New Lipopeptides from the Caribbean Cyanobacterium *Lyngbya majuscula*

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Received September 29, 2000

Four new metabolites have been isolated from a marine red cyanobacterium, *Lyngbya majuscula*, collected at Boca del Drago Beach, Bocas del Toro, Panama. The planar structures were elucidated by 1D and 2D NMR techniques. These compounds were assigned the trivial names pseudodysidenin (**2**), dysidenamide (**3**), nordysidenin (**4**), and dragonamide (**7**).

Cyanophyceae, or blue-green algae, have proven to be a rich source of novel biologically active secondary metabolites. In particular, the genus *Lyngbya* has yielded an impressive array of structurally diverse compounds.[1] As part of ongoing efforts to discover new anticancer compounds from marine sources, the lipophilic extract of a Panamanian variety of the cyanobacterium *Lyngbya majuscula* led us to identify three new polychlorinated metabolites, pseudodysidenin (**2**), dysidenamide (**3**), and nordysidenin (**4**), and a new lipopeptide, dragonamide (**7**). (See Chart 1.)

Compounds **2**−**4** are closely related to dysidenin (**1**)[2] and isodysidenin (**5**)[3] isolated from Australian and New Guinean specimens of *Dysidea herbacea*, respectively. Polychlorinated metabolites have been primarily isolated from some sponges of the genus *Dysidea* which consistently have shown symbiotic association with the filamentous cyanobacterium *Oscillatoria spongeliae* and/or rod-shaped bacteria.[4,5] The isolation of compounds **2**, **3**, and **4** is particularly important on the basis that dysidenin compounds are cellularly localized to cyanobacteria associated with the sponge *Dysidea herbacea*. This is the first reported case of dysidenin-like compounds isolated from a free-living cyanobacterium and thus strengthens the hypothesis that sponge-based dysidenins are biosynthesized from associated cyanobacteria.[6]

Dragonamide (**7**) contains a unique C_8-alkynoate unit. Structurally similar C_8-alkynoate units have been found in marine mollusks (the kulolides,[7] kulomo'opunalides,[8] dolastatin 17,[9] and onchidin),[10] *L. majuscula* collected from various locations (the apramides and yanucamides A and B),[11,12] and *Symploca laete-viridis* (malevamide C).[13]

Results and Discussion

Pseudodysidenin (**2**) was obtained as a white crystalline solid. It displayed a 52:100:80:34:8:1:0.06 ion cluster *m*/*z* 544/546/548/550/552/554/556 on DCIMS (NH_3), indicating six chlorine atoms. The molecular formula of **2**, the most abundant metabolite, was determined to be $C_{17}H_{23}Cl_6N_3O_2S$ based on HRDCIMS data, which indicated five degrees of unsaturation.

The ^{1}H and ^{13}C NMR spectra indicated the presence of a minor conformer in a 5:1 ratio. NMR analysis, however, was not obstructed and was carried out for the major conformer. The ^{13}C NMR spectrum of **2** contained two amide carbonyl signals at δ 170.5 and 171.4 together with bands at δ 169.3, 143.0, and 119.9, resonances that were characteristic of a 2-substituted thiazole ring.[4] This accounted for all five degrees of unsaturation deduced from the molecular formula; thus pseudodysidenin contained only one thiazole ring. Further evidence for chlorine atoms in the molecule was substantiated by two trichloromethyl singlets at δ 104.9 and 105.2 in the ^{13}C NMR spectrum.

The final gross structure of **2** was deduced from its ^{1}H−^{1}H COSY and ^{1}H−^{13}C HMBC NMR spectra and found to be closely related to dysidenin (**1**) and isodysidenin (**5**).[2,3] However, upon closer inspection of the ^{1}H NMR spectrum, it became evident that **2** was an isomer of dysidenin. For instance, H-5 in **2** appeared as a doublet of doublets of doublets (δ 5.10), whereas in dysidenin, H-5 shows as a doublet of doublets (δ 5.27), which suggested a difference in the *N*-methylation pattern. A fragment ion in the mass spectrum occurring at *m*/*z* 171 (15%) due to C-5/C-11 bond cleavage indicated 15 mass units higher in **2** than found in dysidenin.[2] These data strongly support that compound **2** is the N-12 methyl isomer.

