Isolation and Structure Determination of Malevamide E, a Dolastatin 14 Analogue, from the Marine Cyanobacterium *Symploca laete-viridis*[⊥]

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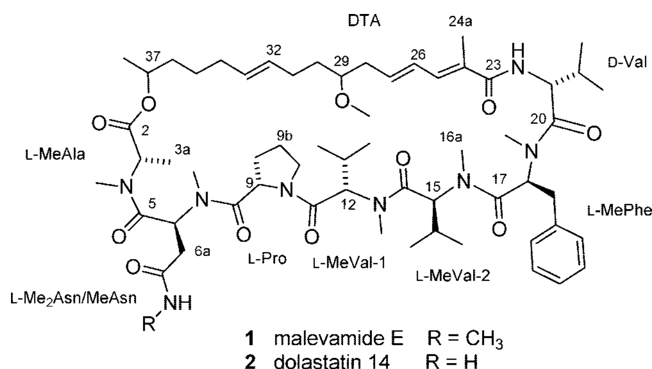
A new depsipeptide, malevamide E (**1**), was isolated from field-collected colonies of the filamentous cyanobacterium *Symploca laete-viridis*. The gross structure of **1** was determined by spectroscopic analyses, including one- and two-dimensional NMR and accurately measured MS/MS. Chiral HPLC analyses of an acid hydrolysate of **1** allowed the stereochemical assignments of its amino acid residues, which include *N*-methyl-L-alanine, α-*N*,γ-*N*-dimethyl-L-asparagine, *N*-methyl-L-phenylalanine, L-proline, D-valine, and *N*-methyl-L-valine. LC-MS/MS analysis of *S. laete-viridis* fractions established the co-occurrence of malevamide E (**1**) and its homologue dolastatin 14 (**2**), which was previously reported in low yield from the sea hare *Dolabella auricularia*. Malevamide E (**1**) demonstrated a dose-dependent (2–45 μM) inhibition of store-operated Ca^{2+} entry in thapsigargin-treated human embryonic kidney (HEK) cells, indicating an inhibitory effect on Ca^{2+} release-activated Ca^{2+} (CRAC) channels.

Cyanobacteria belonging to the genus *Symploca* (Oscillatoriaceae) have recently emerged as productive sources of structurally interesting and biologically active natural products. Notably, extracts of *Symploca* species have yielded an impressive array of new nonribosomally synthesized peptides[1] including symplostatins 1–3,[2] malevamides A−D,[3] tasiamides A and B,[4] tasipeptins A and B,[5] micromide,[6] belamide A,[7] and, most recently, symplocamide A.[8] Several of these compounds are close analogues of the dolastatins, which were first reported from the sea hare *Dolabella auricularia*.[9] In fact, dolastatin 10,[10] one of two *D. auricularia* isolates to reach phase II clinical trials as an investigational anticancer agent,[11] was more recently isolated from a *Symploca* species.[12] The cyanobacterial origin of sea hare isolates (especially from *Lyngbya* and *Symploca* spp.) has been reviewed by Luesch and co-workers.[13] However, the biological source has yet to be identified for many sea hare isolates that carry chemical signatures that suggest microbial origin. This is exemplified by dolastatin 14 (**2**), a highly cytotoxic (nanomolar range) mixed polyketide-peptide isolated by Pettit and co-workers[14] in exceptionally low yield from *D. auricularia*. Since the first report of dolastatin 14 (**2**), there has been considerable interest in its full synthesis, whereas all four diastereomers of its polyketide-derived subunit, dolatrienoic acid (15-hydroxy-7-methoxy-2-methyl-2*E*,4*E*,10*E*-hexadecatrienoic acid), have been synthesized as part of efforts toward the full synthesis of **2** by two independent groups.[15] To date, neither the total synthesis nor the stereochemical configuration of dolastatin 14 (**2**) has been published. Herein, we report the isolation, structure determination, partial stereochemical assignments, and ion channel inhibitory activity of malevamide E (**1**), an *N*-methylated analogue of dolastatin 14 (**2**), and the co-occurrence of **1** and **2** in an extract of *Symploca laete-viridis* Gomont collected in Hawaiian waters.

