ORGANIC LETTERS

2002
Vol. 4, No. 7
1095−1098

# Somocystinamide A, a Novel Cytotoxic Disulfide Dimer from a Fijian Marine Cyanobacterial Mixed Assemblage

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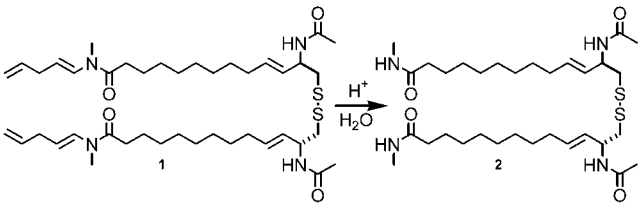
**Received December 19, 2001**

## ABSTRACT




**Bioassay-guided investigation of the extract from a *Lyngbya majusculalSchizothrix* sp. assemblage of marine cyanobacteria led to the discovery of somocystinamide A (1), an extraordinary disulfide dimer of mixed PKS/NRPS biosynthetic origin. Somocystinamide A (1) was highly acid-sensitive, rapidly and completely converting to a characterizable derivative (2). Compound 1 exhibits significant cytotoxicity against mouse neuro-2a neuroblastoma cells ($IC_{50}$ = 1.4 μg/mL), whereas 2 has no activity.**

Marine microalgae have provided a vast array of structurally interesting metabolites, often possessing pharmaceutical and biomedical utility.[1] For example, compounds such as the curacins,[2] microcolins,[3] and brevetoxins[4] are of considerable interest for their antiproliferative, immunosuppressive, and sodium-channel-activating properties, respectively. *Lyngbya majuscula*, in particular, has been extensively studied in our laboratory for its intriguing chemistry and has been shown to produce over 150 different compounds in diverse structural classes. A major theme in *L. majuscula* chemistry is the production of metabolites deriving from a combination of polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) biosynthetic pathways.[1] Examples of such mixed biosynthetic products isolated from our laboratory include the molluscicide barbamide[5] and the potent neurotoxins antillatoxin[6] and kalkitoxin.[7]

A detailed study of a *L. majuscula*/*Schizothrix* sp. mixed assemblage, collected from Somo Somo, Fiji in 1997, led to the isolation of a new lipopeptide, somamide A,[8] a structural homologue of the previously reported molluscan metabolite dolastatin 13.[9] A separate cytotoxic fraction from this extract was also pursued, leading to the discovery of somocystinamide A (**1**) as the active constituent. In this communication we describe the structural characterization and biological activity of this novel cyanobacterial cytotoxin.

The microalgal assemblage was collected by hand (3−6 m depth) and stored at −20 °C in 2-propanol. The material was extracted repeatedly with $CH_2Cl_2$/MeOH (2:1) to

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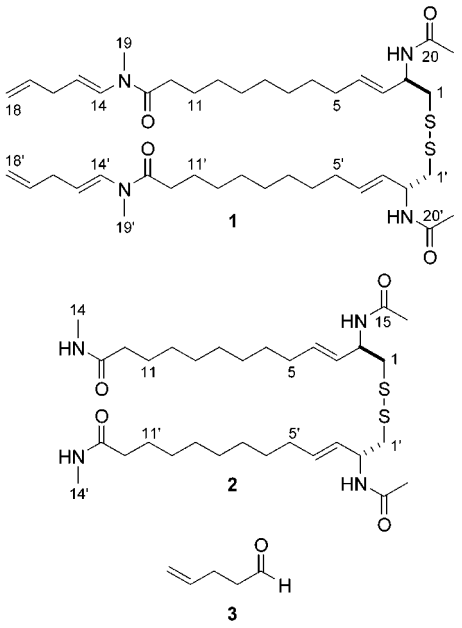
10.1021/ol017275j CCC: $22.00 
**Published on Web 03/09/2002**

produce 1.3 g of viscous black oil, and a portion of this crude extract (1.0 g) was fractionated by silica vacuum liquid chromatography (VLC). Polar fractions eluting with 100% EtOAc and 5% MeOH in EtOAc displayed significant cytotoxic activity against mouse neuro-2a neuroblastoma cells and were thus subjected to $C_{18}$ VLC and reversed-phase HPLC, affording somocystinamide A (**1**) as a white amorphous solid (20.8 mg, 2.1% of extract).[10]




