Yanucamides A and B, Two New Depsipeptides from an Assemblage of the Marine Cyanobacteria *Lyngbya majuscula* and *Schizothrix* Species

Namthip Sitachitta, R. Thomas Williamson, and William H. Gerwick*

College of Pharmacy, Oregon State University, Corvallis, Oregon 97331

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Yanucamides A (**1**) and B (**2**) were isolated from the lipid extract of a *Lyngbya majuscula* and *Schizothrix* sp. assemblage collected at Yanuca Island, Fiji. The structures of compounds **1** and **2** were determined by spectroscopic methods. Both compounds contain a unique 2,2-dimethyl-3-hydroxy-7-octynoic acid, which has previously been described only as a component of kulolide-1 (**3**) and kulokainalide-1 (**4**), metabolites from the marine mollusk *Philinopsis speciosa*. Thus, the isolation of the yanucamides from this cyanobacterial assemblage supports the hypothesis that the kulolides and related metabolites are of cyanobacterial origin.

The marine cyanobacterium (blue-green algae) *Lyngbya majuscula* Gomont (Oscillatoriaceae) is a prolific source of chemically diverse classes of bioactive secondary metabolites. For example, a single extract of *L. majuscula*, collected in Curaçao gave rise to the antimitotic metabolite curacin A;[1] the ichthyotoxins antillatoxin[2] and malyngamide H,[3] the molluscicidal agent barbamide,[4] and the structurally unique linear peptides carmabins A and B.[5] For this reason, we have undertaken the further chemical investigation of *L. majuscula* extracts collected from various locations around the world. Herein, we report the isolation and structure elucidation of two new depsipeptides, yanucamides A (**1**) and B (**2**), from the lipid extract of a mixed assemblage of *L. majuscula* and *Schizothrix* species.

Results and Discussion

The assemblage of cyanobacteria was collected from the north of Yanuca Island, Fiji, and kept cold in 2-propanol until extracted. A portion of the organic extract (800 mg) was subjected to Si gel vacuum liquid chromatography (VLC) using an increasing gradient of EtOAc in hexanes. Purification of a fraction containing the yanucamides utilized C_{18} VLC using a stepwise gradient elution from 60% MeOH in H_2O to 100% MeOH. A yanucamide-containing fraction was subjected to a final purification over reversed-phase HPLC (ODS) to afford compounds **1** and **2**.

Yanucamide A (**1**) was assigned a molecular composition of $C_{33}H_{48}N_3O_7$ by HRFABMS data. The IR spectrum of **1** displayed strong absorption bands at 1732 and 1661 cm^{-1}, indicating the presence of ester and amide functionalities. Of the 12 degrees of unsaturation implied by the molecular formula, nine were defined from ^{13}C NMR and DEPT spectral data as five amide/ester carbonyls and a monosubstituted phenyl group.

Partial structures **1a**−**1d** (Figure 1) of **1** were constructed as valine (Val), *N*-methylphenylalanine (*N*-Me-Phe), *β*-alanine (*β*-Ala), and 2-hydroxyisovaleric acid (Hiv), respectively, using 1D and 2D NMR data (Table 1). The H3−H6 spin system of partial structure **1e** was deduced from the COSY spectrum. Connection between H6 and H8 was equivocal due to the overlapping nature of their proton

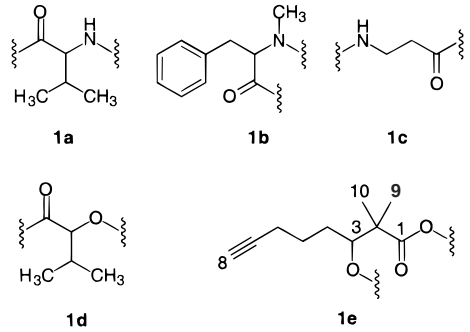

Figure 1. Partial structures **1a**−**1e** of yanucamide A (**1**).

signals (*δ* 2.22). Although DEPT 135 data indicated that C8 (*δ* 70.2) was a methine carbon, it showed no correlation to its corresponding proton in the HMQC spectrum. Instead, an intense one-bond C−H satellite to C8 with a large $^1J_{CH}$ coupling of 243 Hz was observed in the HMBC spectrum, characteristic of an alkyne functionality. The combination of this large C−H coupling value taken together with the ^{13}C NMR chemical shift of C-7 (*δ* 84.5) placed a triple bond next to the C-6 methylene carbon. Geminal dimethyl protons ($H_3$9 and $H_3$10) showed HMBC correlations to a carbonyl carbon at *δ* 179.7 (C-1), a quaternary carbon at *δ* 47.0 (C-2), and an oxygenated tertiary carbon at *δ* 78.7 (C-3), supportive of partial structure **1e** as 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya). This residue was previously reported as part of the kulolide-1 (**3**) and kulokainalide-1 (**4**) structures and fulfilled two of three remaining degrees of unsaturation (Figure 2).

