# Malevamide D: Isolation and Structure Determination of an Isodolastatin H Analogue from the Marine Cyanobacterium *Symploca hydnoides*

F. David Horgen,[†] Emily B. Kazmierski,[†] Hans E. Westenburg,[‡] Wesley Y. Yoshida,[§] and Paul J. Scheuer*[,§]

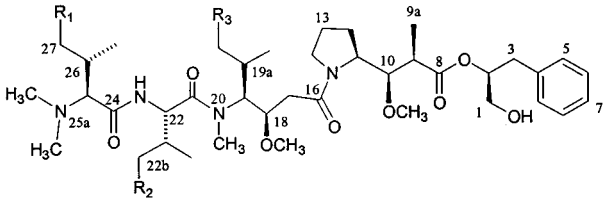
*Natural Sciences, Hawaii Pacific University, Kaneohe, Hawaii 96744, Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822, and Analytical Department, Global Chemical Process R & D, Pharmacia Corporation, Skokie, Illinois 60077*

*Received November 9, 2001*

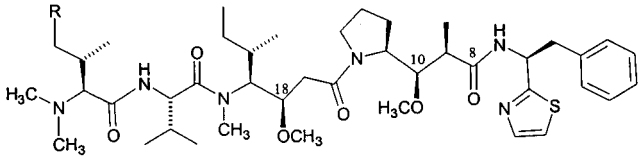
Malevamide D (**1**), a highly cytotoxic peptide ester, and the known compound curacin D (**5**) were isolated from a Hawaiian sample of *Symploca hydnoides*. The structure of **1** was elucidated by spectroscopic analysis including NMR and high-resolution MS/MS. Partial stereochemical assignments of **1** were made by chiral HPLC analysis of acid and base hydrolysates. Malevamide D (**1**) demonstrated toxicity against P-388, A-549, HT-29, and MEL-28 cell lines in the subnanomolar range, while curacin D (**5**) was weakly cytotoxic. Malevamide D (**1**) is closely related to isodolastatin H (**2**), which was previously isolated in low yield from the sea hare *Dolabella auricularia*. A second Hawaiian sample of *S. hydnoides* yielded curacin D (**5**) along with the known dolastatin-10 analogue symplostatin-1 (**3**).

*Symploca hydnoides* Kützing ex Gomont (Oscillatoriaceae) has a wide distribution including the Indian Ocean,[1] the Pacific,[2−5] the east Atlantic,[6] and the Caribbean.[7] Recent reports of its chemical constituents include analogues of dolastatins-10[2] and -13.[8] These reports are notable because the dolastatins were originally isolated in low yield from the sea hare *Dolabella auricularia*, and a number of these compounds possess remarkable biological activity.[9] More recently, dolastatin-10 (**4**), which is in Phase II clinical trials in the United States as a potential anticancer drug,[10] was reported from a *Symploca* species.[11]

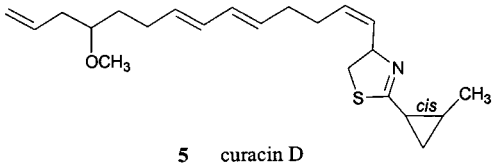
Our attention was drawn to *Symploca* prior to these above reports when an extract of *S. hydnoides* showed strong in vitro cytotoxicity in our ongoing screening of marine organisms. Initial re-collection efforts resulted in a mixed sample of *S. hydnoides* and *S. laete-viridis*, which were morphologically and microscopically similar and from which we originally isolated malevamide D (**1**) (unpublished) and a series of weakly cytotoxic depsipeptides, malevamides A−C. Re-collection and chemical analysis of *S. laete-viridis* identified this species as the source of malevamides A−C.[12] On the other hand, re-collection of *S. hydnoides* from two different sites in waters off Oahu resulted in the reisolation of malevamide D (**1**) from the original collection site off the south shore, confirming the origin of this highly active cytotoxin. Unexpectedly, symplostatin-1 (**3**) was isolated from morphologically identical colonies of *S. hydnoides* collected from a second site off the west shore. Malevamide D (**1**) and symplostatin-1 (**3**), isolated in similar yields ($2.6 \times 10^{-3}$% and $1.4 \times 10^{-3}$% wet wt, respectively), were the major Dragendorff-positive components of their respective samples along with the known compound curacin D (**5**), which was isolated in high yield from both collections.



| | | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|
| **1** | malevamide D | $CH_3$ | H | H |
| **2** | isodolastatin H | H | $CH_3$ | $CH_3$ |



| | | R |
|---|---|---|
| **3** | symplostatin-1 | $CH_3$ |
| **4** | dolastatin-10 | H |



**5** curacin D

## Results and Discussion

Re-collected material of *Symploca hydnoides* (288 g wet wt) from our original collection site off the south shore of Oahu was freeze-dried, ground with mortar and pestle, and extracted with $CH_2Cl_2$. The residue was eluted from a solid-phase extraction cartridge and fractionated by one HPLC step to yield malevamide D (**1**, 7.5 mg) as a clear oil. The isolate was highly cytotoxic, showing $IC_{50}$ values of 0.3−0.7 nM (0.2−0.5 ng/mL) against P-388 (mouse lymphoma), A-549 (human lung carcinoma), and HT-29 (human colon

* Corresponding author. Tel: 808-956-5904. Fax: 808-956-5908. E-mail: scheuer@gold.chem.hawaii.edu.
[†] Hawaii Pacific University.
[‡] Pharmacia Corporation.
[§] University of Hawaii at Manoa.

