Somamides A and B, Two New Depsipeptide Analogues of Dolastatin 13 from a Fijian Cyanobacterial Assemblage of *Lyngbya majuscula* and *Schizothrix* Species

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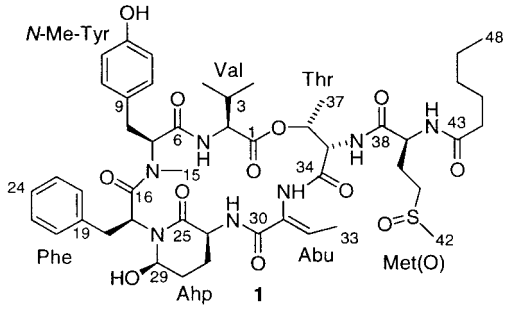
Somamides A (**1**) and B (**2**) were isolated from assemblages of the marine cyanobacteria *Lyngbya majuscula* and *Schizothrix* sp. from the Fijian Islands. These new depsipeptides are analogous in structure to the cyanobacterial metabolite symplostatin 2 (**4**) as well as dolastatin 13 (**3**), originally isolated from *Dolabella auricularia*, further demonstrating the cyanobacterial origin of the dolastatins.

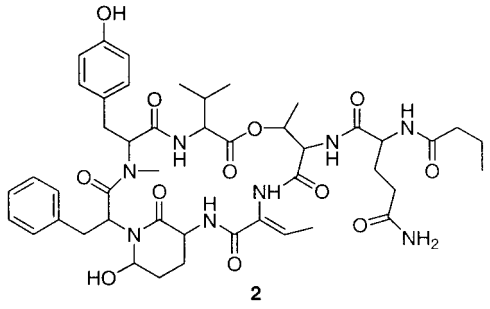
Marine cyanobacteria are known for their ability to produce a great diversity of unique secondary metabolites, especially lipopeptides which incorporate modified amino acid moieties, such as the majusculamides,[1] lyngbyastatins,[2] and hormothamnins.[3] Similarly, the dolastatins are an intriguing group of polypeptides, frequently containing ketide extended residues, which possess exceptional cytostatic and antineoplastic activity.[4] Although originally isolated in minute quantities from the herbivorous mollusc *Dolabella auricularia*, it has become evident that such metabolites truly originate in cyanobacteria.[5] Examination of the lipid extracts from mixed assemblages of the marine cyanobacteria *Lyngbya majuscula* and *Schizothrix* species, collected in the Fijian Islands, led to the discovery of two new depsipeptides (**1**, **2**) with strong structural homology to dolastatin 13 (**3**).[6] Herein we report the isolation and structure elucidation of somamides A (**1**) and B (**2**).

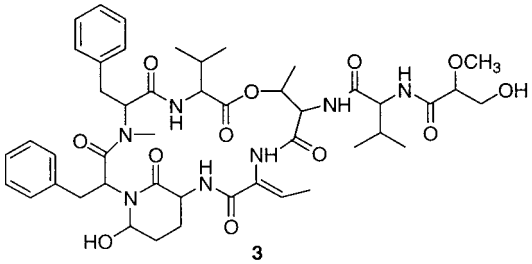
Results and Discussion

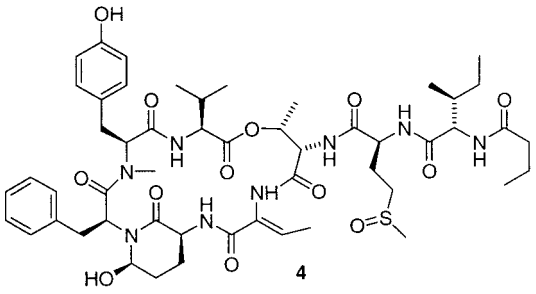
Fractionation of a crude organic extract from a Somo Somo, Fiji, collection of *L. majuscula* and *Schizothrix* sp., followed by C_{18} solid-phase extraction (SPE) and reversed-phase HPLC afforded somamide A (**1**) as a clear oil. The IR spectrum of **1** displayed strong absorption bands at 1730 and 1641 cm^{-1}, indicating the presence of ester and amide functionalities. The molecular composition of **1** was determined as $C_{48}H_{67}N_7O_{12}S$ by HRFABMS (*m*/*z* 948.4587 for $[M + H - H_2O]^+$), and ^1H and ^{13}C NMR spectra were indicative of a depsipeptide (Table 1).

