Structure and Absolute Stereochemistry of Hectochlorin, a Potent Stimulator of Actin Assembly

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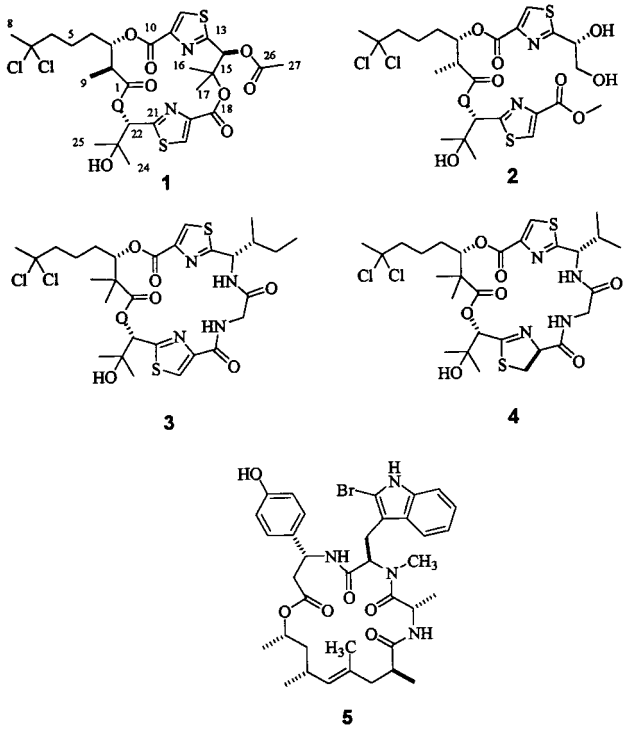
Hectochlorin (**1**) was isolated from marine isolates of *Lyngbya majuscula* collected from Hector Bay, Jamaica, and Boca del Drago Beach, Bocas del Toro, Panama. The planar structure was deduced by one- and two-dimensional NMR spectroscopy. X-ray crystallography was used to determine the absolute stereochemistry of hectochlorin as 2*S*,3*S*,14*S*,22*S*. Hectochlorin is equipotent to jasplakinolide (**5**) in its ability to promote actin polymerization, but unlike jasplakinolide, is unable to displace a fluorescent phalloidin analogue from polymerized actin. In addition, hectochlorin shows both a unique profile of cytotoxicity by the COMPARE algorithm and potent inhibitory activity toward the fungus *Candida albicans*. Structurally, hectochlorin resembles dolabellin and the recently reported lyngbyabellin class of compounds.

Cyanobacteria produce a wide variety of structurally unique and biologically active secondary metabolites.[1] A prevalent structural theme of metabolites isolated from marine cyanobacteria are lipopeptides.[1] Examples include kalkitoxin,[2] the curacins,[3] and the recently reported carmabins.[4] Herein we report the isolation, structure elucidation, absolute stereochemistry and biological properties of hectochlorin (**1**), a unique lipopeptide initially isolated from a cultured strain of *Lyngbya majuscula* (Dillwyn) Harvey (Oscillatoriaceae) collected in Hector Bay, Jamaica, and subsequently from field collections made at Boca del Drago Beach, Bocas del Toro, Panama. Hectochlorin has potent antifungal activity against *Candida albicans* and an intriguing profile of antiproliferative activity in the NCI 60-cell line assay, and it is a strong promoter of actin polymerization. Structurally, hectochlorin resembles dolabellin (**2**)[5] and the recently reported lyngbyabellins A (**3**) and B (**4**).[6]



Results and Discussion

Planar Structure. Hectochlorin (**1**) was isolated from shallow water collections of *L. majuscula* from Hector Bay, Jamaica, and Boca del Drago Beach, Panama. Individual trichomes were cultured from the Jamaica isolate utilizing previously described procedures.[7] A unialgal culture, established through repetitive isolation and subculturing, was maintained at 28 °C with a 16 h light/8 h dark cycle in 10 L of SWBG11 media supplemented with filtered air. To obtain sufficient quantities of algal material for chemical analysis, mass cultures were grown in multiple 15 L sterilized Nalgene pans with each containing 10 L of SWBG11 culture media.[8]

The crude extract (see Experimental Section) was vacuum chromatographed over silica gel with a gradient of EtOAc and hexanes. ^{1}H NMR spectra of the subfractions revealed one fraction in particular that possessed an interesting series of downfield singlet resonances. This fraction was further purified utilizing C_{18} SPE cartridges and RPHPLC to yield hectochlorin as a glassy, pale yellow solid. HR-FABMS established an $[M + H]^+$ for **1** of $C_{27}H_{35}Cl_2N_2O_9S_2$ (*m*/*z* 665.1171, calculated for $C_{27}H_{35}Cl_2N_2O_9S_2$, 665.1161). One-dimensional ^{1}H and ^{13}C NMR showed the presence of four carbonyls and resonances indicative of two thiazole rings, accounting for 10 of the 11 degrees of unsaturation implied by the molecular formula. The remaining degree of unsaturation could be accounted for by an additional ring within the structure of **1**.