Although dissolving readily in $CH_2Cl_2$ and $CHCl_3$, compound **1** was not appreciably soluble in other solvents, and thus NMR experiments were performed in $CDCl_3$.[11] $^1H$ and $^{13}C$ NMR spectra initially indicated that **1** was a small molecule having approximately 21 carbon atoms and existing in two conformations, as shown by an approximate 7:3 ratio of several $^1H$ and $^{13}C$ signals (see Figure 1a and Supporting Information).

A $^1H-^1H$ COSY spectrum of **1** was obtained to begin developing spin systems; however, 2D heteronuclear data acquisition was delayed and the sample remained in $CDCl_3$ for several days. Unexpectedly, marked differences were displayed in the $^1H$ NMR spectrum run at this later time, including the appearance of an aliphatic aldehyde signal ($\delta$ 9.78, t, $J = 1.2$ Hz) and several alterations in the high-field region of the spectrum (Figure 1b). 2D NMR data including HSQC, HSQC−COSY, and HMBC were acquired but proved difficult to interpret due to an absence of key connectivities. Therefore, the sample was again prepared for further NMR experiments.

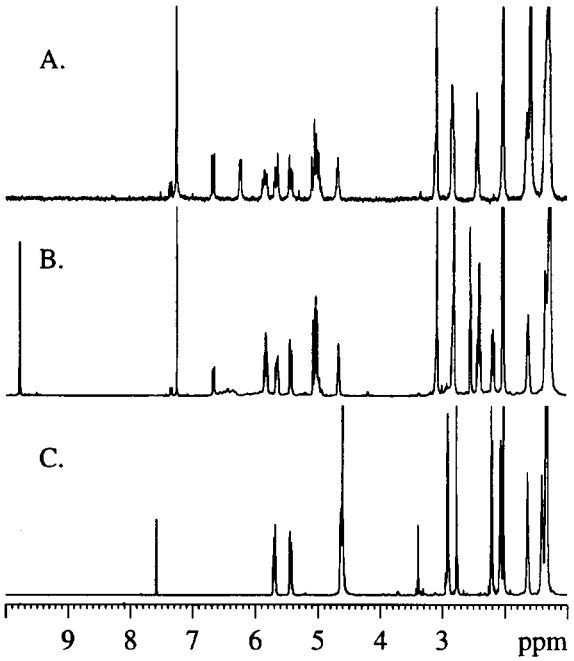



**Figure 1.** $^1H$ NMR spectra of (a) intact somocystinamide A (**1**) in $CDCl_3$, (b) intermediate mixture of **1** and decomposition products **2** and **3** in $CDCl_3$, and (c) stable decomposition product **2** in 1:1 $CDCl_3/CD_3OD$.

Interestingly, the sample was no longer soluble in $CDCl_3$ or $CD_3OD$ but dissolved only in an approximate 1:1 mixture of these two solvents. A $^1H$ NMR spectrum acquired under these new conditions again produced surprising results (Figure 1c). Not only had the aldehyde and high-field signals of spectrum 1b disappeared, but several signals present in the initial $^1H$ NMR spectrum of **1** were additionally absent. $^1H$ and $^{13}C$ NMR data suggested this compound (**2**) was a structurally simplified derivative of the original natural product, and therefore 2D NMR and mass spectral data were obtained.

HR FABMS of **2** produced an $[M + H]^+$ molecular ion at *m/z* 627.3976 for the formula $C_{32}H_{58}N_4O_4S_2$, indicating that **2** was actually a dimeric and symmetric structure.[12] 1D NMR spectra showed that **2** possessed a *trans* double bond and two amide carbonyl carbons.[13] Thus, as a dimer, all six degrees of unsaturation inherent to the molecular formula were assigned, and **2** was shown to be acyclic.