Analysis of HSQC, COSY, HSQC-TOCSY, and HMBC spectra allowed for the construction of eight partial structures which accounted for all of the atoms in **1**. Four standard amino acid residues were deduced as phenylalanine, *N*-methyltyrosine, threonine, and valine. The presence of the more unusual moieties, 2-amino-2-butenoic acid (Abu) and 3-amino-6-hydroxy-2-piperidone (Ahp), were also deduced by 2D NMR and suggested the close relationship of **1** to the previously isolated cyanobacterial metabolite symplostatin 2 (**4**)[7] as well as the depsipeptide dolastatin 13 (**3**).[6] The two remaining partial structures were deduced as hexanoic acid and a methionine sulfoxide, observed as a 1:1 mixture of *R* and *S* sulfoxide diastereomers (Table 1). It is possible that this diastereomeric mixture formed during the isolation process from a naturally occurring methionine residue.









Correlations observed in the HMBC spectrum of **1** allowed for the formation of the partial sequences Val-*N*-Me-Tyr-Phe-Ahp and Thr-Met(O)-hexanoic acid. A ROESY correlation observed between the Abu N_5-H and H_{35} linked

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Table 1. NMR Spectral Data for Somamide A (**1**) in DMSO-d_6

unit	no.	^{1}H (*J* in Hz)	^{13}C	HSQC-TOCSY[a]	HMBC[b]
Val	1		172.5, s		
	2	4.74, d (11.3)	55.5, d	3, N_1	6
	3	2.09, m	30.5, d	2, 4, 5	1, 4, 5
	4	0.75, d (6.9)	16.9, q	3, 5	2, 3, 5
	5	0.88, dd (6.9, 2.3)	18.9, q	3, 4	2, 3, 4
	N_1	7.47, brs			
N-Me-Tyr	6		169.2, s		
	7	4.91, d (11.7)	60.4, d	8	6, 8
	8	3.08, 2.70	32.5, t	7	7, 9, 10/14
	9		127.0, s		
	10/14	6.99, d (8.4)	130.1, d	11/13	8, 11/13, 12
	11/13	6.76, d (8.3)	114.9, d	10/14	9, 12
	12		155.8, s		
	15	2.76, s	30.0, q		7
Phe	16		170.0, s		
	17	4.74, d (11.3)	49.8, d	18	16, 18, 19, 25, 29
	18	2.78, 1.81	34.9, t	17	17, 19, 20/24
	19		136.3, s		
	20/24	6.84, d (7.3)	129.0, d	21/23	18, 21/23, 22
	21/23	7.14, m	127.4, d	20/24, 22	19, 20/24
	22	7.18, m	125.8, d	21/23	20/24
Ahp	25		168.9, s		
	26	3.79, m	47.8, d	27, 28, N_4	
	27	2.41, 1.57	21.5, t	26, 28, N_4	
	28	1.70, 1.57	28.9, t	27, 29	
	29	5.07, brs	73.2, d	28, OH	25
	N_4	7.19			
	OH	6.08, brs			
Abu	30		162.1, s		
	31		129.9, s		
	32	6.53, q (7.1)	131.7, d	33	30, 31, 33
	33	1.50, d (7.1)	12.8, q	32	30, 31, 32
	N_5	9.21, brs			
Thr	34		173.4, s		
	35	4.53, m	55.5, d	36, N_6	
	36	5.52, brs	71.3, d	35, 37	
	37	1.25, d (6.6)	17.5, q	36	35, 36
	N_6	7.89			
Met(O)	38		172.1, s		
	39	4.62, m	51.7, 50.0,[c] d	40, 41, N_7	38
	40	1.95, 1.90, m	24.4, 24.1,[c] t	41	39, 41
	41	2.79, 2.70, m	49.3, 48.9,[c] t	39, 40	39
	42	2.53, 2.51,[c] s	37.7, q		41
	N_7	8.14, brs			
hexanoic acid	43		174.4, s		
	44	2.14, m	34.6, t	45, 46/47, 48	43, 45, 46
	45	1.50	24.5, t	44, 46/47, 48	43, 44, 46, 47
	46	1.28	30.5, t	45, 48	
	47	1.28	21.5, t	48	
	48	0.86, t (7.0)	13.5, q	46/47	47