10.1021/np010560r CCC: $22.00 
Published on Web 03/08/2002

**Table 1.** NMR Assignments of Malevamide D (**1**) in $CD_2Cl_2$[a]

| residue | position | $^{13}C$ | mult | $^{1}H$ | mult | COSY | HMBC |
|---|---|---|---|---|---|---|---|
| PPD | 1 | 63.4 | t | 3.67 | ddd (12.9, 7.4, 2.5) | H: 2, $O_{1a}H$ | C: 2,3 |
| | | | | 3.61 | ddd (13.0, 7.0, 6.0) | | |
| | $O_{1a}H$ | | | 4.66 | br t (7.0) | H: 1 | C: 1 |
| | 2 | 76.4 | d | 5.15 | m | H: 1, 3 | C: 8[b] |
| | 3 | 37.0 | t | 2.93 | dd (13.8, 7.9) | H: 2 | C: 1, 2, 4, 5/5′ |
| | | | | 2.87 | dd (14.0, 6.2) | | |
| | 4 | 137.9 | s | | | | |
| | 5/5′ | 129.9 | d | 7.23 | br d (7.2) | | C: 3, 5/5′, 7 |
| | 6/6′ | 128.6 | d | 7.28 | br t (7.3) | | C: 4, 6/6′ |
| | 7 | 126.8 | d | 7.20 | tt (7.3, 1.8) | | C: 5/5′ |
| DAP | 8 | 173.5 | s | | | | |
| | 9 | 45.9 | d | 2.28 | dq (10.4, 6.9) | H: 9a, 10 | C: 8, 9a, 10, 11 |
| | 9a | 14.9 | q | 1.13 | d (7.0) | H: 9 | C: 8, 9, 10 |
| | 10 | 81.9 | d | 3.96 | dd (10.7, 1.4) | | C: 9, 9a, $O_{10a}Me$, 11, 12 |
| | $O_{10a}Me$ | 61.7 | q | 3.40 | s | | C: 10 |
| | 11 | 60.2 | d | 3.91 | br dd (7.7, 5.4) | H: 12 | C: 12, 13 |
| | 12 | 24.6 | t | 1.82 | m | H: 11 | C: 10, 11, 13, 14 |
| | | | | 1.96 | m | | |
| | 13 | 25.3 | t | 1.76 | m | H: 14 | C: 11, 12 |
| | | | | 2.04 | m | | |
| | 14 | 48.5 | t | 3.45 | m | H: 13 | C: 12, 13, 16[b] |
| MMMAH | 16 | 170.9 | s | | | | |
| | 17 | 38.3 | t | 2.45 | m | H: 18 | C: 16, 18 |
| | | | | 2.34 | m | | |
| | 18 | 78.7 | d | 4.06 | m | H: 17 | |
| | $O_{18a}Me$ | 58.1 | q | 3.31 | s | | C: 18 |
| | 19 | 58.9[c] | | 4.64[d] | | | |
| | 19a | 27.5[e] | d | 1.91 | m | H: 19b, 19c | |
| | 19b | 20.3 | q | 0.82 | d (6.7) | H: 19a | C: 19, 19a, 22 |
| | 19c | 20.3 | q | 1.01 | d (6.5) | H: 19a | C: 19, 19a, 19b |
| | $N_{20}Me$ | 32.2 | q | 3.01 | s | | C: 19, 21 |
| VAL | 21 | 173.8[c] | | | | | |
| | 22 | 54.1 | d | 4.72 | dd (9.0, 6.5) | H: $N_{23}H$, 22a | C: 21, 22a, 22b, 22c, 24 |
| | 22a | 31.3 | d | 1.98 | m | H: 22, 22b, 22c | C: 22, 22b, 22c |
| | 22b | 18.2 | q | 0.93 | d (6.7) | H: 22a | C: 22, 22a, 22c |
| | 22c | 19.7 | q | 0.99 | d (6.7) | H: 22a | C: 22, 22a, 22b |
| | $N_{23}H$ | | | 6.86 | d (8.6) | H: 22 | |
| $Me_2$ILE | 24 | 171.7[c] | | | | | |
| | 25 | 75.2 | d | 2.48 | m | H: 26[f] | |
| | $N_{25a}Me_2$ | 43.2 | q | 2.20 | br s | | C: 25, $N_{25a}Me_2$ |
| | 26 | 34.7 | d | 1.80 | m | H: 25, 27, 29 | C: 24, 25, 27 |
| | 27 | 27.2 | t | 1.52 | m | H: 26, 28 | C: 26, 28, 29 |
| | | | | 1.16 | m | | |
| | 28 | 12.0 | q | 0.91 | t (7.6) | H: 27 | C: 26, 27 |
| | 29 | 15.0 | q | 0.89 | d (6.7) | H: 26 | C: 25, 26, 27 |

