New Alkaloids of the Lamellarin Class from the Marine Ascidian *Didemnum chartaceum* (Sluiter, 1909)

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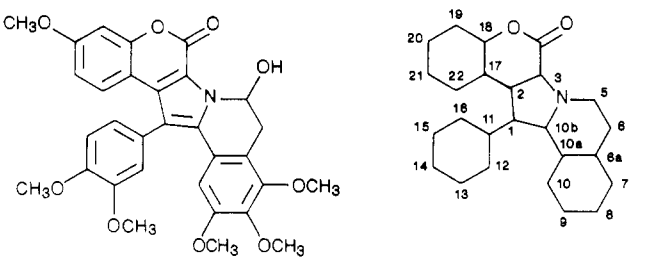
Chemical investigation of the marine ascidian *Didemnum chartaceum* from the Indian Ocean has resulted in the isolation of four new alkaloids of the recently reported lamellarin class. The structure of lamellarin E (**2**) was determined by spectroscopic and X-ray crystallographic methods. The structures of lamellarins F–H (**3–5**) were elucidated by interpretation of NMR spectral data, which relied heavily upon $^{1}J_{C-H}$ and $^{2-3}J_{C-H}$ correlation experiments. Isolation of lamellarins E–H from *D. chartaceum* indicates that lamellarins A–D, isolated from the prosobranch mollusc *Lamellaria* sp., were most likely sequestered from an ascidian in the diet of this mollusc.

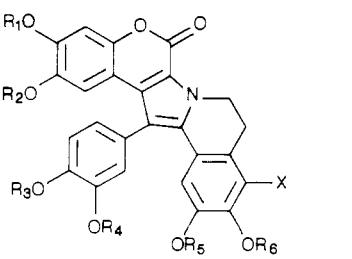
Prior chemical studies have shown marine ascidians to be a rich source of unique and extremely biologically active secondary metabolites.[1] Examples of some of the diverse, biologically active ascidian metabolites are didemnin B, an antineoplastic cyclic depsipeptide from *Tridemnum solidum*,[2] eudistomin C, a potent antiviral carboline from *Eudistoma olivaceum*,[3] and ulithiacyclamide, a cytotoxic cyclic peptide from *Lissoclinum patella*.[4] The biomedical potential for ascidian secondary metabolites has resulted in focused interest in these primitive chordates, but very little work has attempted to demonstrate ecological roles for these compounds in nature.[5] As part of our continuing interest in the chemical adaptations of marine plants and invertebrates, we have recently begun to investigate the secondary metabolites produced by highly diverse species in the class Ascidiacea. In this paper, we report the structures of four new lamellarin class alkaloids, lamellarins E–H (**2–5**), isolated from *Didemnum chartaceum* (Didemnidae, Ascidiacea) collected in the Indian Ocean on the atoll of Aldabra.[6] In addition, the use of direct and long range ^{1}H–^{13}C NMR correlation experiments to determine aromatic hydroxy and methoxy substitution patterns is illustrated. Lamellarins A–D, recently described by Andersen et al.,[7] were isolated from the prosobranch mollusc *Lamellaria* sp. Lamellarin A (**1**), the first example of this novel class of marine alkaloids, was fully defined by X-ray methods.

Purple and brown color morphs of the encrusting, colonial didemnid ascidian *D. chartaceum*, which is devoid of symbiotic algae, were collected near Aldabra Atoll, Republic of the Seychelles, between −10 and −15 m depth. The collection of *D. chartaceum* was stored in isopropyl alcohol for 2 months. Acetone and methanol extracts of the lyophilized ascidian and the lyophilized aqueous residue from the isopropyl alcohol were combined and initially fractionated with vacuum flash chromatography using TLC-grade silica gel and then with Sephadex LH-20 (2:1 CH_2Cl_2/MeOH). Final purification of lamellarins E–H (**2–5**) was accomplished by C-18 reversed-phase HPLC (80:20 methanol/water), yielding lamellarins E–H each as less than 0.05% dry weight of the ascidian.

