Aciculitins A−C: Cytotoxic and Antifungal Cyclic Peptides from the Lithistid Sponge *Aciculites orientalis*

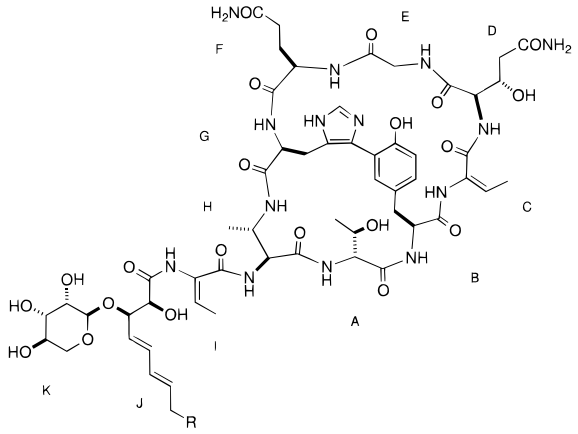
Carole A. Bewley, Haiyin He, David H. Williams, and D. John Faulkner*

Contribution from the Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0212

Received October 30, 1995[⊗]

Abstract: The lithistid sponge *Aciculites orientalis* contains three cyclic peptides, aciculitins A−C (**1**−**3**), that are identical except for homologous lipid residues. The structure of the major peptide, aciculitin B (**2**), was elucidated by interpretation of spectroscopic data. The aciculitins consist of a bicyclic peptide that contains an unusual histidinotyrosine bridge. Attached to the bicyclic peptide are C_{13}−C_{15} 2,3-dihydroxy-4,6-dienoic acids bearing D-lyxose at the 3-position. The structures of aciculitamides A (**4**) and B (**5**), which are artifacts obtained earlier from this sponge, are also presented. The aciculitins **1**−**3** inhibited the growth of *Candida albicans* and were cytotoxic toward the HCT-116 cell line.

Sponges of the order Lithistida produce some of the most interesting bioactive marine natural products.[1,2] Some noteworthy examples, all of which have been the subject of synthesis programs, are the cytotoxic macrolide swinholide A from *Theonella swinhoei*,[3,4] the potent immunosuppressive agent discodermolide from *Discodermia dissoluta*,[5,6] and, from various *Theonella* spp., several novel cyclic peptides including cyclotheonamides A and B,[7] which inhibit serine proteases such as plasmin, thrombin, and trypsin.[8,9] As part of a study of lithistid sponges, we have recently described antifungal cyclic peptides from *Microscleroderma* sp.[10] and *Theonella swinhoei*.[11] We now report the isolation and structural elucidation of aciculitins A (**1**), B (**2**), and C (**3**) from the lithistid sponge *Aciculites orientalis*. The aciculitins are the first glycopeptidolipids obtained from a marine source.



1 R = C_5H_{11}
2 R = C_6H_{13}
3 R = C_7H_{15}

A threonine
B tyrosine
C 2-amino-2-butenoic acid-1
D β-hydroxyglutamine
E glycine
F glutamine
G histidine
H 2,3-diaminobutyric acid
I 2-amino-2-butenoic acid-2
J C_{13}-C_{15} dienoic acids
K lyxose

A specimen of *Aciculites orientalis* was collected by hand using SCUBA (−60 m) at Siquijor Island, the Philippines, and was immediately frozen. The lyophilized sponge was exhaustively extracted with hexanes, followed by ethyl acetate, to obtain extracts that contained mainly triglycerides and sterols, respectively. After removal of all residual solvent, the dry sponge was repeatedly extracted with 50% aqueous acetonitrile until the extracts were inactive when assayed against *Candida albicans*. Approximately half of the acetonitrile was removed *in vacuo*, and the residue was diluted with an equal volume of water, pumped onto a preparative C-18 cartridge, desalted with 0.05% TFA solution, and eluted with a gradient of 20−60% acetonitrile in 0.05% TFA to obtain aciculitins A (**1**, 0.06% dry wt), B (**2**, ca. 1% dry wt), and C (**3**, 0.1% dry wt) as pale yellow powders.

The molecular formulas of aciculitins A−C (**1**−**3**) were established by high resolution mass spectrometry as $C_{61}H_{86}N_{14}O_{21}$, $C_{62}H_{88}N_{14}O_{21}$, and $C_{63}H_{90}N_{14}O_{21}$, respectively, which indicated that they were homologues. A preliminary examination of the spectral data suggested that the aciculitins differed only in the length of an alkyl chain. We therefore chose to direct our attention to the structural elucidation of the major product, aciculitin B (**2**). The IR spectrum contained bands at 3310, 1660, and 1540 cm^{-1} that are typical of peptides. The UV absorptions at 270 nm (ϵ 12 700), 310 (1300), and 330 (1100) were assigned to aromatic systems and a reversible bathochromic shift from 310 nm to 330 on addition of base suggested the presence of a phenol.

The ^{13}C NMR spectrum contained 62 signals, as expected from the molecular formula. The DEPT experiment indicated

[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1996.

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1995**, *12*, 223 and previous reviews in this series.
(2) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793.
(3) Doi, M.; Ishida, T.; Kobayashi, M.; Kitagawa, I. *J. Org. Chem.* **1991**, *56*, 3629.
(4) Paterson, I.; Yeung, K. S.; Ward, R. A.; Cumming, J. G. *J. Am Chem. Soc.* **1994**, *116*, 9391.
(5) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912 (erratum: **1991**, *56*, 1346).
(6) Nerenberg, J. B.; Hung, D. T.; Somers, P. K.; Schreiber, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 12621.
(7) Fusetani, N.; Matsunaga, S.; Matsumoto, H.; Takebayashi, Y. *J. Am. Chem. Soc.* **1990**, *112*, 7053.
(8) Hagihara, M.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6570.
(9) Maryanoff, B. E.; Qiu, X. Y.; Padmanabhan, K. P.; Tulinsky, A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8048.
(10) Bewley, C. A.; Debitus, C.; Faulkner, D. J. *J. Am. Chem. Soc.* **1994**, *116*, 7631.
(11) Bewley, C. A.; Faulkner, D. J. *J. Org. Chem.* **1994**, *59*, 4849.

