Malevamides A−C, New Depsipeptides from the Marine Cyanobacterium *Symploca laete-viridis*

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Three new depsipeptides, malevamides A−C (**1**−**3**), were isolated from the cyanobacterium *Symploca laete-viridis* collected off the south shore of Oahu, Hawaii. Compounds **1**−**3** were identified by spectral methods, and partial stereochemical assignments were made by chiral HPLC of the hydrolyzed compounds. At a concentration of 2 μg/mL, compounds **1**−**3** were inactive against P-388, A-549, and HT-29 cancer cells.

Cyanobacteria are a well-known source of cyclic peptides and depsipeptides, many of which exhibit biological activity and/or contain unusual amino acid residues.[1] However, for members of the genus *Symploca* (Oscillatoriaceae), few reports of their chemistry or biological activity exist in the literature. Cultured *Symploca muscorum* produces geosmin (*trans*-1,10-dimethyl-*trans*-decalin-9-ol), a major cause of undesirable earthy flavor in fish,[2] and the $CHCl_3$ extract of a sample of this species from Enewetak Atoll demonstrated notable in vivo activity in a murine P-388 leukemia model.[3] On the other hand, an organic extract of *Symploca hydnoides* from Palau was inactive in vivo against P-388 leukemia and Ehrlich ascites tumor in mice,[4] and nonpolar extracts from material obtained in the Caribbean showed variable antibacterial activity.[5] In 1998, symplostatin 1 was reported as the cytotoxic constituent of a *S. hydnoides* sample collected on Guam, showing strong selective toxicity toward several solid tumor cell lines.[6] The closely related dolastatin 10 had been isolated in trace yields from the sea hare *Dolabella auricularia*[7] and is currently entering Phase II clinical trials in the United States as an anticancer agent.[8]

Our attention had been drawn to *Symploca* spp., prior to the publication of symplostatin 1,[6] when we found a sample of *S. hydnoides* to possess potent in vitro cytotoxicity. In an attempt to recollect the species, we inadvertently collected the morphologically very similar *Symploca laete-viridis*, which yielded malevamides A−C (**1**−**3**), which are the subject of this report.[9] The potent cytotoxicity of the recollected material suggested that *S. hydnoides* was also present in the sample.[10] Subsequent HPLC and NMR analysis of independent collections of both species showed the presence of malevamides A−C (**1**−**3**) in *S. laete-viridis* and their absence in *S. hydnoides*, thus confirming their biological origin.

Results and Discussion

A sample (325 g wet wt) of the benthic cyanobacterium *S. laete-viridis* (contaminated with *S. hydnoides*) was collected by scuba from a site off the south shore of Oahu. The hexanes and 1-BuOH partition residues of the MeOH extract were fractionated by VLC, followed by normal and reversed-phase HPLC, leading to the isolation of the Dragendorff-positive depsipeptides malevamides A (**1**, 1.3 mg), B (**2**, 4.3 mg), and C (**3**, 4.2 mg).

HRFABMS of malevamide A (**1**) suggested a molecular formula of $C_{54}H_{80}N_8O_{10}$. The ^{1}H NMR spectrum, showing NH resonances (δ 6−9), α-hydrogen signals (δ 4−6), *N*Me singlets ($\sim\delta$ 3), Me doublets and triplets (δ 0.8−1.0), and aromatic multiplets, implied that **1** is a small *N*-methylated peptide containing both aromatic and aliphatic residues. The IR spectrum indicated the presence of both amide (1634−1659 cm^{-1}) and ester (1746 cm^{-1}) functions. Following the interpretation of DEPT, COSY, HMQC, and HMBC NMR experiments ($CDCl_3$), the ^{1}H and ^{13}C NMR signals were assignable to one OMe group, a 2-methylhexanoic acid residue (Mha), and eight α-amino acid residues—two glycines (Gly), two *N*-methylphenylalanines (MePhe), two prolines (Pro), isoleucine (Ile), and *N*-methylvaline (MeVal). Table 1 lists the full chemical shift assignments and key HMBC correlations for **1**.

The COSY and HMBC correlations that led to the identification of the Mha residue are shown in Figure 1. COSY correlations were present for the H-3−H-2−H-4−H-5 spin system, interrupted by overlapping signals for H-5 and H-6 (δ 1.26). A COSY correlation between H-7 and δ 1.26 and HMBC signals between C-1−H-2/H-3, C-5−H-7/H-2, and C-6−H-7 established the Mha substructure.

The residue sequence for **1** was determined by HMBC and NOESY data. The connectivity between Mha and Pro-2 was established by NOESY cross-peaks between H-5 (δ 3.55/3.42) of Pro-2 and both H-2 (δ 2.43) and H-3 (δ 1.01) of Mha. The Pro-2 carbonyl carbon (δ 173.29) exhibited an HMBC correlation with the *N*Me (δ 2.88) of MePhe-2, indicating an amide linkage. In turn, H-2 of the latter residue (δ 5.01) showed a NOESY correlation with the NH signal of Gly-2 (δ 8.84). The H-2 protons (δ 4.09/4.37) and carbonyl (δ 170.34) of Gly-2 gave NOESY and HMBC correlations, respectively, with the *N*Me protons of MeVal (δ 3.01). At the same time, H-2 of MeVal (δ 4.61) correlated in the NOESY spectrum with the NH of the Ile residue (δ 6.62). The connection between Ile and Gly-1 was inferred by a weak HMBC correlation between an H-2 of Gly-1 (δ 4.17/3.84) and C-1 of Ile (δ 170.65), as well as by a NOESY correlation between NH of Gly-1 (δ 6.78) and H-2 of Ile (δ 4.35). Next, HMBC cross-peaks were observed between the *N*Me protons of MePhe (δ 2.97) and C-1 of Gly-1 (δ 168.02). Only a weak NOESY correlation was detected between MePhe (H-2, δ 5.56) and Pro-1 (H-5, δ 3.34), but this assignment was confirmed by the elimination of all other possible sequences. Last, the OMe protons (δ 3.71) showed a strong HMBC correlation with the carbonyl at δ 172.38, indicating the presence of a Me ester on Pro-1.

