-14, and H_2-15, as well as H-9 with H_3-12 and H_3-18, implicated the *cis* orientation of H-9/H-5 and their relative *sin* relationships with the other ethyl groups on the 4,5-fused rings (Figure 1;

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(12) Hippolachnin A (**1**), colorless oil; $[\alpha]_D^{23}$ +27° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 253 (4.11) nm; IR (KBr) υ_{max} 3435, 2927, 1635, 1458, 1375, 1217, 1039, 802 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 329.2092 $[M + Na]^+$ (calcd for $C_{19}H_{30}O_3Na$, 329.2093).

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Figures S15, S16). The NOESY correlation of H-8/H-10 (Figure S17) suggested a *sin* relationship between these two protons oriented to the geometry of 4,5-fused rings. As a result, we proposed a *trans* configuration of H-9 and H-10 on the cyclobutane, owing to the severe unfavorable distortions of the sp^3 atoms involved in the interested rings for the alternative anti-arrangement of the above protons.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of **1** in $CDCl_3$

no.	δ_H, (J in Hz)	δ_C	HMBC	NOESY
1		167.3		
2	4.56, s	83.5	1, 3, 4	H-13a, H_3-14
3		181.1		
4		56.6		
5	2.59, d, 8.0	49.4	3, 4, 6, 7, 8, 9, 10, 13, 15	H-9, 15a, H_3-14
6		104.2		
7a	2.44, q, 7.5	45.1	6, 13, 15	
7b	1.32, m		17	
8	1.98, m	47.5	9, 10	H-10, 17a, 18
9	1.79, m	46.3	4, 10, 11	H-11, H_3-12, 18
10	1.82, m	52.9	5, 8, 9	H-8
11a	1.64, m	24.6	9, 10	H-9, 11b
11b	1.32, m		4, 9, 10	H-11a
12	0.88, 3H, t, 7.5	11.8	4, 10, 11	H-11a, 11b
13a	1.75, m	23.3	3, 5	H-5, H_3-14
13b	1.52, m		3, 5, 10	H-2, H_3-14
14	0.80, 3H, t, 7.5	8.6	4, 13	H-2, 5, 13b
15a	1.82, m	30.8	3, 5, 7, 16	H-5, 7a, 15b
15b	1.67, m		3, 6	H-15a
16	1.02, 3H, t, 7.5	9.6	6, 15	H-5, 7a, 15a
17a	1.33, m	28.3	7, 8, 9, 18	H-5, 8
17b	1.30, m		7, 8, 9	
18	0.88, 3H, t, 7.5	13.0	8, 17	H-7a, 9, 17a
19	3.66, 3H, s	50.4	1	

The calculated ECD method was used to establish the absolute configuration of **1** (Figure 2).[14] The stereostructure of **1** was constructed on the basis of the distance constraints from the key correlations observed in the NOESY spectrum. Using these spectroscopic data, only the stereoisomer shown in Figure 1 and its corresponding mirror image were found to satisfy the observed NOE constraints. Therefore, only two configurations were generated for theoretical calculations to identify the most probable candidate for **1**. A conformational search was then performed using Syby1 8.03 software with a simulated annealing method and the MMFF94 force field. The 24 corresponding minimum geometries were fully optimized using DFT at the B3LYP/6-31G(d) level, as implemented in the Gaussian 03 program package. All of them displayed no imaginary frequencies. Initially, the global minimum energy-minimized structures of two probable configurations of **1** were submitted to ECD calculations using the TDDFT method. The calculated ECD spectrum of **1** was compared with the experimental ECD spectrum to determine the most probable configuration (Figure S21). Finally, with the aid of Multiwfn 3.2 software,[15] the Boltzmann-averaged ECD spectrum of **1** was obtained to provide a clear comparison with the experimental data (Figure 2).

The global minimum geometry was also used for GIAO-based ^{13}C NMR chemical shift calculations to gain further supporting data for the carbon assignments of **1**.[16] The experimental and calculated ^{13}C NMR chemical shifts (relative to TMS-resonance calculated at the same level of DFT) were listed in Table S1 of the SI. The calculated isotropic shielding constants were in good correlation with the experimental ^{13}C NMR chemical shifts (Figure S20 in SI). After linear scaling,[17] the mean absolute error with respect to the experimentally observed chemical shifts was only 1.70 ppm for **1**. The largest absolute error was 3.60 ppm on C10, which might reflect the effect of the strained spirocycle system. Accordingly, the quantum-mechanical GIAO calculations of the ^{13}C NMR chemical shifts provide a reliable basis for the correct carbon assignments in **1**.