Dysidenamide (**3**) was obtained as a colorless amorphous solid. The molecular formula was determined to be $C_{15}H_{23}Cl_6N_3O_3$ based on HRFABMS data. The ^{1}H NMR spectrum displayed a 2:1 ratio; thus analysis was carried out on the major conformer. ^{1}H and ^{13}C NMR data clearly indicated that **3** lacked the thiazole ring present in pseudodysidenin. Instead, a terminal primary amino group was observed based on MS data which showed an intense $[M - NH_2]^+$ ion (64%) at *m*/*z* 489.

Nordysidenin (**4**) was also obtained as a colorless amorphous solid with a molecular formula $C_{16}H_{21}Cl_6N_3O_2S$ based on HRFABMS data. The ^{1}H NMR spectrum of **4** was very similar to pseudodysidenin except for lack of the signal corresponding to the *N*-CH_3 at δ 2.94. Thus, nordysidenin is the N-12 demethyl analogue of **2**.

The relative and absolute configuration of pseudodysidenin (**2**), dysidenamide (**3**), and nordysidenin (**4**) was established by chiral HPLC and optical rotation comparison to other chlorine-containing metabolites. Chiral HPLC analysis of the ozonolysis−acid hydrolysate showed the presence *N*-Me-L-Ala for compounds **2** and **3**, and L-Ala for dysidenamide (**4**), indicating a 12*S* methyl group in all three compounds. The configuration at the remaining stereogenic centers was determined as 2*S*,5*S*,7*S* based on optical rotation comparisons. It is interesting to point out that dysidenin (**1**) and dysideathiazole (**6**)[4] are of identical configuration at C-2, C-5, and C-7 (all *S*) and have negative optical rotations, whereas **5** has the *R* configuration at C-5 and a positive optical rotation.

In addition to the dysidenin-like compounds **2**−**4**, three other peptides were also isolated, the known carbamin A and B[14] and a new linear lipopeptide, dragonamide (**7**). Dragonamide possesses a molecular formula of $C_{37}H_{59}N_5O_5$

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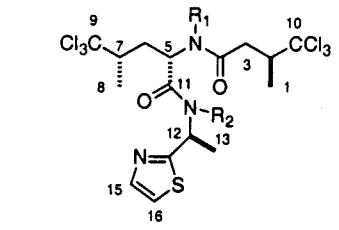
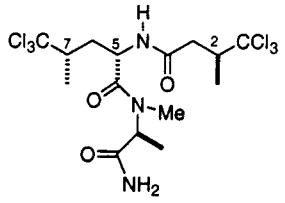
10.1021/np000462q CCC: $20.00
Published on Web 01/26/2001

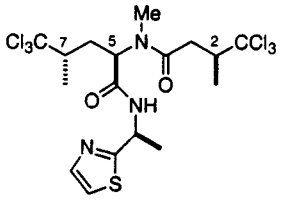
Chart 1



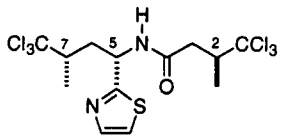
$R_1 = CH_3$ $R_2 = H$ **1** $[\alpha]_D = -98^\circ$
$R_1 = H$ $R_2 = CH_3$ **2** $[\alpha]_D = -96.9^\circ$
$R_1 = H$ $R_2 = H$ **4** $[\alpha]_D = -56.7^\circ$