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Table 1. ^{1}H and ^{13}C NMR Spectral Data (in ppm) for Hectochlorin (**1**) in $CDCl_3$

atom no.	δ ^{1}H (J in Hz)[a]	m	δ ^{13}C[b]	HMBC[c]
1			173.0	
2	3.16 (7.4, 8.9)	dq	42.6	1, 3, 9
3	5.33	m	75.1	2, 4, 5, 9, 10
4a	1.72	m	30.9	2, 3, 5, 6
4b	1.82	m		
5	1.69	m	20.8	3, 4, 6, 7
6a	2.13	m	49.3	4, 5, 7, 8
6b	2.25	m		
7			90.4	
8	2.09	s	37.2	6, 7
9	1.28 (7.4)	d	15.0	1, 2, 3
10			161.1	
11			147.0	
12	8.15	s	128.5	10, 11, 13
13			166.2	
14	6.83	s	74.7	13, 15, 16, 17, 26
15			81.9	
16	1.83	s	24.4	14, 15, 17
17	1.60	s	21.9	14, 15, 16
18			160.4	
19			147.4	
20	7.90	s	127.7	18, 19, 21
21			165.2	
22	5.64	s	77.9	21, 23, 24, 25
23			71.6	
24	1.31	s	26.7	22, 23, 25
25	1.34	s	25.8	22, 23, 24
26			168.7	
27	2.17	s	20.8	26

[a] Recorded at 600.04 MHz. [b] Recorded at 150.14 MHz. [c] Protons showing long-range correlation with indicated carbon.

Inspection of the ^{1}H NMR spectrum of **1** revealed a series of upfield and highly coupled resonances indicative of an aliphatic chain. A downfield methyl resonating at δ 2.09 (H_3-8) showed HMBC correlations to a quaternary carbon at 90.4 ppm (C-7) and a methylene carbon at δ 49.5 (C-6). The chemical shift of C-7 (90.4 ppm) was indicative of a *gem*-dichloro substituent as observed in dolabellin (**2**)[5] and the lyngbyabellins (**3** and **4**)[6] and was consistent with the molecular formula of **1**. HSQC-COSY was used to further extend this moiety to include an additional six carbons (C-1-C-5, and C-9, see Table 1), identifying this unit as 7,7-dichloro-3-acyloxy-2-methyloctanoate (DCAO).

The remaining ^{1}H NMR resonances of hectochlorin (**1**) were all singlets, and therefore, the remaining sequences of atoms were assembled solely from $^{2,3}J_{CH}$ HMBC data and chemical shift comparisons to known compounds. A $^3J_{CH}$ coupling was observed between the carbonyl carbon (C-1, 172.9 ppm) of the DCAO moiety and a proton at 5.64 ppm (H-22). HMBC correlations were also observed from H-22 to both geminal methyl groups (C-24 and C-25) and the downfield quaternary carbon C-23 (71.7 ppm). The chemical shift of C-23 indicated the presence of a tertiary alcohol functionality, suggesting that this five-carbon unit was α,β-dihydroxyisovalerate (DHIV). The pseudo-α proton of the DHIV showed an additional HMBC correlation to a quaternary carbon at δ 165.5 (C-21). Additional HMBC correlations from proton H-20 (7.93 ppm) to this same downfield resonance (C-21) as well as to two additional deshielded quaternary carbon atoms (C-18 and C-19) were diagnostic of a thiazole ring.

A second thiazole ring was assembled using $^{2,3}J_{CH}$ correlations from H-12 (δ 8.16) to C-10 (δ 161.1), C-11 (δ 147.0), and C-13 (δ 166.4). Connection of this thiazole to the DCAO moiety was made via an HMBC correlation from H-3 to C-10. Additionally, this thiazole unit was connected to a second DHIV unit by a $^2J_{CH}$ coupling between the C-14 methine proton resonating at 6.82 ppm and the quaternary carbon at C-13 (δ 166.2). The proton at H-14 also showed HMBC correlations to a quaternary carbon at 82.1 ppm (C-15) bearing geminal methyl groups with chemical shifts of δ 21.9 and 24.4 (C-16 and C-17, respectively), and to the carbonyl of an acetate moiety (168.7 ppm, C-26). In comparison to the first DHIV moiety, the chemical shift of the quaternary carbon bearing the *gem*-dimethyl group was further downfield ($\Delta\delta$ = 10.2 ppm between C-15 and C-23), indicating that C-15 must be attached to a more electronegative substituent. This chemical shift difference was satisfied by an ester linkage between C-15 and C-18. With this ring closure, the 11 degrees of unsaturation required by the molecular formula were satisfied, and, having accounted for all atoms and degrees of unsaturation, the planar structure of hectochlorin (**1**) was complete.

