Tasiamide, a Cytotoxic Peptide from the Marine Cyanobacterium *Symploca* sp.

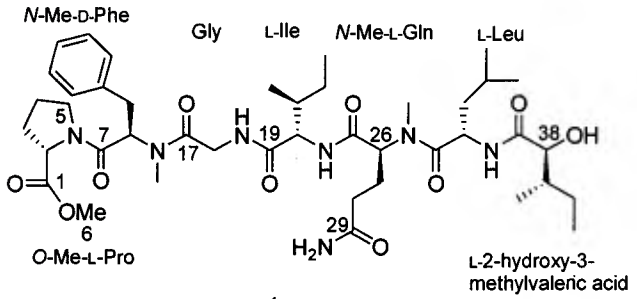
Philip G. Williams,[†] Wesley Y. Yoshida,[†] Richard E. Moore,*[,†] and Valerie J. Paul*[,‡]

Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822, and University of Guam Marine Laboratory, UOG Station, Mangilao, Guam 96913

Received April 19, 2002

The acyclic peptide tasiamide (**1**) has been isolated from the marine cyanobacterium *Symploca* sp. The planar structure and absolute stereochemistry of the peptide were deduced by 2D NMR techniques and chiral HPLC. Tasiamide (**1**) was cytotoxic against KB and LoVo cells with IC_{50} values of 0.48 and 3.47 μg/mL, respectively.

Cyanobacteria are well-known sources of peptides and depsipeptides which display a variety of biological activities.[1] As part of a collaborative effort to discover new antitumor agents effective against solid and/or multidrug-resistant tumors, we began screening cyanobacterial extracts collected in Micronesia.[2] The vast majority of these extracts belonged to the genus *Lyngbya*,[3] but among the samples collected in 1999 was a *Symploca* sp., which displayed potent solid tumor selectivity.[4] Interestingly there are only a few reports of the chemistry and biological activity of this genus in the literature. Cultured *Symploca muscorum* has been reported to produce geosmin, a long chain alcohol that is often responsible for the undesirable earthy flavor in fish,[5] while dihydrochalcone-glucosides have been isolated as the sweet principles of another *Symploca*.[6] More recent collections have produced the malevamides,[7] the symplostatins,[8] and dolastatin 10.[9] The latter was originally isolated from the sea hare *Dolabella auricularia* and is currently in phase II clinic trials as an anticancer agent in the United States.[10] Prompted by these results, the cyanobacterium was recollected in the spring of 2000. We report here the isolation and structure determination of the first metabolite from this *Symploca* sp., the acyclic peptide tasiamide[11] (**1**).



1

NIH304, a *Symploca* sp. collected at Short Drop-off in Palau, was repeatedly extracted with a 4:1 mixture of $CH_3CN-CH_2Cl_2$ to afford a total of 2.67 g of lipophilic extract. Subsequent solvent partitioning and purification by Si gel, C_8, and repeated HPLC yielded tasiamide (**1**) as an amorphous powder (2.1 mg, 0.08% dry extract weight). The optically active powder ($[\alpha]^{21}_D$ +15°, *c* 0.4, $CHCl_3$) displayed IR bands at 1737 and 1643 cm^{-1}, characteristic of ester and amide carbonyl vibrations. The UV spectrum of **1** contained a 254 nm absorbance, characteristic of a benzene chromophore. HRFABMS provided a molecular ion peak at *m/z* 852.4833 ($[M + Na]^+$, Δ 0.8 mmu), which established the molecular formula for **1** as $C_{42}H_{67}N_7O_{10}$. The ^1H NMR data, recorded in $CDCl_3$ (Table 1), revealed seven α-proton signals between 3.94 and 5.52 ppm, while the ^{13}C NMR spectrum showed eight carbonyl signals. Five amide protons signals in the form of two broad singlets (δ_H 5.57, 5.89), two doublets (δ_H 7.05, 7.12), and one doublet of doublets (δ_H 6.96) were visible in the ^1H NMR spectra. The two broad singlets suggested a primary amide, while the doublet of doublets was reminiscent of the coupling of an amide proton of a glycine unit. Other distinctive structural features were two *N*-methylamides (δ_H 2.96 and 3.18) and one methoxy group (δ_H 3.72).

