

Kurahyne, an acetylene-containing lipopeptide
from a marine cyanobacterial assemblage of
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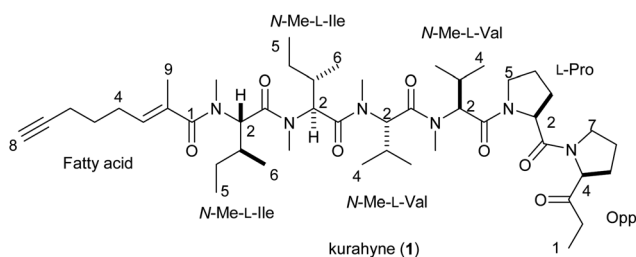
Kurahyne, a new acetylene-containing lipopeptide, was isolated from a cyanobacterial assemblage that mostly consisted of *Lyngbya* sp. Its structure was elucidated by spectroscopic analyses and chiral HPLC analyses of hydrolysis products. Kurahyne inhibited the growth of human cancer cells and induced apoptosis in HeLa cells, and it seemed to localize in mitochondria.

Some peptides derived from marine organisms have attracted attention due to their remarkable bioactivities.¹ Marine cyanobacteria, in particular, produce a variety of novel lipopeptides.² In our continuing search for new bioactive substances from marine cyanobacteria,³ we investigated the constituents of a cyanobacterial assemblage that mostly consisted of *Lyngbya* sp. collected at Okinawa, Japan, and isolated an acetylene-containing lipopeptide, kurahyne (**1**). Structurally, **1** contains a C8-alkynoate moiety and a C7-ketone moiety. The same C8-alkynoate unit has only been found in dragonamide E,⁴ and the same C7-ketone unit has only been found in bisbromoamides.⁵ Kurahyne (**1**) was found to inhibit the growth of human cancer cells and to induce apoptosis in HeLa cells.

The marine cyanobacterial samples⁶ (2.3 kg, wet weight) were collected at Kuraha, Okinawa, and were extracted with methanol. The extract was filtered, concentrated, and partitioned between EtOAc and H₂O. The EtOAc-soluble material 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–H₂O) and reversed-phase HPLC (Cosmosil Cholesterol, MeCN–H₂O; Cosmosil 5C₁₈-MS-II, MeOH–H₂O) to give kurahyne (**1**) (29.9 mg) as a colorless oil. The molecular formula of **1** was found to be C₄₇H₇₈N₆O₇ by HRESIMS (*m/z* 839.5991, calcd for C₄₇H₇₉N₆O₇ [M + H]⁺ 839.6010). The NMR data for **1** are summarized in Table 1.

The ¹H NMR spectrum revealed the presence of four singlets corresponding to *N*-methyl amide substituents (δ 3.38, 3.27, 3.26, 2.86), one vinyl methyl group (δ 1.75), five methine groups corresponding to the α position of the amino acid residue (δ 5.54, 5.53, 5.49, 5.40, 4.50) and nine high-field methyl groups (δ 1.22, 0.96, 0.94, 0.92, 0.90, 0.86, 0.86, 0.79, 0.77). Additionally, the ¹H NMR spectrum indicated an olefinic double triplet (δ 5.38) and a triplet characteristic of a terminal acetylene proton [δ 1.80 (*J* = 2.9 Hz)]. **1** possessed two ¹³C NMR absorptions consistent with a terminal acetylene (δ 83.8 and 69.4), and seven carbonyl signals (δ 208.5, 173.4, 170.91, 170.88, 170.6, 170.3, 169.2). Further analysis of the ¹H NMR, ¹³C NMR, COSY, HMQC and HMBC spectra revealed the presence of proline, two *N*-methylvalines and two *N*-methylisoleucines. Additionally, the presence of residues derived from 2-(1-oxo-propyl)-pyrrolidine (Opp) and 2-methyloct-2-en-7-ynoic acid (fatty acid) was also established. Despite the presence of a terminal acetylene, an IR band of triple bond stretching was not observed (see ESI, S10[†]). The existence of an acetylene group was confirmed based on the magnitude of the coupling constant of C-8 of fatty acid (¹*J*_{C–H} = 253 Hz) on the INEPT spectrum (see ESI, S8 and 9[†]) and comparison of the ¹³C chemical shifts between **1** and dragonamide E⁴ possessing the same fatty acid moiety (see ESI, S20[†]).