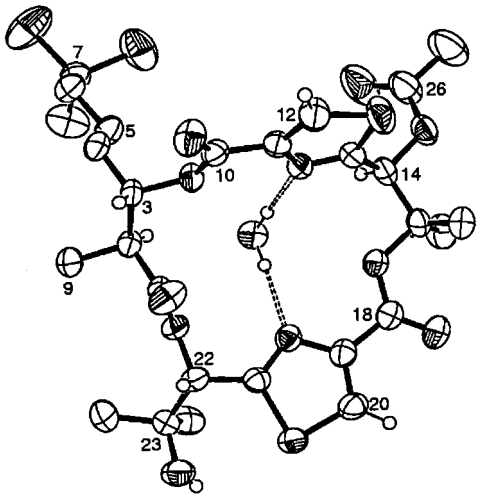

Figure 1. ORTEP representation of hectochlorin (**1**). Absolute stereochemistry was defined from X-ray diffraction analysis utilizing anomalous scattering data. Ellipses are drawn at 50% probability.

X-ray Crystallography and Absolute Stereochemistry. Hectochlorin readily crystallized from a mixture of MeOH and water, forming large prismatic crystals. We therefore undertook structural characterization of hectochlorin by single-crystal X-ray diffraction, which confirmed the structure assignment deduced from the NMR studies. Additionally, due to the presence of several heavy atoms (chlorine and sulfur) and to the fact that all unique data ($0 - h$, $0 - k$, $0 - l$) and Bijvoet pairs were collected, the absolute configuration of the compound was determined. The value of the absolute configuration parameter of 0.02(2) shows that the compound is unambiguously the 2*S*,3*S*,14*S*,22*S* isomer shown as an ORTEP in Figure 1.[9]

During the structural refinement it was found that the crystals were of hectochlorin hydrate, with one molecule of water cocrystallizing with the compound. This molecule of water occupies the central cavity formed by the macrocycle. The water molecule's hydrogen atoms, which could be clearly seen in a Fourier map, form hydrogen bonds to the two imino nitrogens in both thiazole groups. Further, this water molecule acts as a hydrogen bond acceptor toward the hydroxyl group of a neighboring molecule.

Biological Activity. In our initial studies, hectochlorin (**1**) was found to behave similarly to jasplakinolide [**5**, also reported as jaspamide],[10] causing hyperpolymerization of the protein actin.[11] It was recently found that lyngbyabellin A (**3**)[6a] interferes with microfilament formation in cultured cells. On the basis of the strong structural homology between lyngbyabellins A and B (**3** and **4**),[6] we compared the effects of hectochlorin and lyngbyabellin B with those of jasplakinolide on cell growth.

Table 2. Effects of Hectochlorin (**1**), Lyngbyabellin B (**4**), and Jasplakinolide (**5**) on Cell Growth, Actin Polymerization, and Displacement of Fluorescein Isothiocyanate (FITC)-Phalloidin from Actin Polymer

drig	inhibition of cell growth[a] IC_{50} (μM)		stimulation of actin polymerization[b]	displacement of FITC-phalloidin from actin polymer[c]
	CA46	PtK2	EC_{50} (μM) ± SD	EC_{50} (μM) ± SD
hectochlorin (**1**)	0.02	0.3	20 ± 0.6	>60
lyngbyabellin B (**4**)	0.1	1.0	>50	
jasplakinolide (**5**)	0.03	0.3	19 ± 0.5	6.5 ± 1

[a] Cell growth was measured after 24 h at 37 °C with the CA46 cells and after 48 h with the PtK2 cells. Cell number was the parameter measured with the CA46 cells (a suspension line) and cell protein with the PtK2 cells (an attached line). [b] Actin polymerization was measured by the centrifugation assay,[13] and the data presented are averages from three independent experiments. The EC_{50} value represents the concentration of drug inducing a 50% reduction in the protein content of the supernatant compared with a control without drug. [c] Actin and FITC-phalloidin were incubated at 22 °C for 1 h in AMB with 2 μL of PIB per 100 μL reaction mixture. Varying concentrations of phalloidin, hectochlorin, and jasplakinolide in DMSO were added, and the reaction mixtures were incubated for an additional 1 h at 22 °C. Reaction mixtures were centrifuged and fluorescence of the supernatant was measured as described previously.[13] The EC_{50} values represent the drug concentration causing an increase in supernatant fluorescence equal to 50% of the maximum increase obtained with phalloidin.