(10) HPLC isolation conditions were as follows: column, Phenomenex Sphereclone 5 $\mu$m ODS (250 × 10 mm); solvent, MeOH/$H_2O$ (19: 1); flow rate, 1.8 mL/min; detection at 230 nm. Somocystinamide A (**1**): $[\alpha]^{22}_D$ +13.5° (*c* 0.75, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 241 nm ($\epsilon$ 9900), 210 nm ($\epsilon$ 17 700); IR (neat) $\nu_{max}$ 3299, 2922, 2852, 1642, 1553 $cm^{-1}$; FABMS (3-NBA) *m/z* 759 $[M + H]^+$ (56), 412 (6), 379 (23), 347 (15), 333 (13), 197 (19) 98 (100); HRFABMS *m/z* $[M + H]^+$ 759.4925 (calcd for $C_{42}H_{71}N_4O_4S_2$, 759.4917).

(11) NMR spectra were recorded on Bruker AC300 and DRX600 spectrometers with the solvent ($CDCl_3$ or $CD_3OD$) used as an internal standard.

(12) Mass spectral analysis was performed in the positive ion mode on a Kratos MS50TC mass spectrometer with 3-NBA as the matrix.

(13) Derivative **2**: white amorphous solid; $[\alpha]^{23}_D$ −16.7° (*c* 0.15, 1:1 $CHCl_3$/MeOH); UV (MeOH) $\lambda_{max}$ 210 nm ($\epsilon$ 14 600); IR (neat) $\nu_{max}$ 3317, 2918, 2848, 1642, 1542 $cm^{-1}$; $^1H$ NMR (1:1 $CDCl_3/CD_3OD$, 600 MHz) $\delta$ 5.65 (1H, dt, $J = 15.4$, 6.6 Hz, H-4/4′), 5.45 (1H, dd, $J = 15.4$, 6.6 Hz, H-3/3′), 4.62 (1H, q, $J = 6.6$ Hz, H-2/2′), 2.88 (2H, m, $H_2$-1/1′), 2.74 (3H, s, $H_3$-14/14′), 2.21−2.17 (2H, m, $H_2$-12/12′), 2.04 (2H, q, $J = 6.7$ Hz, $H_2$-5/5′), 1.99 (3H, s, $H_3$-16/16′), 1.60 (2H, m, $H_2$-11/11′), 1.35−1.28 (10H, $H_2$-6/6′-$H_2$-10/10′); $^{13}C$ NMR (1:1 $CDCl_3/CD_3OD$, 125 MHz) $\delta$ 175.3 (s, C-13/13′), 170.9 (s, C-15/15′), 133.0 (d, C-4/4′), 127.6 (d, C-3/3′), 50.5 (d, C-2/2′), 43.8 (t, C-1/1′), 35.9 (t, C-12/12′), 31.9 (t, C-5/5′), 28.9 (4C, t, C-7/7′-C-10/10′), 28.7 (t, C-6/6′), 25.6 (t, C-11/11′), 25.4 (q, C-14/14′), 22.0 (q, C-16/16′); FABMS (3-NBA) *m/z* 627 $[M + H]^+$ (68), 346 (14), 313 (36), 281 (22), 267 (29), 225 (20); HRFABMS *m/z* $[M + H]^+$ 627.3976 (calcd for $C_{32}H_{59}N_4O_4S_2$, 627.3979).

**Table 1.** NMR Spectral Data for Somocystinamide A (**1**) in $CDCl_3$ and 1D Spectral Data for **2** in 1:1 $CDCl_3/CD_3OD$

