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Jahanyne, an Apoptosis-Inducing Lipopeptide from the Marine Cyanobacterium *Lyngbya* sp.

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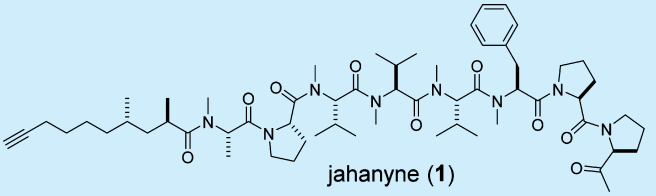
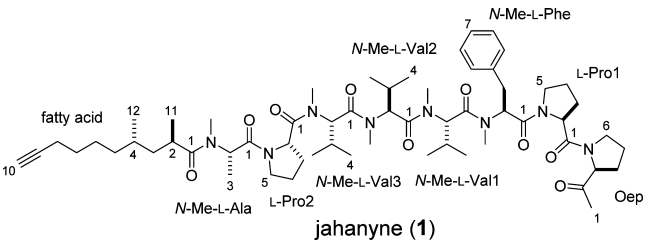
Supporting Information

ABSTRACT: An acetylene-containing lipopeptide, jahanyne, was isolated from the marine cyanobacterium *Lyngbya* sp. Its gross structure was established by spectroscopic analyses, and the absolute configuration was clarified based on a combination of chiral HPLC analyses, spectroscopic analyses, and derivatization reactions. Jahanyne significantly inhibited the growth of human cancer cells and induced apoptosis in HeLa cells.



Various secondary metabolites have been isolated from marine sources over the past several decades. In a series of investigations, marine microorganisms such as cyanobacteria, fungi, and bacteria have been proven to be a rich source of new biologically active substances.[1,2] In particular, marine cyanobacteria have been recognized as prolific producers of novel bioactive peptides,[3] and we have isolated several compounds, including bisebromoamide,[4] kurahamide,[5] kurahyne,[6] and maedamide,[7] from marine cyanobacteria. In our continuing search for novel bioactive substances, we investigated the constituents of the marine cyanobacterium *Lyngbya* sp. and isolated a novel acetylene-containing lipopeptide, jahanyne (**1**). **1** Possessed two rare partial structures, a 2-(1-oxo-ethyl)-pyrrolidine moiety (Oep) and a 2,4-dimethyldec-9-ynoic acid moiety (fatty acid). Although 2-(1-oxo-ethyl)-pyrrolidine itself has been reported to be a constituent of tobacco,[8] **1** is the first discovered compound that contains an Oep moiety as a partial structure. To the best of our knowledge, a 2,4-dimethyl fatty acid moiety containing a terminal acetylene group has been reported in only one other natural product, and the stereochemistry of the fatty acid moiety in the precedent compound, carmabin A,[9] has not been determined to date. Here, we describe the isolation, structure elucidation, and preliminary biological characterization of jahanyne (**1**).

The marine cyanobacterium *Lyngbya* sp. (900 g, wet weight) was collected at the coast near Jahana, Okinawa, and extracted with methanol. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. The organic extract was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation with reversed-phase column chromatography (ODS silica gel, MeOH−H_2O) and repeated reversed-phase HPLC (Cosmosil 5C_{18}-MS-II, MeOH−H_2O; Cosmosil Cholester, MeCN−H_2O) to afford 18.5 mg of jahanyne (**1**).



The molecular formula of jahanyne (**1**) was found to be $C_{60}H_{94}N_8O_9$ by HRESIMS (*m*/*z* 1093.7046, calcd for $C_{60}H_{94}N_8O_9Na$ $[M + Na]^+$ 1093.7041). The NMR data for **1** are summarized in Table 1. The ^1H NMR spectrum revealed the presence of five singlets corresponding to *N*-methyl amide substituents (δ 3.10, 3.03 2.96, 2.92, 2.14), seven α-methines of amino acids (δ 5.88, 5.30, 5.06, 5.03. 4.93, 4.84, 4.72), and five aromatic protons consistent with a phenyl group of a phenylalanine (δ 7.22−7.29). Additionally, **1** possessed two ^{13}C NMR absorptions consistent with a terminal acetylene group (δ 85.0, 69.6) and nine carbonyl signals (δ 208.3, 179.3, 174.7, 172.5, 172.1, 171.8, 171.1, 170.9, 170.5). These data suggested an acetylene-containing peptide structure. Detailed analyses of the ^1H NMR, ^{13}C NMR, COSY, HMQC, and HMBC spectra revealed the presence of two prolines, one *N*-methylphenylalanine, three *N*-methylvalines, and one *N*-methylalanine. Furthermore, the presence of two residues derived from 2-(1-oxo-ethyl)-pyrrolidine and 2,4-dimethyldec-9-ynoic acid (fatty acid) was clarified. The existence of the terminal alkyne moiety was confirmed based on the IR band (2116 cm^{-1}).