We first evaluated the growth inhibitory effects of the various drugs on CA46 cells, a human Burkitt lymphoma line, and found that **1** was as potent as jasplakinolide (**5**), with an IC_{50} value of 20 nM, and was 5 times more potent than lyngbyabellin B (**4**, Table 2). Flow cytometry to measure cellular DNA content by propidium iodide labeling after 24 h treatment at equitoxic concentrations of hectochlorin and lyngbyabellin B (10 times the IC_{50} concentrations, 0.2 and 1.0 μM, respectively) demonstrated a modest accumulation of CA46 cells in the G2/M phase of the cell cycle (37% with hectochlorin and 28% with lyngbyabellin B vs 16% in the untreated control). This result was consistent with the conclusion that the pharmacological target of this group of drugs was the actin component of the cytoskeleton. Additional observations supporting this conclusion were that hectochlorin and lyngbyabellin B had no effect on the polymerization of purified tubulin and no effect on the microtubule component of the cytoskeleton of cultured cells and did not cause accumulation of cells arrested in mitosis (cells with condensed chromosomes).

PtK2 cells were used to study the effects of hectochlorin (**1**) and lyngbyabellin B (**4**) on the actin cytoskeleton in comparison with the effects of jasplakinolide (**5**). To compare the agents at equitoxic concentrations, IC_{50} values for cell growth were first determined (Table 2). The three drugs were similar in their relative activities in the CA46 cells, but on average the IC_{50} values were 10-fold higher. For immunofluorescence studies (Figure 2), cells were examined at the IC_{50} values and at 10-fold higher concentrations. Identical results were obtained with fluorescein isothiocyanate (FITC)-labeled phalloidin and with a FITC-labeled anti-actin antibody, and images obtained with the latter are presented in Figure 2 following 24 h of drug treatment.

Ptk2 cells treated with any of the three drugs showed an increase in binucleated cells when compared with controls (Figure 2A). This is a consequence of arrest at cytokinesis, as is usually observed with actin-active agents. At the IC_{50} concentrations, hectochlorin (Figure 2B) and lyngbyabellin B (Figure 2D) caused an apparent thickening in the microfilaments after 24 h relative to the microfilaments observed in the untreated control cells (Figure 2A). This could result from the bundling of actin filaments, as fewer filaments were present in the center of cells as opposed to the stronger labeling of numerous cortical actin filaments. A similar observation was reported for A-10 smooth muscle cells treated with lyngbyabellin A.[6] In contrast, jasplakinolide caused a much more drastic reorganization of the actin cytoskeleton at its IC_{50} value, with F-actin forming clumps distributed throughout the cytoplasm. On the basis of the potent hypernucleation of actin assembly caused by jasplakinolide (ref 11 and Figure 2F), these cytoskeletal changes have been interpreted as representing numerous patches of short actin filaments.[11]

At concentrations 10-fold higher than the IC_{50} values the effects of hectochlorin (**1**, Figure 2C) and lyngbyabellin B (**4**, Figure 2E) on the actin cytoskeleton were more dramatic. Cells presented a hairy appearance due to cellular protrusions rich in actin filaments. With jasplakinolide there was again a different pattern of actin labeling (Figure 2G). The cytoplasm of the cells retracted extensively, and the labeling of F-actin was concentrated near the nucleus and in small protrusions that gave a spiky appearance to the cells.

As noted above, experiments with purified actin demonstrated that hectochlorin (**1**), like jasplakinolide (**5**), induced actin assembly in the absence of exogenous K^+ ("nonpolymerizing conditions", see Figure 3, curve 1). In a centrifugal assay designed for ease of comparison of stimulatory drugs at multiple concentrations,[13] we found that hectochlorin and jasplakinolide had equivalent activity (Table 2), yielding EC_{50} values of 20 and 19 μM, respectively. Lyngbyabellin B was minimally active in this assay. It should be noted that values obtained in this assay could be viewed as equilibrium values because of the relatively long incubation and sample processing times (total 2.5 h).

Light-scattering studies at 90° were conducted to compare the ability of hectochlorin (**1**) and jasplakinolide (**5**) to stimulate actin polymerization at early time points.[14] The reactions were followed individually for approximately 15 min. In the absence of drug or exogenously added K^+ no actin polymer was formed during the time frame of the experiment (Figure 3, curve 1), while added K^+ caused the expected rapid assembly (curve 2) of actin filaments. Higher concentrations of hectochlorin (curves 3−5) progressively caused more extensive assembly reactions. As the amount of hectochlorin was increased, the lag time became progressively shorter and the apparent rate and extent of polymer formation increased. The same general observation would apply to jasplakinolide (curves 6−8); however, at 10 and 25 μM concentrations the jasplakinolide-induced assembly reactions were much less robust than the hectochlorin-induced reactions (compare curve 3 with 6 and curve 4 with 7). In contrast, the reaction with 50 μM jasplakinolide (curve 8) had an earlier onset and was more rapid, if not more extensive, than the reaction with 50 μM hectochlorin (curve 5).