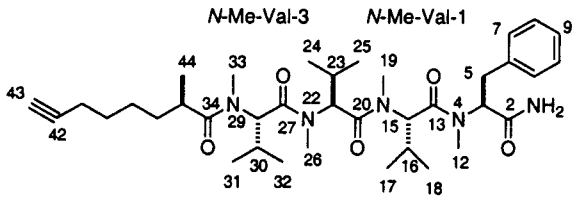
3 $[\alpha]_D = -40.3^\circ$



5 $[\alpha]_D = +47^\circ$



6 $[\alpha]_D = -78.8^\circ$



7

as determined by HRFABMS. ^1H and ^{13}C NMR spectra indicated the presence of a minor conformer (5:1 ratio), however; structural analysis was not obstructed and was carried out for the major conformer.

Singlets at δ 2.39, 2.90, 2.92, and 2.95 in the ^1H NMR spectrum suggested the presence of four *N*-methylated amino acids. In CD_2Cl_2 all *N*-Me resonances were clearly distinguished, and this facilitated the sequencing of partial structures by HMBC. Other features in the ^1H NMR spectrum were a set of three doublets at δ 4.91, 5.05, and 5.16, which indicated the presence of three valine residues, and a triplet at δ 1.90 (J = 2.5 Hz) due to an acetylenic proton. The spin system for another amino acid (*N*-Me-Phe) was deduced from $^1H-^1H$ COSY, HMQC, and HMBC NMR experiments. The presence of five carbonyl carbons (δ 169.6, 170.7, 172.0, 171.2, and 176.8); three aromatic double bonds (δ 128.5, 128.6, 126.9, 136.7) associated with the *N*-Me-Phe amino acid; and a terminal acetylene accounted for all 11 degrees of unsaturation required by the molecular formula.

$^1H-^1H$ COSY and HMBC analysis detected the presence of the fatty acid 2-methyl-7-octynoic acid (Moya): the furthest downfield carbonyl carbon (δ 176.8, C-34) was assigned as an amide linkage to *N*-Me-Val-3 as a result of HMBC correlations from H-28, H-33, and H-44 to C-34. Finally, 16 mass units were unaccounted for, however, easily assigned as a terminal primary amino group. This was corroborated by LRFABMS data, which showed a fragment ion $[M - NH_2]^+$ (5%) at *m/z* 638.

The final sequence of all amino acids and fatty acid was deduced by FABMS as Moya-(*N*-Me-Val-3)-(*N*-Me-Val-2)-(*N*-Me-Val-1)-(*N*-Me-Phe)-NH_2 defining the structure of dragonamide (**7**): a sequential loss of NH_2 (−16), *N*-Me-Phe (−162), 3 × *N*-Me-Val (−114) corroborated the amino acid sequence established by NMR analysis.

Acid hydrolysis of **7** followed by chiral HPLC analysis established that the configuration of all amino acids was L. The only piece of information missing was the absolute configuration at C-35, which can be inferred from optical rotation data comparison to other 2-methylalkanoic acids.[15] Dragonamide (**7**) was hydrogenated over Pd/C followed by acid hydrolysis to liberate 2-methyloctanoic acid.[7] Measurement of the optical rotation ($CHCl_3$) provided a negative sign; thus the absolute configuration at C-35 is *R*.

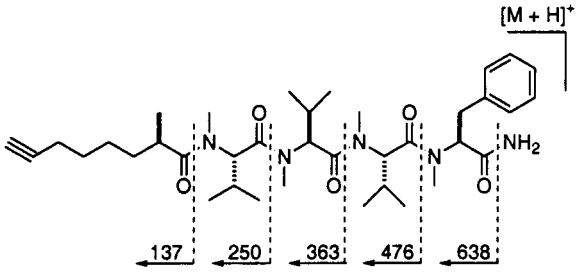


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Figure 1. FAB mass spectral fragmentation pattern of **7**.

The trivial name of dragonamide (**7**) has been coined after the collection site of the organism producing these metabolites. Pseudodysidenin (**2**) and dragonamide (**7**) exhibit cytotoxycity against P-388, A-549, HT-29, and MEL-28 ($IC_{50} > 1$ μg/mL).