[a] Assignments based on $^{13}C$, DEPT, gCOSY, gHMQC, and gHMBC NMR (100/400 MHz) experiments at 25 °C; proton spectrum referenced to residual $CHDCl_2$ ($\delta$ 5.32); carbon spectrum referenced to $CD_2Cl_2$ ($\delta$ 53.8); HMBC data from spectrum optimized for 7 Hz unless otherwise noted; proton chemical shift values and multiplicities from $^{1}H$ NMR spectrum taken at 500 MHz. [b] Correlation observed in HMBC experiment optimized for 3 Hz. [c] Signal broad in $^{13}C$ spectrum; chemical shift assignment based on HMBC correlations to this carbon. [d] Proton gave no correlations in gCOSY, gHMQC, or gHMBC spectra; the chemical shift assigned is from a 1D-gTOCSY experiment (H-18 irradiated). [e] One-bond coupling between H-19a and C-19a did not appear in gHMQC spectrum, but assignment is indicated by gHMBC and gCOSY data. [f] Correlation indicated by 1D-gTOCSY experiment (H-29 irradiated).

carcinoma) cell lines and 0.7 nM against the MEL-28 (human melanoma) cell line.

Taken together, the HRESIMS spectrum and extensive NMR data of **1** suggested a molecular formula of $C_{40}H_{68}N_4O_8$. The $^{1}H$ NMR spectrum of **1** was similar to that of dolastatin-10 (**4**),[13] indicating a similar peptide-like compound, but with the obvious absence of the thiazole moiety. The $^{1}H$ and $^{13}C$ NMR data of **1**[14] suggested the presence of interconverting conformations, as evidenced by signal broadening and the absence of several carbon signals in the one-dimensional carbon spectrum. This is consistent with reports of multiple conformations in $CD_2Cl_2$ for symplostatin-1 (**3**) and dolastatin-10 (**4**).[2,15]

The following residues of **1** were detected by interpretation of gCOSY, DEPT, gHMQC, gHMBC, and one-dimensional gTOCSY experiments: 3-phenyl-1,2-propanediol (PPD), dolaproine (DAP), 3-methoxy-5-methyl-4-(methylamino)hexanoic acid (MMMAH), valine (VAL), and *N*,*N*-dimethylisoleucine ($Me_2$ILE).

Table 1 lists the $^{1}H$ and $^{13}C$ NMR chemical shifts and the interpreted COSY and HMBC data for **1**. Quaternary carbon chemical shifts were assigned by interpretation of HMBC spectra. The carbonyl carbon at $\delta$ 173.5 was assigned to C-8 based on HMBC correlations with H-9 ($\delta$ 2.28) and H-9a ($\delta$ 1.13). The carbon resonating at $\delta$ 170.9 was assigned to C-16 based on an HMBC cross-peak with a proton on C-17 ($\delta$ 2.45). Carbonyl carbons at C-21 and C-24 and methine carbon at C-19 gave broad signals in the $^{13}C$ NMR spectrum, but were clearly detected by HMBC correlations, from which their chemical shifts were assigned. The C-19 proton was not detected in COSY, HMBC, or HMQC experiments; however, irradiation of H-18 ($\delta$ 4.06) in a one-dimensional TOCSY experiment allowed the assignment of H-19 as a broad multiplet at $\delta$ 4.64. This result was consistent with the chemical shift value assigned to H-19 ($\delta$ 4.61) when spectra were taken in $CD_3CN$.

The residue sequence of **1** was determined by HMBC experiments (Table 1). When optimized for long-range

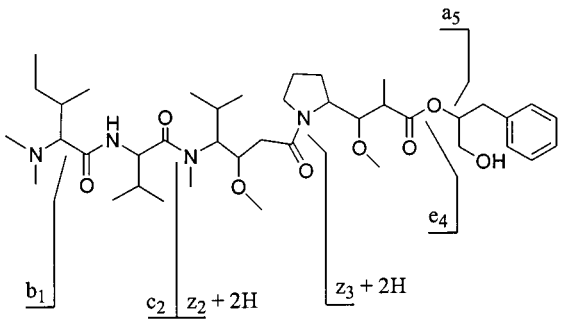



**Figure 1.** Identified sequences for MS/MS fragments of **1**.

coupling of 3 Hz, HMBC correlations were seen between H-2 ($\delta$ 5.15) and C-8 ($\delta$ 173.5) and between H-14 ($\delta$ 3.45) and C-16 ($\delta$ 170.9), allowing the assignment of the partial structure PPD-DAP-MMMAH. The amide bond between MMMAH and VAL was demonstrated by HMBC correlations (optimized to 7 Hz) between the *N*-methyl protons at $\delta$ 3.01 ($N_{20}$Me) and carbons C-19 and C-21 ($\delta$ 58.9 and 173.8, respectively). The final amide bond (VAL−$Me_2$ILE) was indicated by HMBC correlations between H-22 ($\delta$ 4.72) and carbonyl carbons C-21 and C-24 ($\delta$ 173.8 and 171.7, respectively).