S0002-7863(95)03628-6 CCC: $12.00

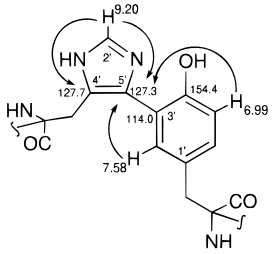


Figure 1. HMBC correlations used to establish connectivity between C-5′ of histidine and C-3′ of tyrosine.

5 methyl, 13 methylene, 25 methine (of which 10 are sp^2), and 19 quarternary carbons; thus, there are 22 exchangeable protons.[12] The DQCOSY[13] and TOCSY[14] spectra indicated the presence of the amino acids glycine, threonine, 2,3-diaminobutyric acid (Dab), and glutamine or glutamic acid. In addition, it was possible to identify a 1,3,4-trisubstituted phenol, a 2,3-dihydroxytetradeca-4,6-dienoic acid, and a C_5 pyranose unit. Analysis of coupling constant and NOE data revealed the (4*E*,6*E*)-geometry of the dienoic acid and indicated that the pyranose unit was lyxose. Two 2-amino-2-butenoic acid (dAbu) units were assigned from the COSY and HMBC[15] data, which provided correlations from the amide signals at δ_H 9.24 and 9.74 to their respective olefinic carbon signals at δ 117.0 and 118.0, that were in turn correlated to two vinyl methyl signals at δ_H 1.83 and 1.81, respectively. The methyl signals at δ 1.83 and 1.81 were coupled to their respective vinyl proton signals at 6.26 and 5.90. ROESY[16] correlations between the signals at δ 6.26 and 5.90 and the −NH signals at 9.24 and 9.74 indicated the (*E*)-geometry for both dAbu units. A β-hydroxyglutamine (Hgln) residue was identified from the DQCOSY and HMBC data: the H-3 signal at δ 4.58 showed correlations to two carbonyl signals at 171.6 and 173.7, the second of which was correlated to two amide NH signals at 7.00 and 7.61.

The unusual combination of tyrosine and histidine residues joined through the 3′-position of tyrosine and the 5′-position of histidine was identified largely by interpretation of the HMBC data (Figure 1). The COSY, HMQC, and HMBC data (Table 1) clearly indicated the presence of a 3′-substituted tyrosine (*m*-Tyr) moiety; in addition, we observed two unexpected HMBC correlations from H-2′ and H-5′ of tyrosine to the same carbon signal at δ 127.3, which was also correlated to an imidazole proton signal at δ 9.20. Further analysis of the spectral data revealed that the carbon signal at δ 127.3 was assigned to C-5′ of a histidine residue. The strong ROE observed between H-2′ of *m*-Tyr and the β protons of His, together with the downfield position of the phenolic proton at δ 12.10, suggests that the tyrosine and histidine rings are approximately coplanar, with hydrogen bonding between the phenolic proton and N-1 of histidine.

Sequencing of Aciculitin B

The sequence of the amino acids and the positions of the fatty acid and lyxose moieties were determined by interpretation of two HMBC experiments (J = 6 and 8 Hz, see Table 1) and a ROESY experiment. The respective two-bond HMBC correlations from the amide NHs of glutamine, glycine, and β-hydroxyglutamine to the carbonyl carbons of glycine, β-hydroxyglutamine, and 2-amino-2-butenoic acid established the sequence of these four amino acids. A three-bond correlation from the α-proton of histidine (δ 5.12) to the carbonyl carbon of glutamine (δ 171.5) allowed the sequence to be extended to dAbu(1)-βOHGln-Gly-Gln-His. The sequence of the remaining four amino acids, *m*-Tyr-Thr-Dab-dAbu(2), was also established from two-bond HMBC correlations; the amide NHs of tyrosine, threonine and the NH-2 of 2,3-diaminobutyric acid were correlated to the carbonyl carbons of threonine, 2,3-diaminobutyric acid, and a second 2-amino-2-butenoic acid residue, respectively. In addition, a three-bond correlation from the α-proton of Dab to C-1 of dAbu(2) confirmed that Dab was coupled to dAbu(2) through NH-2 as opposed to NH-3. A two-bond HMBC correlation between the NH of the same 2-amino-2-butenoic acid and the carbonyl carbon of the 2,3-dihydroxytetradeca-4,6-dienoic acid residue provided the location of the fatty acid group. The lyxose residue could be placed on the 3-hydroxyl group of the 2,3-dihydroxytetradeca-4,6-dienoic acid residue as a result of three-bond correlations between the anomeric proton at δ 4.68 and the carbon signal at δ 76.2 and between the H-3 signal at 4.40 and the anomeric carbon at 96.6. At this stage we required evidence for two additional amide bonds in order to obtain the required bicyclic structure, but no further inter-residue correlations were observed in the HMBC experiments. However, a strong ROESY correlation between the NH (dAbu-1) signal at δ 9.24 and the α-proton signal at 5.18 of the tyrosine residue allowed connectivity between these two amino acids. By default, the final amide bond must be between the histidine residue and NH-3 of 2,3-diaminobutyric acid.

Determination of Absolute Stereochemistry

The absolute configurations of L-glutamine, L-*allo*-threonine, (2*S*,3*R*)-3-hydroxyglutamine,[17] and (2*S*,3*S*)-2,3-diaminobutyric acid[18] were determined by GC-MS analysis using a chiral GC column. Hydrolysis of aciculitin B (**2**) followed by derivatization of the hydrolysate produced a mixture of pentafluoropropionylamide isopropyl esters that were compared with the same derivatives of authentic amino acid standards. Ozonolysis of **2** at −70 °C for 15 min, followed by oxidation, hydrolysis, and derivatization produced slighly greater than 1 equiv of l-aspartic acid, arising from l-histidine. The same procedure was repeated with the modification of continuing the ozonolysis for a period of 1 h to obtain ca. 1.7 equiv of L-aspartic acid that arise from both L-histidine and L-tyrosine. Mild acid hydrolysis followed by esterification with pentafluoropropionic anhydride and analysis by chiral GC-MS gave peaks that were identical to those produced by a D-lyxose standard.