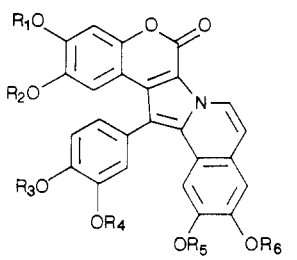
Lamellarin E (**2**) was obtained as white prisms, mp 228–232 °C, by slow evaporation of a methanolic solution of this metabolite. The molecular formula, $C_{29}H_{25}NO_9$, was determined from interpretation of the $M^+ + H$ ion (m/z 532.1563) in the high-resolution FAB mass spectrum in

Chart I





	R_1	R_2	R_3	R_4	R_5	R_6	X
E (**2**)	H	Me	Me	H	Me	Me	OH
F (**3**)	H	Me	Me	Me	Me	Me	OH
G (**4**)	Me	H	Me	H	Me	H	H
(**6**)	Ac	Me	Me	Ac	Me	Me	OAc
(**7**)	Ac	Me	Me	Me	Me	Me	OAc
(**8**)	Me	Ac	Me	Ac	Me	Ac	H



	R_1	R_2	R_3	R_4	R_5	R_6
H (**5**)	H	H	H	H	H	H
(**9**)	Ac	Ac	Ac	Ac	Ac	Ac

(1) Recent examples include: Bloor, S. J.; Schmitz, F. J. *J. Am. Chem. Soc.* **1987**, *109*, 6134. Ishibashi, M.; Ohizumi, Y.; Sasaki, T.; Nakamura, H.; Hirata, Y.; Kobayashi, J. *J. Org. Chem.* **1987**, *52*, 450. Cartê, B.; Faulkner, D. J. *Tetrahedron Lett.* **1982**, *23*, 3863.

(2) Rinehart, K. L., Jr.; Gloer, J. B.; Renis, H. E.; McGovern, J. P.; Swynenberg, E. G.; Stringfellow, D. A.; Kuentzel, S. L.; Li, L. H. *Science (Washington, D.C.)* **1981**, *212*, 933.

(3) Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Hughes, R. G., Jr.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1984**, *106*, 1524.

(4) Ireland, C. M.; Durso, A. R., Jr.; Newman, R. A.; Hacker, M. P. *J. Org. Chem.* **1982**, *47*, 1807.

(5) Views on the ecological roles of transition metals and pH are discussed in the following: Stoecker, D. *Biol. Bull.* **1978**, *155*, 615. Stoecker, D. *Mar. Ecol. Prog. Ser.* **1980**, *3*, 257. Stoecker, D. *Ecology* **1980**, *61*, 1327. Parry, D. L. *Mar. Ecol. Prog. Ser.* **1984**, *17*, 279.

(6) The ascidian was identified as *Didemnum chartaceum* (Sluiter, 1909) by Dr. Françoise Monniot, National Museum of Natural History, Paris, France.

(7) Andersen, R. J.; Faulkner, D. J.; He, C.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5492.

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Table I. ^{1}H NMR Assignments for Lamellarins E–G[a]

C no.	lamellarin E (2)	lamellarin F (3)	lamellarin G (4)	lamellarin H (5)
5	4.55–4.67 (m, 2 H)	4.55–4.67 (m, 2 H)	4.53–4.70 (m, 2 H)	8.98 (d, 1 H, J = 7.2)
6	3.02 (m, 2 H)	3.03 (m, 2 H)	3.00 (m, 2 H)	7.15 (d, 1 H, J = 7.2)
7			6.73 (s, 1 H)	6.96 (s, 1 H)[b]
10	6.34 (s, 1 H)	6.33 (s, 1 H)	6.49 (s, 1 H)	6.57 (s, 1 H)[c]
12	6.89 (s, 1 H)	7.09 (d, 1 H, J = 1.6)	6.83 (s, 1 H)	6.81 (d, 1 H, J = 1.5)
15	7.15 (d, 1 H, J = 7.9)	7.20 (d, 1 H, J = 8.1)	7.13 (d, 1 H, J = 8.0)	6.99 (d, 1 H, J = 8.0)
16	6.89 (d, 1 H, J = 7.9)	7.01 (dd, 1 H, J = 8.1, 1.6)	6.84 (d, 1 H, J = 8.0)	6.72 (dd, 1 H, J = 8.0, 1.5)
19	6.81 (s, 1 H)	6.80 (s, 1 H)	7.01 (s, 1 H)	7.14 (s, 1 H)[b]
22	6.64 (s, 1 H)	6.53 (s, 1 H)	6.68 (s, 1 H)	6.83 (s, 1 H)[c]
R_1			3.81 (s, 3 H)	
R_2	3.38 (s, 3 H)	3.34 (s, 3 H)		
R_3	3.83 (s, 3 H)	3.83 (s, 3 H)	3.86 (s, 3 H)	
R_4		3.75 (s, 3 H)		
R_5	3.29 (s, 3 H)	3.26 (s, 3 H)	3.27 (s, 3 H)	
R_6	3.67 (s, 3 H)	3.67 (s, 3 H)		