The sequence of these partial structures was determined based on the HMBC and NOESY data (Figure 1, Table S1). Five HMBC correlations, *N*-Me of *N*-Me-Phe/C-1 of *N*-Me-

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Table 1. ^{1}H and ^{13}C NMR Data for Jahanyne (1) in CD_3OD

position	δ_C[a]	δ_H[b] (J in Hz)	position	δ_C[a]	δ_H[b] (J in Hz)
Oep			4	20.4, CH_3	0.87, d (6.8)
1	26.8, CH_3	2.18, s	5	18.3, CH_3	0.76, d (6.8)
2	208.3, C		*N*-Me	30.9, CH_3	2.92, s
3	66.8, CH	4.61, dd (9.0, 5.0)	*N*-Me-Val3		
4a	28.7, CH_2	2.23, m	1	172.1, C	
4b		1.90, m	2	60.0, CH	5.06, d (11.2)
5a	25.81, CH_2[c]	2.06, m	3	28.2, CH	2.32, m
5b		1.96, m	4	18.8, CH_3	0.91, d (7.2)
6a	48.3, CH_2	3.84, m	5	19.9, CH_3	0.83, d (6.8)
6b		3.65, m	*N*-Me	30.8, CH_3	3.10, s
Pro1			Pro2		
1	172.5, C		1	174.7, C	
2	59.78, CH[c]	4.72, dd (9.1, 3.8)	2	58.8, CH	4.84, dd (8.9, 4.3)
3a	29.4, CH_2	2.29, m	3a	29.8, CH_2	2.26, m
3b		2.02, m	3b		1.72, m
4a	25.78, CH_2[c]	2.09, m	4a	25.78, CH_2[c]	2.04, m
4b		1.96, m	4b		1.97, m
5a	48.9, CH_2	3.73, m	5a	48.4, CH_2	3.66, m
5b		3.58, m	5b		3.55, m
N-Me-Phe			*N*-Me-Ala		
1	170.5, C		1	171.8, C	
2	56.6, CH	5.88, dd (11.9, 4.5)	2	52.6, CH	5.30, q (7.4)
3a	35.1, CH_2	3.10, m	3	14.2, CH_3	1.27, d (7.4)
3b		3.02, m	*N*-Me	31.4, CH_3	3.03, s
4	138.3, C		fatty acid		
5/9	130.5, CH	7.22, m	1	179.3, C	
6/8	129.6, CH	7.29, m	2	34.8, CH	2.95, m
7	128.1, CH	7.25, m	3a	42.5, CH_2	1.52, m
N-Me	31.3, CH_3	2.96, s	3b		1.30, m
N-Me-Val1			4	31.8, CH	1.43, m
1	171.1, C		5a	37.5, CH_2	1.33, m
2	60.0, CH	5.03, d (10.8)	5b		1.12, m
3	28.1, CH	2.15, m	6a	27.1, CH_2	1.48, m
4	20.2, CH_3	0.83, d (6.8)	6b		1.33, m
5	18.0, CH_3	0.64, d (7.2)	7	29.8, CH_2	1.48, m
N-Me	30.2, CH_3	2.14, s	8	18.9, CH_2	2.17, m
N-Me-Val2			9	85.0, C	
1	170.9, C		10	69.6, CH	2.19, m
2	59.83, CH[c]	4.93, d (11.1)	11	17.8, CH_3	1.07, d (6.8)
3	28.2, CH	2.31, m	12	20.1, CH_3	0.89, d (6.4)

[a]Measured at 100 MHz. [b]Measured at 400 MHz. [c]These carbon signals are interchangeable, respectively.