The assignment of the absolute configuration of the 2,3-dihydroxy-4,6-dienoic acid unit proved somewhat difficult. Catalytic hydrogenation of aciculitin B (**2**) cleanly reduced the diene of the 2,3-dihydroxy-4,6-dienoic acid unit to the corresponding saturated 2,3-dihydroxy acid moiety. The reduced peptide was hydrolyzed and the resulting components were esterified with isopropanol followed by acylation with pentafluoropropionic anhydride to obtain derivatives suitable for chiral GC-MS analysis. The isopropyl esters of racemic *threo*- and

(12) It was necessary to add approximately 0.5% TFA to the NMR samples of aciculitins in DMF-d_7 to prevent tautomerization between NH-1 and NH-3 of the imidazole ring.

(13) (a) Piantini, U.; Sorenson, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800. (b) Rance, M; Sorenson, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wuthrich, K. *Biochem. Biophys. Res. Comm.* **1983**, *117*, 479.

(14) Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521.

(15) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285.

(16) (a) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811. (b) Bax, A. and Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207.

(17) Blaskovich, M. A.; Lajoie, G. A. *J. Am. Chem. Soc.* **1993**, *115*, 5021.

(18) Schmidt, U.; Mundinger, K.; Riedl, B.; Haas, G.; Lau, R. *Synthesis* **1992**, 1201.

Table 1. ^{1}H and ^{13}C NMR Assignments for Aciculitin B (**2**)[a]

amino acid	δ ^{13}C	δ ^{1}H	*m*	*J* (Hz)	HMBC[b]	ROESY[c]
			Threonine Amino Acid 3			
1	172.4					
2	57.8	4.27	t	8.8	172.4, 169.6, 67.2	8.00
3	67.2	4.23	m		172.4, 57.8	8.00
4	31.8	1.29	d	10.5	67.2, 57.8	8.00
NH		8.00	d	8.8	169.6	8.78, 4.50, 4.27, 4.23, 3.98, 1.29
			Tyrosine			
1	171.6					
2	56.4	5.18	m		172.4, 171.6	9.24, 8.96, 7.59, 7.58
3	38.0	2.90	dd	15.6, 5.9	132.4, 130.2, 129.0, 56.4	7.58, 7.15
		3.24	dd	15.6, 9.3		7.58
1′	129.0					
2′	130.2	7.58	d	1.9	154.4, 132.4, 127.7	5.18, 3.38, 3.24, 3.05, 2.90
3′	114.0					
4′	154.4					
5′	115.8	6.99	d	8.3	154.4, 129.0, 114.0	7.15
6′	132.4	7.15	dd	8.3, 1.9	154.4, 130.2	6.99, 2.90
NH		8.96	d	6.8		5.18
OH		12.10	br s			
			dAbu-1[d]			
1	167.5					
2	132.4					
3	117.0	6.26	q	7.3	167.5, 132.4, 13.4	1.83
4	13.4	1.83	d	7.3	132.4, 117.0	8.45, 6.26
NH		9.24	br s			5.18
			β-Hydroxyglutamine			
1	171.6					
2	59.0	4.48	m		171.6, 167.5, 67.3, 39.5	8.45, 7.58
3	67.3	4.58	m		173.7, 171.6, 39.5	
4	39.5	2.54	m		173.7, 67.3, 59.0	8.45
5	173.7					
NH		8.45	d	7.8	167.5	4.48, 2.54, 1.83
NH_2		7.00	br s		173.7	
		7.61	br s		173.7	
			Glycine			
1	171.7					
2	42.8	3.74	dd	16.6, 4.9	171.7	8.39, 7.59
		3.98	dd	16.6, 5.9	171.7	8.39, 8.00, 7.59
NH		7.59	br t	5.5	171.6	5.18, 4.48, 3.98, 3.74
			Glutamine			
1	171.5					
2	54.0	4.43	m		171.5	8.39, 8.27, 1.94
3	27.7	1.94	m		54.0, 31.2	4.43
4	31.2	2.18	dt	15.4, 7.8	176.4, 54.0, 27.7	7.61
		2.35	dt	15.4, 7.8	176.4, 54.0, 27.7	7.61
5	176.4					
NH		8.39	d	6.8	171.7	4.43, 3.98, 3.74
NH_2		7.61	br s		176.4	2.35, 2.18
			Histidine			
1	169.8					
2	50.8	5.12	m		171.5, 169.8, 28.0	8.27
3	28.0	3.05	dd	15.6, 9.3	169.8, 127.7, 50.8	7.58
		3.38	dd	15.6, 2.9	127.7, 50.8	7.58
2′	133.0	9.20	s		127.7, 127.3	
4′	127.7					
5′	127.3					
NH		8.27	d	7.8		5.12, 4.43
			2,3-Diaminobutyric Acid			
1	169.6					
2	58.9	4.50	m		166.7, 45.8	8.78, 8.00, 4.83
3	45.8	4.83	m			4.50
4	17.9	1.22	d	6.7	58.9, 45.8	8.78, 1.81
NH-2		8.78	d	6.8	166.7	8.00, 4.50, 1.81, 1.22
NH-3		7.80	br s			