We were intrigued that the 50 μM concentrations of jasplakinolide or hectochlorin caused more intense light scattering than was observed with K^+-induced assembly (compare curve 2 with curves 5 and 8 in Figure 3). In view of the thick filaments present in the PtK2 cells treated with hectochlorin at the IC_{50} value, we speculated that this

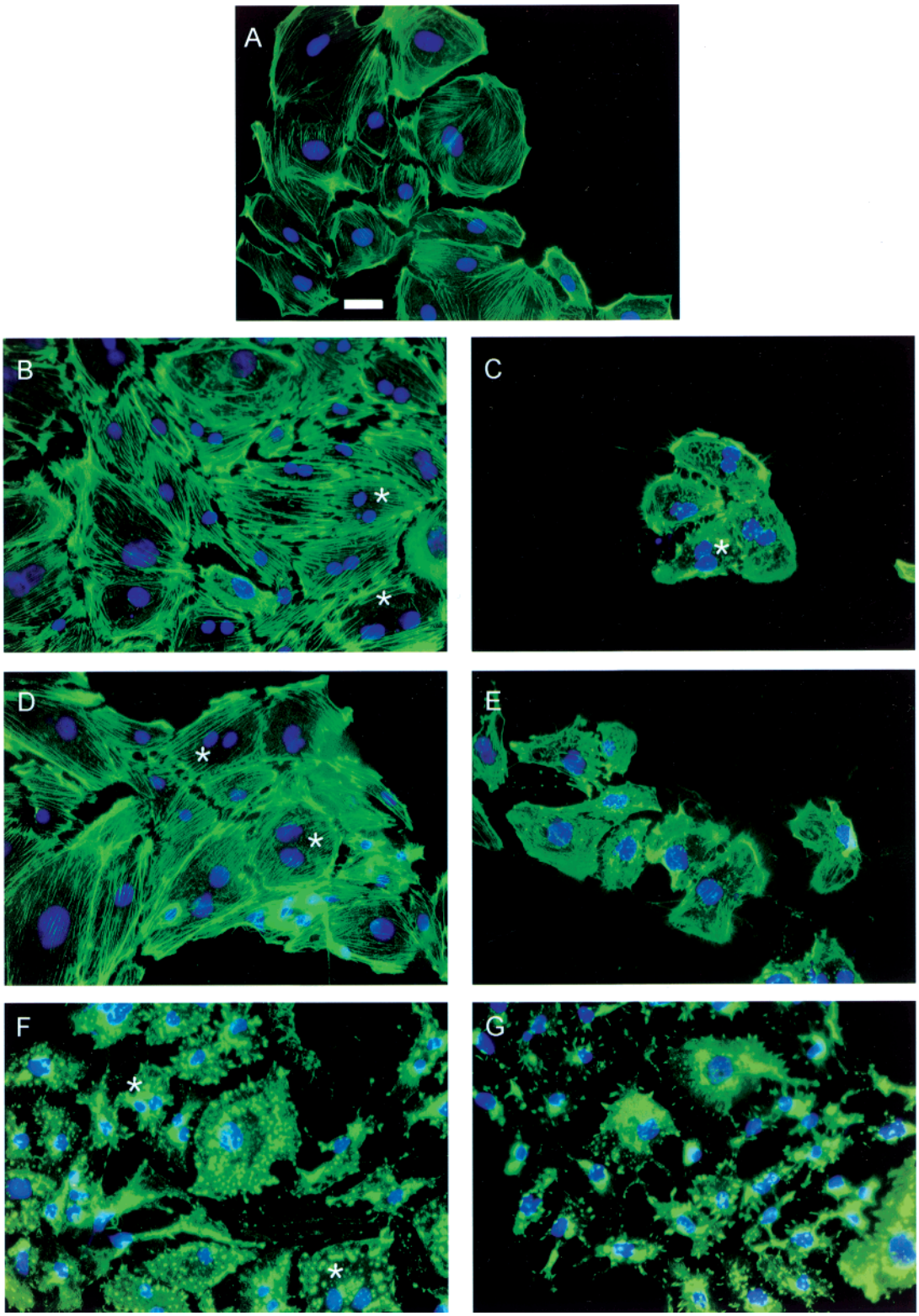

Figure 2. Effects of hectochlorin (**1**), lyngbyabellin B (**4**), and jasplakinolide (**5**) on the actin cytoskeleton of PtK2 cells. After 24 h at 37 °C cells were processed as described previously and exposed to an FITC-labeled anti-β-actin antibody (visualized as green in the figure) and to the DNA-reactive compound DAPI (visualized as blue).[12] Cells were examined under a 40× oil objective (N.A. 1.30), and the white bar in panel A indicates 30 μm. Asterisks indicate binucleated cells, presumably arrested at cytokinesis. (A) No drug. (B) Hectochlorin at 0.3 μM. (C) Hectochlorin at 3.0 μM. (D) Lyngbyabellin B at 1.0 μM. (E) Lyngbyabellin B at 10 μM. (F) Jasplakinolide at 0.3 μM. (G) Jasplakinolide at 3.0 μM.