The residue sequence of **1** was confirmed by high-resolution ESITOFMS/MS analysis. Figure 1 shows the assignment of observed secondary ions of the $MH^+$ ion (*m*/*z* 733.5) that were unambiguously assigned by HR mass determination (Table 2). Fragments $b_1$ and $c_2$ confirm the $Me_2$ILE-VAL sequence, while fragment $a_5$ is fully consistent with the C-terminal position of the diol. Secondary ions $z_2$ + 2H and $z_3$ + 2H confirm the MMMAH-DAP-PPD sequence and therefore eliminate any other possible sequences. The fully deduced structure indicates that malevamide D (**1**) is an analogue of isodolastatin H (**2**), a cytotoxin isolated is low yield from *D. auricularia*.[17]

The stereochemistry of the VAL, $Me_2$ILE, and PPD residues was determined by chiral HPLC of the acid and base hydrolysates. The PPD residue of **1** was isolated by preparative TLC of the base hydrolysate. Co-injection of this residue with standard (*R*/*S*)- and (*S*)-3-phenyl-1,2-propanediol on a chiral phenyl column indicated that the PPD residue of **1** has *S*-stereochemistry. Acid hydrolysis of **1** under standard conditions (6 N HCl at 109 °C for 17.5 h), followed by HPLC fractionation, yielded the dipeptide *N*,*N*-dimethylisoleucylvaline ($Me_2$ILE-VAL). Free VAL and $Me_2$ILE were present in only trace amounts in the hydrolysate of **1** and its HPLC fractions, and unambiguous assignment of their stereochemistries could not be made. Isoleucylvaline is expected to be resistant to standard peptide hydrolysis conditions,[18] and $Me_2$ILE-VAL was previously found to resist hydrolysis.[19] Therefore, the dipeptide obtained was subjected to extended hydrolyses for up to 168.5 h, and the hydrolysates were analyzed by chiral HPLC; the results indicated the presence of L-$Me_2$ILE and L-VAL and the absence of all other stereoisomers.

The stereochemistry at the other chiral centers in malevamide D (**1**) is assigned as 9*R*, 10*R*, 11*S*, 18*R*, and 19*S*, which is consistent with the configurations of dolastatin-10 (**4**), symplostatin-1 (**3**), and isodolastatin H (**2**). The relative stereochemistries of the DAP residue of malevamide D (**1**) and isodolastatin H (**2**) are equivalent, as suggested by nearly identical $^1$H NMR coupling constants (in benzene-$d_6$) for both compounds at positions-9, -10, and -11.[17,20] The *threo* orientation between positions-9 and -10 of **1** was confirmed by an NOE experiment in benzene-$d_6$, wherein irradiation of H-9a protons ($\delta$ 1.22) gave enhancement of signals for H-10 ($\delta$ 4.26) and $O_{10a}$Me ($\delta$ 3.25). Furthermore, all discernible benzene-$d_6$ $^1$H NMR signals of DAP and PPD residues of compounds **1** and **2** gave virtually identical chemical shifts.[17,20] Considering that the configuration at position-2 of **1** was determined by chiral HPLC to be *S*—identical to isodolastatin H (**2**)—these data strongly suggest that both compounds have identical absolute stereochemistry in the DAP residue. The chemical shift equivalency of DAP residue signals of **1** and **2** also implies that the other adjacent residues (dolaisoleuine for **2** and MMMAH for **1**) also share identical absolute stereochemistry.

The stereochemistry proposed is also consistent with the similarity between the cytotoxicity of **1** and that of **2** and **3**.[2,17] This evidence is strengthened by cytotoxicity SAR studies of dolastatin-10 analogues, which show 100-fold or higher decrease in cytotoxicity for analogues with variable stereochemistries at positions-9, -10, -18, and -19,[21,22] suggesting that malevamide D (**1**) has the same stereochemistry at these positions as compared with **2** and **3**.

Like symplostatin-1 (**3**), malevamide D (**1**) contains a $Me_2$ILE residue, but the thiazole ring of symplostatin-1 (**3**) and dolastatin-10 (**4**) is reduced to an alcohol as in isodolastatin H (**2**). In both **1** and **2**, the C-terminal residue is connected to DAP by an ester bond (**1**: IR $\nu_{max}$ 1732 cm$^{-1}$) rather than an amide, but **1** and **2** maintain a phenyl group with a two-carbon spacer, which was shown to be essential for maximum activity of dolastatin-10 analogues in SAR studies.[23] The third residue of **1**, a new naturally occurring $\gamma$-amino acid, differs from that of **2**−**4**, in that a *sec*-butyl group is replaced by an isopropyl group.

The chemical profile of *Dolabella auricularia* is variable, particularly with respect to geographical location.[9,17,24,25] This variability may reflect not only differences in the cyanobacterial portion of the sea hares' diet, known to be the diet of a general herbivore, but also differences in metabolite production within cyanobacterial species. In fact, the yields of the dolastatins from *D. auricularia* suggest that the presence of these substances in extracts of *D. auricularia* may not be a case of sequestration or accumulation, as is often stated, but simply due to the presence of undigested or partially digested cyanobacteria in the digestive gland of the animal. By comparing the yield of dolastatin-10 (**4**) from *D. auricularia* ($2.9 \times 10^{-6}$% wet wt)[13] and its analogue symplostatin-1 (**3**) from our Hawaiian collections of *S. hydnoides* ($1.4 \times 10^{-3}$% wet wt), the yield of the sea hare isolate is 1/500 that of the cyanobacterial isolate; if only 0.2% of the weight of the sea hare consisted of *S. hydnoides* in the gut, the metabolite might only exist substantially in the digestive organ. The terms sequestration and accumulation may not apply here. Similarly, isodolastatin H (**2**) from *D. auricularia* ($9 \times 10^{-7}$% wet wt)[17] was isolated in about 1/3000 the yield of its analogue malevamide D (**1**) from *S. hydnoides* ($2.6 \times 10^{-3}$% wet wt). Again, by analogy, a minor component of cyanobacterium in the gut might explain the reported yield of isodolastatin H (**2**). This argument is strengthened by the recent isolation of dolastatin-10 (**4**) from a *Symploca* species (very possibly *S. hydnoides*) in equivalent yield ($10^{-2}$% dry wt)[11] to malevamide D (**1**) and symplostatin-1 (**3**) from *S. hydnoides*.