HMBC and COSY experiments (Table 1) established the presence of *N*-methylphenylalanine, glycine, isoleucine, leucine, and 2-hydroxy-3-methylvaleric acid units. The latter was easily distinguished from isoleucine by the characteristic signal at 76.5 ppm for C-38. No proton or carbon correlations from H-38 or C-38 to an exchangeable proton were evident in the COSY or HMBC spectra. A correlation in the COSY spectrum from H-26 to H-27 and from H-27 to H-28 established an isolated C_3H_5 chain which was connected to an *N*-methylamide at δ_H 3.18 (H-30) by an HMBC correlation from C-26. Further HMBC cross-peaks to H-27 from two carbonyls (C-25 and C-29) expanded this to an aminodicarboxy unit. The two broad exchangeable proton signals at 5.57 and 5.87 ppm, along with the lack of a resonance indicative of a carboxylic acid at 10 ppm in the ^1H NMR spectrum, implied the presence of a *N*-Me-Gln unit, although no HMBC correlations could be seen between the primary amide protons and C-29.

The final carbonyl signal at δ_C 172.5 (C-1) showed HMBC correlations to a methoxy singlet at δ_H 3.72, a methine at δ_H 4.39 (H-2), and a pair of geminal protons at δ_H 2.12 (H-3a) and 1.87 (H-3b). COSY correlations from H-3a to H-4 and H-4 to H-5 established the vicinal relationship of these protons. The downfield chemical shift of C-5 (δ_C 46.8) indicated that this carbon was attached to a nitrogen, which suggested that it was in a *O*-methylproline or *O*-methylornithine unit. The diastereotopic nature of the H-5 protons (δ_H 3.29 and 3.39) suggested restricted rotation in the unit, and this seemed to favor proline; however, no HMBC correlations were observed across the pyrrole nitrogen to support this conclusion (i.e., C-5 to H-2). The molecular formula of **1** implied 13 degrees of unsaturation, of which 12 had already been accounted for in the form of three carbon−carbon double bonds, eight carbonyls, and

* To whom correspondence should be addressed. (R.E.M.) Tel: (808) 956-7232. Fax: (808) 956-5908. E-mail: moore@gold.chem.hawaii.edu. (V.J.P) Tel: (671) 735-2186. Fax: (671) 734-6767. E-mail: vpaul@uog9.uog.edu.
† University of Hawaii at Manoa.
‡ University of Guam Marine Laboratory.

10.1021/np020184q CCC: $22.00
Published on Web 07/20/2002

Table 1. NMR Spectral Data for Tasiamide (**1**) in $CDCl_3$

C/H no.	N-HSQC[a]	δ_H[b] (J in Hz)	δ_C[c,d]	COSY[b]	HMBC[e,f]	ROESY[b]
1			172.5, s		2, 3, 6	
2		4.39, dd (8.7, 6.4)	58.9, d	3a, 3b	3, 4	3
3		2.12, m	28.8, t		2, 4, 5	
		1.87, m				
4		1.93, m	24.9, t	3a		
		1.81, m		3a		
5		3.36, m	46.8, t	4a, 4b	3	3, 8
		3.29, m		4a, 4b		
6		3.72, s	52.3, q			
7			167.9, s		8, 9a, 9b	
8		5.52, dd (8.7, 6.8)	56.3, d	9a, 9b	16	5, 9, 11
9		3.29, dd (−13.5, 8.7)	35.1, t	8, 9b	8	4, 11
		2.82, dd (−13.5, 6.8)		8, 9a	8	
10			136.8, s		8, 9a, 9b, 12	
11/15		7.22, d (7.5)	129.4, d	12	9a, 9b, 11, 13	
12/14		7.26, dd (7.5, 7.1)	128.4, d	11, 13	12	
13		7.20, t (7.1)	126.8, d	12	11	
16		2.96, s	29.7, q		8	
17			167.6, s		16, 18	
18		4.07, dd (−17.5, 4.8)	41.1, t	18a, NH-Gly		16, 23, 24, NH-Gly
		3.82, dd (−17.5, 3.6)		18b, NH-Gly		16, 23, 24, NH-Gly
NH-Gly	−270.9	6.96, dd (4.8, 3.6)		18a, 18b		21
19			171.4, s		18, 20, NH-Gly	
20		4.31, dd (8.9, 6.4)	57.7, d	21, NH-Ile	24	8, NH-Gly
21		1.84, m	37.1, d	22, 24	20	NH-Gly
22		1.41, m	24.7, t	21, 22b, 23	20, 21, 23, 24	
		1.11, m		22a, 23		
23		0.87, t (7.6)	11.3, q	22	22	
24		0.88, d (6.8)	15.6, q	21	20, 22	
NH-Ile	−264.0	7.05, d (8.9)		20		
25			169.7, s		20, 26, 27, NH-Ile	
26		5.06, dd (7.5, 7.3)	56.2, d	27a, 27b	27, 30	3, 27, 30, NH-Ile
27		2.31, m	22.9, t	26, 27b, 28a, 28b	26	30
		2.00, m		27a, 28a, 28b		30
28		2.23, m	32.2, t	27b, 28b	26	
		2.19, m		28a		
29			174.1, s		27, 28	
29-NH	−276.4	5.89, br s				29-NHb
	−276.4	5.57, br s				29-NHa, 18b, 38
30		3.18, s	31.0, q		26	27a, 27b, 33, 34, 36
31			174.4, s		26, 30, 32	
32		4.97, dt (8.4, 7.3)	47.1, d	33, NH-Leu	34	33, 34
33		2.23, m	41.0, t		32, 34, 35, 36	30
		1.60, m				
34		1.60, m	24.9, d	35	33	30
35		0.96, d (6.4)	22.1, q	34	34, 36	30
36		0.95, d (7.1)	23.0, q	34	34	30
NH-Leu	−260.4	7.12, d (8.4)		32		
37			174.0, s		38, NH-Leu	
38		3.94, d (3.9)	76.5, d	39	40, 42	39, 40a, 42, NH-Leu
39		1.84, m	38.3, d	40, 42	38, 40	
40		1.46, m	23.7, t	39, 40b, 41	39, 41, 42	
		1.23, m		39, 40a	38, 39	
41		0.90, t (7.3)	11.8, q	40a	40	
42		0.97, d (6.9)	15.5, q	39	38, 40	