might be due to actin filament bundle formation or, possibly, formation of polymers of aberrant morphology (previous experiments with jasplakinolide had confirmed that unbundled actin filaments of normal morphology were formed under the conditions used here).[13]

We explored these possibilities using centrifugation and electron microscopy. We were unable to pellet any significant amount of actin polymer by low-speed centrifugation (20000*g* for 30 min at 22 °C). Electron microscopy of samples containing actin and hectochlorin showed numerous unbundled actin filaments identical to those induced by K^+ or jasplakinolide (data not shown).

We observed one further biochemical difference between hectochlorin and jasplakinolide. As shown previously,

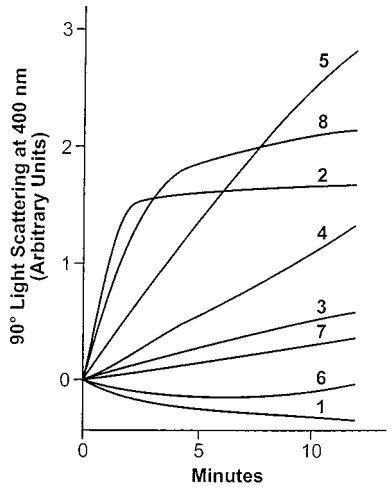

Figure 3. Stimulation of actin polymerization by hectochlorin (**1**) or jasplakinolide (**5**). Actin assembly was followed by 90° light scattering as described in the text. The figure represents a composite of each reaction mixture followed individually. Curve 1: no addition (actin only). Curve 2: assembly induced with PIB. Curve 3: 10 μM hectochlorin. Curve 4: 25 μM hectochlorin. Curve 5: 50 μM hectochlorin. Curve 6: 10 μM jasplakinolide. Curve 7: 25 μM jasplakinolide. Curve 8: 50 μM jasplakinolide.

jasplakinolide readily displaces FITC-phalloidin from actin polymer.[13,15] Hectochlorin was unable to do this (Table 2). Hectochlorin was also unable to inhibit FITC-phalloidin binding to polymer when it was added prior to addition of the fluorescent drug (data not presented). These results with hectochlorin are similar to our observations with dolastatin 11, which also promotes actin polymerization.[13]

We have shown that hectochlorin is more potent than lyngbyabellin B (**4**) in its effects on purified actin and as a cytotoxic compound, but the two agents appear to have the same basic mode of action on the actin cytoskeleton. Hectochlorin (**1**) was quantitatively similar to jasplakinolide, particularly as a cytotoxic agent. These compounds all promote actin polymerization, but the actin cytoskeleton rearrangements in cells are different. The major biochemical difference between hectochlorin and jasplakinolide is the inability of hectochlorin to displace FITC-phalloidin from actin filaments or even prevent the binding of FITC-phalloidin to the filaments. Although hectochlorin resembles dolastatin 11 in the inability to interfere with FITC-phalloidin binding to F-actin, dolastatin 11 induces morphological effects on the cellular actin cytoskeleton that are closer to those of jasplakinolide than to those of hectochlorin.[13] Thus far, none of these drugs appear to have a significant effect on actin filament morphology when observed by electron microscopy. Thus, their different effects on the cellular actin cytoskeleton may result from altered interactions of actin-associated proteins with actin filaments in drug-treated cells.[16]

Hectochlorin (**1**) was also provided to the National Cancer Institute for cytotoxicity testing in the in vitro panel of 60 different cancer cell lines. Of the nine tumor classes of cell lines (leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian, renal, prostate, and breast), hectochlorin showed greatest potency against several cell lines in the colon, melanoma, ovarian, and renal panels and had an average GI_{50} against the 60 cell lines of 5.1 μM. The flat shape of the dose−response curves of hectochlorin against most of these cell lines was characteristic of compounds that are antiproliferative but not cytotoxic [e.g., inhibitors of microtubule or actin processes (see Supporting Information), consistent with the biochemical findings presented above].

