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Pitiamide A, a New Chlorinated Lipid from a Mixed Marine Cyanobacterial Assemblage

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Abstract: Pitiamide A, a new chloro-lipid, was isolated from an extract of a mixed assemblage of *Lyngbya majuscula* and a *Microcoleus* sp. found growing on intact colonies of the hard coral *Porites cylindrica* on Guam. The structure was determined by interpretation of 2D-NMR spectra.
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Black band disease, a cyanobacterial infection of scleractinian hard corals by *Phormidium corallyticum* has been documented to cause coral colony destruction in the western Atlantic and elsewhere.[2] Cytotoxic metabolites, the nakienones A-C and nakitriol, have been isolated from a *Synechocystis* sp. found overgrowing (or infecting) an Okinawan species of hard coral.[3] We have recently observed a thin blue-gray tuft-forming assemblage of *Lyngbya majuscula* and *Microcoleus* sp. (approx. 4:1) overgrowing the apical tips of otherwise healthy, intact branches of the yellow reef-building coral *Porites cylindrica* at various locations throughout Micronesia.[4]

Small cyanobacteria tufts (1-4 cm^3) were carefully removed from coral tips and stored at -20° until extracted with CH_2Cl_2/MeOH (1:1). The crude extract strongly deterred feeding by the yellow-banded parrotfish (*Scarus schlegeli*), a common reef herbivore found throughout Micronesia.[5] 2D-TLC analysis of the extracts showed the presence of several UV-active, orange and purple-charring (H_2SO_4, heat) relatively non-polar secondary metabolites. A 752.4 mg portion of the crude extract (1.31 g, dark oil, 28.7 g dry marc) was fractionated by silica gel column chromatography with a gradient of hexanes to EtOAc to CH_2Cl_2 to MeOH. The fractions eluting with 40% EtOAc/hexanes to 100% EtOAc (v/v) were combined (242.0 mg) and a portion (210.9 mg) was separated by Sephadex LH-20 chromatography (50% (v/v) CH_2Cl_2 in MeOH), and further purified by repetitive NP-HPLC (75 and 65% EtOAc in hexanes). The two major compounds were decolorized with activated charcoal. Final purification by NP-HPLC (2% (v/v) MeOH in CH_2Cl_2 provided pitiamide A (**1**, 8.3 mg) and pitiamide B (**2**, 15.1 mg).[6]

Low resolution EIMS examination suggested the presence of a chlorine in the structure of **1** (m/z 381, 8.6% rel. abundance $[M]^+$; 383, 3.1% rel. abundance $[M+2]^+$). Analysis of **1** by ^{13}C NMR and High resolution EIMS (381.2551; calc. for 381.2434; 70 eV) provided a molecular formula for $[M]^+$ of $C_{22}H_{36}ClNO_2$ (calc. for 5° unsaturation). Compound **1** was optically active $[\alpha]_D$ = -10.3° (c = 3.0, $CHCl_3$). Examination of **1** by IR (neat, υ = 3650-3100, 2959, 2930, 2873, 1709, 1645, 1553 cm^{-1}), UV (λ_{max} 223 nm, MeOH), and ^1H-NMR data (Table 1) revealed the presence of a ketone, an amide-linkage, a conjugated diene, and two methyl branches.

Three ^1H spin systems were assembled by ^1H-^1H COSY (Figure 1) and two carbonyl carbons were confirmed by ^{13}C-NMR. The ^1H-^{13}C HMBC spectrum facilitated the attachment of spin systems **a-c** (Figure 1) and the carbonyl moieties. Specifically, 2 and 3-bond couplings from carbonyl carbon C8 (δ 214 ppm) to $H_2$9 of partial structure **b** and H7 and $H_3$23 of **a** allowed for the attachment of **a** and **b** through a ketone moiety. The C14 carbonyl (δ 172.6 ppm) showed $^2J_{CH}$ and $^3J_{CH}$ couplings to $H_2$15 and $H_2$16 of **c** and H12a and H12b of **b**. Attachment of a vinyl chloride to C1 completed the planar structure of **1**. Additionally, a pronounced

fragmentation ion, observed by EIMS (m/z 267, 69% rel. abundance, 70 eV) was consistent with a McLafferty rearrangement and cleavage between C5 and C6 of structure **1**.