[a] Recorded at 50 MHz. [b] Recorded at 500 MHz. [c] Recorded at 125 MHz. [d] Multiplicity deduced by HSQC. [e] Protons showing long-range correlation with indicated carbon. [f] Correlations were observed for $^nJ_{CH} = 7$ Hz.

one ring. The final degree of unsaturation had to be another ring since no unaccounted sp^2 carbons remained. Careful consideration of the fragments suggested two possible structures, one with proline and another in which the C-terminus was an *O*-methylornithine moiety cyclized through the ϵ-amino group and C-29 of glutamine. One of the exchangeable protons at 5.89 or 5.57 ppm must then be the alcohol proton on C-38, and the other a secondary amide proton at C-29. A ^{15}N-HSQC experiment determined which exchangeable protons were attached to nitrogens. The data (Table 1) indicated the δ 5.89 and 5.57 protons were attached to the same amide nitrogen at −276.4 ppm.[12] Also acetylation of 100 μg of **1** with excess acetic anhydride in pyridine overnight at room temperature resulted in a downfield shift of H-38 from 3.95 to 5.02 ppm without elimination of the exchangeable protons at 5.89 and 5.57 ppm. These two experiments ruled out the possibility of a structure that contained *O*-methylornithine.

The HMBC data provided the majority of the peptide sequence. Correlations to the *N*-methylamide signal at δ_H 2.96 (H-16) from C-8 and C-17 connected the nitrogen of phenylalanine to the carbonyl of glycine. Cross-peaks to the second *N*-methylamide signal (H-30) from C-26 and C-31 established the glutamine-leucine linkage. The majority of the remaining sequence was obtained from HMBC correlations to the amide proton signals of glycine, isoleucine, and leucine from C-19, C-25, and C-37, respectively. The location of *O*-methylproline was discerned by a ROESY experiment that showed strong cross-peaks between H-5 and the α-proton signal of Phe (H-8) as well as a weak

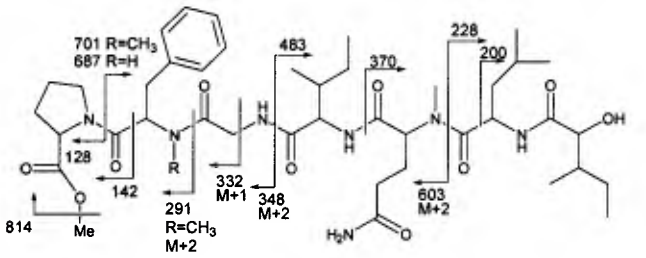


Figure 1. FABMS fragmentation of **1**.

correlation between H-3 and H-26 of the glutamine unit. On the basis of the relative strength of these correlations the seventh amino acid was attached to *N*-Me-Phe at C-7.

The FAB mass spectrum of **1** provided support for the amino acid sequence in the expanded structure (Figure 1). Key fragmentations include *m/z* 348, 291, and 128 from the cleavage of the amide bond between glycine and isoleucine followed by sequential loss of glycine and *N*-Me-Phe, respectively. Key "N-terminus"-containing fragments were 483, 370, and 228 resulting from cleavage of the same glycine-isoleucine amide bond and loss of isoleucine and glutamine, respectively. Cleavage of the proline-phenylalanine bond with loss of proline resulted in a prominent *m/z* of 701, supporting the location of the proline unit.