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer model 243 polarimeter. UV and IR spectra were recorded on a Beckman DU 640B UV spectrophotometer and a Nicolet 510 spectrophotometer, respectively. NMR spectra were recorded on Bruker DRX 600 and General Electric GN 500 instruments. Chemical shifts for data reported in $CDCl_3$ are reported relative to residual $CHCl_3$ as an internal standard (Table 1). HRMS were obtained on a Kratos MS50TC. X-ray diffraction data were collected on a Siemens P-4 X-ray diffractometer with HiStar area detector (Cu Kα radiation). Structure solution and refinement was carried out using SHELXS and SHELXL, respectively. HPLC was performed using a Waters 515 pumps and a Waters 996 photodiode array spectrophotometer. TLC grade (10−40 μm) Si gel was used for vacuum chromatography, and Merck aluminum-backed TLC sheets (Si gel 60 F_{254}) were used for TLC.

Collection and Culture Conditions. The marine cyanobacterium *L. majuscula* was collected by hand from shallow water (2 m) on August 22, 1996, at Hector Bay, Jamaica. The bulk of the sample was preserved in IPA, and a small portion was stored in native seawater for culturing. A voucher sample is available from WHG as collection number JHB-22 Aug 96-01C2. Following transport to Oregon, the culture sample was freed from competing cyanobacteria and other contaminants using standard techniques.[7] The new unialgal cultures were maintained in a 28 °C temperature-controlled room with 16/8 light/dark cycle provided by Sylvania 40 W cool white fluorescent lights (4.67 μmol photons s^{-1} m^{-2}). The liquid culture media used for the isolation procedure consisted of SWBG11 and ESW. When a sufficient cell mass had grown, usually 6−7 weeks after inoculation, the cells were harvested according to the technique of Rossi et al.[8]

Alternatively, the cyanobacterium *L. majuscula* (122998-BDD-1) was collected from shallow waters adjacent to Boca del Drago Beach (1−2 m), Bocas del Toro, Panama, in December 1998. The alga resembles *L. majuscula* observed in waters around the island of O'ahu, Hawaii, except that the Panamanian variety has a reddish coloration.

Extraction and Isolation. Approximately 114.2 g (wet weight) of the harvested algal material was repetitively extracted with CH_2Cl_2/MeOH (2:1) to yield 1.1 g of crude extract. The crude extract was fractionated using vacuum liquid chromatography (VLC, 9.5 cm × 4 cm) on TLC grade Si gel. Fractions eluting between 50% EtOAc in hexanes and 80% EtOAc in hexanes were recombined and further purified over a C_{18} SPE cartridge (gradient elution from 50% MeOH in H_2O to 100% MeOH), in which fractions eluting in 70−80% MeOH in H_2O were subsequently subjected to RPHPLC. An isocratic elution profile in 82% MeOH in H_2O (Phenomenex SPHERECLONE ODS, 250 × 10 mm, 5 μm) yielded pure hectochlorin (**1**, 35.2 mg, 3.2% of crude extract).

Alternatively, freeze-dried preparations of the field collected algae (290 g) were extracted with CH_2Cl_2/2-propanol (IPA) (3 × 1.0 L), filtered, and concentrated under reduced pressure to yield 6.1 g of crude extract. The extract was loaded on a Sephadex LH-20 column (33 × 5.0 cm) equilibrated in CH_2Cl_2. The column was eluted using a gradient profile as follows: (1) CH_2Cl_2 (1.0 L), (2) CH_2Cl_2/acetone (1:1, 2.0 L), (3) methanol (2.0 L). Ten major fractions (A−J) were collected and concentrated to dryness. Fractions A and B were combined and further separated by reversed-phase flash chromatography (TMC-ODS 120 Å, 33 × 3.0 cm) eluted with 80% aqueous MeCN. Several fractions were collected and pooled together according to their TLC behavior, resulting in seven major fractions (1−7). Reversed-phase HPLC (Phenomenex Ultracarb 30 ODS 5; 250 × 10 mm; 30% aqueous MeCN at 2.0 mL/min and monitoring at 220 nm) of fraction 1 afforded pure hectochlorin (3.8 mg).

Hectochlorin (1): glassy, pale yellow solid; $[\alpha]_D^{25}$ −8.7 (*c* 1.04, MeOH); IR (neat) 3459, 3119, 2983, 2939, 2882, 1756, 1746, 1729, 1713, 1572, 1484, 1244, 1091 cm^{-1}; ^1H and ^{13}C