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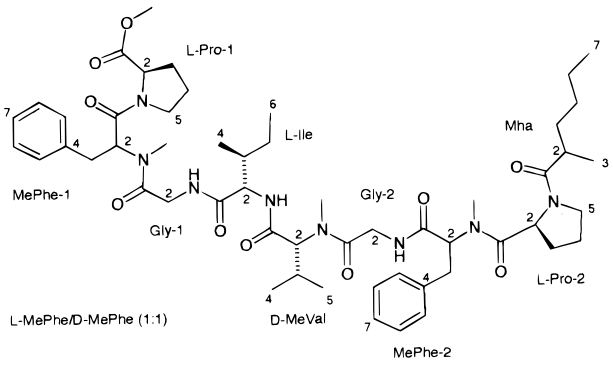
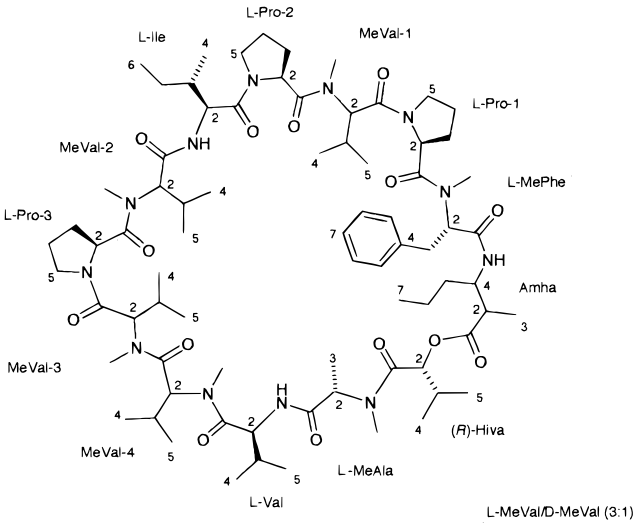


1 Malevamide A



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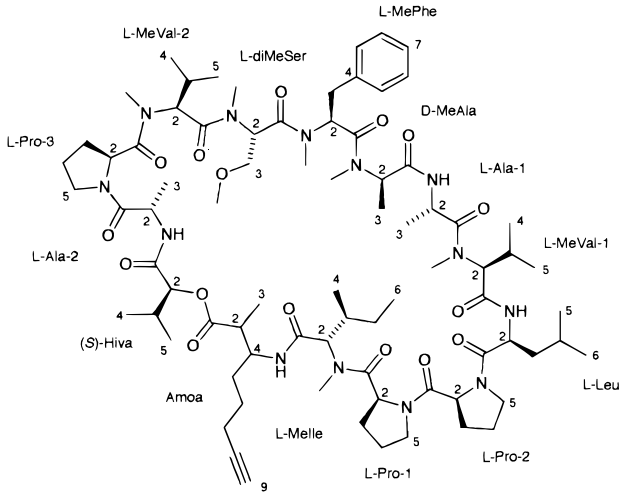

3 Malevamide C

The stereochemistry of the amino acid residues of malevamide A (**1**) was determined by chiral HPLC of the acid hydrolysate, which indicated the presence of L-Ile, D-MeVal, L-Pro, and both D- and L-MePhe (in a 1:1 ratio). The stereochemistry of Mha was not determined.

Malevamide B (**2**), also a peptide as suggested by its ^{1}H NMR spectrum, displayed a HRFABMS pseudomolecular ion that indicated a molecular formula of $C_{76}H_{124}N_{12}O_{14}$. The IR data indicated a depsipeptide (ester, 1728 cm^{-1}; amide, 1633−1659 cm^{-1}). Detailed analysis of ^{1}H and ^{13}C NMR, DEPT, COSY, TOCSY, HMQC, and HMBC NMR experiments ($CDCl_3$) proved the presence of a 3-amino-2-methylhexanoic acid residue (Amha), a 2-hydroxyisovaleric acid residue (Hiva), and 11 α-amino acid residues, including four MeVal, three Pro, and one each Ile, methylalanine (MeAla), MePhe, and valine (Val). Table 2 lists the NMR assignments of malevamide B (**2**) and selected HMBC correlations. The Amha substructure was evident from sequential COSY correlations between H-3−H-2−H-4(−NH)−H-5 and H-6−H-7, and HMBC cross-peaks between C-1−H-2/H-3, C-5−H-7, and C-6−H-7 (Figure 1).

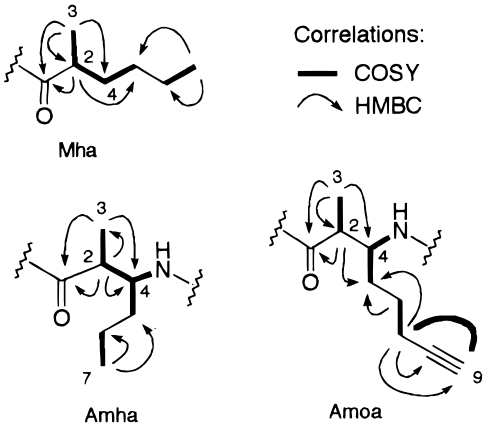

Figure 1. COSY and HMBC correlations for Mha, Amha, and Amoa residues, respectively, of malevamides A−C (**1**−**3**).