| position | $^{13}C$ of **1** | $^{1}H$ of **1** (*J* in Hz) | HSQC−COSY[a] of **1** | HMBC[b] of **1** | $^{13}C$ of **2**[c] | $^{1}H$ of **2**[c] |
|---|---|---|---|---|---|---|
| 1/1′ | 44.7 | 3.08, dd (13.4, 6.2)<br>2.83[d] | 4.67 | 50.8, 127.6 | 43.8 | 2.88 |
| 2/2′ | 50.8 | 4.67, brp (6.5) | 2.83, 3.08, 5.43, 6.34 | 44.7, 127.6, 133.7, 169.7 | 50.5 | 4.62 |
| 3/3′ | 127.6 | 5.43, dd (15.5, 6.5) | 5.66 | 32.2, 44.7, 50.8 | 127.6 | 5.45 |
| 4/4′ | 133.7 | 5.66, dt (15.5, 6.6) | 2.02, 5.43 | 29.3, 32.2, 50.8 | 133.0 | 5.65 |
| 5/5′ | 32.2 | 2.02, q (6.7) | 1.33, 5.66 | 29.3, 127.6, 133.7 | 31.9 | 2.04 |
| 6/6′ | 29.3 | 1.33[d] | 2.02 | | 28.7 | 1.35−1.28 |
| 7/7′-9/9′ | 29.2 | 1.33[d] | | | 28.7 | 1.35−1.28 |
| 10/10′ | 28.9 | 1.33[d] | 1.64 | | 28.9 | 1.35−1.28 |
| 11/11′ | 25.0 | 1.64, p (7.1) | 1.33, 2.43 | 28.9, 171.7 | 25.6 | 1.60 |
| 12/12′ | 33.8 | 2.43, m | 1.64 | 25.0, 28.9, 171.7 | 35.9 | 2.21−2.17 |
| 13/13′ | 171.7/171.4[e] | | | | 175.3 | |
| 14/14′ | 129.2<br>128.1[e] | 6.67, d (13.9)<br>7.36[e], d (14.6) | 4.99 | 29.7, 34.7, 109.0, 171.7 | 25.4 | 2.74 |
| 15/15′ | 109.0<br>108.4[e] | 4.99, m<br>4.97[e] | 2.83, 6.67 | 34.7, 129.2, 137.0 | 170.9 | |
| 16/16′ | 34.7 | 2.83[d] | 4.99 | 109.0, 115.4, 129.2, 137.0 | 22.0 | 1.99 |
| 17/17′ | 137.0/137.4[e] | 5.84, m | 5.06 | 34.7 | | |
| 18/18′ | 115.4/115.0[e] | 5.06, m | 5.84 | 137.0 | | |
| 19/19′ | 29.7/32.1[e] | 3.07, s/3.08[e], s | | 129.2, 171.7 | | |
| NH | | 6.34 | | 50.8, 169.7 | | |
| 20/20′ | 169.7 | | | | | |
| 21/21′ | 23.4 | 2.01, s | | 169.7 | | |

[a] Proton showing COSY correlation to indicated proton. [b] Proton showing HMBC correlation to indicated carbon. [c] See ref 13 for proton coupling constants. [d] Obscured. [e] Minor conformer.

HSQC−COSY and HMBC were used to connect the low-field methine signal CH-2/2′ to an acetylated amine moiety (C-15/15′, $CH_3$-16/16′), the *trans* double bond (CH-3/3′, CH-4/4′) and a mid-field methylene signal ($CH_2$-1/1′). This methylene showed no further correlations, and its proton and carbon shifts were consistent with the presence of a sulfur substituent. 2D NMR data were then utilized to extend outward from the double bond to a methylene ($CH_2$-5/5′) followed by a $(CH_2)_5$ envelope and a high-field methylene. This latter methylene ($CH_2$-11/11′) was connected to yet another mid-field $CH_2$ unit ($CH_2$-12/12′) that, by HMBC, terminated with the carbonyl of an N-methylated amide. Dimerization of this linear chain through a disulfide functionality accounted for all atoms in **2**, thus completing its planar structure (see ref 13 and supporting data).

To our fortune, additional somocystinamide A (**1**) was detected in side fractions associated with its original isolation. Realizing the apparent instability of the compound, mass spectra and NMR data in acid-neutralized $CDCl_3$ were quickly and carefully collected, and the structure of this unique metabolite, somocystinamide A (**1**), was finally resolved.