Results and Discussion

A freeze-dried sample of *S. laete-viridis* was extracted sequentially with CH_2Cl_2 and MeOH. Following partition of the MeOH



extract between hexane and 1-BuOH, the 1-BuOH and CH_2Cl_2 residues were combined and fractionated by repeated reversed-phase HPLC, leading to the isolation of the new depsipeptide malevamide E (**1**, 0.50 mg).[16]

Compound **1** gave a protonated molecular ion of *m/z* 1103.7145 in ESITOFMS. When interpreted in conjunction with the NMR data, the accurate mass suggested a molecular formula of $C_{60}H_{94}N_8O_{11}$ (Δ +2.2 ppm), and the isotopic peak abundance of the protonated molecular ion was consistent with predicted values (data not shown). The presence of residues of the following amino acids and 16-carbon unit were determined by interpretation of ^{1}H, ^{13}C, COSY, 1D-TOCSY, HMQC, and HMBC NMR experiments: *N*-methylalanine (MeAla), α-*N*,γ-*N*-dimethylasparagine (Me_2Asn), *N*-methylphenylalanine (MePhe), proline (Pro), valine (Val), *N*-methylvaline (MeVal; ×2), and 15-hydroxy-7-methoxy-2-methyl-2*E*,4*E*,10*E*-hexadecatrienoic acid (dolatrienoic acid, DTA). Table 1 lists the carbon and proton chemical shift assignments and HMBC and ROESY correlations for malevamide E (**1**).

The residue sequence of **1** was determined by analysis of HMBC and ROESY data. HMBC correlations of *N*-methyl hydrogens and α-hydrogens with carbonyl carbons of the neighboring *N*-terminal residue established the partial sequences Val-MePhe-MeVal-MeVal and Me_2Asn-MeAla. Correlations between C-23 (carbonyl of DTA) and both H-22 (NH of Val) and H-24a (α-methyl of DTA) were used to place the position of DTA on the *N*-terminus of Val. The position of Pro between MeVal-1 and Me_2Asn was determined by considering ROESY cross-peaks between H-12 (α-hydrogen of MeVal-1) and both H-9c protons (δ-hydrogens of Pro), along with ROESY correlations between H-9 (α-hydrogen of Pro) and H-7a (α-*N*-methyl of Me_2Asn). The ester linkage between C-2 and C-37

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Table 1. NMR Assignments of Malevamide E (**1**) in $CDCl_3$[a]

residue	position	δ_c	*m*	δ_H	mult. (*J* values)	HMBC[c]	ROESY
MeAla	2	170.7	0			H-3, H-3a	
	3	55.6	1	4.87	q (8.0)	H-4a	H-6
	3a	16.0	3	1.48	d (7.3)	H-3	H-4a
	4a	29.8	3	2.78	s		H-3a
Me_2Asn	5	169.3	0			H-4a, H-6, H-6a	
	6	49.1	1	5.87	dd (4.8, 9.2)	H-7a	H-3
	6a	35.6	2	2.98, 2.39	m, dd (4.4, 14.6)		
	6b	170.2	0			H-6a, H-6d	
	6c			5.61	br s		
	6d	26.3	3	2.75	d (5.0)		
	7a	30.2	3	2.95	s		H-9
Pro	8	171.0	0			H-7a	
	9	57.1	1	4.72	m		H-7a
	9a	28.3	2	2.01, 1.71	m, m		
	9b	24.6	2	1.75, 1.85	m, m		
	9c	47.3	2	3.58, 3.77	m, m		H-12
MeVal-1	11	168.6	0			H-12	
	12	59.8	1	4.89	d (10.6)	H-12b, H-12c, H-13a	H-9c, H-12b, H-12c
	12a	27.0	1	2.09	m	H-12b, H-12c	H-13a
	12b	18.0	3	0.50	d (6.6)	H-12c	H-12, H-12c
	12c	19.7	3	0.93	d (6.2)	H-12b	H-12, H-12b
	13a	30.1	3	2.66	s	H-12	H-12a, H-15
MeVal-2	14	170.3	0			H-12, H-13a, H-15	
	15	58.5	1	5.01	d (10.6)	H-15b, H-15c, H-16a	H-13a, H-15b
	15a	27.5	1	2.17		H-15, H-15b, H-15c	H-16a
	15b	18.3	3	0.77	d (6.6)	H-15c	H-15
	15c	19.5	3	0.78	d (6.6)	H-15, H-15b	
	16a	29.6	3	2.68	s	H-15	H-15a, H-18
MePhe	17	169.2	0			H-15, H-16a, H-18, H18a	
	18	54.9	1	5.76	dd (7.3, 8.1)	H-18a, H-19a	H-16a
	18a	35.5	2	2.83, 3.28	dd (6.8, 13.8), dd (8.8, 13.7)		
	18b	136.9	0			H-18a, H-18d,d′	
	18c,c′	129.6	1	7.25	br d (6.6)	H-18a, H-18e	
	18d,d′	128.3	1	7.20	br t (7.3)		
	18e	126.6	1	7.14	br t (7.0)	H-18c,c′	
	19a	30.8	3	3.13	s	H-18	H-21
Val	20	172.7	0			H-18, H-19a, H-21	
	21	54.8	1	4.68	t (7.0)	H-21b, H-21c	H-19a, H-21b, H-21c
	21a	30.5	1	1.94	m	H-21b, H-21c	
	21b	18.9	3	0.68	d (6.6)	H-21c	H-21, H-21c
	21c	18.5	3	0.90	d (6.6)	H-21b	H-21, H-21b
	22			6.02	d (9.9)		H-24a
DTA	23	169.2	0			H-22, H-24a, H-25	
	24	127.8	0			H-24a	
	24a	12.7	3	1.88	br s	H-25	H-22, H-26
	25	134.6	1	6.73	d (12.1)	H-24a	H-27
	26	127.5	1	6.30	dd (11.2, 14.8)	H-28	H-24a
	27	137.5	1	5.96	m	H-28	H-25
	28	36.8	2	2.48, 2.31	m, m		
	29	79.6	1	3.25	m	H-29a	
	29a	56.8	3	3.35	s		
	30	34.1	2	1.47, 1.53	m		
	31	29.3	2	2.05	m, m	H-32	
	32	130.7	1	5.39	m[b]	H-30, H-31, H-34	
	33	130.1	1	5.37	m[b]	H-31, H-34, H-35	
	34	32.8	2	1.95	m		
	35	26.2	2	1.27	m		
	36	35.3	2	1.44, 1.52	m, m	H-37a	
	37	72.7	1	4.78	m	H-37a	
	37a	19.7	3	1.19	d (6.2)		