The sequence of these partial structures was determined based on HMBC and NOESY data (Table 1 and Fig. 1). A NOESY



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[†] Electronic supplementary information (ESI) available: ¹H, ¹³C, COSY, NOESY, HMQC, HMBC and INEPT NMR spectra in C₆D₆ for kurahyne (**1**). IR spectrum. HPLC chromatograms for determination of absolute configurations. Detailed experimental procedures. See DOI: 10.1039/c4ra00132j

Table 1 NMR Data for kurahyne (1) in C₆D₆^a

Unit	Position	δ_{C}^b	δ_{H}^c (J in Hz)	COSY	HMBC	NOESY
Opp	1	7.7	0.96, 3H, dd (7.8, 7.8)	2a, 2b	2, 3	2a, 2b
	2a	33.44 ^c	2.29, 1H, dq (18.7, 7.8)	1, 2b	1, 3	1, 2b, 4
	2b		2.07, 1H, dq (18.7, 7.8)	1, 2a	1, 3	1, 2a, 4
	3	208.5				
	4	64.1	4.41, 1H, dd (8.6, 4.7)	5a, 5b	5, 6	2a, 2b, 5a, 5b
	5a	27.77 ^d	1.35, 1H, m	4, 5b, 6a, 6b	3, 4, 6, 7	4
	5b		1.18, 1H, m	4, 5a, 6a, 6b	3, 6, 7	4
	6a	25.01 ^e	1.43, 1H, m	5a, 5b, 6b, 7a, 7b	4, 5, 7	7b
	6b		1.31, 1H, m	5a, 5b, 6a, 7a, 7b	4, 5, 7	7a
	7a	46.7	3.45, 1H, m	6a, 6b, 7b	4, 5, 6	6b, 7b, 2 (Pro)
	7b		3.05, 1H, m	6a, 6b, 7a	5, 6	6a, 7a, 2 (Pro)
Pro	1	170.3				
	2	58.1	4.50, 1H, dd (8.5, 4.0)	3a, 3b	3, 4	3a, 3b, 7a (Opp), 7b (Opp)
	3a	28.6	1.82, 1H, m	2, 3b, 4a, 4b	1, 2, 4, 5	2, 4b, 5b
	3b		1.64, 1H, m	2, 3a, 4a, 4b	1, 2, 4, 5	2, 4a
	4a	24.96 ^e	1.81, 1H, m	3a, 3b, 4b, 5a, 5b	2, 3, 5	3b
	4b		1.43, 1H, m	3a, 3b, 4a, 5a, 5b	2, 5	3a, 5a
	5a	47.9	4.03, 1H, m	4a, 4b, 5b	2, 3, 4	4b, 5b, 2 (N-Me-Val-1)
	5b		3.72, 1H, m	4a, 4b, 5a	3, 4	3a, 5a, 2 (N-Me-Val-1), 4 (N-Me-Val-1)
N-Me-Val-1	1	169.2				
	2	59.7	5.40, 1H, d (11.2)	3	1, 3, 4, 5, N-Me	3, 4, 5, N-Me, 5a (Pro), 5b (Pro)
	3	28.1	2.47, 1H, m	2, 4, 5	1, 2, 4	2, 4, 5, N-Me
	4	19.7	1.22, 3H, d (6.5)	3	2, 3, 5	2, 3, 5b (Pro)
	5	18.6	0.77, 3H, d (7.0)	3	2, 3, 4	2, 3, N-Me
	N-Me	30.9	3.38, 3H, s		2, 1 (N-Me-Val-2)	2, 3, 5, 2 (N-Me-Val-2)
N-Me-Val-2	1	170.88 ^f				
	2	58.6	5.49, 1H, d (10.7)	3	1, 3, 4, 5, N-Me (N-Me-Val-1)	3, 4, 5, N-Me, N-Me (N-Me-Val-1)
	3	27.71 ^d	2.55, 1H, m	2, 4, 5	2, 4, 5	2, 4, 5, N-Me
	4	19.9	0.94, 3H, d (6.4)	3	2, 3, 5	2, 3
	5	18.0	0.79, 3H, d (7.0)	3	2, 3, 4	2, 3, N-Me
	N-Me	30.70 ^g	3.27, 3H, s		2, 1 (N-Me-Ile-1)	2, 3, 5, 2 (N-Me-Ile-1)
N-Me-Ile-1	1	170.6				
	2	57.0	5.53, 1H, d (11.2)	3	1, 3, 4, 6, N-Me (N-Me-Val-2)	3, 4a, 5, 6, N-Me (N-Me-Val-2)
	3	33.42 ^h	2.37, 1H, m	4a, 4b, 6	2	2, 5, 6, N-Me
	4a	24.1	1.35, 1H, m	3, 4b, 5	3, 5, 6	2
	4b		1.03, 1H, m	3, 4a, 5	3, 5, 6	
	5	10.9	0.86, 3H, m	4a, 4b	3, 4	2, 3, N-Me
	6	15.77	0.86, 3H, m	3	2, 3, 4	2, 3
	N-Me	30.63 ^g	3.26, 3H, s		2, 1 (N-Me-Ile-2)	3, 5, 2 (N-Me-Ile-2)
N-Me-Ile-2	1	170.91 ^f				
	2	56.6	5.54, 1H, d (11.2)	3	1, 3, 4, 6, N-Me (N-Me-Ile-1), 1 (fatty acid)	3, 4a, 4b, 5, 6, N-Me (N-Me-Ile-1)
	3	33.1 ^h	2.37, 1H, m	4a, 4b, 6	2	2, 4a, 5, 6, N-Me
	4a	24.6	1.39, 1H, m	3, 4b, 5	2, 3, 5, 6	2, 3, 4b, N-Me
	4b		1.19, 1H, m	3, 4a, 5	3, 5, 6	2, 4a
	5	11.0	0.90, 3H, m	4a, 4b	3, 4	2, 3
	6	15.83	0.92, 3H, d (8.5)	3	2, 3, 4	2, 3
	N-Me	31.9	2.86, 3H, s		2, 1 (fatty acid)	3, 4a, 3 (fatty acid), 9 (fatty acid)
Fatty acid	1	173.4				
	2	133.6				
	3	129.1	5.38, 1H, dt (7.5, 1.6)	4, 9	1, 4, 9	4, 5, N-Me (N-Me-Ile-2)
	4	26.6	1.96, 2H, m	3, 5	2, 3, 5, 6	3, 9, 5
	5	28.0	1.34, 2H, m	4, 6	3, 4, 6, 7	4, 6
	6	18.2	1.93, 2H, dt (7.3, 2.9)	5, 8	4, 5, 7, 8	5
	7	83.8				
	8	69.4	1.80, 1H, t (2.9)	6		
	9	14.4	1.75, 3H, br s	3	1, 2, 3	4, N-Me (N-Me-Ile-2)