NMR data in $CDCl_3$, see Table 1; ^1H NMR data in C_6D_6 (500 MHz) δ 7.67 (1H, s, H-12), 7.48 (1H, s, H-20), 6.97 (1H, s, H-14), 5.63 (1H, s, H-22), 5.48 (1H, m, H-3), 3.01 (1H, m, H-2), 2.02 (1H, m, H-6a), 1.91 (1H, m, H-6b), 1.83 (3H, s, H-8), 1.70 (1H, m, H-5a), 1.68 (3H, s, H-17), 1.60 (1H, m, H-5b), 1.58 (3H, s, H-16), 1.56 (1H, m, H-4a), 1.50 (3H, s, H-27), 1.42 (1H, m, H-4b), 1.15 (3H, s, H-25), 1.07 (3H, s, H-24), 1.03 (3H, d, J = 7.0 Hz, H-9); ^{13}C NMR data in C_6D_6 (125 MHz) δ173.0 (C-1), 168.0 (C-26), 166.5 (C-13), 165.5 (C-22), 161.3 (C-10), 160.7 (C-18), 147.9 (C-19), 147.7 (C-11), 128.4 (C-12), 128.0 (C-20), 91.0 (C-7), 81.7 (C-15), 78.4 (C-22), 75.5 (C-3), 75.3 (C-14), 71.4 (C-23), 49.6 (C-6), 43.1 (C-2), 37.0 (C-8), 31.1 (C-4), 26.8 (C-24), 25.6 (C-25), 24.3 (C-17), 21.8 (C-16), 21.1 (C-5), 19.95 (C-27), 15.0 (C-9); HRFABMS (in 3-NBA) $[M + H]^+$ m/z 665.1171 (cald for $C_{27}H_{35}Cl_2N_2O_9S_2$, 665.1161).

X-ray Crystallography. Five milligrams of hectochlorin was dissolved in a vial in 1 mL of MeOH followed by the careful addition of 1 mL of H_2O, creating two distinct solvent phases. The vial was sealed and monitored for crystal growth over the course of several days. By the third day large prismatic crystals formed that were visible to the naked eye. From a mass of crystals, a well-shaped crystal of dimensions 0.30 × 0.20 × 0.20 mm^3 was selected and mounted on the tip of a thin glass fiber using epoxy glue. Crystallographic parameters were determined as reported elsewhere.[17] Relevant data collection parameters and results of structural refinement for this structural determination are given in the Supporting Information.[18]

Antimicrobial Assay. The antimicrobial activity of hectochlorin was evaluated using standard paper sensitivity disk-agar plate methodology (disk diameter, 6 mm). Hectochlorin gave a 16 mm zone of inhibition at 100 μg/disk and a 11 mm zone of inhibition at 10 μg/disk against *Candida albicans* (ATCC 14053); however, it was inactive against *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 11775), *Salmonella choleraesuis* subsp. *choleraesuis* (ATCC 14028), *Bacillus subtilis* (ATCC 6051), and *Staphylococcus aureus* (ATCC 12600).

Actin Studies. Purified rabbit muscle actin was obtained from Cytoskeleton (Denver, CO), phalloidin and antifade mounting solution from Molecular Probes (Eugene, OR), PtK2 cells (normal kidney cells of the kangaroo rat *Potorous tridactylis*) and CA46 cells (human Burkitt lymphoma cells) from the American Type Culture Collection (Manassas, VA), 4′,6-diamidino-2-phenylindole (DAPI), FITC-conjugated phalloidin, and FITC-conjugated β-anti-actin monoclonal antibody (clone Ac-15) from Sigma (St. Louis, MO), and the chambered coverglass system from Nalge Nunc International (Naperville, IL). Jasplakinolide was generously provided by the Drug Synthesis & Chemistry Branch, National Cancer Institute (Rockville, MD).

Methodologies for maintenance of PtK2 and CA46 cells in culture, measurement of drug effects on cell growth, direct immunofluorescence (actin and DNA), flow cytometry, electron microscopy, measurement of the displacement of FITC-phalloidin from F-actin, and measurement of actin polymerization by centrifugation were described previously.[3d,13,19]

Actin assembly was also measured by 90° light scattering (400 nm) in a fluorometer (Photon Technology International, Lawrenceville, NJ) at 22 °C. Each 100 μL (final volume) reaction mixture contained 25 μM actin, 5% (v/v) DMSO, and drug or 2 μL of polymerization inducing buffer (PIB = 2.5 M KCl, 100 mM $MgCl_2$, 50 mM ATP), as indicated, in actin monomer buffer (AMB = 5 mM Tris-HCl (pH 8.0), 0.2 mM $CaCl_2$, 0.2 mM ATP, 5 mM dithiothreitol). Actin in AMB was added to the cuvette, and light scattering was measured for 3 min to establish a background. At this point DMSO, drug in DMSO, or PIB + DMSO was added to the cuvette. The cuvette contents were rapidly mixed, and light scattering was measured.

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Note Added in Proof: A report on the total synthesis of hectochlorin has recently appeared: Cetusic, J. R. P.; Green, F. R., III; Graupner, P. R.; Oliver, M. P. *Org. Lett.* **2002**, *4*, 1307−1310.

Supporting Information Available: Copies of ^1H, ^{13}C, $^1H-^{13}C$ HSQC, $^1H-^{13}C$ HMBC NMR data, NCI 60-cell line cytotoxicity data, and X-ray crystallographic data for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44(0)1223-336033 or email deposit@ccdc.cam.ac.uk).
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