[a] Assignments based on ^1H, ^{13}C, DEPT, COSY, HMQC, and HMBC NMR (125/500 MHz) experiments at room temperature; proton spectrum referenced to residual C*H*Cl_3 (δ 7.24); carbon spectrum referenced to *C*DCl_3 (δ 77.0). [b] Signals for H-32 and H-33 are better resolved in C_6D_6 and show a coupling constant of 15 Hz. [c] Correlation from carbon to indicated hydrogen.

is not indicated by HMBC or ROESY data, but the occurrence of an ester not only fulfills the balance of double-bond equivalents but is consistent with the chemical shifts of C-2 (δ_C 170.7) and H-37 (δ_H 4.78) and was supported by the ester carbonyl peak in the IR spectrum (1730 cm^{-1}). The assigned gross structure is a *N*-methyl analogue of dolastatin 14 (**2**), and the similarity was confirmed by comparison of ^{13}C NMR chemical shifts of **1** with those of **2**[14] (in $CDCl_3$ for **1** and CD_2Cl_2 for **2**), where all chemical shifts are within 0.8 ppm (averaging −0.3 ppm), with the exception of C-6b (Δ −1.9 ppm) and the presence of an additional *N*-methyl carbon signal in **1** (δ_c 26.3), both in keeping with the presence of a γ-*N*-methyl group.

The residue sequence of **1** was supported by ESITOFMS/MS analysis. Accurate masses were determined for product ions from collisionally activated dissociation (CAD) of the protonated molecular ions. Fragments were assigned on the basis of predictable cleavage sites where expected fragment masses agreed with experimental accurate mass measurements (Figure 1). Table 2 shows the measured mass, fragment assignment, and the mass accuracy for each product ion. As mentioned above, the cyclization of the

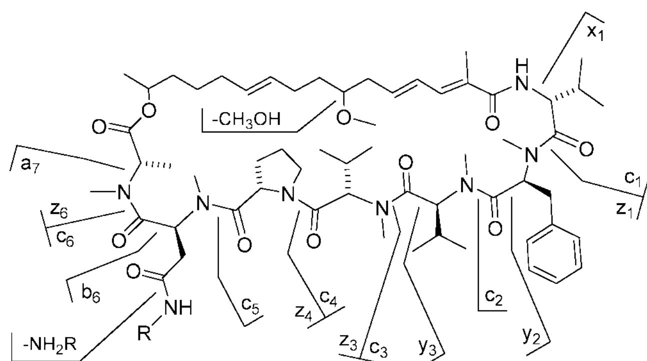

Figure 1. Cleavage sites for MS/MS product ions of malevamide E (**1**, R = CH_3) and dolastatin 14 (**2**, R = H).