[a] Proton showing TOCSY correlation to indicated proton. [b] Proton showing HMBC correlation to indicated carbon. [c] Indicates the presence of multiple conformers.

this moiety to the Thr residue. However, FABMS fragmentation was instrumental in completion of the planar structure of somamide A. A peak at *m*/*z* 503, corresponding to the fragment *N*-Me-Tyr-Phe-Ahp-Abu, and a peak at *m*/*z* 295 for the fragment Thr-Abu-Ahp were both observed, thereby placing the Abu unit between Thr and Ahp. Fragment ions at *m*/*z* 420 and 348, corresponding to *N*-Me-Tyr-Phe-Ahp and Thr-Met(O)-hexanoic acid, respectively, were also detected and further supported the HMBC sequence assignments. The final degree of unsaturation of **1** required a connection between the Val carbonyl (C_1) and the Thr hydroxyl (C_{36}−OH), thereby completing the planar structure of somamide A.

The absolute stereochemistries of the residues in somamide A were determined by Marfey's analysis[8] and indicated that the Val, *N*-Me-Tyr, Phe, Thr, and Met (analyzed as the sulfoxide) were all of *L* configuration. The absolute configuration of C_{26} (Ahp) could not be determined by Marfey's analysis despite several attempts to isolate the corresponding Glu residue following oxidation and acid hydrolysis of **1**.[7] However, proton and carbon chemical shifts for the Ahp unit of **1** were nearly identical to those observed in symplostatin 2 (**4**). In addition, ROESY correlations observed throughout the Ahp ring and between H_{17} (Phe) and H_{29} (Ahp) supported a 26*S*,29*R* configuration, analogous to the stereochemical assignments in symplostatin 2 (**4**).[7] Hence, in summary somamide A (**1**) was determined to possess 2*S*,7*S*,17*S*,26*S*,29*R*,35*S*,36*R*,39*S* stereochemistry.

The N_5-H of the Abu residue did not show a ROESY correlation to either the C_{32} methine or the C_{33} methyl protons, preventing determination of the double bond geometry by homonuclear dipolar coupling. However, this was circumvented by application of a 1D HSQMBC experiment from which a coupling constant of 3.5 Hz between the methine proton, H_{32} (6.53), and C_{30} (162.1) was measured, thus establishing the geometry as *Z*.[9]

A second organic extract from a similar *L. majuscula*/*Schizothrix* sp. cyanobacterial assemblage, collected near Taveuni Island, Fiji, was fractionated over LH-20 and

Table 2. NMR Spectral Data for Somamide B (**2**) in DMSO-d_6

unit	no.	^{1}H (*J* in Hz)	^{13}C	HSQC-TOCSY[a]	HMBC[b]
Val	1		174.9, s		
	2	4.71, d (11.6)	55.7, d	N_1	6
	3	2.06	30.7, d	4, 5	1, 5
	4	0.75, d (6.8)	17.1, q	5	2, 3, 5
	5	0.86	18.9, q	3, 4	2, 3, 4
	N_1	7.44, brs			
N-Me-Tyr	6		169.4, s		
	7	4.89, d (11.3)	60.4, d	8	6
	8	3.08, 2.70	32.5, t	7	7, 9, 10/14
	9		127.3, s		
	10/14	6.97, d (8.3)	130.1, d	11/13	8, 11/13, 12
	11/13	6.75, d (8.3)	115.0, d	10/14	9, 12
	12		156.0, s		
	15	2.75, s	30.1, q		7, 16
Phe	16		170.3, s		
	17	4.73, d (11.3)	49.9, d	18	16, 25, 29
	18	2.86, 1.81	35.0, t	17	17, 19, 20/24
	19		136.6, s		
	20/24	6.83, d (7.2)	129.0, d	21/23	18, 22
	21/23	7.17, m	127.5, d	20/24, 22	19, 20/24
	22	7.13, m	125.9, d	21/23	20/24
Ahp	25		168.8, s		
	26	3.78, m	47.9, d	27, N_4	
	27	2.39, 1.54	21.7, t	26, 28	
	28	1.68, 1.54	28.9, t	27, 29	
	29	5.07, brs	73.5, d		
	N_4	7.11			
	OH	6.06, brs			
Abu	30		162.5, s		
	31		129.9, s		
	32	6.51, q (7.1)	131.5, d	33	30, 31, 33
	33	1.49, d (7.1)	12.8, q	32	31, 32
	N_5	9.18, brs			
Thr	34		175.6, s		
	35	4.54, m	55.5, d	36, N_6	34
	36	5.42, brs	71.4, d	37	
	37	1.22, d (6.2)	17.9, q	36	35, 36
	N_6	7.88			
Gln	38		171.3, s		
	39	4.38, m	51.9, d	40, 41, N_7	43
	40	1.94, 1.76	22.4, t	39, 41	38, 39, 42
	41	2.11	31.2, t	40	39, 42
	42		173.6, s		
	N_7	8.04, brs			
butanoic acid	43		172.3, s		
	44	2.11, m	36.8, t	45, 46	43, 45, 46
	45	1.52, m	18.4, t	44, 46	43, 44, 46
	46	0.85	13.4, q	45	44, 45