[a] ^{1}H NMR spectra were recorded at 360 MHz in DMSO-d_6. Assignments for lamellarins E–G were aided by spin-decoupling experiments, DEPT sequence experiments, and $^1J_{C-H}$ and $^{2-3}J_{C-H}$ correlation experiments. Assignments for lamellarin H were based on a comparison of ^{1}H NMR spectral data for lamellarin G, spin-decoupling experiments, and DEPT sequence experiments. J values are reported in hertz and chemical shifts are given in δ units (downfield of Me_4Si). [b,c] Assignments may be reversed.

conjunction with ^{1}H and ^{13}C NMR data (Tables I and II). The molecular formula indicated a high degree of unsaturation for this molecule. Extended conjugation was apparent from the UV spectrum [330 (sh), 310 (ϵ 23 000), 278 (38 000), 268 (sh), and 206 nm (48 000)], and bathochromic shifts were observed upon the addition of base (1 drop, 1 N NaOH). These data, together with eight ^{13}C NMR resonances downfield of 140 ppm, were indicative of a phenolic compound. Of the 21 ^{13}C signals downfield of 104 ppm, the resonance at δ 154.2 was assigned to a conjugated ester by correlation with a strong IR absorption at 1695 cm^{-1}. The remaining 20 aromatic carbons accounted for 10 degrees of unsaturation, leaving six rings in lamellarin E.

The proton NMR spectrum of **2** revealed the presence of four methoxy groups at δ 3.29, 3.38, 3.67, and 3.83 (all s, 3 H) and two mutually coupled methylenes, δ 3.02 and 4.55–4.67 (both m, 2 H). The aromatic protons of the triacetate **6**, produced by acetylation of **2** (excess acetic anhydride in pyridine), were well dispersed in the ^{1}H NMR spectrum and revealed all couplings among these protons. For the triacetate three aromatic singlets were observed at δ 6.71, 6.83 and 7.14 (all 1 H). Also, three protons characteristic of a 1,3,4-trisubstituted benzene ring were seen at δ 7.08 (dd, J = 7.8, 1.9 Hz), 7.15 (d, J = 1.9 Hz), and 7.23 (d, J = 7.8 Hz).

From the close similarities between the HRMS, ^{1}H NMR, and UV spectra of **2** with those of the recently reported marine alkaloids, lamellarins A–D,[7] we assigned **2** as a dihydrolamellarin. Lamellarin E (**2**) differed from the previously described dihydrolamellarin, lamellarin C, in the number of aromatic methyl ethers. Assuming as before,[7] that the phenyl group attached at C1 shields the protons at C10 and C22 and the methyl groups of the methoxys attached at C9 and C21, we were able to place two of the four methoxys, δ 3.29 and 3.38, at C9 and C21 in lamellarin E. However, the confident assignment of the other aromatic methyl ethers to any of the five remaining possible sites of attachment could not be made without additional information.

The problem of determining the methoxy substitution pattern of lamellarin E was solved by two techniques, an analysis of the direct ($^1J_{C-H}$) and long range ($^{2-3}J_{C-H}$) ^{1}H–^{13}C couplings, including $^3J_{C-H}$ couplings through oxygen, and by an X-ray crystallographic study.

Results from the 2D $^1J_{C-H}$ correlation experiments for lamellarins E–G are given in Table I. The results from 2D $^{2-3}J_{C-H}$ correlation experiments for lamellarins E–G are listed in Table III. For each compound, a 2D $^{2-3}J_{C-H}$ correlation experiment(s) was performed, optimized for observing 3.5, 6, or 8 Hz J_{C-H} couplings.

Table II. ^{13}C NMR Assignments for Lamellarins E–H[a]

C no.	lamellarin E (2)	lamellarin F (3)	lamellarin G (4)	lamellarin H (5)
1	115.0	115.0	114.2	121.0
2	127.2	127.4	126.7	125.4
3	112.5	112.7	112.4	108.5
5	41.6	41.6	41.9	116.9
6	21.1	21.2	27.4	109.3
6a	114.2	114.2	127.0	123.7
7	147.2	147.2	115.2	112.2
8	136.3	136.3	146.9	146.1
9	150.7	150.7	145.8	145.4
10	100.8	100.7	109.0	109.3
10a	122.2	122.2	117.8	117.7
10b	135.0	135.1	135.9	133.9
11	127.2	127.1	127.1	128.8
12	117.7	114.2	117.6	117.6
13	147.4	149.6	147.3	147.1
14	147.5	148.6	147.3	146.8
15	113.3	112.7	113.3	112.5
16	121.4	123.0	121.3	121.4
17	108.4	108.4	110.0	106.1
18	146.7[b]	146.9[c]	144.5[d]	145.3
19	103.5	103.5	100.6	103.2
20	145.5[b]	145.5[c]	147.7[d]	148.2
21	144.3	144.4	142.6	142.1
22	104.9	104.8	108.2	109.3
23	154.2	154.2	154.2	154.4
R_1			55.7	
R_2	55.0	54.9		
R_3	56.0	55.8	55.6	
R_4		55.8		
R_5	54.6	54.5	54.5	
R_6	60.2	60.2		