molecule via an ester bond was not directly detected by NMR, but is supported by the MS/MS fragment c1/z6 (and c1/z6-MeOH). At the same time, the position assigned to Pro was confirmed by the overlapping fragments c6/z1 and c4/z1, which restrict the position of the Pro to one of two positions between Ala and MeVal-1, while its placement next to MeVal-1 was indicated by fragment c5/z3. Taken together, the assigned fragments confirm the entire amino acid/fatty acid sequence of **1**.

The structural similarity of malevamide E (**1**) and dolastatin 14 (**2**) suggests a common biosynthetic origin. In order to determine whether dolastatin 14 (**2**) was produced by the present local field-collected strain of *S. laete-viridis*, the parent fraction of **1** was subjected to LC-MS/MS analysis. In fact, a compound with an accurate mass consistent with that of dolastatin 14 (**2**) (*m/z* 1089.7017, Δ +5.3 ppm) eluted shortly before malevamide E (**1**). The pattern of CAD fragmentation of **2** was nearly identical to that of malevamide E (**1**) and demonstrated several fragments that were 14 mass units less than corresponding peaks for **1**, as expected for fragments that differ by a NH_2 versus a NHMe group on the asparagine (Asn) side chain (see Table 2). Together, the fragments c6/z4 and $M^+ - NH_2/M^+ -$ NHMe confirmed that the difference in the two molecules lies with the Asn residue at the γ-position. These data clearly demonstrate that dolastatin 14 (**2**) co-occurs with malevamide E (**1**) in *S. laete-viridis*. Based on integration of selected ion chromatograms, the molar ratio between **1** and **2** in the sample is 13:1, indicating a dolastatin 14 (**2**) yield of 4×10^{-5}% (wet wt) in *S. laete-viridis* compared with 1.2×10^{-7}% (wet wt) in *D. auricularia*.[14]

The configuration and conformation of the double and amide bonds of **1** were determined by ROESY correlations and coupling constants. A coupling constant of 15 Hz between olefinic hydrogens H-26 and H-27 indicated an *E*-configuration for Δ^{26}. A ROESY correlation between the olefinic methyl hydrogens H-24a and the olefinic hydrogen H-26 suggested both an *E*-configuration for Δ^{24} and an expected *s-trans*-conformation of the 24,26-diene. Furthermore, the ROESY resonance correlating the olefinic methyl H-24a with the NH of Val (H-22) requires both an *s-cis*-conformation between the C-23 carbonyl and Δ^{24} and *trans*-conformation of the C-23/N-22 amide bond. An *E*-configuration of Δ^{32} was assigned on the basis of the coupling constant between H-32 and H-33 (J = 15 Hz) as observed in C_6D_6, a solvent in which the signals were better resolved.[17] Finally, all amide bonds in the sequence Val-MePhe-MeVal-MeVal-Pro-Me_2Asn are *trans*, as indicated by ROESY cross-peaks between the α-hydrogen of each residue with the *N*-methyl protons (or H-9c protons for Pro) of the adjacent C-terminal amide, while a correlation between H-3 (α-hydrogen of MeAla) and H-6 (α-hydrogen of Me_2Asn) requires a *cis*-conformation of the C-5/N-4 amide bond.

The configurations of the amino acid residues of **1** were determined by chiral ligand-exchange HPLC of the acid hydrolysate of **1**, which indicated the presence of D-Val, L-Pro, L-MeAla, L-MeVal, L-MePhe, and L-*N*-methylaspartic acid (the acid hydrolysis product expected for L-Me_2Asn). The similarity between the optical rotations[14] and ^{13}C NMR chemical shifts of malevamide E (**1**) and dolastatin 14 (**2**) and their co-occurrence in *S. laete-viridis* strongly suggest that both compounds share the same stereochemical configurations. Contrary to what was previously suggested,[14] dolastatin 14 (**2**) therefore contains a D-amino acid, a common feature among cyanobacterial depsipeptides.[13]