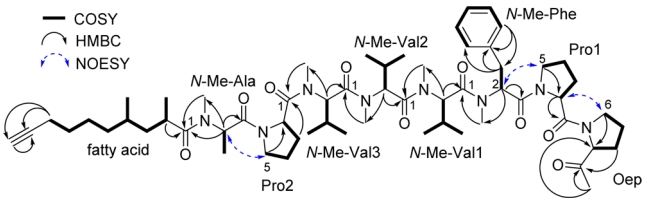

Figure 1. Gross structure of jahanyne (**1**), based on 2D NMR correlations.

Val1, *N*-Me of *N*-Me-Val1/C-1 of *N*-Me-Val2, *N*-Me of *N*-Me-Val2/C-1 of *N*-Me-Val3, *N*-Me of *N*-Me-Val3/C-1 of Pro2, and *N*-Me of *N*-Me-Ala/C-1 of the fatty acid, revealed two partial structures, *N*-Me-Phe-*N*-Me-Val1-*N*-Me-Val2-*N*-Me-Val3-Pro2 and *N*-Me-Ala-Fatty acid. Furthermore, three NOESY correlations, H-6a of Oep/H-2 of Pro1, H-5 of Pro1/H-2 of *N*-Me-Phe, and H-5 of Pro2/H-2 of *N*-Me-Ala, allowed us to determine the gross structure of **1** as shown in Figure 1.

The absolute configurations of all of the amino acid moieties in **1** were assigned to be L-form by a comparison of the results of chiral HPLC of the acid hydrolysate of **1** with D and L amino acid standards (see Supporting Information, pp S10−S13). With regard to the Oep moiety, a direct acid hydrolysis of **1** can result in the epimerization of C-3 of the Oep moiety. To prevent this epimerization, reduction of ketone with $NaBH_4$ followed by acid hydrolysis afforded 2-(1-hydroxyethyl)-pyrrolidine derived from the Oep moiety as a mixture of two diastereomers. The absolute stereochemistry of Oep was determined by reversed-phase HPLC analysis based on a comparison of the retention times of Marfey derivatives[10] of the obtained diastereomeric alcohols to those of authentic samples. As a result, the absolute configuration of the Oep moiety was elucidated to be 3*S* (see SI, pp S14−S15).

The absolute configuration of C-2 of the fatty acid moiety was determined by applying the PGME method.[11] Catalytic reduction of **1** followed by acid hydrolysis afforded 2,4-

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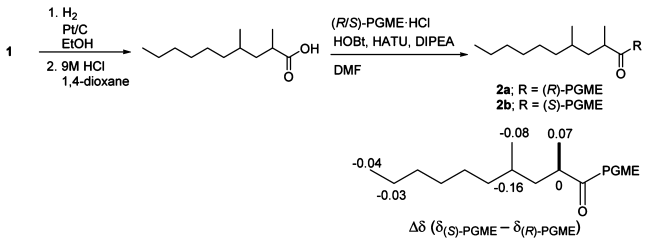
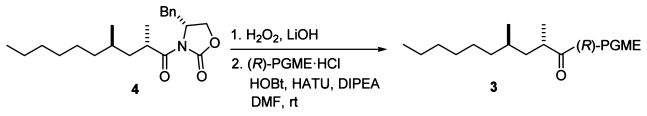
dimethyldecanoic acid derived from **1**. The obtained fatty acid was condensed with (*R*)- or (*S*)-phenylglycine methyl ester (PGME) to afford **2a** and **2b**, respectively. The ^{1}H NMR spectra of the obtained diastereomers were compared, and calculated $\Delta\delta$ values revealed that the stereochemistry of C-2 was 2*R* (Scheme 1; see SI, pp S16−S19).

Scheme 1. Preparation of the PGME Amides of the Fatty Acid from Jahanyne (1)



The remaining stereochemistry at C-4 of the fatty acid was established based on the differences in ^{1}H NMR chemical shifts between two geminal protons at C-3. According to Schmidt et al.,[12] it is possible to determine the relative configuration of 1,3-dimethyl-substituted alkyl chains by interpreting ^{1}H NMR shift differences between two geminal protons sandwiched between two methyl groups. The $\Delta\delta$ value of H-3a and H-3b in **1**, 0.22 ppm, apparently indicated that the relative stereochemistry of the fatty acid moiety was *anti*, (2*R*,4*S*). To confirm this stereochemistry, we synthesized the (*R*)-PGME amide of (2*S*,4*R*)-2,4-dimethyldecanoic acid (**3**), the enantiomer of **2b**, for comparison purposes. Hydrolysis of the known amide **4**[13] followed by condensation with (*R*)-PGME afforded **3** (Scheme 2). The ^{1}H NMR spectrum of **3** was identical to that of **2b** (see SI, pp S20−S21). Therefore, the absolute stereochemistry of jahanyne was established as shown in **1**.