## Experimental Section

**Isolation of Malevamide D (1) and Curacin D (5).** *S. hydnoides* was collected in waters adjacent to Ala Moana Beach Park (depth: 2−5 m) on July 1, 1999 (288 g wet wt). Earlier collections of the organism were identified by Dr. G. M. L. Patterson. The freeze-dried material (53.15 g) was

**Table 2.** Identified Product Ion MS/MS Fragments of Malevamide D (**1**)

| $MH^+$ exptl[a] | $MH^+$ calcd | MMA[b] (ppm) | assigned sequence[c] | molecular formula |
|---|---|---|---|---|
| 701.4815 | 701.4853 | 5.5 | $-OCH_3$ | $C_{39}H_{65}N_4O_7$ |
| 567.4122 | 567.4122 | 0.0 | $a_5$ w/demethoxylation | $C_{30}H_{55}N_4O_6$ |
| 493.3257 | 493.3278 | 4.2 | $z_2 + 2H$ | $C_{27}H_{45}N_2O_6$ |
| 322.1989 | 322.2018 | 9.1 | $z_3 + 2H$ | $C_{18}H_{28}NO_4$ |
| 241.1909 | 241.1916 | 2.9 | $c_2$ | $C_{13}H_{25}N_2O_2$ |
| 170.1195 | 170.1181 | 8.2 | $z_3/e_4 + H$ | $C_9H_{16}NO_2$ |
| 114.1308 | 114.1283 | 22.1 | $b_1$ | $C_7H_{17}N$ |

[a] Using $MH^+$ *m/z* 733.5115 for lock mass correction. [b] Mass measurement accuracy. [c] Nomenclature used for mass spectrometric fragmentation as described by Tuinman and Pettit.[16]

extracted with 500 mL of $CH_2Cl_2$. After removal of solvent, the extract residue (284 mg) was eluted from an ODS solid-phase extraction cartridge (Waters 20805) with MeOH and subjected to HPLC in 3 portions [Waters Cosmosil, H14159, 20 × 250 mm; mobile phase: MeOH/$H_2O$/TFA (67:33:0.05); 9 mL/min; UV detection at 220 nm]. The peaks eluting at 17 min were combined and dried under reduced pressure and partitioned between $CH_2Cl_2$/$H_2O$ after adjusting the aqueous phase to pH 9 with aqueous $NH_4OH$. The organic phase was dried with anhydrous $Na_2SO_4$ and filtered, and the solvent was removed to yield malevamide D[26] (**1**, 7.5 mg, 0.014% dry wt). The peaks eluting at 25 min were combined and solvent was removed under reduced pressure to give curacin D (**5**, 40 mg, 0.075% dry wt).

**Malevamide D (1):** clear oil; $C_{40}H_{68}N_4O_8$; $[\alpha]^{26}_D$ −55° (MeOH, *c* 0.10); UV $\lambda$ max ($\epsilon$) 206 (21 800), 258 shoulder (649) nm; IR $\nu$ max (film on NaCl) 3307 br, 3032, 2965, 1732, 1682, 1667, 1651, 1643, 1634, 1622, 1455, 1256, 1099, 749, 701 $cm^{-1}$; HRESIMS *m/z* 733.5114, $MH^+$ ($C_{40}H_{69}N_4O_8$ requires 733.5115, Δ +0.1 mmu); HRESIMS/MS, see Table 2; $^1H$ and $^{13}C$ NMR data ($CD_2Cl_2$), see Table 1.

**Curacin D (5):** pale yellow oil; $C_{22}H_{33}NOS$; $[\alpha]^{29}_D$ +43° ($CHCl_3$, *c* 0.56) [lit. value $[\alpha]_D$ +33° ($CHCl_3$, *c* 0.14)];[27] HRESIMS *m/z* 360.2390, $MH^+$ ($C_{22}H_{34}NOS$ requires 360.2361, Δ −2.9 mmu); $^1H$ and $^{13}C$ NMR identical to published values.[27] Curacin D (**5**) demonstrated an $IC_{50}$ of 1 $\mu$g/mL (3 $\mu$M) against the P-388 cell line and 2 $\mu$g/mL (6 $\mu$M) against A-549 and HT-29 cell lines. Curacin D was previously isolated from the cyanobacterium *Lyngbya majuscula*.[27]

**Isolation of Symplostatin-1 (3) and Curacin D (5).** On April 21, September 20, and October 1, 1998, a total of 679 g (wet wt) of *S. hydnoides* was collected in waters near Kahe Point on the west coast of Oahu (depth: 2−6 m). Fresh material from each collection was extracted exhaustively in MeOH, and the extracts were combined (27.2 g) and subjected to normal-phase VLC (silica, Fisher S733-1). The fractions eluting with $CH_2Cl_2$/MeOH (85:15 and 80:20) were combined and further fractionated by Sephadex LH-20 CC (2.5 × 104 cm; mobile phase: MeOH; 1.2 mL/min). The fractions eluting between 371 and 471 min were subjected repeatedly to reversed-phase VLC (ODS, YMC AA12IA4; mobile phase: MeOH/$H_2O$ mixtures) and silica HPLC [Rainin Microsorb, 80-199-C5; mobile phase: isocratic and gradient mixtures of $CH_2Cl_2$/MeOH (80:20 to 100:0)] to yield symplostatin-1 (**3**, 9.4 mg, $1.4 \times 10^{-3}$% wet wt). Curacin D (**5**) was detected in VLC fractions from this sample but not isolated.