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 400/500 and 100/125 MHz, respectively, using residual solvent signals as internal references. The HMQC experiments were optimized for $^1J_{CH} = 140$

Table 1. ^{1}H NMR Data for Dysidenin (**1**),[11] Pseudodysidenin (**2**), Dysidenamide (**3**), and Nordysidenin (**4**) in $CDCl_3$

no.	^{1}H (mult; J = Hz) **1**	^{1}H (mult; J = Hz)[a] **2**	^{1}H (mult; J = Hz)[b] **3**	^{1}H (mult; J = Hz)[b] **4**
1	1.36 (d; 7.0)	1.35 (d; 6.5)	1.37 (d; 6.8)	1.37 (d; 6.5)
2	3.30 (m)	3.20 (m)	3.19 (m)	3.21 (m)
3	2.50 (m)	2.34 (dd; 10, 15)	2.29 (dd; 10, 15)	2.32 (dd; 10, 15)
		3.07 (dd; 2.5, 15)	3.00 (dd; 3, 15)	3.05 (dd; 3.0, 15)
4				
5	5.27 (dd; 4, 11)	5.10 (ddd; 2.4, 9, 11)	5.06 (ddd; 2.4, 9, 11)	4.69 (dt; 2.1,11)
6	1.94 (m)	1.67 (m)	1.64 (ddd; 2.4, 11, 14)	1.84 (ddd; 3.3, 10, 13)
	3.10 (m)	2.35 (br t; 13)	2.31 (m)	2.39 (dt; 1, 13)
7	2.20 (m)	2.60 (m)	2.60 (m)	2.54 (m)
8	1.33 (d; 7)	1.48 (d; 6.3)	1.49 (d; 6.6)	1.41 (d; 6.5)
9				
10				
11				
12	5.20 (dq; 7, 8)	6.15 (q; 6.9)	5.11 (q; 7.3)	5.39 (dq; 7, 7)
13	1.56 (d; 7)	1.66 (d; 7)	1.38 (d; 6.8)	1.62 (d; 7)
14				
15	7.60 (d; 3.5)	7.73 (d; 3.3)		7.72 (d; 3.3)
16	7.26 (d; 3.5)	7.33 (d; 3.3)		7.29 (d; 3.3)
N_5-H		7.07 (d; 9)	6.74 (d; 9)	7.03 (br s)
N_5-Me	3.04 (s)			
N_{12}-Me		2.94 (s)	3.05 (s)	
N_{12}-H	6.86 (bd; 8)			5.53 (br s)

[a] Recorded at 400 MHz. [b] Recorded at 500 MHz.

Hz, and the HMBC experiments for $^nJ_{CH}$ = 7 Hz. High-resolution mass spectra were determined in the DCI and FAB modes. Optical rotations were measured on a Jasco DIP-700 instrument using CH_2Cl_2 or $CHCl_3$ at 20 °C at the sodium D line (589 nm).

Biological Material. Cyanobacterium 122998-BDD-1 was a *L. majuscula* strain collected in waters adjacent to Boca del Drago Beach (depth: 2−5 ft), Bocas del Toro, Panama, in December 1998. In life, the alga resembles *L. majuscula* observed in waters around the island of O'ahu, Hawaii, except for the reddish coloration of the Panamanian variety. The organisms was identified by Dr. G. M. L. Patterson.