Correlations observed in the HMBC spectrum of **1** from the α-CH's to the neighboring carbonyl carbons were used to deduce a sequence of Dhoya/Val/Hiv. The correlation shown from *N*-CH_3 of **1b** to the carbonyl carbon of **1d** linked partial structure **1b** to the Hiv end of the depsipeptide sequence. Three-bond coupling from H3 of Dhoya to the carbonyl carbon of *β*-Ala completed the planar and cyclic structure of yanucamide A (**1**), thus accounting for the remaining unassigned degree of unsaturation. Acid hydrolysis of **1** followed by Marfey analysis revealed the L-configuration of valine and D-configuration of *N*-methylphenylalanine.[6] The absolute configuration of the L-Hiv residue was determined by analysis of the corresponding methyl ester derivative of L-Hiv following hydrolysis of **1**

* To whom correspondence should be addressed. Tel.: (541) 737-5801. Fax: (541) 737-3999. E-mail: bill.gerwick@orst.edu.

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Table 1. NMR Data for Yanucamide A at 150 MHz (^{13}C) and 600 MHz (^{1}H) in MeOH-d_4

unit	C no.	^{13}C	DEPT	^{1}H mult (J = Hz)	HMBC[a]
Dhoya	1	179.7	C		
	2	47.0	C		
	3	78.7	CH	5.25 dd (10.7, 2.20)	C: 1, 2, 9, 10, 31
	4a	29.6	CH_2	1.56 m	C: 3, 5, 6
	4b			1.76 m	C: 5, 6
	5	26.6	CH_2	1.43 m	C: 3, 4, 6, 7
	6	18.7	CH_2	2.22 m	C: 4, 5, 7, 8
	7	84.5	C		
	8	70.2	CH	2.22 m	C: 6, 7
	9	17.1	CH_3	1.32 s	C: 1, 2, 3, 10
	10	25.1	CH_3	1.11 s	C: 1, 3, 9
L-Val	11	174.3	C		
	12	60.5	CH	3.88 d (6.8)	C: 1, 11, 13, 14, 15
	13	30.4	CH	2.14 m	C: 11, 12, 14, 15
	14	19.4	CH_3	1.05 d (6.8)	C: 12, 13, 14
	15	19.9	CH_3	1.06 d (6.9)	C: 12, 13, 15
L-Hiv	16	172.1	C		
	17	76.6	CH	4.94 d (3.5)	C: 11, 16, 18, 19, 20
	18	30.2	CH	0.71 m	C: 19, 20
	19	17.0	CH_3	0.66 d (6.2)	C: 17, 18, 20
	20	19.8	CH_3	0.77 d (6.5)	C: 17, 18, 19
D-*N*-Me Phe	21	171.6	C		
	22	64.1	CH	4.74 dd (9.7, 4.7)	C: 21, 23, 24, 30
	23a	36.2	CH_2	3.30 dd (14.0, 4.7)	C: 21, 24, 25, 29
	23b			3.03 dd (14.0, 9.7)	C: 21, 24, 25, 29
	24	139.2	C		
	25, 29	130.6	CH	7.22 m	C: 23, 27
	26, 28	129.9	CH	7.30 m	C: 24
	27	128.1	CH	7.25 m	
	30	30.6	CH_3	2.90 s	C: 16, 22
β-Ala	31	173.2	C		
	32a	33.4	CH_2	2.59 ddd (18.8, 11.3, 3.7)	C: 31, 33
	32b			2.79 ddd (18.8, 3.9, 2.3)	C: 31
	33	36.5	CH_2	3.32 m	C: 21, 31, 32

[a] HMBC optimized for 8 Hz coupling.