[a] ^{13}C NMR spectra were recorded at 50 MHz in DMSO-d_6. Assignments for lamellarins E–G were aided by DEPT sequence experiments, $^1J_{C-H}$ and $^{2-3}J_{C-H}$ correlation experiments, and the X-ray structure of lamellarin E. Assignments for lamellarin H were based on a comparison of the ^{13}C NMR assignments for lamellarin G and DEPT sequence experiments. Chemical shifts are given in δ units (downfield of Me_4Si). [b–d] Assignments may be reversed.

For lamellarin E (Table III), the proton at δ 6.34 exhibited correlations to a distinct group of ^{13}C NMR resonances, which included δ 150.7 and 136.3, the carbons of attachment for the two methoxys in the ^{1}H NMR spectrum at δ 3.29 and 3.67. Since $^4J_{C-H}$ couplings are not observed, the correlations between the C10 proton and two methoxy-bearing carbons allowed the assignment of methoxy

Table III. Combined Results of Three $^{2-3}J_{C-H}$ Correlation Experiments for Lamellarins E–G[a]

	long range correlations to C no.		
^{1}H at C no.	J = 8 Hz	J = 6 Hz	J = 3.5 Hz
	Lamellarin E (2)		
R_2	21		
R_3	14		
R_5	9		
R_6	8		
10	6a, 8, 9, 10a, 10b	NP[b]	NP
12, 16	1, 14, 16		
19	17, 18, 20, 21		
22	2, 18, 20		
	Lamellarin F (3)		
R_2	21	21	
R_3	14	2	
R_4	13	13	
R_5	9	9	
R_6	8	8	
10	8, 9, 10b, 12	8, 9, 10a, 10b, 12	8, 10a, 10b
12	1, 11, 13, 14, 16	1, 11, 13, 14, 16	1, 11
15	11, 13, 14, 16	11, 13, 14, 16	11
16		1, 12, 14	1
19	17, 18, 20, 21	17, 21	17, 20
22	2, 18, 20, 21	2, 18, 20, 21	2, 18, 20
	Lamellarin G (4)		
R_1	20	20	
R_3	14	14	
R_5	9		
7	9, 10a	9, 10a	
10	6a, 8, 9, 10b	6a, 8, 10b	
12, 16	1, 14, 16	1, 13, 16	NP
15		11, 13, 14	
19	17, 18, 20, 21	17, 18, 21	
22	2, 18, 20, 21	2, 18, 20, 21	

[a] $^{2-3}J_{C-H}$ correlation experiments were performed on lamellarin E, optimized for observing 8 Hz coupling; lamellarin F, optimized for observing 8, 6, and 3.5 Hz couplings; and lamellarin G, optimized for observing 8 and 6 Hz couplings. All $^{2-3}J_{C-H}$ correlation experiments were recorded at 50 MHz in DMSO-d_6. [b] $^{2-3}J_{C-H}$ correlation experiments optimized for observing those $^{2-3}J_{C-H}$ values were not performed.

at C8 and not at C7. In addition, the relative upfield shift, δ 136.3, of the methoxy-bearing C8 carbon also supported this assignment. The two singlet protons, δ 6.64 and 6.81, showed correlations to a second group of ^{13}C NMR signals, which included δ 144.3, the carbon of attachment for the shielded methyl ether group at δ 3.38.

The last methyl ether resonance (δ 3.83) to be assigned was placed at either C13 or C14 on the basis of its correlation to the carbon resonance at δ 147.5. A meta orientation for the oxygens on this phenyl ring was discounted since none of its protonated carbons showed the upfield shift in the ^{13}C NMR spectrum, which would be expected for a meta orientation of hydroxys and/or methoxys. Because of the overlap of the C13 and C16 protons in the 2D $^{2-3}J_{C-H}$ NMR correlation spectrum, analysis of these correlations could not distinguish between placement of methoxy at C13 or C14.

The final assignments of the methoxy positions were made from the X-ray structure of lamellarin E (Figure 1), which placed the δ 3.83 methoxy group at C14. The X-ray structure also confirmed the assignments of the δ 3.29, 3.38, and 3.67 methoxys at C9, C21, and C8, respectively. With the X-ray structure and the $^{1-3}J_{C-H}$ correlation data, complete assignment of the ^{1}H and ^{13}C NMR spectra was possible (Tables I and II).