The residue sequence for **2** was determined by HMBC (in $CDCl_3$ and CD_3CN) and ROESY correlations (in $CDCl_3$). HMBC correlations were observed between the *N*Me group, NH group, and/or H-2 proton of a given residue and C-1 of its neighboring residue (Table 2) to establish the following three partial structures: (1) MeVal-3−MeVal-4−Val−MeAla−Hiva−Amha−MePhe−Pro-1; (2) MeVal-1−Pro-2; and (3) Ile−MeVal-2−Pro-3. ROESY correlations were present between the H-2 of MeVal-1 (δ 4.92) and H-5 of Pro-1 (δ 3.59/3.73) and between the equivalent signals of MeVal-3/Pro-3 (δ 5.04, 4.07/3.71) and Ile/Pro-2 (δ 4.59, 3.84/3.70). This established the gross structure as **2**.

Chiral HPLC analysis of **2** indicated the presence of L-Ile, L-MeAla, L-MePhe, D- and L-MeVal (1:3 ratio), L-Pro, L-Val, and (*R*)-Hiva. The stereochemistry of Amha was not determined.

The observed HRFABMS pseudomolecular ion for compound **3** agreed with the NMR data which suggested a compound with a molecular formula of $C_{79}H_{125}N_{13}O_{16}$ and contained signals consistent with an *N*-methylated peptide. The IR spectrum indicated both amide (1634, 1644 cm^{-1}) and ester (1738, 1732 cm^{-1}) functions. Analysis of ^{1}H, ^{13}C, DEPT, COSY, TOCSY, HMQC, and HMBC NMR (CD_3CN) experiments allowed the proton and carbon signals of **3** to be assigned to a Hiva residue and 12 α-amino acid residues, including three Pro, two alanines (Ala), two MeVal, *N*,*O*-dimethylserine (diMeSer), Leu, MeAla, *N*-methylisoleucine (MeIle), and MePhe. Additionally, a 3-amino-2-methyl-7-octynoic acid (Amoa) was proposed on the basis of COSY and HMBC correlations (Figure 1). Sequential COSY (CD_3CN) correlations were observed between H-3−H-2, H-4(−NH)−H-5, and H-6−H-7. These three fragments could be connected as a result of HMBC (CD_3CN) correlations between C-5−H-2/H-6/H-7 and C-4−H-3; the CD_2Cl_2 COSY spectrum displayed propargylic coupling between H-9 (δ 1.96 t, J = 2.5 Hz) and H-7 (δ 2.19 m). One-dimensional TOCSY experiments (CD_3CN)supported the Amoa assign-

Table 1. NMR Assignments of Malevamide A (**1**)[a]

residue	position	^{13}C	mult.	^{1}H	mult. (*J* value)	HMBC correlation to C	residue	position	^{13}C	mult.	^{1}H	mult. (*J* value)	HMBC correlation to C
Pro-1	1	172.38	0			H-2, H-3, OMe	MeVal	3	25.52	1	2.32	m	H-5
	2	58.94	1	4.39	m	H-3	(cont.)	4	18.48	3	0.851	d (6.5)	H-5
	3	28.81	2	2.13,1.86	m, m	H-2, H-4, H-5		5	19.71	3	0.97	d (6.5)	H-3, H-4
	4	24.99	2	1.82,1.93	m, m	H-3		NMe	30.08	3	3.01	s	H-2
	5	46.70	2	3.34	m	H-3	Gly-2	1	170.34	0			H-2, MeVal: NMe
	OMe	52.16	3	3.71	s			2	41.79	2	4.09,4.37	dd (5.0, 16.5), m	
MePhe-1	1	167.89	0			H-2		NH		1	8.84	br t (5.0)	
	2	56.34	1	5.56	dd (6.5, 8.5)	H-3, NMe	MePhe-2	1	170.11[b]	0			
	3	34.98	2	2.81,3.30	m, m	H-2		2	62.56	1	5.01	dd (3.0, 12.0)	NMe
	4	138.20[c]	0					3	34.04	2	3.23,3.00	dd (3.3, 14.3), m	
	5,5′	129.50[d]	1	7.1	m			4	137.23[c]	0			
	6,6′	128.75[e]	1	7.2	m			5,5′	129.43[d]	1	7.1	m	
	7	126.76[f]	1	7.2	m			6,6′	128.36[e]	1	7.2	m	
	NMe	29.67	3	2.97	s	H-2		7	126.60[f]	1	7.2	m	
Gly-1	1	168.02	0			H-2, MePhe-1: NMe		NMe	29.15	3	2.88	s	
	2	41.21	2	4.17	dd (5.0, 18.0)		Pro-2	1	173.29	0			H-3, MePhe-2: H-2, NMe
				3.84	dd (3.0, 17.5)			2	55.00	1	4.27	dd (5.5, 8.0)	
	NH		1	6.78	m			3	28.28	2	0.92,0.86	m, m	
Ile	1	170.65	0			H-2, Gly-2: H-2		4	25.16	2	2.00,1.60	m, m	
	2	57.90	1	4.35	dd (5.5, 8.0)	H-3		5	47.42	2	3.55,3.42	m, m	H-4
	3	36.24	1	2.02	m	H-2	Mha	1	175.46	0			H-2, H-3
	4	11.52	3	0.92	d (6.5)			2	37.56	1	2.43	m	H-3
	5	24.65	2	1.12,1.42	m, m	H-6		3	16.63	3	1.01	d (6.5)	
	6	15.77	3	0.90	d (5.0)			4	33.28	2	1.62,1.29	m, m	H-2, H-3
	NH		1	6.62	m			5	29.30	2	1.26	m	H-2, H-7
MeVal	1	170.01	0			H-2		6	22.61	2	1.26	m	H-7
	2	63.00	1	4.61	d (10.0)	H-4, H-5, NMe		7	13.99	3	0.852	t (6.5)	