The co-occurrence of malevamide E (**1**) and dolastatin 14 (**2**) in *S. laete-viridis* and the apparent predominance of dolastatin 14 (**2**) in *D. auricularia* are interesting. Neither compound is a plausible isolation artifact of the other, while partial metabolism of malevamide E (**1**) in the gut or other tissues of the grazing sea hare is unlikely to yield dolastatin 14 (**2**), but rather its aspartic acid analogue if the γ-amide of Asn were preferentially hydrolyzed. We speculate that methylation of the γ-amide of Asn occurs either on the Asn precursor prior to the core biosynthesis of malevamide E (**1**) or by direct methylation of dolastatin 14 (**2**). In either case, the relative yields of **1** and **2** would likely be controlled independent of the core biosynthesis: in the first case by the pool of α-*N*,γ-*N*-dimethylasparagine/α-*N*-methylasparagine and in the second by the expression and activity of a peptidyl-asparagine methylase. The latter is an attractive hypothesis since peptidyl-asparagine methylases are known in cyanobacteria.[18]

The Me_2Asn residue of malevamide E (**1**) is an unusual amino acid. Although α-*N*-methylation of amino acids is common in cyanobacterial nonribosomal peptides, γ-*N*-methylation of Asn is rare. Notably, methylation of the γ-*N*-position of Asn is a known conserved post-translational modification of phycobiliproteins in cyanobacteria and red algae[18] and has been reported for Asn residues of polytheonamides A and B from the sponge *Theonella swinhoei*.[19] Methylation at both α-*N*- and γ-*N*-positions of Asn, however, appears to be unprecedented in nature.

As part of a screening effort to find cellular targets of marine extracts and isolates, we tested malevamide E (**1**) in a number of ion-channel specific bioassays. As a result we found that malevamide E (**1**) had a dose-dependent (2–45 μM) inhibitory effect on the store-operated Ca^{2+} entry in thapsigargin-treated human embryonic kidney (HEK) cells (Figure 2). Store-operated Ca^{2+} entry or Ca^{2+} release-activated Ca^{2+} (CRAC) channels[20] allow the entry of extracellular Ca^{2+} in order to refill depleted intracellular Ca^{2+} stores. Depletion of these stores is either the consequence of physiological stimuli (e.g., receptor-regulated Ca^{2+} release) or, as in our bioassay, the blockade of the sarco-endoplasmic reticulum Ca^{2+}-ATPases (SERCA) with a selective inhibitor like thapsigargin.[21] Although the concept of store-operated Ca^{2+} channels has long been established,[20,22] only recently have the molecular components mediating this conductance been identified as members of the Stim and Orai/CRACM gene families.[23] Identifying malevamide E (**1**) as a possible modulator of these channels adds a new chemical entity to a small number of known CRAC channel inhibitors.[24]

Experimental Section

General Experimental Procedures. The optical rotation was measured on a JASCO DIP-370 polarimeter. The UV spectrum was recorded on a Perkin-Elmer Lambda EZ 210 spectrophotometer. The IR spectrum was recorded on a Perkin-Elmer Spectrum RX1 FTIR spectrometer. The NMR experiments were performed on a General Electric GN Omega 500 spectrometer operating at 500 and 125 MHz, for ^{1}H and ^{13}C, respectively, or a Varian Unity INOVA 400 spectrometer operating at 400/100 MHz. Samples were dissolved in $CDCl_3$ or C_6D_6. HMQC and HMBC spectra were optimized for J_{CH} = 142 Hz and $^nJ_{CH}$ = 7 Hz, respectively. COSY experiments were double quantum filtered. Mass spectra (MS and MS^2) were measured by ESITOF on a Micromass QTOF API US or by ESI-ion trap on a Thermo Finnigan LCQ Deca XP Max.