On February 24, 1999, 352 g (wet wt) of *S. hydnoides* was collected near Kahe Point (depth: 2−6 m). The freeze-dried material (44.2 g dry wt) was extracted exhaustively with $CH_2Cl_2$ to yield 500 mg of extract residue that was fractionated by VLC (silica, Fisher S733-1; mobile phase: $CH_2Cl_2$/MeOH mixtures). Symplostatin-1 (**3**) was isolated from the fraction eluting with $CH_2Cl_2$/MeOH (90:10) by colleagues.[11] A portion (83 mg) of the fraction (144 mg) eluting with $CH_2Cl_2$/MeOH (98:2) was subjected to two successive preparative TLC separations [silica, Analtech 02013; mobile phase I: $CH_2Cl_2$/MeOH (99:1); mobile phase II: hexane/EtOAc (7:25)] to give curacin D (**5**, 5.5 mg, $2.7 \times 10^{-3}$% wet wt).

**Symplostatin-1 (3):** amorphous solid; $C_{43}H_{70}N_6O_6S$; HRESIMS *m/z* 799.5144, $MH^+$ ($C_{43}H_{71}N_6O_6S$ requires 799.5156, Δ +1.2 mmu); $^1H$ NMR identical to published values.[2]

**Alkaline Hydrolysis of 1.** Malevamide D (**1**, 1.0 mg) was dissolved in 1.35 mL of MeOH. While stirring, 1.0 mL of $H_2O$ and 15 mL of 10 N NaOH were added. After stirring for 3 h, the solution was acidified to pH 5 with dilute HCl and extracted three times with $CH_2Cl_2$. The combined $CH_2Cl_2$ phases were dried with anhydrous $Na_2SO_4$, and the solvent was removed under reduced pressure. 3-Phenyl-1,2-propanediol was isolated by preparative TLC [silica, Supelco 5-6444; mobile phase: $CH_2Cl_2$/MeOH (95:5), $R_f$ 0.25].

**Chiral HPLC of 3-Phenyl-1,2-propanediol (PPD) from 1.** PPD obtained from alkaline hydrolysis of **1** and standard *R*/*S*- and *S*-PPD was chromatographed on a chiral phenyl column (Daicell, Chiralcel OD, 10 × 250 mm; mobile phase: hexane/2-propanol (75:25); 1.0 mL/min]. [Standard *S*- and *R*/*S*-PPD were obtained by reduction of the corresponding 3-phenyllactic acid (Aldrich 11,306-9: L-phenyllactic acid and Sigma: DL-$\beta$-phenyllactic acid, respectively) with $LiAlH_4$ in THF, followed by preparative TLC as described above.] (*S*)-3-Phenyl-1,2-propanediol eluted at 23.7 min, while the *R*-enantiomer eluted at 22.7 min. PPD from **1** eluted at 23.7 min, indicating *S*-stereochemistry. This was confirmed by the coelution of standard *S*-PPD and the PPD sample obtained from **1**.

**Acid Hydrolysis of 1.** Malevamide D (**1**, 10 mg)[28] was hydrolyzed in 1.0 mL of 6 N HCl (Pierce 24309) at 109 °C for 17.5 h, then dried under a stream of $N_2$ and further dried under vacuum overnight. The hydrolysate was eluted from an ODS solid-phase extraction cartridge (Phenomenex, Strata C18-E) using MeOH/$H_2O$ (10:90) to yield 8.0 mg of residue. This residue was chromatographed by reversed-phase HPLC (Cosmosil, 20 × 250 mm) using a MeOH/$H_2O$ gradient [3:97 (2 min), 3:97 to 15:85 (over 15 min), 15:85 (2 min), 15:85 to 50:50 (over 11 min); 8.0 mL/min]. The fraction eluting at 23 min gave *N*,*N*-dimethylisoleucylvaline ($Me_2$ILE-VAL, 1.5 mg, 43% yield from **1**): amorphous solid; $C_{13}H_{26}N_2O_3$; $[\alpha]^{25}_D$ −7° (MeOH, *c* 0.069); ESIMS/MS, primary ions *m/z* 259 $MH^+$, 281 $MNa^+$; secondary ion of *m/z* 281, *m/z* 114; $^1H$ NMR ($CD_3OD$) $\delta$ 0.920 d (3H, $J$ = 6.7 Hz), 0.922 d (3H, $J$ = 7.1 Hz), 0.93 t (3H, $J$ = 7.2 Hz), 0.97 d (3H, $J$ = 6.9 Hz), 1.16 m (1H), 1.61 m (1H), 1.87 m (1H), 2.16 m (1H), 2.36 s (6H), 2.81 m (1H), 4.24 d (1H, $J$ = 4.7 Hz) ppm.