The absolute stereochemistry of **1** was deduced after chiral HPLC analysis of the degradation products of the acid hydrolyzate. The HPLC trace of the hydrolyzate was compared with authentic standards to establish the stereochemistry of the amino acid-derived units as L-Pro, *N*-Me-D-Phe, L-Ile, L-Leu, and L-2-hydroxy-3-methylvaleric acid. The *N*-Me-L-Gln unit was detected as *N*-Me-L-Glu by chiral HPLC after acid hydrolysis.

Tasiamide is an acyclic peptide that contains several structural features common among cyanobacterial peptides, including a hydroxy acid, two *N*-methylamides, and an ester. The closest structurally related metabolite is the linear peptide malevamide A, which shares the amino acid sequence from *O*-methylproline through isoleucine. Tasiamide displayed cytotoxicity against KB and LoVo cells with IC_{50} values of 0.48 and 3.47 μg/mL, respectively.

Experimental Section

General Experimental Procedures. The UV spectrum of **1** was determined on a Hewlett-Packard 8453 spectrophotometer, and the IR spectrum was recorded on a Perkin-Elmer 1600 FTIR instrument as a film on a NaCl disk. The optical rotation was measured on a Jasco-DIP-700 polarimeter at the sodium D line (589 nm). FABMS and HRFABMS were recorded in the positive mode on a VG ZAB2SE spectrometer. The NMR spectra of **1** were recorded in $CDCl_3$ on a GE GN Omega 500 operating at 500 and 125 MHz using the residual solvent signal as the internal reference. HPLC separations were performed on a Beckman 110B apparatus coupled to a Applied Biosystems 759A absorbance detector.

Biological Material. The cyanobacterium was collected at Short Drop-off in Palau during May of 2000. The sample was identified by V. J. Paul, and a voucher is maintained in formalin at UOG.

Extraction and Isolation. The freeze-dried cyanobacterium (250 g) was thrice extracted with a 4:1 mixture of $CH_3CN-CH_2Cl_2$, providing 2.67 g of total lipophilic extract. After partitioning between hexane and 80% aqueous methanol the aqueous residue was loaded on to a Si flash column and was eluted with increasing amounts of MeOH in CH_2Cl_2. The 8% fraction (50 mg) was passed over a C_8 column, and the 50% aqueous CH_3CN residue (9.2 mg) was subsequently rechromatographed on an Econosil cyanopropyl column (10 × 250 mm, solvent system of a linear gradient from 30 to 70% CH_3CN in H_2O over 40 min; flow rate 3 mL/min; PDA detection) to give a 6.0 mg fraction (t_R = 8.0 min). This fraction was further purified on a YMC-AQ column (10 × 250 mm, solvent system of a MeOH−0.01% TFA linear gradient from 65 to 100% MeOH over 30 min; flow rate 3 mL/min; PDA detection) providing 2.1 mg of tasiamide (t_R = 15.3 min).

Tasiamide (1): amorphous powder; $[\alpha]^{21}_D$ +15° (c 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.27), 254 (2.52) nm; IR (film) ν_{max} 3311, 1737, 1643, 1453 cm^{-1}; ^1H NMR, ^{13}C NMR, $^1H-^1H$ COSY, and HMBC data, see Table 1; FAB *m/z* (intensity) $[M + Na]^+$ 852 (15), $[M + H]^+$ 830 (16), 814 (8), 701 (56), 687 (13), 603 (6), 483 (10), 370 (71), 348 (4), 332 (6), 291 (46), 228 (43), 200 (66), 142 (20), and 128 (36); HRFABMS *m/z* $[M + Na]^+$ 852.4833 (calcd for $C_{42}H_{67}N_7O_{10}Na$ 852.4841).