A HR FABMS $[M + H]^+$ molecular ion at *m/z* 759.4295 identified the molecular composition of **1** as $C_{42}H_{70}N_4O_4S_2$, indicating that **1** was also a symmetric, acyclic dimer. 1D NMR spectra showed that each symmetric unit of **1** possessed a terminal olefin, two *trans*-disubstituted double bonds, and two amide carbonyls, accounting for all 10 degrees of unsaturation. 2D NMR data confirmed that the intact structure of **2** was conserved within **1** and assembled the remaining $C_{10}H_{10}$ component missing from **2** as two five-carbon 1,4-diene moieties. These units were connected to the terminal amide nitrogens by the key HMBC correlation from H-14/14′ ($\delta$ 6.67) to C-13/13′ ($\delta$ 171.7, see Table 1), thus completing the planar structure of somocystinamide A (**1**). The mixture of conformers of **1** is presumed to arise from *cis/trans* isomerization of these tertiary amides.

The absolute stereochemistry of **1** was determined by ozonolysis, acid hydrolysis, and oxidation to release the corresponding cysteic acid residues. An HPLC comparison and co-injection of this product with standard D- and L-cysteic acid revealed only L-cysteic acid; hence somocystinamide A (**1**) is of 2*R*,2′*R* absolute configuration.[14]

It appears that trace quantities of HCl and $H_2O$ present in the original NMR solvent initiated the cleavage of **1** leading to the formation of **2** and the release of the C14−C18 and C14′−C18′ units as aldehyde **3**. Although this fragment was not isolated and was likely evaporated during the second preparation of the NMR sample, the presence of **3** is strongly supported by the 2D NMR data obtained on the crude decomposition mixture (see Figure 1b and Supporting Information).

(14) A stream of ozone was bubbled through **1** (0.3 mg) dissolved in $CH_2Cl_2$ (2 mL) at −78 °C until the solution turned a pale blue color (ca. 2 min). The solvent was removed under a stream of $N_2$, and 6 N HCl (1 mL) was added. This solution was microwaved in an Ace high-pressure tube (1 min) and again dried under $N_2$. Formic acid (500 $\mu$L) and 30% $H_2O_2$ (10 $\mu$L) were added, and the solution was stirred at room temperature (30 min) and then dried under $N_2$. The material was resuspended in $H_2O$ (50 $\mu$L) and analyzed by reversed-phase chiral HPLC [Phenomenex, Chirex phase 3126 (4.6 × 50 mm), 2 mM $CuSO_4$−$CH_3CN$ (95:5), 1.0 mL/min, detection at 254 nm]. The retention times of the authentic amino acids were 3.5 and 4.1 min for L- and D-cysteic acid, respectively. Injection of the product from **1** under these HPLC conditions produced only a peak at 3.5 min, and co-injection of this material with the standards confirmed the presence of L-cysteic acid.

Somocystinamide A (**1**) exhibits potent cytotoxicity to mouse neuro-2a neuroblastoma cells with an $IC_{50}$ of 1.4 $\mu$g/mL.[15] While derivative **2** displayed no cytotoxicity, its insoluble nature may contribute to this apparent inactivity. Somocystinamide A (**1**) represents a new and unique cyanobacterial metabolite class of likely mixed PKS/NRPS biosynthetic origin. We envisage L-cysteine is ketide extended with five malonyl CoA-derived acetate units, followed by linkage of an *N*-methyl glycine moiety and then further extension by two additional acetates. Decarboxylation to produce the terminal olefin and dimerization completes the proposed biosynthesis of **1**.

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**Acknowledgment.** Financial support from the National Institute of Health (CA52955 and GM63554) is gratefully acknowledged. We also thank Dr. R. T. Williamson and the OSU Department of Biochemistry and Biophysics for NMR assistance, Dr. T. Okino for conducting cytotoxicity assays, B. Arbogast and OSU Environmental Health Sciences Center for mass spectral data, and the Government of Fiji for permission to make the cyanobacterial collection.

**Supporting Information Available:** NMR and mass spectral data for compounds **1** and **2** and intermediate product mixture. This material is available free of charge via the Internet at http://pubs.acs.org.

OL017275J