Biological Material. A sample of *S. laete-viridis* Gomont (97 g wet wt) was collected at a depth of 1–3 m from coastal waters of Oahu

Table 2. Identified MS/MS Product Ions of Malevamide E (**1**) and Dolastatin 14 (**2**)

exptl (**1**)[a]	exptl (**2**)[a]	assigned sequence[b]	molecular formula	calcd exact mass	MMA[c] (ppm)
1103.7145		M + H	$C_{60}H_{95}N_8O_{11}$	1103.7120	+2.3/ −
	1089.7017	M + H	$C_{59}H_{93}N_8O_{11}$	1089.6964	− /+4.9
1072.6697	1072.6659	M − NH_2R	$C_{59}H_{90}N_7O_{11}$	1072.6698	−0.1/−3.6
627.3870		c6/z1	$C_{33}H_{51}N_6O_6$	627.3870	0.0/ −
	613.3691	c6/z1	$C_{32}H_{49}N_6O_6$	613.3714	− /−3.7
477.3303	477.3316	c1/z6	$C_{27}H_{45}N_2O_5$	477.3328	−5.2/−2.5
445.3054	445.3039	c1/z6 − CH_3OH	$C_{26}H_{41}N_2O_4$	445.3066	−2.7/−6.1
388.2600	388.2600	c4/z1	$C_{22}H_{34}N_3O_3$	388.2600	0.0/0.0
353.2209		c6/z3	$C_{17}H_{29}N_4O_4$	353.2189	+5.7/ −
	339.2060	c6/z3	$C_{16}H_{27}N_4O_4$	339.2032	− /+8.3
275.1773	275.1783	c3/z1	$C_{16}H_{23}N_2O_2$	275.1760	+4.7/+8.4
255.1703	255.1708	c4/y2	$C_{13}H_{23}N_2O_3$	255.1709	−2.4/−0.4
244.1334	244.1334	c2/x1 − H_2	$C_{15}H_{18}NO_2$	244.1338	−1.6/−1.6
240.1332	240.1336	a7/z4 − NH_2R	$C_{11}H_{18}N_3O_3$	240.1348	−6.7/−5.0
240.1332		c6/z4	$C_{11}H_{18}N_3O_3$	240.1348	−6.7/ −
	226.1181	c6/z4	$C_{10}H_{16}N_3O_3$	226.1192	− /−4.9
211.1436	211.1433	c5/z3	$C_{11}H_{19}N_2O_2$	211.1447	−5.2/−6.6
142.0863	142.0866	c4/y3 and/or c3/y2	$C_7H_{12}NO_2$	142.0868	−3.5/−1.4

[a] Measured accurate mass of protonated molecule and product ions from collisionally activated dissociation. [b] Nomenclature based on Tuinman and Pettit.[26] Observed product ions are protonated. R = CH_3 and H for **1** and **2**, respectively. [c] Mass measurement accuracy for **1** and **2**, respectively.

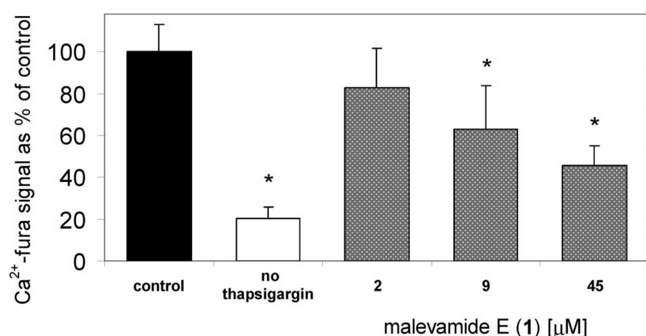

Figure 2. Malevamide E (**1**) attenuates the CRAC-mediated rise of intracellular [Ca^{2+}] in a dose-dependent manner. Depletion of intracellular Ca^{2+} stores in a Ca^{2+}-free KRH and in the presence of 2 μM thapsigargin results in the activation of CRAC. Upon addition of 4 mM Ca^{2+}, CRAC activity can be visualized as the rapid increase of Ca^{2+}-fura-2 fluorescence. The change of the intracellular [Ca^{2+}] is quantified as the change of the ratio of Ca^{2+}-bound over Ca^{2+}-free fura-2. Preincubation of the cells with malevamide E (**1**) attenuates the rise of the Ca^{2+}-fura-2 signal in a dose-dependent manner. Omitting thapsigargin results in the almost complete suppression of Ca^{2+}-influx and serves as a baseline for our assay. The bar graphs represent mean values ± StDev from 2 to 3 independent experiments with duplicate or triplicate measurements for each condition. Data are normalized to the rise of the Ca^{2+}-fura-2 signal under control conditions (see Experimental Section). The data for 9 and 45 μM malevamide E (**1**), as well as for "no thapsigargin", are significantly different from controls with *P*-values of >99.9% (*) as determined by Student's *t* test. The data for 2 μM malevamide E (**1**) show a trend, but do not reach significance (*P*-value is <95%).

near Ala Moana Beach Park on June 24, 1999. Colonies, which grow in indirect light on overhanging rocky substrate, were collected by hand. A dried voucher specimen (080104-ALA-2) is on file in the College of Natural Sciences, Hawaii Pacific University.