[a] In $CDCl_3$; proton spectrum referenced to residual $CHCl_3$ (δ 7.26); carbon spectrum referenced to $CDCl_3$ (δ 77.00); spectra taken at 125 and 500 MHz for carbon and proton, respectively. Assignments based on ^{1}H, ^{13}C, DEPT, COSY, NOESY, HMQC, and HMBC experiments. HMBC optimized for 3 or 7 Hz. Coupling values given in Hz; chemical shift values given in ppm. [b] Assignments not confirmed by HMBC correlations. [c−f] Assignments may be reversed.

Table 2. NMR Assignments of Malevamide B (**2**)[a]

residue	position	^{13}C	mult.	^{1}H	mult. (*J* value)	HMBC correlation to C	residue	position	^{13}C	mult.	^{1}H	mult. (*J* value)	HMBC correlation to C
Amha	1	174.37	0			H-2, H-3; Hiva: H-2	MeVal-2	1	169.33	0			H-2; Ile: H-2[b], NH[b]
	2	42.16	1	2.95	qd (7.2, 3.9)			2	61.89	1	4.77	d (10.5)	H-4, H-5, NMe
	3	8.39	3	0.96	d (7.2)	H-2		3	26.06	1	2.26	m	H-2, H-4, H-5
	4	50.98	1	4.67	m	H-2, H-3		4	18.43	3	0.79	d (6.7)	
	5	30.07	2	1.46	m	H-7		5	20.36	3	1.03	d (6.1)	
	6	19.99	2	1.45	m	H-7		NMe	30.82	3	2.96	s	H-2
	7	13.78	3	0.97	t (7.5)		Pro-3	1	173.24	0			H-3; MeVal-2: H-2, NMe
	NH			8.55	d (8.4)			2	56.45	1	4.68	br t (6.7)	
MePhe	1	169.02	0			H-2; Amha: NH[b]		3	28.80	2	1.83,2.14	m, m	H-2
	2	62.75	1	4.85	m	H-3, NMe		4	25.28	2	1.84,2.10	m, m	
	3	34.08	2	3.18	dd (2.8, 14.4)	H-5,5′		5	47.65	2	4.07,3.71	m, m	
				3.09	dd (11.7, 14.9)		MeVal-3	1	169.14	0			H-2
	4	138.17	0					2	59.01	1	5.04	d (11.1)	H-4, H-5, NMe
	5,5′	129.48	1	7.13	d (7.2)			3	27.64	1	2.25	m	H-2, H-4, H-5
	6,6′	128.83	1	7.29	t (7.2)			4	18.24	3	0.73	d (6.7)	
	7	126.87	1	7.23	t (7.2)			5	18.89	3	0.97	d (7.8)	
	NMe	28.91	3	2.85	s			NMe	30.46	3	3.10	s	H-2
Pro-1	1	173.07	0			H-3; MePhe: H-2[b], NMe	MeVal-4	1	171.26	0			H-2; MeVal-3: H-2, NMe
	2	54.94	1	4.27	br t (6.9)			2	57.87	1	5.19	d (11.1)	H-3, NMe
	3	28.46	2	0.70,0.90	m, m			3	27.29	1	2.30		H-3, H-4, H-5
	4	25.28	2	1.51,1.93	m, m	H-3		4	17.90	3	0.77	d (6.7)	
	5	48.09	2	3.59,3.73	td (10.4, 7.7), m			5	19.68	3	0.81	d (4.4)	
MeVal-1	1	169.61	0			H-2		NMe	30.17	3	3.05	s	
	2	59.14	1	4.92	d (10.5)	H-4, H-5, NMe	Val	1	171.84	0			H-2; MeVal-4: H-2[b], NMe
	3	27.76	1	2.10	m	H-2, H-4		2	54.30	1	4.73	dd (6.7, 9.4)	H-4, H-5
	4	18.55	3	0.83	d (7.2)			3	30.17	1	2.00	m	H-2, H-4, H-5
	5	19.09	3	0.84	d (6.7)			4	17.41	3	0.89	d (6.7)	
	NMe	30.04	3	3.08	s	H-2		5	19.59	3	0.93	d (7.2)	
Pro-2	1	173.40	0			H-3; MeVal-1: H-2, NMe		NH			6.70	d (9.4)	
	2	56.25	1	4.85	m		MeAla	1	170.67	0			H-2, H-3; Val: H-2, NH[b]
	3	28.91	2	2.15,1.76	m, m			2	51.75	1	5.23	q (7.2)	H-3, NMe
	4	25.17	2	1.93,2.11	m, m			3	13.36	3	1.30	d (6.7)	
	5	47.37	2	3.84,3.70	m, m			NMe	30.52	3	3.04	s	
Ile	1	168.94	0			H-2	Hiva	1	171.26	0			H-2, H-3; MeAla: H-2, NMe
	2	54.73	1	4.59	t (9.2)	H-4		2	75.41	1	4.82	d (6.7)	H-3, H-4, H-5
	3	37.08	1	1.83	m	H-2, H-4, H-6		3	30.40	1	2.20	m	
	4	15.50	3	0.88	d (6.1)			4	18.24	3	1.01	d (7.2)	
	5	24.27	2	1.07,1.45	m, m	H-2, H-4, H-6		5	18.43	3	1.11	d (6.6)	
	6	10.80	3	0.82	t (7.5)								
	NH			6.96	d (10.0)								