^a ¹H-¹³C connectivities were determined by HMQC method. ^b Measured at 100 MHz. ^c Measured at 400 MHz. ^d These carbon signals are interchangeable, respectively. ^e These carbon signals are interchangeable, respectively. ^f These carbon signals are interchangeable, respectively. ^g These carbon signals are interchangeable, respectively. ^h These carbon signals are interchangeable, respectively.





its growth inhibitory activity (IC_{50} : $85 \pm 13 \mu\text{M}$, HeLa cells). The staining sites of **2** seemed to be included in those of Mito Tracker Red in HeLa cells on the basis of fluorescence microscopic analysis (Fig. 3B). From this result, it is likely that the mechanism of action of **1** is mitochondrially-targeted.

In conclusion, kurahyne (**1**), a novel acetylene-containing lipopeptide, was isolated from a marine cyanobacterial assemblage that mostly consisted of *Lyngbya* sp. The structure of **1** was established by spectroscopic analysis and HPLC analysis of acid hydrolysates. The structural features of **1** are the presence of an Opp moiety and a 2-methyloct-2-en-7-ynoic acid moiety. To our knowledge, **1** is the first reported compound which possesses both of these moieties. **1** inhibited the growth of both HeLa cells and HL60 cells, with IC_{50} values of $3.9 \pm 1.1 \mu\text{M}$ and $1.5 \pm 0.1 \mu\text{M}$, respectively. Furthermore, **1** was revealed to induce apoptosis in HeLa cells, and it seemed to localize in mitochondria. The detailed bioactive investigation of **1** is ongoing.

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- 6 The most cyanobacterium was morphologically classified into the genus *Lyngbya* because it was composed of short cells with a thick sheath. From the phylogenetic tree inferred from 959 bp of 16S rRNA gene sequences (see ESI, S16†) revealed that the present cyanobacterium (Maeda 130904A, accession no. AB857842) formed a clade with *Oscillatoria miniata* NAC8-50 (GU724208), and closely related with *Trichodesmium* spp.⁹ The identification described above was carried out using the same cyanobacterium collected at the same location in September 2013, and we confirmed that its extract contained **1**. Despite our efforts, other minor cyanobacteria in the assemblage were not identified by 16S rRNA gene analysis. A detailed classification will be reported later.
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