Table 1 (Continued)

amino acid	δ ^{13}C	δ ^{1}H	*m*	*J* (Hz)	HMBC[b]	ROESY[c]
			dAbu-2			
1	166.7					
2	132.4					
3	118.0	5.90	q	6.3	166.7, 132.4, 13.4	9.74, 1.81
4	13.4	1.81	d	6.3	132.4, 118.0	8.78, 5.90, 1.22
NH		9.74	s		171.9, 166.7	5.90, 4.40, 4.08, 3.69
			Dhtda[e]			
1	171.9					
2	75.0	4.08	d	2.4	171.9	9.74, 5.65, 4.40
3	76.2	4.40	dd	9.2, 2.4	136.0, 96.6	9.74, 6.30, 4.68, 4.08, 3.42
4	126.6	5.65	dd	15.1, 9.2	129.8, 75.0	6.15, 4.68, 4.08
5	136.0	6.30	dd	15.1, 10.3	136.8, 76.2	5.85, 4.68, 4.40
6	129.8	6.15	dd	14.6, 10.3	136.0, 126.6, 32.5	5.65
7	136.8	5.85	m		136.0, 32.5, 29.1	6.30
8	32.5	2.10	m		136.8, 129.8, 29.8	
9	29.1	1.40	m		32.5, 29.1	
10	29.8	1.30	m		32.5, 31.7	
11	29.1	1.29	m			
12	31.7	1.29	m			
13	22.5	1.29	m		13.7	
14	13.7	0.88	t	7.0	31.7, 22.5	
			Lyxose			
1	96.6	4.68	d	1.5	76.2, 72.0, 71.2, 63.5	6.30, 5.65, 4.40, 3.42
2	71.2	3.70	*f*		72.0	
3	72.0	3.69	*f*		68.1	9.74
4	68.1	3.68	*f*		71.2	3.42
5	63.5	3.42	br s		96.6, 72.0, 68.1	4.68, 4.40, 3.68

[a] All spectra referenced to residual solvent signal of DMF-d_7 (δ_H 2.91, δ_C 39.1) at 25 °C and recorded at 125 MHz for ^{13}C and 500 MHz for ^{1}H. [b] Optimized for $J = 6$ and $J = 8$ Hz. [c] Mixing time = 100 ms with $B_{1(eff)} = 1.9$ KHz. [d] dAbu = 2-amino-2-butenoic acid. [e] 2,3-Dihydroxytetradeca-4,6-dienoic acid. [f] Multiplicities were unassignable in DMF-d_7 due to severe overlap. See Experimental Section for assignments in MeOH-d_4.

erythro-2,3-dihydroxytetradecanoic acids were synthesized and then acylated with pentafluoropropionic anhydride to obtain the same derivatized standards for chiral GC-MS analysis. The *threo* and *erythro* isomers were cleanly separated on the chiral column and indicated that the natural material contained a *threo*-diol; however, the chiral support failed to separate the optical enantiomers. The absolute configuration of the *threo*-2,3-dihydroxytetradecanoic acid residue could only be determined by preparing a chiral ester. The reduced peptide was hydrolyzed, and the resulting components were esterified with (S)-*sec*-butanol to form chiral (*S*)-*sec*-butyl esters and acylated with pentafluoropropionic anhydride. Comparison by chiral GC-MS of the derivatized hydrolysate with derivatives of both enantiomers of *threo*-2,3-dihydroxytetradecanoic acid, prepared as shown in Figure 2,[19,20] showed that the absolute configuration was (2*S*,3*R*).

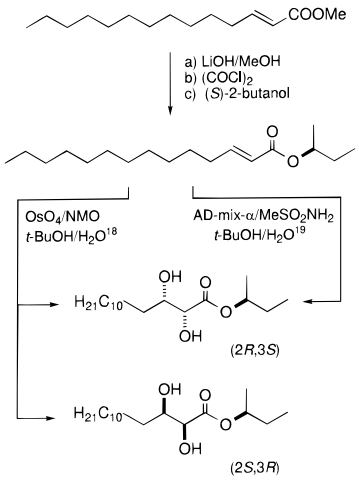


Figure 2. Synthesis of the (2*R*,2′*S*,3*S*) and (2*S*,2′*S*,3*R*) isomers of *sec*-butyl 2,3-dihydroxytetradecanoate.

Aciculitins A (**1**) and C (**3**) exhibit the same profile of biological acitivity as aciculitin B (**2**), and analysis of the spectral data show that **1**−**3** differ only in the length of the alkyl chains of the dihydroxydienoic acids. The similarity between all other spectral data of **1**−**3** suggests that the stereochemistry of acicultins A and C is identical to that of aciulitin B. Aciculitins A−C (**1**−**3**) contain new structural units, namely histidinotyrosine and the 3-lyxose C_{13}−C_{15} 2,3-dihydroxy-4,6-dienoic acids. *β*-Hydroxyglutamine has been reported as a constituent of cyclic peptide antibiotics such as the lipopeptins[21] and neopeptins[22] from unidentified strains of terrestrial species of *Streptomyces*. (2*S*,3*S*)-Diaminobutyric acid occurs in several different linear and basic peptides in which the 3-amino group is a primary amine; examples include antrimycin[23] from *S. xanthocidicus* and lavendomycin from *S. levendulae*.[24]

Aciculitamides A (4) and B (5)

In a previous study of *Aciculites orientalis*, we had isolated and partially identified[25] two metabolites, aciculitamides A and B, that we now believe are artifacts of the isolation procedure. During isolation we had observed loss of biological activity (see

(19) Van Rheenan, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973.
(20) Kolb, H. C.; Van Nieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.
(21) Nishi, M.; Isono, K.; Izaki, K. *Agric. Biol. Chem.* **1981**, *45*, 895.
(22) Ubukata, M.; Uramoto, M.; Isono, K. *Tetrahedron Lett.* **1984**, *25*, 423.
(23) Shimada, N.; Morimoto, K.; Naganawa, H.; Takita, T.; Hamada, M.; Maeda, K.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1981**, *34*, 1613.
(24) Komori, T.; Ezaki, M.; Kino, E.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1985**, *38*, 691.