Extraction and Isolation. The sample was freeze-dried, ground, and extracted sequentially with CH_2Cl_2 and MeOH. The MeOH extract was partitioned between hexane and 1-BuOH. The 1-BuOH (185 mg) and CH_2Cl_2 (60 mg) residues were combined and fractionated in multiple portions by reversed-phase HPLC [Phenomenex Luna C_{18}(2), 10 μm, 21.2 × 250 mm; mobile phase: MeOH/H_2O (90:10); flow rate: 7.5 mL/min; UV detection: 230 nm]. LC-MS analysis of resulting fractions [Phenomenex Luna C_{18}(2), 5 μm, 2.0 × 250 mm; mobile phase: MeCN/H_2O gradient with 0.1% formic acid; flow rate: 0.25 mL/min; ESI-ion trap MS detection] indicated the presence of a compound with a molecular weight of 1102.7 (*m/z* 1103.7 MH^+ and *m/z* 1125.7 MNa^+) in a broad peak eluting at 17 min (fraction A). Similar fractions were combined from each run (totaling 2.2 mg) and subjected to further HPLC in several portions [Phenomenex Luna C_{18}(2), 5 μm, 4.6 × 250 mm; mobile phase: MeCN/H_2O gradient with 0.1% formic acid; flow rate: 1.0 mL/min; UV detection: 230 nm; ESI-ion trap MS detection from low flow from a 1:9 static flow splitter] to yield malevamide E (**1**, 0.50 mg).[16]

Malevamide E (1): amorphous, white solid; $[\alpha]^{25}_D$ −100 (*c* 0.057, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 204 (4.6), 261 (4.1) nm; IR (film on AgCl plate) ν_{max} 3310, 2961, 2930, 2871, 1730, 1659, 1651, 1644, 1633, 1484, 1470, 1461, 1454, 1408, 1390, 1385, 1291, 1258, 1206, 1092, 745, 700 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Table 1; ESITOFMS MH^+ *m/z* 1103.7145 ($C_{60}H_{95}N_8O_{11}$ requires 1103.7120).

Chiral HPLC Analyses of Hydrolysate of 1. Malevamide E (**1**, 150 μg) was hydrolyzed in 6 N HCl at 109 °C for 21 h, then dried under a stream of N_2 at 50 °C. Residual HCl was removed from the sample by repeatedly reconstituting the sample with several hundred microliters of water followed by evaporation under a stream of N_2. The resulting hydrolysate residue was eluted from a C_{18} SPE cartridge (Waters 20805) using MeOH/H_2O (10:90). The eluate was dried under vacuum and reconstituted with 75 μL of H_2O prior to chiral ligand-exchange HPLC analyses [Phenomenex Chirex D-penicillamine, 4.6 × 250 mm; flow rate: 0.9 mL/min; mobile phase I: 1.7 mM $CuSO_4$ in MeCN/H_2O (14:86); mobile phase II: 1.9 mM $CuSO_4$ in MeCN/H_2O (5:95); UV detection: 245 nm; injected amount: 9 nmol]. The hydrolysate was chromatographed alone and co-injected with standards to confirm peak assignments. The following standards coeluted with peaks from the hydrolysate: L-MeAla, L-MeAsp (the hydrolysis product of Me_2Asn), L-MePhe, L-Pro, L-MeVal, and D-Val. Mobile phase I elution times: L-MeVal (9.7 min), D-MeVal (11.5 min), L-Val (12.7 min), D-Val (15.3 min), D-MeAsp (31.2 min), L-MeAsp (37.1 min), L-MePhe (62.4 min), and D-MePhe (74.8 min). Mobile phase II elution times: L-MeAla (9.4 min), D-MeAla (10.1 min), L-Pro (13.2 min), L-Val (23.2 min), D-Pro (26.6 min), and D-Val (31.3 min). All standard and *N*-methyl amino acid standards were obtained commercially (as separate L- and D-enantiomers or as L-enantiomer and racemate), except DL-MeAsp and L-MeAsp, which were kindly provided by Dr. Philip Williams (Department of Chemistry, University of Hawaii at Manoa).