[a] Proton showing TOCSY correlation to indicated proton. [b] Proton showing HMBC correlation to indicated carbon.

further purified by C_{18} SPE and reversed-phase HPLC to yield a small quantity (<0.5 mg) of somamide B (**2**). The molecular formula of **2** was determined as $C_{46}H_{62}N_8O_{12}$ by HRFABMS (*m*/*z* 901.4473 for $[M + H - H_2O]^+$). Extensive analysis of 2D NMR spectra again allowed deduction of eight partial structures corresponding to Gln, Phe, *N*-Me-Tyr, Val, Thr, Ahp, Abu, and butanoic acid (Table 2). Correlations observed in the HMBC allowed for the formation of a Val-*N*-Me-Tyr-Phe-Ahp sequence and a Gln-butanoic acid sequence. ROESY correlations observed between the Val N_1-H and *N*-Me-Tyr H_7, the *N*-Me-Tyr H_7 and Phe H_{17}, and the Phe H_{17} and Ahp H_{29} confirmed this partial sequence. Likewise, ROESY correlations observed between the Ahp N_4-H and Abu N_5-H, the Abu N_5-H and Thr H_{35}, and the Thr N_6-H and Gln H_{39} provided the sequence Val-*N*-Me-Tyr-Phe-Ahp-Abu-Thr-Gln-butanoic acid. Therefore, the ring closure of Val-Thr once again satisfied the molecular formula and completed the planar structure of somamide B (**2**). A ROESY correlation was observed between the Abu N_5-H and the C_{33} methyl protons, indicating a *Z* geometry for the double bond in this residue. Due to the minute quantity of somamide B, Marfey's analysis to determine absolute stereochemistry was not performed.

Somamides A (**1**) and B (**2**) demonstrate the extensive biosynthetic capabilities of marine cyanobacteria to produce secondary metabolites of novel structure, in particular, products of the nonribosomal peptide synthetase pathway (NRPS). Similar metabolites (e.g., dolastatin 13 (**3**))[6] have previously been reported from marine invertebrates, including the sea hare, *D. auricularia*, well known for its ability to sequester compounds acquired from its diet of cyanobacteria.[5] Thus, isolation of these depsipeptides provides further evidence that such metabolites are truly of cyanobacterial origin.

Experimental Section

General Experimental Procedures. All NMR spectral data 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 (DMSO-d_6 at δ 2.49 and 39.51). Mass spectra were recorded on a Kratos MS50TC mass spectrometer. IR spectra were recorded on a Nicolet 510 Fourier transform IR spectro-

photometer, and optical rotations were measured on a Perkin-Elmer 141 polarimeter. HPLC isolation of compounds **1** and **2** was performed using a Waters Millipore Lambda-Max model 480 LC spectrophotometer with a Waters Millipore model 590 pump.