**Acid Hydrolysis of *N*,*N*-Dimethylisoleucylvaline.** The dipeptide (100 $\mu$g) was hydrolyzed in 0.5 mL of 6 N HCl, and aliquots were removed after 48.5 (150 $\mu$L), 94.5 (150 $\mu$L), and 168.5 (200 $\mu$L) h. After drying under a stream of $N_2$, the residue from each aliquot was reconstituted with $H_2O$ and dried under $N_2$. The residues were eluted from an ODS solid-phase extraction cartridge (Alltech 255100, 100 mg) and reconstituted with 100 $\mu$L of $H_2O$ prior to analysis [Chiralpak MA(+), 4.6 × 50 mm; UV detection at 254 nm; injected amount 8 nmol; mobile phase: 2.0 mM aqueous $CuSO_4$; 0.5 mL/min]. The hydrolysate residues from each timed aliquot were chromatographed alone and co-injected with standards to confirm assignments. L-, D-, L-*allo*-, and D-*allo*-*N*,*N*-dimethylisoleucine standards were obtained from the corresponding stereoisomers of isoleucine by established methods.[29] Both L-valine and L-*N*,*N*-dimethylisoleucine coeluted with peaks from the hydrolysate. The relative peak area for the L-valine and L-*N*,*N*-dimethylisoleucine peaks in the chromatogram for each hydrolysate aliquot increased with increased hydrolysis time. Elution times were as follows: D-$Me_2$ILE (8.7 min), D-VAL (9.4

min), D-*allo*-$Me_2$ILE (9.5 min), L-$Me_2$ILE (11.2 min), L-*allo*-$Me_2$ILE (13.3 min), and L-VAL (17.8 min).

**Cytotoxicity Testing.** Cytotoxicity assays were performed by Instituto Biomar S.A., Madrid, Spain.

**Acknowledgment.** We thank the following institutions for financial support: Sea Grant College Program, Instituto Biomar S.A., and the National Science Foundation. Authors F.D.H. and E.B.K. also acknowledge support from the Hawaii Pacific University Trustee's Scholarly Endeavors Program. We thank Drs. Winklet Gallimore, Jorge Jimenez, and Arleen Regala and Ms. Jessica Haapkyla for assistance in collecting the biological material, Dr. Adrian Franke for MS/MS analysis of $Me_2$ILE-VAL (acknowledging NCI/NIH CCSG support, #CA71789-03), Dr. G. M. L. Patterson for identifying the organism, and Drs. M. Tius and R. E. Moore for use of chiral HPLC columns.