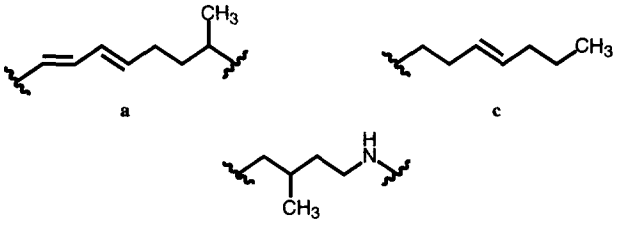
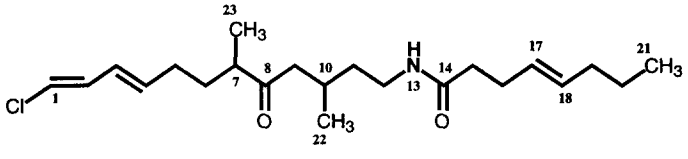


Figure 1. Partial structures **a** through **c**.

The C3-C4 and C17-C18 olefins were assigned *E* configurations, based upon their large vicinal coupling constants ($^3J_{HH}$ = 15.3, Table 1). The strong electron withdrawing effect of the C1 vinyl chloride significantly reduces the magnitude of the vicinal H1-H2 coupling.[7] Therefore, the $^3J_{HH}$ value of 13.3 Hz was indicative of an *E* geometry for the C1-C2 olefin. The absolute configuration at C7 and C10 was not assigned.



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NMR and low resolution EIMS analysis of **2** was consistent with a structurally related chlorinated analog to **1** with an additional methylene (m/z 395, 6.7% rel. abundance $[M]^+$; 397, 2.5% rel. abundance $[M+2]^+$). Compound **2** was unstable and underwent isomerization to a mixed product as evidenced by the presence of 1-*(E)*- **2** ($^3J_{HH}$ = 13.1 Hz, H1-H2), 1-*(Z)*- **2** ($^3J_{HH}$ = 7 Hz, H1-H2), and a trace dechloro-**2** product formation (^{1}H-NMR). The relative instability of these compounds bring into question the chemical nature of the true metabolite profile present in the intact cyanobacterial filaments.

The simple aliphatic compound 1-chlorotridec-1*(E)*-ene-6*(R)*,8*(R)*-diol represents the only other terminally substituted vinyl chloride-containing lipid previously reported from marine cyanobacteria.[8] This compound, like **1** was isolated from cyanobacteria found in mixed assemblage. The pitiamides may represent acyclic analogs to the malyngamides which have previously been isolated from *L. majuscula*.[9]

Table 1. NMR Data for Compound **1**.[a]

Position[#]	^{13}C δ	^{1}H δ	^{1}H-^{1}H COSY[b]	^{1}H-^{13}C HMBC[c]
1	118.99	6.10 d 13.3	H2	C2, C3
2	133.49	6.39 dd 13.3, 10.7	H3	C1, C3, C4
3	126.81	5.97 bdd 15.3, 10.7	H4	C1, C2, C5
4	134.72	5.64 dt 15.3, 7.1	H5	C2, C5, C6
5	30.27	2.04 bm	H6a, H6b	C3, C4, C6, C7
6	31.84	a) 1.4 m	H6b, H7	C4, C5, C7, C8, C23
		b) 1.76 m	H7	C4, C5, C7, C8, C23
7	45.86	2.48 m	H23	C5, C6, C8, C23
8	214	---	---	---
9	48.34	2.37 dd 6.6, 4.2	H10	C8, C10, C11, C22
10	25.90	2.06 bm	H11a, H11b	C9, C11, C12, C22
11	36.38	a) 1.37 m	H11b, H12a, H12b	C9, C10, C12, C22
		b) 1.42 m	H12a, H12b	C9, C10, C12, C22
12	37.26	a) 3.19 m	H12b, NH13	C10, C11, C14
		b) 3.28 m	NH13	C10, C11, C14
13	---	5.71 bm	---	---
14	172.60	---	---	---
15	36.73	2.23 bt 7.2	H16	C14, C16, C17
16	28.64	2.32 bq 6.6	H17	C14, C15, C17, C18
17	128.50	5.40 ddd 15.3, 8.8, 6.4	H18	C15, C16, C18, C19
18	131.65	5.47 ddd 15.3, 8.6, 6.5	H19	C16, C17, C19, C20
19	34.57	1.95 bq 6.8	H20	C17, C18, C20, C21
20	22.53	1.35 bq	H21	C18, C19, C21
21	13.60	0.87 t 7.4	---	C19, C20
22	20.74	0.91 d 6.6	---	C9, C10, C11
23	16.37	1.06 d 7.1	---	C7, C8