Collection. The mixed assemblages of *Lyngbya majuscula* and *Schizothrix* sp. marine cyanobacteria (voucher specimens available from WHG as collection numbers VSO-8 Feb 97-2 and VTI-9 Feb 97-2) were collected near Somo Somo, Fiji (3−6 m depth), and Taveuni Island, Fiji (12−18 m depth), on February 8 and 9, 1997. The material was stored in 2-propanol at reduced temperature until workup.

Extraction and Isolation. Approximately 43 g (dry wt) of the algal material from Somo Somo was repetitively extracted with CH_2Cl_2/MeOH (2:1) to yield 1.3 g of crude extract. The extract was fractionated by VLC over Si gel. Fractions eluting between 90% EtOAc in hexanes and 5% MeOH in EtOAc were further purified, first by C_{18} SPE cartridge (MeOH/H_2O, 7:3) followed by reversed-phase HPLC (MeOH/H_2O, 3:1, Phenomenex Sphereclone ODS, 250 × 10 mm, 5μ), to yield 3.1 mg of somamide A (**1**). Similarly, 87 g (dry wt) of the Taveuni Island collection was extracted, producing 2.1 g of crude extract. This extract was fractionated over LH-20 (3 × 40 cm column) using 100% MeOH. An early eluting fraction was further purified, first by C_{18} SPE cartridge (MeOH/H_2O, 7:3) and then by reversed-phase HPLC (MeOH/H_2O, 3:1, Phenomenex Sphereclone ODS, 250 × 10 mm, 5μ), to yield 0.5 mg of somamide B (**2**).

Somamide A (1): glassy oil; $[\alpha]^{22}_D$ −2.5° (*c* 0.08, MeOH); IR (neat) 3370, 3270, 2930, 2852, 1730, 1641, 1535, 1516, 1202, 1025 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS (in 3-nba) *m*/*z* 988 (85), 948 (100), 884 (6), 703 (6), 503 (7), 420 (24), 348 (9), 295 (8), 246 (36), 226 (38), 150 (63), 99 (18), 56 (96); HRFABMS *m*/*z* $[M + H - H_2O]^+$ 948.4587 (calcd for $C_{48}H_{66}N_7O_{11}S$, 948.4541).

Somamide B (2): glassy oil; $[\alpha]^{22}_D$ −10.2° (*c* 0.05, MeOH); IR (neat) 3365, 3296, 3271, 2927, 2858, 1731, 1640, 1534, 1446, 1203, 1075 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; FABMS (in 3-nba) *m*/*z* 941 (62), 901 (46), 420 (14), 329 (46), 219 (42), 176 (100); HRFABMS *m*/*z* $[M + H - H_2O]^+$ 901.4473 (calcd for $C_{46}H_{61}N_8O_{11}$, 901.4460).

Absolute Configuration of Amino Acids in Somamide A (1). Somamide A (0.5 mg) was hydrolyzed with 6 N HCl at 110 °C for 16 h. The hydrolysate was evaporated to dryness and dissolved in H_2O (50 μL). A 0.1% 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (Marfey's reagent) solution in acetone (50 μL) and 20 μL of 1 N $NaHCO_3$ were added, and the mixture was heated at 40 °C for 1 h. The solution was cooled to room temperature, neutralized with 10 μL of 2 N HCl, and evaporated to dryness. The residue was resuspended in 100 μL of DMSO/H_2O (1:1), and the solution was analyzed by reversed-phase HPLC (Waters Nova-Pak C_{18}, 3.9 × 150 mm, UV detection at 340 nm) using a linear gradient (10% CH_3CN in H_2O containing 0.05% TFA to 50% CH_3CN).

The retention times (t_R, min) of the derivatized amino acids in the hydrolysate of somamide A matched those of L-Met(O) (18.6), L-Thr (18.9), L-Val (33.0), and L-Phe (38.5) but not D-Met(O) (19.3), D-Thr (23.6), L-allo-Thr (19.4), D-allo-Thr (21.0), D-Val (37.9), or D-Phe (42.7).

A second linear gradient using CH_3CN in 50 mM NH_4OAc (10% to 50% CH_3CN over 60 min) was also performed. The retention time for the derivatized *N*-Me-Tyr residue matched that of *N*-Me-L-Tyr (12.8) but not *N*-Me-D-Tyr (13.9).

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