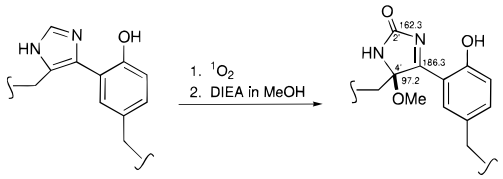
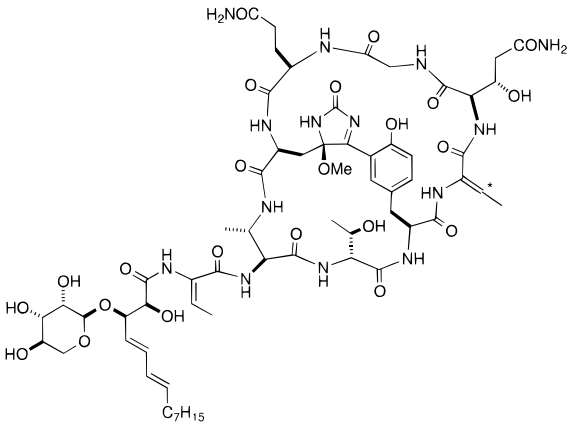


Figure 3. Photooxidation and methanolysis of the imidazole of histidino-tyrosine of aciculitin B (**2**) produces the previously undescribed ring system present in aciculitamides A (**4**) and B (**5**). The ^{13}C chemical shifts shown are for aciculitamide A (**4**) recorded in 2:1 acetone-d_6/DMSO-d_6 (see Experimental Section).

below) and therefore suspected that the peptides were reacting with methanol, which was used as an extraction and chromatographic solvent. Armed with knowledge gained during the structural elucidation of the aciculitins, we have elucidated the structures of aciculitamides A (**4**) and B (**5**) by interpretation of spectral data and by chemical interconversion.



4 * (*E*)-isomer
5 * (*Z*)-isomer

Aciculitamides A (**4**) and B (**5**) were isolated after extraction of *A. orientalis* with methanol and have the same molecular formula, $C_{63}H_{90}N_{14}O_{23}$, which differs from that of the major natural product aciculitin B (**2**) by 46 amu (CH_2O_2). This difference corresponds to the addition of oxygen and methanol and loss of water. The work of Foote and co-workers[26] on the singlet oxygen oxidation of imidazoles provided a mechanistic analogy that allowed us to propose the structures of aciculitamides A (**4**) and B (**5**). [4 + 2]-Cycloaddition of singlet oxygen across the imidazole ring, followed by cleavage of the peroxide bond and replacement of the hydroxyl group by methoxyl, would produce the previously undescribed ring system shown in Figure 3. Analysis of the ^1H and ^{13}C NMR data for aciculitamides resulted in the assignments shown therein. The signals that had previously been most difficult to assign due to the paucity of HMBC correlations were the carbon signals at δ 185.6 and 162.1, but the chemical shifts of these signals and the observed HMBC and ROESY correlations are all compatible with the proposed structure.[26] In order to confirm the structure of aciculitamide A (**4**), aciculitin B (**2**) was reacted with singlet oxygen in 5% DMSO in dichloromethane solution, and the intermediate peroxide was treated with methanol and a hindered base to obtain a product having the same retention time as aciculitamide A (**4**) on reversed-phase HPLC and the same molecular ion at $m/z = 1412$ in the mass spectrum. Comparison of the ^1H NMR spectra also suggested that the synthetic material was identical to that of aciculitamide A (**4**). Interestingly, only one of two possible diastereoisomers of **4** was formed: observation of a ROESY correlation between the methoxy signal and the methyl signal of the diaminobutyric acid unit allowed the stereochemistry of methanol addition to be defined as (*R*). The structure of aciculitamide B (**5**) is the same as that of **4** except that the geometry of the dAbu-1 unit is (*Z*) rather than (*E*), as demonstrated by NOEDS experiments.

Biological Activity

Aciculitins A−C (**1**−**3**) are cytotoxic to the human-colon tumor cell line HCT-116 with an IC_{50} of 0.5 μg/mL and inhibit the growth of *Candida albicans* at a loading of 2.5 μg/disk in the standard disk assay.[27] In contrast, aciculitamide A (**4**) shows no cytotoxicity to the HCT-116 cell line and does not inhibit the growth of *C. albicans* at loadings less than 500 μg/disk. Aciculitamide A differs from the aciculitins only in the histidino-tyrosine unit: the complete loss of both cytotoxicity and antifungal activity that accompanied the oxidation of the imidazole ring emphasized the importance of the histidine residue for bioactivity.

Experimental Section

Instrumentation. UV and IR spectra were recorded on Perkin Elmer Lambda 3B and 1600 Series FT-IR spectrophotometers, respectively. Optical rotations were measured on a Rudolph Research Autopol III polarimeter (*c* g/100mL) at 589 nm. NMR spectra were recorded on a Varian Unity 500 spectrometer. The mixing times and field strengths of the spin locks used in the TOCSY and ROESY experiments were 45 ms at 9.2 kHz and 100 ms at 1.95 kHz, respectively. Two ROESY experiments were acquired with spectral widths of 5890 and 5950 Hz with transmitter offsets of 0 Hz. Chiral GC-MS experiments were performed on a Hewlett Packard 5890A gas chromatograph fitted with an Alltech Chirasil-Val capillary column (0.32 mm × 25 m) and interfaced to a Hewlett Packard 5988A mass spectrometer. FAB mass spectra were measured on a VG ZAB spectrometer at the Mass Spectrometry Facility, UC, Riverside.

Isolation of Aciculitins A−C (1−3). The sponge *Aciculites orientalis* (SIO acquisition number 94−001) was collected by hand (depth 60 m) from the small island of Siquijor, located southeast of the large island of Negros, Philippines. The sponge was immediately frozen and shipped to La Jolla over dry ice where it was freeze dried upon receipt. A portion of the freeze-dried sponge (80 g) was extracted exhaustively with hexanes to yield a bright yellow solid (316 mg) and ethyl acetate to yield a green oil (390 mg). The sponge was dried *in vacuo* to remove any residual EtOAc before extracting with 50% aqueous acetonitrile to give 3 L of crude aqueous extract. Approximately half of the acetonitrile was removed *in vacuo*, and the remaining portion was diluted with an equal volume of water and pumped onto a prepartive C-18 cartridge (Waters Bondapak, 4.5 × 30 cm). The column was washed with 4 column volumes (2 L) of 0.05% TFA before eluting with a gradient of 20−60% acetonitrile in 0.05% TFA in 60 min. Three products were collected and several fractions of the major product, corresponding to aciculitin B, were taken. All fractions were lyophilized separately to yield aciculitins A (**1**, 48 mg, 0.06% dry weight), B (**2**, ca. 750 mg, 1% dry weight), and C (**3**, 80 mg, 0.1% dry weight).