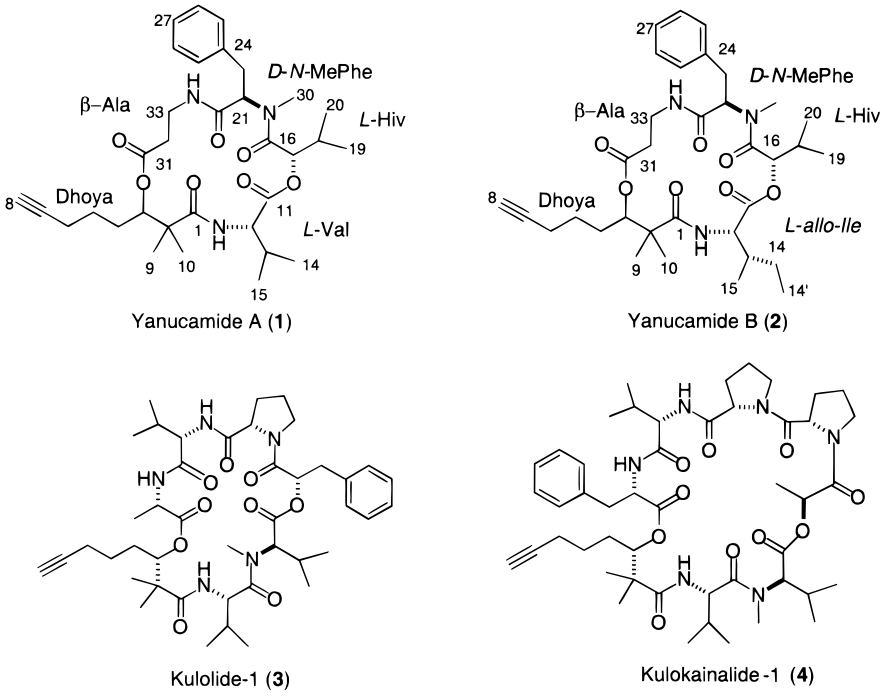

Figure 2. Structures of yanucamides A (**1**) and B (**2**), kulolide-1 (**3**), and kulokainalide-1 (**4**).

using chiral GC−MS under optimized conditions and in comparison with standards. Due to the limited supply of **1**, the absolute configuration of the Dhoya unit was not determined.

The HRFABMS of yanucamide B (**2**) established its molecular formula as $C_{34}H_{50}N_3O_7$, one methylene unit more than that of yanucamide A (**1**). Further confirmation of this relationship between **1** and **2** was provided by the ^{1}H NMR spectrum of **2**, which was similar to that of **1**, except for two new signals at δ 1.40 (H14a) and 1.64 (H14b). These two new resonances were assigned to a methylene carbon (δ 27.1) based on HSQC data. Analysis of 1D and 2D NMR data of **2** indicated that the additional methylene carbon belonged to an isoleucine (Ile) residue that replaced L-Val

Table 2. NMR Data for Yanucamide B at 150 MHz (^{13}C) and 600 MHz (^{1}H) in MeOH-d_4

unit	C no.	^{13}C	DEPT	^{1}H mult (J = Hz)	HMBC[a]
Dhoya	1	179.6	C		
	2	47.1	C		
	3	79.4	CH	5.28 brd d (10.7)	C: 1, 2, 9, 10, 31
	4a	29.2	CH_2	1.64 m	
	4b			1.79 m	
	5	26.2	CH_2	1.46 m	C: 6, 7
	6	18.7	CH_2	2.22 m	C: 4, 5, 7, 8
	7	84.5	C		
	8	70.2	CH	2.22 m	C: 6, 7
	9	17.1	CH_3	1.33 s	C: 1, 2, 3, 10
	10	24.7	CH_3	1.12 s	C: 1, 2, 3, 9
L-*allo*-Ile	11	174.0	C		
	12	58.7	CH	4.04 d (6.0)	C: 1, 11, 14, 15
	13	36.8	CH	1.91 m	C: 11, 14
	14a	27.1	CH_2	1.40 m	C: 13, 14′, 15
	14b			1.64 m	C: 13, 14′, 15
	14′	11.6	CH_3	0.95 t (7.3)	C: 13, 14
	15	15.9	CH_3	1.08 d (6.8)	C: 12, 14
L-Hiv	16	172.0	C		
	17	76.5	CH	4.95 d (2.0)	C: 11, 16, 19, 20
	18	30.2	CH	0.67 m	
	19	17.0	CH_3	0.64 d (4.5)	C: 17, 18, 20
	20	19.8	CH_3	0.78 d (5.8)	C: 17, 18, 19
D-*N*-Me Phe	21	171.6	C		
	22	63.8	CH	4.75 dd (9.5, 4.6)	C: 21, 23
	23a	35.8	CH_2	3.03 dd (13.8, 9.5)	C: 21, 22, 24, 25, 29
	23b			3.44 dd (13.8, 4.6)	C: 21, 22, 24, 25, 29
	24	139.2	C		
	25, 29	130.6	CH	7.24 m	C: 23
	26, 28	129.9	CH	7.32 m	C: 24
	27	128.1	CH	7.27 m	
	30	30.1	CH_3	2.97 s	C: 16, 22
β-Ala	31	173.2	C		
	32a	33.0	CH_2	2.59 brd dd (18.5, 3.9)	C: 31
	32b			2.81 ddd (18.5, 12.2, 2.8)	C: 31
	33	36.2	CH_2	3.32 m	C: 21, 31, 32