LC-MS/MS Analysis of 1 and *S. laete-viridis* Fraction A. In order to confirm the sequence assignment of **1** and assess whether dolastatin 14 (**2**) co-occurs in the organism, purified malevamide E (**1**) and a small aliquot of fraction A from *S. laete-viridis* were subjected to reversed-phase HPLC with MS/MS detection [Grace Vydac C_{18} Mass Spec column, 5 μm, 2.1 × 250 mm; flow rate: 0.2 mL/min; mobile phase A: 0.2% formic acid in H_2O; mobile phase B: 0.2% formic acid in MeCN; gradient: 0–75% B over 70 min; detector: ESITOFMS/MS in the positive ion mode and a cone voltage of 50 V]. Malevamide E (**1**) and dolastatin 14 (**2**) eluted at 63.7 and 61.8 min, respectively. Accurately measured MS/MS data of protonated molecular ions and the respective product ions are listed in Table 2. The integration of the selective ion chromatograms for **1** and **2** from LC/MS analysis of both fraction A and the CH_2Cl_2 extract indicated a ratio of 13:1, respectively.

Store-Operated Ca^{2+} Entry Assay. HEK-293 cells were grown in 75 cm^2 culture flasks with DMEM media, supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL streptomycin, in a humidified incubator (5% CO_2 at 37 °C). Changes in cytosolic [Ca^{2+}] were monitored as changes in fluorescence using the Ca^{2+}-indicator dye fura-2[25] and measured with a scanning fluorometer (FLEXstation I, Molecular Devices). In detail, HEK cells (50 000 cells/well) were plated in poly L-lysine-coated 96-well plates (Greiner, μ-Clear). The culture medium was completely removed at [16–20 h] post plating and replaced with the fura-2 loading-buffer: Krebs-Ringer-HEPES (KRH) buffer (135 mM NaCl, 5 mM KCl, 1.5 mM $MgCl_2$, 20 mM HEPES), including 2 mM probenecid, 0.1% glucose, 2 μM fura-2-acetoxymethyl ester (Invitrogen, F-1201), and 0.1% Pluronic F-127. After a 45 min incubation at 37 °C a final of 2 μM thapsigargin (Tocris, 1138) was added to the loading buffer, and incubation continued for another 10 min. The loading buffer was then aspirated; the cells were washed once with KRH before the addition of assay buffer (KRH). The plates were then transferred to the prewarmed FLEXstation (37 °C), which also contained a compound plate holding malevamide E (**1**) and $CaCl_2$ solutions in separate wells. Cells were initially incubated with malevamide E (**1**) or vehicle (KRH buffer) for ~10 min prior to addition of $CaCl_2$ (final concentration 4 mM). Vehicle-receiving cells (and pretreated with thapsigargin) served as positive controls for the activation of store-operated Ca^{2+}-channels, while cells not treated with thapsigargin served as baseline reference for the effective activation of store-operated channels. On average, we observed a 4-fold increase of the intracellular [Ca^{2+}] in vehicle-receiving cells compared to cells not treated with thapsigargin, which defined the assay window. Following the addition of 4 mM $CaCl_2$, the intracellular Ca^{2+}-fura-2 signal was monitored for 1 min as the fluorescence intensity measured at 510 nm after excitation at both 340 nm (Ca^{2+}-bound fura-2) and 380 nm (Ca^{2+}-free fura-2). The ratio of the signal at these two wavelengths (340/380 nm) was used to calculate the fold-change in $[Ca^{2+}]_i$. In order to compare data from different assay days, fold-changes in $[Ca^{2+}]_i$ were expressed as percent of vehicle-treated cells (controls) from the same assay plate/day. Each malevamide E (**1**) test concentration was tested on 2 or 3 assay days.

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References and Notes

(1) These compounds are putatively biosynthesized independent of ribosomal peptide synthesis based on the presence of non-proteinogenic amino acid residues having *N*-methylation, D-configuration, and/or β-and γ-amino substituents.

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(16) Malevamide E (**1**) was also isolated from a mixed collection of *S. laete-viridis* and *S. hydnoides* (see ref 3a for details on the collected material), providing additional quantities (0.8 mg) for the spectroscopic and chemical analyses described here.

(17) The signals for H-32 and H-33 in C_6D_6 are 5.69 dt ($J = 15$, 6.6 Hz) and 5.50 m (splitting pattern obscured by overlap with H-37).

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