Extraction and Isolation. The freeze-dried algae (290 g) were extracted with CH_2Cl_2−2-propanol (IPA) (3 × 1.0 L), filtered, and concentrated under reduced pressure to yield 6.1 g of crude extract. The crude extract was loaded on a Sephadex LH-20 column (33 × 5.0 cm) equilibrated in CH_2Cl_2. The column was eluted using a gradient profile as follows: (1) CH_2Cl_2 (1.0 L), (2) CH_2Cl_2−acetone (1:1, 2.0 L), (3) methanol (2.0 L). Ten major fractions (A−J) were collected and concentrated to dryness. Fractions A and B were combined and further separated by reversed-phase flash chromatography (YMC-ODS 120 Å, 33 × 3.0 cm) eluting with 80% aqueous MeCN. Several fractions were collected and pooled together according to their TLC behavior into seven major fractions (1−7). Reversed-phase HPLC (Phenomenex Ultracarb 30 ODS 5; 250 × 10 mm; 65% aqueous MeCN to 80% aqueous MeCN in 40 min at 2.5 mL/min and monitoring at 220 nm) of fraction 2 afforded carbamin A (18.3 mg), carbamin B (2.3 mg), dragonamide (12.9 mg), and dysidenamide (2.4 mg), and fraction 3 yielded pseudodysidenin (154 mg) and nordysidenin (2.6 mg).

Pseudodysidenin (2): white crystalline solid, 154 mg (0.053% dry wt); $[\alpha]_D$ −96.9° (*c* 0.032, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Tables 1 and 2, assignments were made by interpretation of COSY, HMQC, and HMBC data; HRDCIMS (NH_3) *m/z* obsd 543.97204 $[M + H]^+$ ($C_{17}H_{23}{}^{35}Cl_6N_3O_2S$, Δ −2.9 ppm).

Dysidenamide (3): colorless amorphous solid, 2.4 mg (0.00083% dry wt); $[\alpha]_D$ −40.3° (*c* 0.96, CH_2Cl_2); ^{1}H and ^{13}C NMR data, see Tables 1 and 2, assignments were made by interpretation of COSY, HMQC, and HMBC data; HRFABMS *m/z* obsd 525.976828 $[M + Na]^+$ ($C_{15}H_{23}{}^{35}Cl_6N_3O_3$ + Na, Δ −4.7 ppm).

Nordysidenin (4): colorless amorphous solid, 2.6 mg (0.0009% dry wt); $[\alpha]_D$ −56.7° (*c* 0.76, CH_2Cl_2); ^{1}H and ^{13}C NMR data, see Tables 1 and 2, assignments were made by interpretation of COSY, HMQC, and HMBC data; HRFABMS *m/z* obsd 529.95639 $[M + H]^+$ ($C_{16}H_{21}{}^{35}Cl_6N_3O_2S$, Δ 3.2 ppm).

Table 2. ^{13}C NMR Data for Dysidenin (**1**),[11] Pseudodysidenin (**2**), Dysidenamide (**3**), and Nordysidenin (**4**) in $CDCl_3$

no.	**1**[a]	**2**[b]	**3**[c]	**4**[c]
1	17.3*	16.8	16.8	16.9
2	51.4**	51.6	51.6	51.8
3	37.4§§	40.1	40.1	40.1
4	171.2+	170.5	170.5	170.8
5	54.0**	47.4	47.3	47.5
6	31.0§§	36.7	36.7	36.6
7	51.9**	51.6	51.7	51.6
8	16.2*	16.5	16.5	16.7
9	105.5§	105.9	105.2	105.3
10	105.1§	104.9	104.9	104.8
11	171.9+	171.4	172.0	171.1
12	47.3**	50.5	52.2	51.2
13	21.8	16.2	13.5	22.0
14	168.2+	169.3	172.4	170.1
15	142.3	143.0		142.4
16	118.9	119.9		119.2
N_5-H				
N_5-Me		29.8	30.6	
N_{12}-Me	30.8			
N_{12}-H				

[a] *, **, +, §, §§ assignments of these signals may be interchanged. [b] Recorded at 100 MHz. [c] Recorded at 125 MHz