[a] HMBC optimized for 8 Hz coupling.

in **1**. The absolute stereoconfigurations of L-*allo*-isoleucine and D-*N*-MePhe in **2** were determined by Marfey analysis, whereas the L-configuration of the Hiv residue was again established based on GC−MS analysis of the corresponding methyl ester derivative. The Dhoya stereochemistry was not determined.

Both yanucamides A (**1**) and B (**2**) exhibited strong brine shrimp toxicity (LD_{50}, 5 ppm). Interestingly, the Dhoya unit has previously been found only in kulolide-1 (**3**) and kulokainalide-1 (**4**), metabolites isolated from the marine mollusk *Philinopsis speciosa*.[7,8] A study by Scheuer and coworkers has shown that *P. speciosa* preys on the herbivorous sea hare *Stylocheilus longicaudus*, an organism well-known to sequester secondary metabolites from its diet of mat-forming cyanobacteria.[9] Thus, the discovery of the yanucamides from a field-collected marine cyanobacterium substantiates the hypothesis that marine cyanobacteria are the probable source of the kulolides and their related metabolites. Moreover, based on this reasoning, we speculate that the unassigned stereocenter in yanucamides A and B is the same (3*S*) as in kulolide-1 (**3**).

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker DRX600 spectrometer operating at a proton frequency of 600.01 MHz and a carbon frequency of 150.90 MHz, with the solvent used as an internal standard (MeOH-d_4 at δ 4.87 for ^{1}H and δ 48.15 for ^{13}C). Mass spectra were recorded on a Kratos MS50TC mass spectrometer. GC−MS data were obtained on a Hewlett-Packard 5890 Series II GC connected to a Hewlett-Packard 5971 mass spectrometer. Ultraviolet (UV) spectra were recorded on Hewlett-Packard 8452A UV−vis spectrometer. Infrared (IR) spectra were run as neat films with a Nicolet 510 Fourier transform IR spectrophotometer. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter. HPLC separations were performed with a Waters M-6000A pump, a Rheodyne 7010 injector, and a Waters Lambda-Max 480 UV detector. Merck aluminum-backed TLC sheets (Si gel F_{254}) were used for TLC. VLC was performed with Merck Si gel G for TLC or with Baker Bonded Phase−octadecyl (C_{18}). All solvents were distilled from glass prior to use.

Collection. The mixed assemblage of marine cyanobacteria *Schizothrix* sp. and *Lyngbya majuscula* (voucher specimen available from WHG as collection number VYI-5 Feb 97-1) was collected from shallow water (2−3 m) at Yanuca Island, Fiji, in February 1997, and stored in 2-propanol at reduced temperature until workup.

Isolation and Purification. Approximately 1 L wet wt of preserved alga was extracted with CH_2Cl_2−MeOH (2:1) twice to give 1.29 g of crude organic extract and 67.8 g dry wt of extracted algal material. A portion of the organic extract (800 mg) was subjected to Si gel VLC with a stepped gradient elution from 100% hexanes to 100% EtOAc to 50% MeOH in EtOAc, giving five distinctive fractions. Fraction 4 was purified by reversed-phase (C_{18}) VLC followed by ODS HPLC [Phenomenex Spherisorb ODS (2), MeOH−H_2O (3:1), flow rate 3 mL/min, detection at 215 nm, t_R, 23 min for **1** and 30 min for **2**] to yield yanucamide A (**1**, 7.5 mg) and yanucamide B (**2**, 5.5 mg).