Aciculitin A (1): pale yellow powder; $[\alpha]_D$ −35 (*c* 0.27, 1:1 CH_3CN/H_2O); UV (MeOH) λ_{max} 210 nm (ϵ 12 100), 270 (12 700), 310 (1300) and 330 (1100) [addition of KOH to the solution caused a reversible bathochromic shift from 310 (1300) to 330 (1300)]; IR (KBr) 3310, 1660, 1540 cm^{-1}; HRFABMS (NBA), *m/z* 1351.6132 $(M + H)^+$,

(25) Tentative structures for aciculitamides A and B were proposed at the 7th International Symposium on Marine Natural Products (Capri, 1993), but by the time the proceedings were published we had gathered enough data to indicate that a portion of the proposed structure would have to be revised. The majority of the structural assignment remains unaltered. Faulkner, D. J.; He, H.; Unson, M. D.; Bewley, C. A.; Garson, M. J. *Gazz. Chim. Ital.* **1993**, *123*, 301.

(26) Ryang, H.-S.; Foote, C. S. *J. Am. Chem. Soc.* **1979**, *101*, 6683.

(27) Acar, J. F.; Goldstein, F. W. In *Antibiotics in Laboratory Medicine*, 2nd ed.; Lorian, V., Ed.; Williams & Wilkins: Baltimore, MD, 1986; Chapter 2.

$C_{61}H_{87}N_{14}O_{21}$ requires *m/z* 1351.6170 (Δ −2.8 ppm). Aciculitin A (**1**) differs from aciculitin B (**2**) by the presence of a tridecadienoic acid rather than a tetradecadienoic acid; chemical shifts for **1** are identical to those of **2** (Table 1) with the exception of the tridecadienoic acid residue: ^{1}H NMR (500 MHz, DMF-d_7 + 0.1% TFA) δ 0.88 (3H, d, *J* = 6.5 Hz, H-13), 1.28−1.29 (6H, envelope, H-10 − H-12), 1.40 (2H, m, H-9), 2.10 (2H, m, H-8), 4.08 (1H, d, *J* = 2.4 hz, H-2), 4.40 (1H, dd, *J* = 9.2, 2.4 Hz, H-3), 5.65 (1H, dd, *J* = 15.1, 9.2 Hz, H-4), 5.85 (1H, m, H-7), 6.15 (1H, dd, *J* = 14.6, 10.3 Hz, H-6), 6.30 (1H, dd,*J* = 15.1, 10.3 Hz, H-5); ^{13}C NMR (125 MHz, DMF-d_7 + 0.1% TFA) δ 13.7 (q), 22.1 (t), 29.5 (t, 2C), 29.3 (t), 32.5 (t), 75.0 (d), 76.2 (d), 126.6 (d), 129.8 (d), 136.0 (d), 136.8 (d), 171.9 (s).

Aciculitin B (2): pale yellow powder; $[\alpha]_D$ −37 (*c* 0.35, 1:1 CH_3CN/H_2O); UV and IR data are identical to those reported for aciculitin A (**1**) above; ^{1}H NMR (500 MHz, DMF-d_7 + 0.1% TFA) see Table 1; ^{13}C NMR (125 MHz, DMF-d_7 + 0.1% TFA) see Table 1; HRFABMS (NBA), *m/z* 1365.6307 $(M + H)^+$, $C_{62}H_{89}N_{14}O_{21}$ requires *m/z* 1365.6327 (Δ −1.4 ppm).

Aciculitin C (3): pale yellow powder; $[\alpha]_D$ −34 (*c* 0.27, 1:1 CH_3CN/H_2O); UV and IR, identical to those reported for aciculitin A (**1**) above; HRFABMS (NBA), *m/z* 1379.6501$(M + H)^+$, $C_{63}H_{91}N_{14}O_{21}$ requires *m/z* 1379.6483 (Δ 1.3 ppm). Aciculitin C (**3**) differs from aciculitin B (**2**) by the presence of a pentadecadienoic acid rather than a tetradecadienoic acid; chemical shifts for **3** are identical to those of **2** (Table 1) with the exception of the pentadecadienoic acid residue: ^{1}H NMR (500 MHz, DMF-d_7 + 0.1% TFA) δ 0.88 (3H, d, *J* = 6.5 Hz, H-15), 1.28−1.29 (10H, envelope, H-10−H-14), 1.40 (2H, m, H-9), 1.40 (2H, m, H-9), 2.10 (2H, m, H-8), 4.08 (1H, d, *J* = 2.4 hz, H-2), 4.40 (1H, dd, *J* = 9.2, 2.4 Hz, H-3), 5.65 (1H, dd, *J* = 15.1, 9.2 Hz, H-4), 5.85 (1H, m, H-7), 6.15 (1H, dd, *J* = 14.6, 10.3 Hz, H-6), 6.30 (1H, dd, *J* = 15.1, 10.3 Hz, H-5); ^{13}C NMR (125 MHz, DMF-d_7 + 0.1% TFA) δ 13.7 (q), 22.0 (t), 29.7 (t, 3C), 29.9 (t), 30.1 (t), 32.5 (t), 75.0 (d), 76.2 (d), 126.6 (d), 129.8 (d), 136.0 (d), 136.8 (d), 171.9 (s).