[a] In $CDCl_3$; proton spectrum referenced to residual $CHCl_3$ (δ 7.26); carbon spectrum referenced to $CDCl_3$ (δ 77.00); spectra taken at 125 and 500 MHz for carbon and proton, respectively. Assignments based on ^{1}H, ^{13}C, DEPT, COSY, TOCSY, ROESY, HMQC, and HMBC experiments. Coupling values given in Hz; chemical shift values given in ppm. [b] Correlation observed in the spectrum taken in CD_3CN.

ment by detecting the continuous spin system [δ 2.71 qd (H-2), 1.19 d (H-3), 4.20 m (H-4), 1.38−1.58 m (H-5/H-6), 2.15 m (H-7), 2.13 br s (H-9), 6.43 d (NH)]. Table 3 lists full proton and carbon chemical shift assignments and important HMBC correlations for malevamide C (**3**).

The residue sequence for **3** was determined by HMBC correlations (in CD_2Cl_2 and CD_3CN) and ROESY correlations (in CD_2Cl_2). HMBC correlations (CD_3CN) between C-1 of a given residue with the NH group, *N*Me group, and/or H-2 of the adjacent residue (Table 3) supported the following two partial structures: (1) MeVal-1−Ala-1−MeAla−MePhe−diMeSer−MeVal-2−Pro-3 and (2) Ala-2−Hiva−Amoa−MeIle. An HMBC correlation in CD_2Cl_2 between the NH of Leu (δ 7.50) and the carbonyl carbon of MeVal-1 (δ 170.10) established the amide bond between the two residues. ROESY cross-peaks between C-5 protons of Pro-3 (δ 3.72/3.64) and the α-proton of Ala-2 (δ 4.62) is evidence of the connection between the two residues and connects the above two partial structures. Furthermore, ROESY correlations between *N*Me of MeIle (δ 3.05) and H-2 of Pro-1 (δ 4.86) suggest connectivity between the two residues. In turn, the α-hydrogen of Pro-2 (δ 4.56) correlated with H-5 of Pro-1 (δ 3.88/3.64). The amide bond between Leu and Pro-2 is indicated by a ROESY correlation between H-5 of Pro-2 (δ 3.68/3.56) and a proton resonating at δ 4.73−4.74 (CD_2Cl_2, Leu H-2, or Pro-3 H-2), which is assignable to Leu H-2 since the position of Pro-3 has already been established in a distant part of the molecule. This assignment fulfills the final degree of unsaturation required by the molecular formula and establishes the gross structure as a cyclic depsipeptide.

Chiral HPLC analysis showed the presence of L-Ala, L-diMeSer, L-Leu, D-MeAla, L-MeIle, L-MePhe, L-MeVal, L-Pro, and (*S*)-Hiva. The stereochemistry of Amoa was not determined. Interestingly, Hiva and MeAla of **3** have the opposite stereochemistry compared with the corresponding residues of **2**.

To confirm that the source of malevamides A−C (**1**−**3**) was *S. laete-viridis*, the organism was recollected from the original location, and *S. hydnoides* was carefully excluded. The sample was freeze-dried and extracted with CH_2Cl_2 and MeOH. Reversed-phase HPLC of the extracts showed peaks consistent with the malevamides A−C (**1**−**3**) standards. These peaks were collected, and ^{1}H NMR analysis confirmed the presence of these compounds. (The absence of **1**−**3** in *S. hydnoides* was indicated by a recollection from the same location and similar analysis of that species.)

Malevamides A−C (**1**−**3**) failed to inhibit 50% of cell growth of the following cell lines at the highest concentration tested (2 μg/mL): P-388 mouse lymphoma (ATCC CCL 46), A-549 human lung carcinoma (ATCC CCL8), and HT-29 human colon carcinoma (ATCC HTB 38) cell lines.

Malevamides A−C (**1**−**3**) contain several structural features that are common among cyanobacterial peptides, including *N*-methylation, β-amino acids, and α-hydroxy acids.[1] Such peptides appear to be frequently accumulated in herbivorous marine mollusks and their predators as suggested by the isolation of symplostatin 1 (a dolastatin 10 analogue) from *S. hydnoides*,[6] dolastatin 12 (another *Dolabella auricularia* isolate) from an assemblage of *Lyngbya majuscula*/*Schizothrix calcicola*,[11] and kulolide-1 from the mollusk *Philinopsis speciosa* and its prey *Stylocheilus longicaudus*.[12]

The isolation of malevamide C (**3**) from a cyanobacterium adds to the above premise. The unusual β-amino acid residue, Amoa, was reported once previously as a component of the symmetrical depsipeptide onchidin, isolated from a marine mollusk of the genus *Onchidium*.[13] An asymmetric synthesis of (2*S*,3*S*)- and (2*R*,3*R*)-3-amino-2-methyl-7-octynoic acid has been achieved.[14] Additionally, the 2-nor-analogue of Amoa is part of dolastatin 17 from *D. auricularia*,[15] and a number of 3-hydroxy-7-octynoic acid-containing peptides, with varying degrees of C-2 methylation, have been reported from *P. speciosa*[12] and *Onchidium* sp.[16] Similarity among these residues is suggestive of a common biosynthesis and supports the supposition that these compounds are obtained by the mollusks via their diets.