Yanucamide A (1): colorless amorphous solid; $[\alpha]^{20}_D$ −33° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ϵ) 204 nm (13 000); IR (KBr) 3400 (br), 3315, 2963, 1732, 1661, 1379, 1174 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; FABMS *m*/*z* 599 $[M + 1]^+$ (64), 507 (8), 460 (9), 356 (19), 329 (12), 307 (18), 244 (64), 154 (100), 135 (76), 120 (15), 107 (25), 89 (24), 77 (27), 69 (22), 55 (30); HRFABMS *m*/*z* 598.3491 (calcd for $C_{33}H_{48}N_3O_7$, 598.3489).

Yanucamide B (2): colorless amorphous solid; $[\alpha]^{20}_D$ −31° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (ϵ) 204 nm (13 000); IR (KBr) 3356 (br), 3307, 2964, 2926, 2875, 1732, 1660, 1467, 1453, 1412, 1380, 1196 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; FABMS *m*/*z* 613 $[M + 1]^+$ (16), 612 (25), 585 (19), 401 (14), 369 (20),

244 (100), 134 (47), 91 (16), 86 (26), 73 (9), 69 (22), 55 (19); HRFABMS *m*/*z* 612.3651 (calcd for $C_{34}H_{50}N_3O_7$, 612.3653).

Acid Hydrolysis and Marfey Analysis of Yanucamides A and B. Yanucamides A (**1**, 1 mg) and B (**2**, 1 mg) were separately hydrolyzed with 6 N HCl, at 105 °C for 12 h. A portion of the hydrolysate was extracted with EtOAc, and the aqueous layer was added to 50 μL of 0.1% 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide solution in acetone and 100 μL of 0.1 N $NaHCO_3$, followed by heating at 80 °C for 5 min. After cooling to room temperature, the reaction mixture was neutralized with 50 μL of 0.2 N HCl and diluted with CH_3CN-H_2O-TFA (50:50:0.05). The solution was analyzed by reversed-phase HPLC [MICROSORB-MV (Rainin), C_{18}, UV detection at 340 nm] using a CH_3CN in H_2O linear gradient (20−50% CH_3CN over 30 min and 50% CH_3CN for 10 additional min). The retention time (t_R, min) of the derivatized amino acids in the hydrolysate of **1** matched those of L-Val (17.4; D-Val, 21.7) and D-*N*-MePhe (23.3; L-*N*-MePhe, 22.1). The retention time of derivatized amino acids in the hydrolysate of **2** matched those of L-*allo*-Ile (20.9; D-*allo*-Ile, 26.8; L-Ile, 23.7; D-Ile, 27.0) and D-*N*-MePhe (23.3, L-*N*-MePhe, 22.1).

Absolute Stereochemistry of 2-Hydroxyisovaleric Acid (2-Hiv) by Chiral GC−MS. Excess HCl from a portion of the 6 N HCl hydrolysate of **1** was removed under a stream of dry N_2. The hydrolysate was diluted in 0.5 mL of diethyl ether and treated with diazomethane for 10 min. Excess CH_2N_2 was removed with a stream of N_2. Capillary GC−MS analyses were carried out using a Chiralsil-Val column (Alltech, 25 m × 0.25 mm). The following conditions were used for GC: a 11 psi initial head pressure and a column temperature held at 40 °C for 10 min after injection of the sample, then increased from 40 °C to 100 °C at a rate of 3 °C/min, then from 100 °C to 150 °C at a rate of 15 °C/min, and finally held at 150 °C for 5 min. The retention time found for the yanucamide A-derived 2-Hiv was found at 7.7 min. Standards of D- and L-Hiv were also converted to the corresponding methyl derivatives by the same procedure (D-Hiv, 8.4 min; L-Hiv, 7.7 min).

Brine Shrimp Toxicity Assay. In a method slightly modified from the original description,[10] about 15 newly hatched brine shrimp (*Artemia salina*) in ca. 0.5 mL artificial seawater were added to each well in a 140-well plate containing different concentrations of the sample in 50 μL EtOH and 4.5 mL artificial seawater to make a total volume of ca. 5 mL. Samples and controls were run in duplicate. After 24 h at 28 °C, the brine shrimp were observed, and the number dead and alive were counted with a dissecting light microscope to generate LD_{50} values.

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Supporting Information Available: Spectra of yanucamides A and B. This material is available free of charge via the Internet at http://pubs.acs.org.

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