Determination of Absolute Configurations of Amino Acids. **(a) Hydrolysis with 5.0 N HCl.** A solution of peptide or reaction products (0.5−1 mg) in degassed 5.0 N HCl (ca. 500 μg/800 μL) was heated to 100 °C in a sealed tube for 16 h and then cooled. The solvent was removed in a stream of dry N_2, with heating, followed by high vacuum.

(b) Derivatization and Analysis by Chiral GC-MS. A premixed solution (0.5−0.8 mL) of acetyl chloride (1 part) in isopropyl alcohol (4 parts) was added to each of the hydrolysates in a 1 mL thick-walled reaction vial and the vial was securely capped. The solution was heated to 100 °C for 45 min, cooled and the solvent removed in a stream of dry N_2. Pentafluoropropionic anhydride (PFPA, 400 μL) in CH_2Cl_2 (400 μL) was added to the residue, and the solution was heated at 100 °C for 15 min, followed by cooling and removal of the solvent in a stream of dry N_2. The residue was dissolved in CH_2Cl_2 (100 μL) and immediately analyzed by GC-MS using an Alltech Chirasil-Val capillary column (0.32 mm × 25 m). The oven temperature was ramped from 65 °C to 210 °C at 4 °C/min and a mass range of 50−600 Da was recorded every 1.96 s. The identity of each peak was confirmed by coinjection with a solution of a standard that had been derivatized in the same manner. Retention times in min: 12.90, D-Thr; 13.14, L-Thr; 16.78, D-*allo*-Thr; 17.11, L-*allo*-Thr; 18.74, D-*threo*-β-OHGln; 19.27, L-*threo*-β-OHGln; 22.78, D-*erythro*-β-OHGln; 23.20, L-*erythro*-β-OHGln; 24.02, 2*R*,3*R*-DAB; 24.06, 2*S*,3*S*-Dab; 24.09, D-Asp; 24.25, L-Asp; 25.97, 2*R*,3*S*-Dab; 26.38, 2*S*,3*R*-DAB; 26.48, D-Gln; 26.80, L-Gln.

(c) Ozonolyis and Oxidation of Aciculitin B (2). A solution of **2** (500 μg) in MeOH (2 mL) was cooled to −70 °C, and a stream of ozone in argon was bubbled into the cooled solution for 12 min. The solution was dried under a stream of N_2 while warming to room temperature, and the residue was oxidized by dissolving in a solution of 1:2 50% H_2O_2:HCO_2H and warming to 70 °C, with stirring, for 20 min. Excess solvents were removed under high vacuum, and the residue was hydrolyzed, derivatized and analyzed by chiral GC-MS as described in (a) and (b) above. A new peak in the chromatogram was observed corresponding to 1.1 equiv of L-aspartic acid and must be derived from the histidine portion. The ozonolysis procedure was repeated with the following modifications: a stream of ozone in argon was bubbled into a cooled (−70 °C) solution of **2** in MeOH (1 mg/5 mL) for 45 min after which bubbling was continued while warming to room temperature and until the solvent was evaporated. The residue was oxidized, hydrolyzed and derivatized as described above to yield 1.7 equiv of L-aspartic acid and must be derived from the combined oxidations of the histidino-tyrosine unit. The same procedure was performed on an authentic sample of L-tyrosine and yielded ca. 60% L-aspartic acid by chiral GC-MS and ^{1}H NMR. The absolute configurations of His and Tyr are therefore L.

Analysis for Lyxose. A solution of aciculitin B (**2**, 1 mg) in degassed 4 N HCl (800 μL) was heated to 70 °C for 16 h. Excess HCl was removed under high vacuum, and the hydrolysate was derivatized with PFPA (400 μL) in CH_2Cl_2 (400 μL) at 100 °C for 15 min. Excess reagents were removed under a stream of dry N_2, and the derivative was analyzed by chiral GC-MS. Peaks identical to D-lyxose were observed.

Synthesis of 2,3-Dihydroxytetradecanoic Acids: **Methyl Tetradec-2-enoate.** Methyl 2-(triphenylphosphoranylidene)acetate (2.27 g, 1.5 equiv) was added to a stirred solution of dodecyl aldehyde (lauraldehyde) (1 mL, 835 mg, 4.5 mmol) in toluene (10 mL), and the mixture was refluxed under nitrogen for 3 h. The solution was allowed to cool to room temperature, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica using ethyl acetate/hexane (3:97) as eluent to afford the *E* isomer as an oil (0.668 g, 62%) and a mixture of the *E*:*Z* isomers (1:4, 0.22 g, 21%, R_f 0.28, 0.36 respectively).

Methyl (*E*)-tetradec-2-enoate: IR ($CHCl_3$) 2930, 2850, 1713, 1654 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 7 Hz), 1.25 (16H, br s), 1.41 (2H, m), 2.16 (2H, br q, *J* = 7 Hz), 3.71 (3H, s), 5.78 (1H, br d, *J* = 16 Hz), 6.94 (1H, dt, *J* = 16, 7 Hz); EIMS *m/z* = 240 (M^+, 2), 209 (31), 208 (22), 166 (32), 113 (100).

Methyl (*Z*)-tetradec-2-enoate: ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.87 (3H, t, *J* = 7 Hz), 1.25 (16H, br s), 1.41 (2H, m), 2.64 (2H, br q, *J* = 7 Hz), 3.70 (3H, s), 5.75 (1H, br d, *J* = 11 Hz), 6.23 (1H, dt, *J* = 11, 7 Hz).

(*E*)-Tetradec-2-enoic Acid. Aqueous lithium hydroxide solution (4 mL, 0.5 N) was added to a solution of methyl (*E*)-tetradec-2-enoate (453 mg, 1.89 mmol) in methanol (20 mL), and the mixture was refluxed for 2 h, after which time no starting material was observed by TLC. The solution was diluted with dichloromethane (150 mL) and washed with aqueous hydrochloric acid (25 mL, 0.1 N), water (2 × 20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4), evaporated, and recrystallized from ethyl acetate/hexane (349 mg, 82%): mp 33−34 °C; IR ($CHCl_3$) 2930, 2850, 1697, 1653 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 7 Hz), 1.25 (16H, br s), 1.42 (2H, m), 2.22 (2H, br q, *J* = 7 Hz), 5.81 (1H, br d, *J* = 16 Hz), 7.07 (1H, dt, *J* = 16, 7 Hz); EIMS *m/z* = 226 (M^+, 3), 209 (6), 208 (25), 166 (50), 113 (100).