Scheme 2. Synthesis of the (*R*)-PGME Amide of (2*S*,4*R*)-2,4-Dimethyldecanoic Acid (3)



To evaluate the growth-inhibitory activities of jahanyne (**1**), an MTT assay with HeLa cells and HL60 cells was used. The cells were treated in 96-well plates with various concentrations of the compounds (0.01−10 μM for HeLa cells, 0.001−10 μM for HL60 cells) for 72 h. The data from these assays revealed that **1** inhibited the growth of both HeLa cells and HL60 cells, with IC_{50} values of 1.8 μM and 0.63 μM, respectively. Additionally, **1** showed cytotoxicity against HeLa cells, as determined using the trypan blue dye exclusion assay (Figure 2A). Furthermore, the cell death of these cells induced by **1** was suppressed in the presence of Z-VAD-FMK, an irreversible and cell-permeable inhibitor of caspases (Figure 2A). Significant DNA laddering in these cells was observed in the presence of **1** (Figure 2B). These results indicated that **1** induced apoptosis in HeLa cells.

In conclusion, jahanyne (**1**), a novel acetylene-containing lipopeptide, was isolated from the marine cyanobacterium *Lyngbya* sp. The gross structure of **1** was established by spectroscopic analyses, and the absolute configurations of

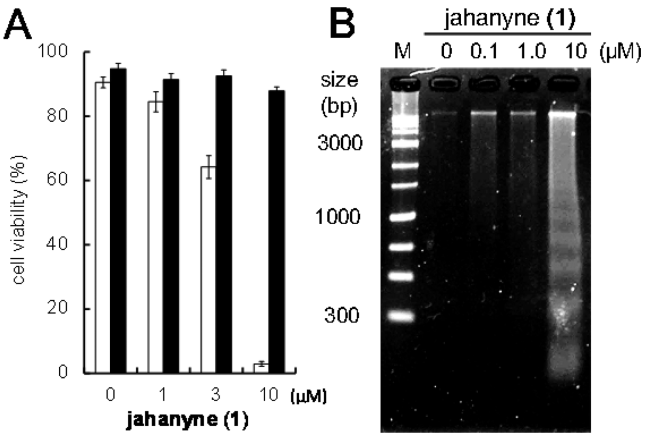


Figure 2. Induction of apoptosis by jahanyne (**1**) in HeLa cells. (A) HeLa cells were preincubated (solid column) or not (open column) with 50 μM of Z-VAD-FMK. They were then treated with the indicated concentrations of **1**. After further incubation for 48 h, cell viability was determined based on trypan blue dye exclusion. Values are the mean ± SD of quadruplicate determinations. (B) HeLa cells were incubated with the indicated concentrations of **1** for 24 h. Cellular DNA was then extracted and electrophoresed on agarose gels.

amino acids were clarified based on the chiral HPLC analyses of the acid hydrolysate. The absolute stereochemistry of the characteristic 2,4-dimethyl fatty acid moiety was established based on a combination of the PGME method, a spectroscopic analysis developed by Schmidt et al., and a synthetic method. Through these experiments, we demonstrated that the method by Schmidt et al.[12] was effective for determining the structures of natural products. Additionally, **1** is the first discovered compound that contains a 2-(1-oxo-ethyl)-pyrrolidine as a partial structure, and it is indicated that the marine cyanobacterium *Lyngbya* sp. is the prolific source of natural products possessing characteristic structures. With respect to biological activity, **1** significantly inhibited the growth of both HeLa cells and HL60 cells, with IC_{50} values of 1.8 μM and 0.63 μM, respectively. Furthermore, **1** was revealed to induce apoptosis in HeLa cells. According to the previous paper,[14] the structure-related compound, carmabin A, exhibited good antimalarial activity. The acyclic nature and the presences of a 2,4-dimethyldec-9-ynoic acid moiety and an aromatic amino acid moiety are consistent with each other. Therefore, jahanyne (**1**) may exhibit antimalarial activity. A detailed investigation of the bioactivity of **1** is in progress.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, COSY, NOESY, HMQC, and HMBC NMR spectra in CD_3OD for jahanyne (**1**). HPLC chromatograms for determination of the absolute configurations. Detailed experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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