Experimental Section

General Experimental Procedures. NMR experiments were performed on either a General Electric GN Omega 500 spectrometer operating at 500 and 125 MHz or a Varian Unity INOVA 400 spectrometer operating at 400 and 100 MHz. HMQC and HMBC spectra were optimized for $J_{CH} = 142$ Hz and $^nJ_{CH} = 7$ or 3 Hz, respectively. COSY experiments were double quantum filtered. Optical rotations were measured on a JASCO DIP-370 polarimeter. HRFABMS were measured on a VG ZAB2SE mass spectrometer. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer.

Biological Material. Mixed samples of *S. laete-viridis* Gomont and *S. hydnoides* Kützing ex Gomont (containing primarily the former) were collected in waters adjacent to Ala Moana Beach Park (depth: 1−3 m) on November 3 (230 g wet wt), November 11 (35 g), and November 20 (60 g), 1997. The morphologically and microscopically similar organisms were identified by Dr. G. M. L. Patterson.

Extraction and Isolation. The fresh material from each collection was extracted exhaustively with MeOH, followed by removal of solvent under reduced pressure. The combined extracts (11.9 g, largely salt) were reconstituted with MeOH/H_2O and partitioned against hexanes. After removal of MeOH under reduced pressure, the aqueous phase was further partitioned against 1-BuOH. The hexanes and 1-BuOH dried residues (290 and 500 mg, respectively) were combined and subjected to normal-phase VLC (29 g Si gel, Fisher S733-1; 60 mL fritted glass funnel; sample adsorbed to 2 g Si gel). The sample was eluted with hexanes/$CHCl_3$ mixtures, $CHCl_3$, and $CHCl_3$/MeOH mixtures. The fractions eluting with $CHCl_3$/MeOH (98:2 to 92:8) were combined (157 mg) and eluted from a small Si gel (2 g, Fisher S733-1) column with EtOAc followed by $CHCl_3$/MeOH (90:10). The EtOAc eluate residue (76 mg) was chromatographed by normal-phase HPLC (Econosil, Alltech 6259; mobile phase: hexane/EtOAc mixtures and EtOAc); two broad overlapping peaks eluting with EtOAc were subjected to reversed-phase HPLC [Cosmosil, Waters H12288; mobile phase: MeOH/H_2O (70:30 and 90:10)], leading to the isolation of **2** (4.3 mg, 0.0013% of wet wt). The $CHCl_3$/MeOH (90:10) eluate residue (63 mg) from the small Si gel column was fractionated by reversed-phase HPLC (Cosmosil, Waters H12288), eluting with a MeOH/H_2O gradient (55−100% MeOH, 19 fractions collected). Further reversed-phase HPLC of fraction 16 [Cosmosil, Waters H12288; mobile phase: MeOH/H_2O (80:20)] yielded **3** (4.2 mg, 0.0013% of wet wt). Fraction 15 yielded **1** (1.3 mg, 0.00040% of wet wt) after subsequent reversed-phase HPLC [Cosmosil, Waters H12288; mobile phase: MeOH/H_2O (70:30)] followed by Sephadex LH-20 chromatography (1.5 × 104 cm column; mobile phase: MeOH). Prior to optical rotation determination and bioassay, compound **3** was further purified by Sephadex LH-20 using the same conditions.

Recollection and HPLC of *Symploca laete-viridis*. A sample of *S. laete-viridis* (97 g wet wt), free of *S. hydnoides*, was recollected from the same location on June 24, 1999. The sample was freeze-dried, ground with mortar and pestle, and extracted with CH_2Cl_2 followed by MeOH. The MeOH extract was partitioned between H_2O and 1-BuOH; the 1-BuOH phase was dried over anhydrous Na_2SO_4 and filtered. The CH_2Cl_2

Table 3. NMR Assignments of Malevamide C (**3**)[a]