As shown in Figure 2, there was satisfactory agreement between the stimulated and experimental ECD spectrum of **1**. The computed ECD spectrum for the structure at 4*S*, 5*R*, 6*R*, 8*R*, 9*R*, 10*R* clearly reproduced both the sign and shape of the measured ECD spectrum. Thus, the absolute configuration of **1** was unambiguously established as 4*S*, 5*R*, 6*R*, 8*R*, 9*R*, 10*R*.

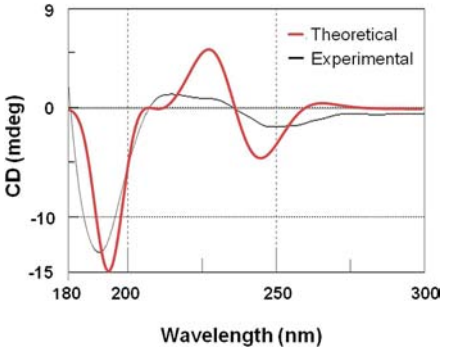

Figure 2. Experimental and theoretical ECD spectra for hippolachnin A (**1**).

A plausible biogenetic pathway for Hippolachnin A (**1**) is proposed in Scheme 1. The ethyl branches are hypothesized to be assembled in part from butyrate units.[18] Four butyrate units and an acetate unit are required to construct

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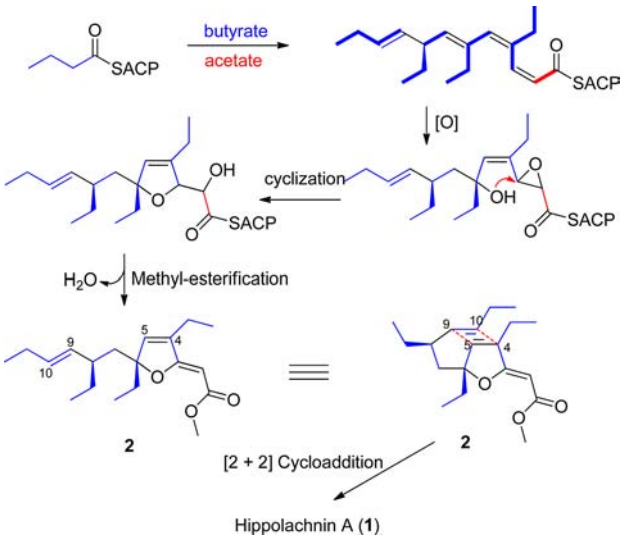
Scheme 1. Plausible Biosynthetic Pathway of **1**



the polyketide skeleton. Following a series of oxidation, cyclization, elimination reactions and methyl-esterification at C-1, the intermediate **2** is produced. A four-membered ring was formed via an intramolecular [2 + 2] cycloaddition reaction between $\Delta^{4,5}$ and $\Delta^{9,10}$ of **2**. Finally, the metabolite **1** was generated via methyl-esterification at C-1.

Hippolachnin A (**1**) was evaluated for antifungal activity against seven pathogenic fungi using ketoconazole (KCZ), voriconazole (VCZ), fluconazole (FCZ), and terbinafine (TBF) as positive controls (Table 2). Hippolachnin A exhibited potent antifungal activity against *Cryptococcus neoformans*, *Trichophyton rubrum*, and *Microsporum gypseum*, with a MIC value of 0.41 μM for all three species and moderate activity against four other fungi strains. The results showed that hippolachnin A (**1**) is a promising new lead compound for antifungal therapy. The cytotoxic activity of **1** on human cancer cell lines HCT-116, A549, and HeLa was also evaluated using the MTT method[19] but showed no significant inhibitory activity on these cancer lines.

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Table 2. Antifungal activity of hippolachnin A (**1**) against seven pathogenic fungi

		MIC (μM)			
fungi	**1**	KCZ	VCZ	FCZ	TBF
Cryptococcus neoformans	0.41	0.12	0.18	3.27	13.7
Candida albicans	13.1	0.47	0.18	6.54	3.44
Candida glabrata	1.63	0.12	0.18	6.54	3.44
Cryptococcus parapsilosis	1.63	1.88	0.18	6.54	27.5
Aspergillus fumigatus	13.1	7.53	5.73	209	3.44
Trichophyton rubrum	0.41	0.12	0.09	1.63	0.43
Microsporum gypseum	0.41	0.24	0.18	0.82	0.43

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Supporting Information Available. Experimental procedures, HRESIMS, IR, 1D NMR, and 2D NMR spectra of hippolachnin A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.