A computer-generated perspective drawing of the final X-ray model of lamellarin E (2) is shown in Figure 1. The X-ray structure showed that the crystal was racemic, so the atropisomer shown is accompanied by an equal amount of its enantiomer. The racemic nature of lamellarins E–H

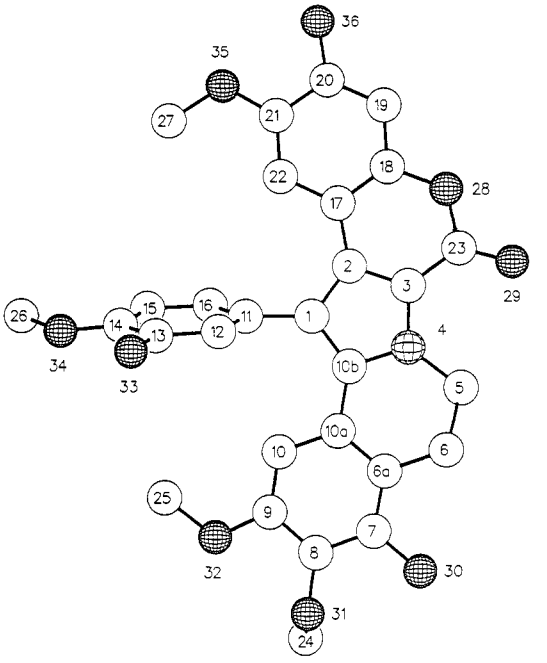

Figure 1. Computer-generated perspective drawing for the final X-ray structure of lamellarin E (2). The compound is racemic and the enantiomer shown is accompanied by equal amounts of its enantiomer.

was also indicated by their lack of optical activity. The structure of lamellarin E (2) is divided into two planar portions, and the angle between them is 100°.

Lamellarin F (3) crystallized as white prisms from DMSO, mp 273–276 °C dec. It differed from lamellarin E by an additional methoxy group as indicated by an increase of 14 mass units for the $M^+ + H$ ion in the HRFAB mass spectrum (m/z 546.1789, $C_{30}H_{28}NO_9$, requires 546.1764) and by the appearance of additional signals at δ 3.75 (s, 3 H) and 55.8 in the ^{1}H and ^{13}C NMR spectra. Lamellarins E and F showed virtually identical UV spectra, indicating no change in the chromophore. Acetylation of lamellarin F yielded the expected diacetate 7.

The analysis of $^1J_{C-H}$ and $^{2-3}J_{C-H}$ NMR correlation data for lamellarin F allowed the relative positions of methoxys and hydroxys to be determined (Chart I) and resulted in complete assignment of both the ^{1}H and ^{13}C NMR spectra. Because excellent resolution of all the aromatic protons in the J_{C-H} correlation experiments was obtained for lamellarin F, the individual correlation groupings allowed specific structural questions to be addressed. First, $^3J_{C-H}$ correlations between the protons at C12 and C16 to C1 established that oxygenation of the phenyl ring was at C13 and C14 and not at C12 or C16. Secondly, the $^3J_{C-H}$ correlation between the C16 proton and C14, bearing the δ 3.83 methoxy group, allowed assignment of the new methyl resonance (δ 3.75) to a methyl ether at C13. Also, observation of an ^{1}H NMR NOE enhancement of the methyl resonance at δ 3.75 upon irradiation of the doublet proton (J = 1.6 Hz) at δ 7.09, and observation of an NOE enhancement of the methyl resonance at δ 3.83 upon irradiation of the doublet proton (J = 8.1 Hz) at δ 7.01, confirmed these assignments.

Lamellarin G (4) was obtained as white prisms, mp 263–265 °C dec, from DMSO. The molecular formula $C_{28}H_{23}NO_8$, calculated from the molecular ion at m/z 501.1373 in the HRFAB mass spectrum, revealed the loss of an oxygen atom relative to lamellarins E and F. The appearance of an additional aromatic singlet in the ^{1}H

NMR spectrum indicated the oxygen at C7 had been replaced by a proton, as was found for lamellarin D.[7] This change is shown in the ^{13}C NMR spectrum by the change of an oxygenated aromatic carbon resonance to a protonated aromatic carbon at δ 115.2. Of the three methyl resonances in the ^1H NMR spectrum, only one showed the upfield shift due to the shielding effect of the phenyl group at C1. The methoxy, corresponding to a methyl resonance at δ 3.27, was placed at C9 due to the $^1J_{C-H}$ and $^{2-3}J_{C-H}$ correlations observed for the C6a–C10a aromatic system. The δ 3.81 methoxy was assigned to C20 since it did not experience the shielding effect of the phenyl group at C1. Further, J_{C-H} correlations established that this methoxy belonged on the C17–C22 aromatic system. Finally, the δ 3.86 methoxy was positioned at C14 and not C13 because of the relative upfield shift of the methoxy at C13 in lamellarin F.