residue	position	^{13}C	mult.	^{1}H	mult. (*J* value)	HMBC correlation to C	residue	position	^{13}C	mult.	^{1}H	mult. (*J* value)	HMBC correlation to C
Amoa	1	175.19	0			H-2, H-3; Hiva: H-2	Ala-1	3	18.90	3	1.16	d (6.8)	H-2
	2	44.78	1	2.71	qd (7.2, 2.8)	H-3	(cont.)	NH			6.87	d (7.3)	
	3	14.90	3	1.19	d (7.3)	H-2	MeAla	1	170.14	0			H-2; Ala-1: NH
	4	51.00	1	4.20	m	H-3		2	53.59	1	5.26	q (7.1)	H-3, NMe
	5	32.18	2	1.52	m	H-2, H-6, H-7		3	13.50	3	1.25	d (7.3)	H-2
	6	26.68	2	1.53,1.42	m			NMe	31.01	3	2.75	s	H-2
	7	18.74	2	2.15	m		MePhe	1	171.53	0			H-2; MeAla: H-2, NMe
	8	85.34	0			H-7[c]		2	54.55	1	5.92	dd (4.9, 11.0)	NMe
	9	70.35	1	2.13	br s	H-7[c]		3	35.55	2	3.12	dd (11.4, 14.5)	H-2
	NH			6.43	d (10.0)						2.94	dd (4.3, 14.5)	
MeIle	1	171.25	0			H-2; Amoa: NH		4	138.95	0			
	2	62.02	1	4.75	d (11.0)	NMe		5,5′	130.96	1	7.24	m	
	3	32.40	1	2.02	m	H-2		6,6′	129.60	1	7.27	m	
	4	16.63	3	0.89	d (7.3)	H-2		7	127.82	1	7.19	tt (1.1, 7.0)	
	5	25.40	2	1.38,0.93	m, m	H-4, H-6		NMe	30.84	3	2.99	s	H-2
	6	11.28	3	0.82	t (7.6)		diMeSer	1	169.96	0			H-2; MePhe: H-2, NMe
	NMe	31.81	3	3.01	s	H-2		2	54.50	1	6.27	dd (4.9, 9.5)	H-3, NMe
Pro-1	1	175.12	0			MeIle: H-2, NMe		3	70.51	2	3.42	dd (5.1, 10.5)	OMe
	2	57.29	1	4.82	dd (3.3, 8.2)						3.48	t (10.3)	
	3	30.21	2	2.05,1.68	m, m			NMe	30.12	3	2.08	s	
	4	26.36	2	1.90,2.12	m, m			OMe	60.27	3	3.25	s	
	5	48.46	2	3.75,3.58	m, m		MeVal-2	1	170.85	0			H-2; diMeSer: H-2, NMe
Pro-2	1	171.74[b]	0					2	59.83	1	4.86	d (10.5)	NMe
	2	58.79	1	4.52	dd (5.6, 8.3)			3	27.30	1	2.28	m	H-2, H-5
	3	29.52	2	2.09,1.71	m, m	H-2		4	19.13	3	0.72	d (6.8)	H-2
	4	26.36	2	1.89,2.06	m, m	H-2		5	20.86	3	0.83	d (6.6)	H-2
	5	48.21	2	3.63,3.52	m, m			NMe	31.59	3	2.85	s	
Leu	1	171.57[b]	0				Pro-3	1	172.97	0			H-2; MeVal-2: H-2, NMe
	2	58.26	1	4.72	td (10.5, 2.4)	NH		2	50.23	1	4.73	m	
	3	40.25	2	1.81,1.35	m, m	H-5, H-6		3	29.83	2	2.14,1.66	m, m	H-2, H-5
	4	26.31	1	1.57	m, m			4	25.69	2	1.93	m, m	H-2, H-5
	5	21.80	3	0.83	d (6.6)			5	48.34	2	3.70,3.57	m, m	
	6	23.75	3	0.87	d (6.6)		Ala-2	1	171.25	0			H-2, H-3
	NH			7.36	d (9.3)			2	47.73	1	4.56	quint (7.0)	H-3
MeVal-1	1	170.77	0			H-2; Leu: NH[c]		3	18.27	3	1.26	d (6.8)	H-2
	2	63.99	1	4.67	d (11.2)	NMe		NH			6.64	d (7.1)	
	3	27.18	1	2.14	m	H-2, H-4, H-5	Hiva	1	169.49	0			H-2; Ala-2: NH
	4	19.00	3	0.73	d (6.6)			2	78.48	1	5.02	d (4.2)	
	5	19.43	3	0.79	d (6.6)			3	31.69	1	2.23	m	
	NMe	30.92	3	3.00	s	H-2		4	17.35	3	0.91	d (6.8)	H-2, H-5
Ala-1	1	174.26	0			H-2, NH; MeVal-1: H-2, NMe		5	19.53	3	0.92	d (6.8)	H-2, H-4
	2	47.17	1	4.64	quint (6.8)	H-3							

[a] In CD_3CN; proton referenced to residual CHD_2CN(δ 1.93); carbon referenced to CD_3CN (δ 118.69); spectra taken at 125 and 500 MHz for carbon and proton, respectively, except one-dimensional TOCSY with gradients, which was taken at 400 MHz. Assignments based on ^{1}H, ^{13}C, DEPT, COSY, TOCSY, ROESY, HMQC, and HMBC experiments. Coupling values given in Hz; chemical shift values given in ppm. [b] Assignments may be reversed. [c] Correlation observed in the spectrum taken in CD_2Cl_2.

extract and 1-BuOH phase residues were subjected to reversed-phase HPLC [Cosmosil, Waters H12288; mobile phase: MeOH/H_2O/TFA (85:15:0.05)]. The peak eluting at 18 min clearly contained malevamides B (**2**) and C (**3**) based on ^{1}H NMR. The peak eluting at 10 min was further chromatographed [Cosmosil, Waters H12288; mobile phase: MeOH/H_2O (80:20)]. ^{1}H NMR of the broad peak eluting at 23 min revealed the presence of malevamide A (**1**).

Malevamide A (1): amorphous white solid; $C_{54}H_{80}N_8O_{10}$; $[\alpha]^{28}_D$ +25° (MeOH, *c* 0.13); $[\alpha]^{27}_D$ +26° ($CHCl_3$, *c* 0.13); UV λ max (log ϵ) 209 (4.48), 259 (2.55) nm; IR ν max (film on NaCl) 3483 br, 3395 br, 3306, 3062, 3029, 2962, 2933, 2874, 1746, 1659, 1651, 1644, 1634, 1538, 1455, 1287, 1200, 1175, 1094, 753, 702 cm^{-1}; HRFABMS *m*/*z* 1001.6122, MH^+ ($C_{54}H_{81}N_8O_{10}$ requires 1001.6075, Δ −4.7 mmu), ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 1.