a) Data reported for **1** in $CDCl_3$. NMR spectra recorded at 500 Mhz for ^{1}H and 125 Mhz for ^{13}C. Data presented as δ in ppm, multiplicity, J in Hz. ^{1}H spectra referenced to the residual $CDCl_3$ (7.26 ppm). ^{13}C chemical shifts are referenced to the center peaks of $CDCl_3$ (77.0 ppm). Assignments based on ^{1}H-^{13}C HMQC spectra. b) ^{1}H-^{1}H COSY data presented in non-redundant format from top to bottom. c) ^{1}H-^{13}C HMBC optimized for J_{CH} = 7.0 Hz.

The occurrence of overgrowth or infection of scleractinian corals by this type of cyanobacterial assemblage has not yet been well documented. Nothing is known of specific interactions between these cyanobacteria and *P. cylindrica*. Herbivore feeding deterrence may play some role in the ability of these organisms to colonize exposed coral tips. Relative instability, due to isomerization has made these compounds difficult to study in ecologically relevant field-based experiments. Pitiamide A (**1**) and B (**2**) are structurally new representatives of a growing set of unusual biologically active natural products produced by cyanobacteria found in mixed assemblages.[8,10] Ypaoamide, a recently described cyanobacterial metabolite has been shown to act as a feeding deterrent to both vertebrate and invertebrate herbivore species.[10,11] Ypaoamide, like compounds **1** and **2**, is produced by a strain of *L. majuscula* found in mixed assemblage with other cyanobacteria.

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References and Notes

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4. Tufts of cyanobacteria were found on the tips of *P. cylindrica* colonies (-1 to -2 meters in depth) on the shallow fringe reefs of Guam, Yap, Chuuk, and Pohnpei throughout 1995 and 1996. Voucher specimen on file with William H. Gerwick's herbarium (Oregon State University College of Pharmacy).
5. The crude cyanobacterial extract was tested at concentration of 4.6% (w/w) in laboratory aquarium assays.[a,b] The crude extract significantly reduced grazing by 91% relative to control food strips (control mean = 76 squares consumed (mean standard error 11.8); treated mean = 7.0 squares consumed (mean standard error 2.8); N = 13, $p \approx 0.0001$. Data analyzed by a paired *t*-test. Differences were tested for normality by a Wilk-Shapiro/Rankit plot test (Statisix 4.0). a) Hay, M.E.; Kappel, Q.E.; Fenical, W. *Ecology* **1994**, 75, 1714-1726. b) Lumbang, W.A. and Paul, V.J. *J. Exp. Mar. Biol. Ecol.* **1996**, 201, 185-195.
6. Compounds **1** and **2** are named to reflect the site of collection (Piti Bomb Holes, Guam).
7. For CH_2CHCl: $^3J_{HH}$ *cis* = 1.3, $^3J_{HH}$ *trans* = 14.6 Hz; Günther, H. *NMR Spectroscopy-Basic principles, concepts, and applications in chemistry*, 2nd ed.; John Whiley: New York, 1995; pp. 519.
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