Lamellarin H (**5**), the most polar and major metabolite from *D. chartaceum*, was isolated as an amorphous solid. A fraction containing significant amounts of this metabolite was initially chromatographed over Florisil, which resulted in significant loss. The UV spectrum of lamellarin H [391 (ϵ 12000), 373 (9000), 340 (6000), 324 (sh), 303 (sh), 281 (24000), and 208 nm (31000)] indicated a more extensively conjugated chromophore. The molecular formula $C_{25}H_{15}NO_8$ was calculated from the parent ion (M^+, m/z 451.0787) in the HRFAB mass spectrum of lamellarin H and also reflected this increase in unsaturation. The ^1H and ^{13}C NMR spectra of lamellarin H showed neither methyl ether nor aliphatic resonances. Nine signals were observed in the ^1H NMR spectrum; four singlets at δ 6.56, 6.79, 6.94, and 7.11, resonances at δ 6.99 (d, 1 H, J = 7.9 Hz), 6.80 (d, 1 H, J = 1.9 Hz), and 6.71 (dd, 1 H, J = 7.9, 1.9 Hz) were assigned to C13, C16, and C12, respectively, and the two remaining doublets (J = 7.3 Hz) at δ 8.98 and 7.12 were assigned to C5 and C6. The C5–C6 double bond was first observed in lamellarins B and D.[7] Acetylation of lamellarin H yielded the expected hexaacetate **9**. Lamellarin H is unique among this group of alkaloids in that it is the sole metabolite totally lacking methoxy functionalities.

The evolutionary trend in some opisthobranch molluscs toward a reduction in shell size or loss of the shell has been correlated with the presence of bioactive secondary metabolites.[8] Chemically rich sponges, bryozoans, and coelenterates[9,10] have been shown to be the dietary sources of certain biologically active compounds isolated from opisthobranchs of the order Nudibranchia. In other cases, chemically rich algae of all three major divisions have been implicated as the source of bioactive compounds sequestered by sea hares (order Anaspidae) and ascoglossans (order Ascoglossa).[9] These physically vulnerable molluscs are believed to use these dietary-derived compounds as a chemical defense against potential predators.[8,10]

Prosobranch molluscs of the family Lamellariidae resemble opisthobranch molluscs in that they possess reduced shells covered completely or in part by a fleshy mantle.[11,12] Members of the family Lamellariidae have been described as specific predators of colonial ascidians,[11-13] as have many nudibranchs.[12] These molluscs, preying on ascidians, appear to have followed a similar evolutionary pattern as the numerous opisthobranchs preying on chemically rich sponges, bryozoans, coelenterates, and algae. This report of lamellarins E–H from *D. chartaceum* indicates that *Lamellaria* sp. most likely acquired lamellarins A–D from a similar ascidian food source.

Experimental Section

General. General instrumentation and procedures in common use have been described in an earlier contribution.[14] Direct and long range J_{C-H} 2D NMR correlation experiments were performed on a Bruker WP-200 spectrometer at 50 MHz in DMSO-d_6. A 256w (^1H) × 4K(^{13}C) data matrix was used for all J_{C-H} correlation experiments. The $^1J_{C-H}$ correlation experiments were acquired by using the pulse sequence described by Bax and Morris[15] and the $^{2-3}J_{C-H}$ correlation experiments were acquired by using the pulse sequence proposed by Kessler et al.[16]

Collection, Extraction, and Isolation Procedures. The purple and brown color morphs of *D. chartaceum* were collected by using SCUBA (−10 to −15 m) off Picard Island, Aldabra Atoll, Republic of the Seychelles, in March 1985. Both color morphs were found to contain similar secondary metabolites by in situ analysis of the extracts by thin layer chromatography. The ascidian was preserved in isopropyl alcohol. The alcohol was subsequently removed by rotary evaporation to leave an aqueous residue that was then lyophilized along with the remaining frozen animal. The lyophilized aqueous residue and the ascidian were combined (67.8 g dry weight) and extracted first with acetone (3 × 300 mL) followed by methanol (3 × 300 mL). The combined acetone and methanol extracts were chromatographed by silica gel vacuum flash chromatography using hexane–ethyl acetate mixtures. The column was rinsed finally with acetone and methanol. Further purification of the alkaloids was accomplished by Sephadex LH-20 (2:1 CH_2Cl_2/MeOH) with final purification by reversed-phase HPLC silica-ODS using 20% water–methanol as the eluting solvent. Yields of lamellarins E–H (**2**–**5**) were 30.7 (0.045% dry wt), 30.0 (0.044% dry wt), 20.2 (0.030% dry wt), and 9.0 mg (0.013% dry wt), respectively.