**Supporting Information Available:** General experimental procedures including details of HRMS/MS analyses and $^1$H, $^{13}$C, DEPT, gCOSY, 1D-gTOCSY, gHMBC, and gHMQC spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Desikachary, T. V. *Cyanophyta*; Indian Council of Agricultural Research: New Delhi, 1959; p 335.
(2) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod.* **1998**, *61*, 1075−1077.
(3) Martinez, M. R. *A Checklist of Blue-green Algae of the Philippines*; University of the Philippines Los Baños: Laguna, 1984; p 85.
(4) Sammarco, P. W. *Mar. Ecol. (Prog. Ser.)* **1983**, *13*, 1−14.
(5) Kashiwagi, M.; Mynderse, J. S.; Moore, R. E.; Norton, T. R. *J. Pharm. Sci.* **1980**, *69*, 735−738.
(6) Donze, M. *Blumea* **1968**, *16*, 159−192.
(7) Robles-Centeno, P. O.; Ballantine, D. L.; Gerwick, W. H. *Hydrobiologia* **1996**, *326/327*, 457.
(8) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J. *J. Nat. Prod.* **1999**, *62*, 655−658.
(9) Pettit, G. R. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Spring-Verlag: Vienna, 1997; Vol. 70, pp 1−79.
(10) Anonymous. National Cancer Institute PDQ Clinical Trials Database: http://cancernet.nci.nih.gov/trialsrch.shtml, August 14, 2001.
(11) Luesch, H.; Moore, R. E.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H. *J. Nat. Prod.* **2001**, *64*, 907−910.
(12) Horgen, F. D.; Yoshida, W. Y.; Scheuer, P. J. *J. Nat. Prod.* **2000**, *63*, 461−467.
(13) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, D. B.; Bontems, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 6883−6885.
(14) NMR spectra were taken in a number of deuterated solvents. The sharpest signals for **1** were observed in $CD_3CN$ and $CD_2Cl_2$. Full NMR analyses were carried out in both solvents. The data in $CD_2Cl_2$ are reported since these data were slightly more informative, although the data in $CD_3CN$ helped resolve several ambiguous HMBC correlations. One-dimensional $^1$H and NOE NMR experiments were carried out in benzene-$d_6$.
(15) Benedetti, E.; Carlomagno, T.; Fraternali, F.; Hamada, Y.; Hayashi, K.; Paolillo, L.; Shioiri, T. *Biopolymers* **1995**, *36*, 525−538.
(16) Tuinman, A. A.; Pettit, G. R. *Int. J. Peptide Protein Res.* **1990**, *36*, 331−334.
(17) Sone, H.; Shibata, T.; Fujita, T.; Ojika, M.; Yamada, K. *J. Am. Chem. Soc.* **1996**, *118*, 1874−1880.
(18) Bodansky, M.; Bodansky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1984; p 262.
(19) Pais, M.; Marchand, J.; Ratle, G.; Jarreau, F.-X. *Bull. Soc. Chim. Fr.* **1968**, 2979−2984.
(20) Proton chemical shifts and coupling constant assignments of **1** in benzene-$d_6$ were made by $^1$H, 1D-NOE, and 1D-TOCSY NMR experiments (500 MHz; residual benzene-$d_5$ referenced to 7.16 ppm): PPD: $\delta$ 3.78 (br dd, 1H, $J$ = 12.2, 6.3 Hz, H-1), 3.90 (br dd, 1H, $J$ = 12.2, 4.6 Hz, H-1), 5.53 (m, 1H, H-2), 2.78 (dd, 1H, $J$ = 13.8, 6.5 Hz, H-3), 2.93 (dd, 1H, $J$ = 13.8, 7.7 Hz, H-3), 7.02−7.14 (5H, H-5/5′, -6/6′, and -7); DAP: $\delta$ 2.51 (dq, 1H, $J$ = 10.6, 6.9 Hz, H-9), 1.22 (d, 3H, $J$ = 6.9 Hz, H-9a), 4.26 (br d, 1H, $J$ = 11.5 Hz, H-10), 3.25 (s, 3H, $O_{10a}$Me), 4.19 (m, 1H, H-11), 1.6 (m, 1H, H-12), 1.92 (m, 1H, H-12), 1.20 (m, 1H, H-13), 1.6 (m, 1H, H-13), 2.84 (m, 1H, H-14), 3.00 (m, 1H, H-14); MMMAH: $\delta$ 1.99 (m, 1H, H-17), 2.15 (m, 1H, H-17), 4.13 (m, 1H, H-18), 3.26 (s, 3H, $O_{18a}$Me), 4.89 (m, 1H, H-19), 1.66 (m, 1H, H-19a), 0.86 (d, 3H, $J$ = 6.6 Hz, H-19b), 0.98 (d, 3H, $J$ = 6.7 Hz, H-19c), 2.76 (s, 3H, $N_{20}$Me); VAL: $\delta$ 4.89 (m, 1H, H-22), 2.06 (m, 1H, H-22a), 1.01 (br s, 3H, H-22b), 1.05 (d, 3H, $J$ = 6.8 Hz, H-22c), 6.98 (m, 1H, $N_{23}$H); $Me_2$ILE: $\delta$ 2.55 (m, 1H, H-25), 2.20 (br s, 6H, $N_{25a}Me_2$), 1.80 (m, 1H, H-26), 1.34 (m, 1H, H-27), 1.80 (m, 1H, H-27), 0.95 (t, 3H, $J$ = 7.3 Hz, H-28), 0.99 (d, 3H, $J$ = 6.4 Hz, H-29). Data were compared with literature values for **2**.[17]
(21) Bai, R. L.; Roach, M. C.; Srirangam, J. K.; Barkoczy, J.; Pettit, G. R.; Ludueña, R. F.; Hamel, E. *Biochem. Pharmacol.* **1993**, *45*, 1503−1515.
(22) Pettit, G. R.; Srirangam, J. K.; Barkoczy, J.; Williams, M. D.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Hogan, F.; Bai, R.; Chapuis, J.-C.; McAllister, S. C.; Schmidt, J. M. *Anti-Cancer Drug Des.* **1998**, *13*, 243−277.
(23) Miyazaki, K.; Kobayashi, M.; Natsume, T.; Gondo, M.; Mikami, T.; Sakakibara, K.; Tsukagoshi, S. *Chem. Pharm. Bull.* **1995**, *43*, 1706−1718.
(24) Sone, H.; Nemoto, T.; Ojika, M.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8445−8448.
(25) Pettit, G. R.; Xu, J.-P.; Hogan, F.; Cerny, R. L. *Heterocycles* **1998**, *47*, 491−496.
(26) "Malewa" (ma lé va) is Hawaiian for "to sway " (Pukui, M. K.; Elbert, S. H. *Hawaiian Dictionary*; revised and enlarged edition; University of Hawaii Press: Honolulu, 1986; p 232), which describes the habit of both this cyanobacterium and *S. laete-viridis*, resembling reddish tufts of fur which gracefully sway back and forth in the wave surge.
(27) Márquez, B.; Verdier-Pinard, P.; Hamel, E.; Gerwick, W. H. *Phytochemistry* **1998**, *49*, 2387−2389.
(28) Additional quantities of malevamide D (**1**) were available following re-collection and reisolation, which were carried out to supply our collaborators, Pharmamar S.A., with additional material for preclinical evaluation. Freeze-dried samples were extracted with $CH_2Cl_2$/MeOH (95:5) and subjected to two reversed-phase HPLC steps [Cosmosil column; mobile phase I: MeOH/$H_2O$/TFA (67:33:0.05); mobile phase II: MeCN/20 mM $NH_4OAc$ (pH 4.8) gradient (20:80 to 80:20)].
(29) Bowman, R. E.; Stroud, H. H. *J. Chem. Soc.* **1950**, 1342−1345.

NP010560R