Chiral HPLC Analysis of Hydrolysate of 1. Malevamide A (**1**, 100 μg) was hydrolyzed in 6 N HCl at 109 °C for 20 h, then dried under a stream of N_2 and further dried under vacuum. The hydrolysate was eluted from a C_{18} Sep-Pak (Waters 20805) column using MeOH/H_2O (10:90). The eluate was dried under vacuum and reconstituted with 100 μL of H_2O prior to analysis [Chirex (D) Penicillamine, Phenomenex 00G-3126-E0, 4.6 × 250 mm; detection: UV 245 nm; injected amount 7 nmol; mobile phase I: 1.7 mM $CuSO_4$ in MeCN/H_2O (14:86), flow rate 0.8 mL/min; mobile phase II: 1.9 mM $CuSO_4$ in MeCN/H_2O (5:95), flow rate 1.0 mL/min; mobile phase III: 2.0 mM $CuSO_4$ in H_2O, flow rate 1.0 mL/min]. The hydrolysate was chromatographed alone and co-injected with standards to confirm assignments. The following standards coeluted with peaks from the hydrolysate: Gly, L- and D-MePhe (1:1 ratio), L-Pro, D-MeVal, and L-Ile. A peak corresponding to D-Pro integrated to 13% of the L-Pro peak; the presence and predominance of l-Pro was therefore confirmed by chiral GC/MS using established methods.[17] Mobile phase I elution times: Gly (5.6 min), L-MePhe (41.5 min), D-MePhe (46.5 min). Mobile phase II elution times: L-Pro (10.7 min), L-MeVal (12.1 min), D-MeVal (16.8 min), D-Pro (21.9 min), L-*allo*-Ile (35.4 min), L-Ile (41.8 min), D-*allo*-Ile (45.6 min), D-Ile (55.4 min).

Malevamide B (2): amorphous white solid; $C_{76}H_{124}N_{12}O_{14}$; $[\alpha]^{28}_D$ −193° (MeOH, *c* 0.19); $[\alpha]^{27}_D$ −178° ($CHCl_3$, *c* 0.19); UV λ max (log ϵ) 208 (4.72), 236 sh (3.98) nm; IR ν max (film on NaCl) 3492 br, 3387 br, 3296, 3062, 3030, 2963, 2934, 2875, 1728, 1659, 1651, 1644, 1633, 1470, 1462, 1454, 1445, 1409, 1294, 1203, 1120, 1091, 703, 640 cm^{-1}; HRFABMS *m*/*z* 1429.9357, MH^+ ($C_{76}H_{125}N_{12}O_{14}$ requires 1429.9437, Δ +8.0 mmu), ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 2.

Chiral HPLC Analysis of Hydrolysate of 2. The malevamide B (**2**, 190 μg) hydrosylate (6 N HCl, 190 °C, 15 h) was worked up and analyzed as described above for the malevamide A hydrolysate. The following residues coeluted with malevamide B hydrolysate peaks: L-MePhe, (*R*)-Hiva, D-MeAla, L-Pro, L- and D-MeVal (3:1 ratio), L-Val, L-Ile. Mobile phase I elution times: L-MePhe (42.6 min), D-MePhe (48.3 min), (*S*)-Hiva (52.8 min), (*R*)-Hiva (83.2 min). Mobile phase II elution times: L-MeAla (7.9 min), D-MeAla (8.3 min), L-Pro (10.4 min), L-MeVal (11.6 min), D-MeVal (16.2 min), L-Val (18.1 min), D-Pro (20.7 min), D-Val (24.1 min), L-*allo*-Ile (37.4 min), L-Ile (44.1 min), D-*allo*-Ile (48.3 min), D-Ile (59.0 min).

Malevamide C (3): amorphous white solid; $C_{79}H_{125}N_{13}O_{16}$; $[\alpha]^{28}_D$ −199° (MeOH, *c* 0.15); $[\alpha]^{27}_D$ −198° ($CHCl_3$, *c* 0.15); UV λ max (log ϵ) 207 (4.72), 236 sh (3.97) nm; IR ν max (film on NaCl) 3480, 3401, 3343, 3084, 3062, 2962, 2933, 2874, 2115 (weak), 1738, 1732, 1644, 1634, 1454, 1411, 1266, 1204, 1167, 1124, 1102, 701, 638 cm^{-1}; HFABMS *m*/*z* 1512.9377, MH^+ ($C_{79}H_{126}N_{13}O_{16}$ requires 1512.9446, Δ +6.9 mmu), ^{1}H and ^{13}C NMR data (CD_3CN), see Table 3.

Chiral HPLC Analysis of Hydrolysate of 3. The malevamide C (**3**, 200 μg) hydrosylate (6 N HCl, 190 °C, 15 h) was worked up and analyzed as described above for the malevamide A hydrolysate. The following residues coeluted with components of the malevamide C hydrolysate: L-Leu, L-MePhe, (*S*)-Hiva, L-Ala, D-MeAla, L-Pro, L-MeVal, L-diMeSer, and L-MeIle. Mobile phase I elution times: L-Leu (20.8 min), D-Leu (22.6 min), L-MePhe (42.4 min), D-MePhe (47.2 min), (*S*)-Hiva (50.9 min), (*R*)-Hiva (81.6 min). Mobile phase II elution times: L-Ala (7.2 min), L-MeAla (7.8 min), D-MeAla (8.2 min), D-Ala (8.7 min), L-Pro (10.3 min), L-MeVal (12.6 min), D-diMeSer (13.3 min), L-diMeSer (14.0 min), D-MeVal (17.7 min), D-Pro (20.5 min), L-*allo*-MeIle (27.1 min), L-MeIle (28.5 min), D-*allo*-MeIle (42.5 min), and D-MeIle (42.5 min). Mobile phase III elution times: L-MeAla (15.3 min) and D-MeAla (16.1 min).

***N*-Methyl Amino Acid Standards.** *N*-Methyl amino acid standards were obtained commercially (Sigma: DL-MeAla, L-MeAla, D- and L-MePhe, DL-MeVal; Schweizerhall: L-MeVal) except for the isomers of diMeSer and MeIle, which were prepared from *N*-benzyloxycarbonyl-Ser and *N*-benzyloxycarbonyl-Ile, respectively, by an established method.[18]

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

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