Lamellarin E (2): white prisms (methanol); mp 228–232 °C; UV (MeOH) 330 (sh), 310 (ϵ 23000), 278 (38000), 268 (sh), 206 (48000); UV (MeOH + NaOH) 323 (ϵ 23000), 288 (28000), 265 (sh), 205 (64000); IR (Nujol) 3400, 1695, 1460, 1375 cm^{-1}; HRFABMS, obsd (M^+ + H) m/z 532.1563, $C_{29}H_{26}NO_9$ requires 532.1608.

Lamellarin F (3): white prisms (DMSO); mp 273–276 °C dec; UV (MeOH) 330 (sh), 312 (ϵ 28000), 278 (31000), 268 (sh), 210 (47000); UV (MeOH + NaOH) 322 (ϵ 31000), 290 (28000), 207 (52000); IR (Nujol) 3390, 1690, 1460, 1375 cm^{-1}; HRFABMS, obsd (M^+ + H) m/z 546.1789, $C_{30}H_{28}NO_9$ requires 546.1764; NOESY and difference NOE correlations δ 7.20–3.83, 7.09–3.75, 6.53–3.34, 6.33–3.26.

Lamellarin G (4): white prisms (DMSO); mp 263–265 °C dec; UV (MeOH) 334 (ϵ 21000), 314 (21000), 276 (25000), 267 (sh), 206 (46000); UV (MeOH + NaOH) 368 (sh), 346 (ϵ 8000), 324 (12000), 278 (12000), 204 (55000); IR (Nujol) 3350, 1710, 1690, 1460, 1375 cm^{-1}; HRFABMS, obsd (M^+) m/z 501.1373, $C_{28}H_{23}NO_8$ requires 501.1424.

Lamellarin H (5): amorphous solid; UV (MeOH) 391 (ϵ 12000), 373 (9000), 340 (6000), 324 (sh), 303 (sh), 281 (24000), 208 (31000); UV (MeOH + NaOH) 405 (ϵ 13000), 373 (sh), 311 (sh), 298 (20000), 205 (44000); HRFBMS, obsd (M^+) m/z 457.0787, $C_{25}H_{15}NO_8$ requires 457.0797.

Preparation of Acetates 6–9. In a typical procedure, acetic anhydride (0.5 mL) was added to a solution of **2** (4 mg) in pyridine and stirred for 24 h at room temperature. Reagents were evaporated under high vacuum and the residue was purified by preparative TLC to obtain the diacetate **6** (2.5 mg) as a colorless oil.

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Triacetate 6: oil; ^{1}H NMR (360 MHz, DMSO-d_6) δ 2.00 (s, 3 H), 2.25 (s, 3 H), 2.27 (s, 3 H), 3.13 (m, 2 H), 3.26 (s, 3 H), 3.38 (s, 3 H), 3.77 (s, 3 H), 3.84 (s, 3 H), 4.67 (m, 1 H), 4.78 (m, 1 H), 6.71 (s, 1 H), 6.83 (s, 1 H), 7.08 (dd, 1 H, J = 7.8, 1.9 Hz), 7.14 (s, 1 H), 7.15 (d, 1 H, J = 1.9 Hz), 7.23 (d, 1 H, J = 7.8 Hz).

Diacetate 7: oil; ^{1}H NMR (360 MHz, DMSO-d_6) δ 2.25 (s, 3 H), 2.38 (s, 3 H), 2.96 (m, 2 H), 3.31 (s, 3 H), 3.34 (s, 3 H), 3.70 (s, 3 H), 3.78 (s, 3 H), 3.86 (s, 3 H), 4.62 (m, 1 H), 4.70 (m, 1 H), 6.67 (s, 1 H), 6.77 (s, 1 H), 7.08 (dd, 1 H, J = 7.8, 1.9 Hz), 7.16 (d, 1 H, J = 1.9 Hz), 7.23 (d, 1 H, J = 7.8 Hz), 7.30 (s, 1 H).