Chiral HPLC of Hydrolysate of 2, 3, and 4. The dragonamides [A (**2**, 3.0 mg); B (**3**, 0.25 mg); C (**4**, 0.25 mg)] were dissolved in CH_2Cl_2 (5.0 mL) and cooled to −78 °C. Ozone was bubbled through the solution until a bluish coloration persisted. Solvent was removed under a stream of nitrogen and the residue hydrolyzed in 6 N HCl at 110 °C for 20 h. The product mixture was dried and analyzed by chiral HPLC, comparing the retention times with those of authentic standards [Phenomenex Chirex (D) Penicillamine, 4.6 × 250 mm); solvent 2.0 mM $CuSO_4$−MeCN (95:5); flow rate 0.6 mL/min; detection 245 nm]. The retention times (t_R, min) of the authentic amino acids were *N*-Me-L-Ala (11.0), *N*-Me-D-Ala (11.8), L-Ala (11.5), and D-Ala (12.0). The following residue coeluted with **2** and **3** hydrolysate peaks: *N*-Me-L-Ala, and L-Ala for **4**.

Dragonamide (7): colorless amorphous solid, 12.9 mg (0.0044% dry wt); $[\alpha]_D$ −260.8° (*c* 2.6, CH_2Cl_2); ^{1}H and ^{13}C NMR data, see Table 3, assignments were made by interpretation of COSY, HMQC, and HMBC data; HRFABMS *m/z* obsd 654.459446 $[M + H]^+$ ($C_{37}H_{59}N_5O_5$, Δ 0.4 ppm).

Chiral HPLC of Hydrolysate of 7. Dragonamide (**7**, 1.0 mg) was dissolved in 6 N HCl (0.5 mL) and the solution heated

Table 3. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for Dragonamide (**7**) in $CDCl_3$

unit	no.	^{13}C[a]	^{1}H (mult, J = Hz)[b]	COSY	HMBC
NH_2	1		6.04 (s)		
	2	172.0			
	3	56.3	5.55 (dd; 5.5, 11)	5	2, 5, 6, 12, 13
	5	33.3	3.22 (dd; 5.3, 15) 3.00 (dd; 11, 15)	3	5, 6, 7, 11
N-Me-Phe	6	136.7			
	7	128.5	7.13 (m)		6
	8	128.6	7.27 (m)		5, 7, 9, 11
	9	126.9	7.22 (m)		7, 8, 10, 11
	10	128.6	7.27 (m)		5, 7, 9, 11
	11	128.5	7.13 (m)		6
	12	30.6	2.90 (s)		3, 13
N-Me-Val-1	13	171.2			
	14	58.4	5.05 (d; 10.5)	16	13, 20, 19, 16,17,18
	16	27.0	2.26 (m)	14, 17, 18	
	17	19.9	0.85 (d; 6.4)	16	14, 16, 18
	18	17.3	0.63 (d; 6.6)	16	14, 16, 17
	19	29.6	2.39 (s)		14, 20
N-Me-Val-2	20	169.6			
	21	57.8	4.91 (d; 10.7)	23	20, 23, 24, 25, 27
	23	27.0	2.22 (m)	21, 24, 25	
	24	19.6	0.69 (d; 6.8)	23	21, 23, 25
	25	17.6	0.68 (d; 6.4)	23	21, 23, 24
	26	30.3	2.92 (s)		21, 27
N-Me-Val-3	27	170.7			
	28	57.8	5.16 (d; 10.7)	30	27, 30, 31, 32, 33, 34
	30	27.0	2.31 (m)	28, 31, 32	
	31	19.4	0.79 (d; 6.4)	30	28, 30, 32
	32	17.4	0.77 (d; 6.8)	30	28, 30, 31
	33	30.1	2.95 (s)		28, 34
Moya	34	176.8			
	35	36.1	2.67 (m)	36, 44	36
	36	33.5	1.66 (m)	35, 37	35
	37	26.7	1.31 (m)		
	38	26.7	1.31 (m)		
	39	26.7	1.31 (m)		
	40	28.3	1.45 (m)	41	
	41	18.2	2.12 (dt; 2.5, 7)	40, 43	42, 43
	42	84.1			
	43	68.3	1.90 (dt; 0.5, 2.5)	41	42
	44	17.6	1.08 (d; 6.6)	35	34, 35, 36