Absolute Stereochemistry. The acid hydrolyzate of **1** (300 μg of **1** in 6 N HCl at 118 °C for 16 h) was analyzed by chiral HPLC, and the retention times were compared with authentic standards [CHIRALPAK MA(+), 4.6 × 50 mm, Diacel Chemical Industries, Ltd.; solvent 2 mM $CuSO_4$ for Leu, Ile, and Pro, 2 mM $CuSO_4$−MeCN (90:10) for *N*-Me-Phe, and 2 mM $CuSO_4$−MeCN (85:15) for 2-hydroxy-3-methylvaleric acid; flow rate 0.8 mL/min; detection at 254 nm except *N*-Me-Glu, which was analyzed on a Chirex Phase 3126 (D), 4.6 × 250 mm, Phenomenex; solvent 2 mM $CuSO_4$−MeCN (90:10); flow rate 0.8 mL/min; detection at 254 nm]. The retention times (t_R, min) of the authentic standards were L-Pro (11.6), D-Pro (6.2), L-Ile (34.8), L-allo-Ile (26.0), D-Ile (16.8), D-allo-Ile (13.5), L-Leu (33.0), D-Leu (15.8), *N*-Me-L-Phe (32.2), *N*-Me-D-Phe (26.1), L-2-hydroxy-3-methylvaleric acid (65.3), L-allo-2-hydroxy-3-methylvaleric acid (52.8), D-2-hydroxy-3-methylvaleric acid (40.1), D-allo-2-hydroxy-3-methylvaleric acid (33.8), *N*-Me-L-Glu (48.2), and *N*-Me-D-Glu (22.9). The retention times of the amino acid components of the hydrolyzate were L-Pro (11.6), L-Leu (33.0), L-Ile (34.8), *N*-Me-D-Phe (26.1), *N*-Me-L-Glu (48.2), and L-2-hydroxy-3-methylvaleric acid (66.0).

Acknowledgment. We would like to thank the Republic of Palau for the marine research permit and the National Cancer Institute for providing support through NCNPDDG grant CA53001 and R01 grant CA12623. The upgrade of the 500 MHz NMR spectrometer used in this research was funded by grants from the CRIF Program of the National Science Foundation (CHE9974921) and the upgrade of the 300 MHz NMR spectrometer by the Air Force Office of Scientific Research (F49620-01-1-0524). The cyanobacterium was collected by S. Belliveau, J. Biggs, I. Kuffner, B. Thacker, E. Cruz-Rivera, and H. Luesch. Bioassays were carried out by G. Tien at the University of Hawaii, Department of Chemistry. Mass spectral analyses were performed by the UCR Mass Spectrometry Facility, Department of Chemistry, University of California at Riverside, and M. Burger at the University of Hawaii, Department of Chemistry.

References and Notes

(1) Burja, A. M.; Banaigs, B.; Abou-Mansour, E.; Burgess, J. G.; Wright, P. C. *Tetrahedron* **2001**, *57*, 9347−9377.
(2) Moore, R. E.; Corbett, T. H.; Patterson, G. M. L.; Valeriote, F. A. *Curr. Pharm. Des.* **1996**, *2*, 317−330.
(3) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Corbett, T. H. *J. Am. Chem. Soc.* **2001**, *123*, 5418−5423, and references therein.
(4) Corbett, T. H.; Valeriote, F. A.; Polin, L.; Panchapor, C.; Pugh, S.; White, K.; Lowichik, N.; Knight, J.; Bissery, M.-C.; Wozniak, A.; LoRusso, P.; Biernat, L.; Polin, D.; Knight, L.; Biggar, S.; Looney, D.; Demchik, L.; Jones J.; Jones, L.; Blair, S.; Palmer, K.; Essenmacher, S.; Lisow, L.; Mattes, K. C.; Cavanaugh, P. F.; Rake, J. B.; Baker, L. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*; Valeriote, F. A., Corbett, T. H., Baker, L. H., Eds.; Kluwer Academic Publishers: Norwell, 1992; pp 35−87.
(5) Slater, G. P.; Blok, V. C. *Wat. Sci. Technol.* **1983**, *15*, 181−190.
(6) Tanaka, T.; Yamasaki, K.; Kohda, H.; Tanaka, O.; Mahato, S. B. *Planta Med.* **1980**, (Suppl), 81−83.
(7) Horgan, D. F.; Yoshida, W. Y.; Scheuer, P. J. *J. Nat. Prod.* **2000**, *63*, 461−467.
(8) (a) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H. *J. Nat. Prod.* **2002**, *65*, 16−20. (b) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J. *J. Nat. Prod.* **1999**, *62*, 655−658, and references therein.

(9) Luesch, H.; Moore, R. E.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H. *J. Nat. Prod.* **2001**, *64*, 907−910.
(10) Da Rocha, A. B.; Lopes, R. M.; Schwartsmann, G. *Curr. Opin. Pharmacol.* **2001**, *4*, 364−369.
(11) Tasi (Chamorro) meaning ocean.
(12) Witanowski, M.; Webb, G. A. *Nitrogen NMR*; Plenum Press: New York, 1973; p 188.

NP020184Q