Triacetate 8: oil; ^{1}H NMR (360 MHz, DMSO-d_6) δ 2.20 (s, 3 H), 2.24 (s, 3 H), 2.27 (s, 3 H), 3.10 (m, 2 H), 3.29 (s, 3 H), 3.83 (s, 3 H), 3.88 (s, 3 H), 4.56 (m, 1 H), 4.84 (m, 1 H), 6.59 (s, 1 H), 6.83 (s, 1 H), 7.14 (s, 1 H), 7.25 (s, 1 H), 7.26 (d, 1 H, J = 1.8 Hz), 7.32 (dd, 1 H, J = 8.0, 1.8 Hz), 7.38 (d, 1 H, J = 8.0 Hz).

Hexaacetate 9: amorphous solid; ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.25 (s, 3 H), 2.26 (s, 3 H), 2.30 (s, 3 H), 2.31 (s, 3 H), 2.32 (s, 3 H), 2.37 (s, 3 H), 7.08 (s, 1 H), 7.13 (d, 1 H, J = 7.4 Hz), 7.30 (s, 1 H), 7.39 (d, 1 H, J = 1.9 Hz), 7.41 (d, 1 H, J = 1.9 Hz), 7.42 (s, 1 H), 7.45 (s, 1 H), 7.58 (s, 1 H), 9.32 (d, 1 H, J = 7.4 Hz); HRDEIMS, obsd (M^+) m/z 709.1416, $C_{37}H_{27}NO_{14}$ requires 709.1432.

Single-Crystal X-ray Analysis of Lamellarin E (2). Crystals of lamellarin E were grown from a methanol solution by slow evaporation. Crystals formed in the triclinic class with a = 8.165 (3), b = 13.228 (4), and c = 17.9892 (4) Å, α = 59.143 (14)°, β = 89.732 (16)°, γ = 110.495(15)°. The crystal density (1.3 g/cm^3) indicated that two units of composition $C_{29}H_{25}O_9N\cdot CH_3OH$ formed the unit cell. The space group had to be either P_1 (Z = 2) or $P\bar{1}$ (Z = 1).

All unique diffraction maxima with $2\theta < 114°$ were collected by using graphite-monochromated Cu Kα radiation (1.54178 Å) and variable speed, 1° ω scans. After correction for Lorentz, polarization, and background effects, 3040 reflections (83%) were judged observed ($F_o > 3\sigma(F_o)$). A phasing model was found in space group $P\bar{1}$ using the MULTAN series of programs.[17] All non-hydrogen atoms were found by recycling plausible molecular fragments. Hydrogen atoms were located on a ΔF synthesis following partial refinement. Block-diagonal least-squares refinements with anisotropic non-hydrogen atoms and fixed isotropic hydrogens have converged to a current residual of 0.073 for the observed data. Additional crystallographic information is available as described in the paragraph entitled Supplementary Material Available at the end of this paper.

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Supplementary Material Available: Tables of fractional coordinates, thermal parameters, interatomic distances, and interatomic and torsional angles for lamellarin E (7 pages). Ordering information is given on any current masthead page.

(17) All crystallographic calculations were done on a PRIME 9950 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were FOBS, a data reduction program by G. D. Van Duyne, Cornell University, 1987; MULTAN80 and RANTAN80, systems of computer programs for the automatic solution of crystal structures from X-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, University of York, England, 1980; BLS78A, an anisotropic block-diagonal least-squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLUTO78, a locally modified crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1987; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu and G. Van Duyne, Cornell University, 1985.

Sesterterpene Sulfates from a Sponge of the Family Halichondriidae

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A marine sponge of the family Halichondriidae from California contained a sulfated sesterterpene hydroquinone and five sulfated sesterterpenes. The structures of halisulfates 1–5 (**1–5**) were elucidated by interpretation of spectral data and a structure is proposed for halisulfate 6 (**6**).

Among the wide variety of secondary metabolites that have been isolated from marine sponges,[1] relatively few sulfate esters have been described. The most frequently encountered sulfate esters are the steroidal sulfates[2] and phenolic sulfates.[3] During a search for biologically active sponge metabolites, we have found five sulfated sesterterpene furans and a sulfated sesterterpene hydroquinone in a Californian sponge of the family Halichondriidae. In this paper we report the structural elucidation of a sulfated sesterterpene hydroquinone, halisulfate 1 (**1**), and five sulfated sesterterpene furans, halisulfates 2–6 (**2–6**) (Chart I).

The dark brown sponge was collected at −30 m in Scripps Canyon, La Jolla, CA. The dichloromethane-soluble material from a methanol extract was chromatographed on Sephadex LH-20 with 1:1 dichloromethane–methanol as eluant to obtain two fractions that inhibited *Staphylococcus aureus* and *Candida albicans*. Halisulfate 1 (**1**), mp >300 °C (darkens at ~200 °C), crystallized from the more polar fraction. The less polar fraction was separated on reversed-phase HPLC using 30% aqueous am-

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