[a] Recorded at 125 MHz. [b] Recorded at 500 MHz.

at 110 °C for 20 h. The product mixture was dried and analyzed by chiral HPLC, comparing the retention times with those of authentic standards [Phenomenex Chirex (D) Penicillamine, 4.6 × 250 mm); solvent 2.0 mM $CuSO_4$−MeCN (95:5); flow rate 0.8 mL/min; detection 245 nm]. The retention times (t_R, min) of the authentic amino acids were *N*-Me-L-Val (13.0), *N*-Me-D-Val (17.8), *N*-Me-L-Phe (35.5), and *N*-Me-L-Val (38.8). The following residue coeluted with **7** hydrolysate peaks: *N*-Me-L-Val and *N*-Me-L-Phe.

Cytotoxicity Testing. Cytotoxicity assays were carried out by Instituto Biomar, S. A., Madrid, Spain.

Acknowledgment. We thank NSF, the Sea Grant College Program, Instituto Biomar, S. A., and PharmaMar, S. A., for financial support. The cyanobacterium was identified by Dr. Gregory M. L. Patterson, Department of Chemistry, University of Hawaii. We also thank Mr. Wesley Y. Yoshida for conducting NMR experiments and the University of California, Riverside, and University of Illinois, Urbana−Champaign, for mass spectral measurements.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for all new compounds are available free of charge via the Internet at http://pubs.acs.org.

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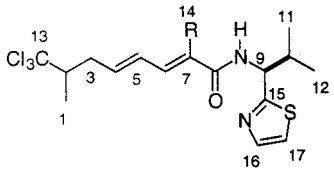
(6) An additional polychlorinated compound was also isolated from this cyanobacterium. Herbamide B (**9**) was isolated as a minor component and is similar in structure to **8**, which was isolated from *D. herbacea* (Clark, W. D.; Crews, P. *Tetrahedron Lett.* **1995**, *36*, 1185−1188). Unfortunately, it decomposed before any MS data could be obtained to confirm the structure assigned by NMR analysis. Standard amino acid analysis revealed L-valine. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30



(d; J = 6.5 Hz; H_3-1), 2.59 (m; H-2), 2.98 (m; H-3a), 2.22 (m; H-3b), 5.97 (ddd; J = 6, 9, and 15 Hz; H-4), 6.41 (dd; J = 11 and 15 Hz; H-5), 6.94 (d; J = 11 Hz; H-6), 5.32 (dd; J = 6 and 9 Hz; H-9), 2.35 (m; H-10), 0.98 (d; J = 7 Hz; H_3-11), 0.93 (d; J = 7; H_3-12), 2.01 (s; H_3-14), 7.74 (d; J = 3.3 Hz; H-16), 7.26 (d; J = 3.3 Hz; H-17), and 6.70 (d; J = 9 Hz; NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.2 (C-1), 54.7 (C-2), 36.8 (C-3), 137.0 (C-4), 128.4 (C-5), 133.5 (C-6), 129.1 (C-7), 168.4 (C-8), 56.1 (C-9), 33.8 (C-10), 18.2 (C-11), 19.1 (C-12), 105.3 (C-13), 13.0 (C-14), 170.1 (C-15), 142.3 (C-16), and 118.6 (C-17).

(7) In kulolide, the substituted octynoic acid moiety is unstable under the acidic conditions required for hydrolysis. Reese, M. T.; Gulavita, N. K.; Nakao, Y.; Hamann, M. T.; Yoshida, W. Y.; Coval, S. J.; Scheuer, P. J. *J. Am. Chem. Soc.* **1996**, *118*, 11081−11084.

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NP000462Q