Tetradec-2-enoic Acid. The preceeding procedure was performed using a 1:1 mixture of the (*E*) and (*Z*) isomers of methyl tetradec-2-enoate (127 mg, 0.53 mmol) to yield a 1:1 mixture of (*E*)- and (*Z*)-tetradec-2-enoic acids (104 mg, 87%) that were not purified further.

(*Z*)-Tetradec-2-enoic acid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 7 Hz), 1.25 (16H, br s), 1.41 (2H, m), 2.64 (2H, br q, *J* = 7 Hz), 5.78 (1H, br d, *J* = 11 Hz), 6.34 (1H, dt, *J* = 11, 7 Hz).

Isopropyl (*E*)-Tetradec-2-enoate. To a stirred solution of (*E*)-tetradec-2-enoic acid (20 mg, 0.088 mmol) in dry dichloromethane (0.5 mL) at 0 °C under argon was added oxallyl chloride (23 μl, 3 equiv) dropwise over 2 min followed by one drop of dry DMF. The solution was stirred for 30 min, evaporated under reduced pressure, diluted with dry dichloromethane (0.5 mL), and treated with isopropyl alcohol (20 μl, 3 equiv). The solution was stirred at room temperature for 5 min followed by the addition of pyridine (35 μl, 5 equiv). After 1 h, the solution was diluted with dichloromethane (50 mL), washed with water (10 mL) and brine (10 mL), dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography on silica with ethyl acetate/hexane (3:97, R_f 0.34) as eluent to afford isopropyl (*E*)-tetradec-2-enoate as an oil (18 mg, 77%): IR ($CHCl_3$) 2930, 2855, 1706, 1653 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 7 Hz), 1.25 (16H, br s), 1.25 (6H, d, *J* = 6 Hz), 1.40 (2H, m), 2.17 (2H, br q, *J* = 7 Hz), 5.04 (1H, m, *J* = 6 Hz), 5.77 (1H, br d, *J* = 16 Hz), 6.93 (1H, dt, *J* = 16, 7 Hz); EIMS *m/z* = 268 (M^+, 1), 227 (90), 209 (100), 208 (42).

Isopropyl (*Z*)-Tetradec-2-enoate. The previous procedure was performed on a mixture (1:1) of the (*E*) and (*Z*) isomers of tetradec-2-enoic acid (7 mg, 0.03 mmol) to yield a mixture (1:1) of the (*E*) and (*Z*) isomers of 2-propyl tetradec-2-enoate (6 mg, 72%) which were purified by flash column chromatography with ethyl acetate/hexane (3:97) as eluent (R_f 0.34 and 0.43, respectively).

Isopropyl (*Z*)-tetradec-2-enoate: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, $J = 7$ Hz), 1.25 (16H, br s), 1.25 (6H, d, $J = 6$ Hz), 1.42 (2H, m), 2.67 (2H, br q, $J = 7$ Hz), 5.03 (1H, m, $J = 6$ Hz), 5.71 (1H, br d, $J = 11$ Hz), 6.18 (1H, dt, $J = 11$, 6 Hz).

(*S*)-*sec*-Butyl (*E*)-Tetradec-2-enoate. The procedure used for the synthesis of isopropyl (*E*)-tetradec-2-enoate was followed with the exception that (*S*)-*sec*-butyl alcohol was employed to yield (*S*)-*sec*-butyl (*E*)-tetradec-2-enoate as an oil [8 mg, 74%, $R_f = 0.36$; ethyl acetate/hexane (3:97)]: IR ($CHCl_3$) 2930, 2855, 1700, 1653 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, $J = 7$ Hz), 0.88 (3H, t, $J = 6$ Hz), 1.20 (3H, d, $J = 6$ Hz), 1.25 (16H, br s), 1.44 (2H, m), 1.60 (2H, m), 2.17 (2H, br q, $J = 7$ Hz), 4.89 (1H, m, $J = 6$ Hz), 5.78 (1H, dt, $J = 16$, 1.5 Hz), 6.94 (1H, dt, $J = 16$, 7 Hz); EIMS *m/z* = 282 (M^+, 0.1), 227 (100), 209 (65).

Isopropyl (*threo*)-2,3-Dihydroxytetradecanoate. A mixture of isopropyl (*E*)-tetradec-2-enoate (11 mg, 0.041 mmol), 4-methylmorpholine *N*-oxide (18 μl of 60% in H_2O by wt, 2 equiv) in *tert*-butyl alcohol/water (1:1, 1 mL) and osmium tetroxide (2 drops of 2.5% in 2-methyl-2-propanol) was stirred until TLC revealed the absence of starting material. Sodium sulfite was added to the mixture (9 mg) which was stirred for 30 min. The mixture was diluted with ethyl acetate (40 mL), washed with water (2 × 10 mL) and brine (10 mL), dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography with ethyl acetate/hexane (25:75, $R_f = 0.36$) to yield isopropyl (*threo*)-2,3-dihydroxytetradecanoate as a white solid (10.5 mg, 85%): mp 49−50 °C; IR ($CHCl_3$) 2930, 2855, 1724 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, $J = 7$ Hz), 1.25 (18H, br s), 1.27 (6H, d, $J = 6$ Hz), 1.55 (2H, m), 1.81 (1H, d, $J = 9$ Hz, OH), 3.01 (1H, d, $J = 5$ Hz, OH), 3.83 (1H, m), 4.03 (1H, dd, $J = 5$, 2 Hz), 5.14 (1H, m, $J = 6$Hz); EIMS *m/z* = 215 (3), 118 (100).

Isopropyl (*erythro*)-2,3-Dihydroxytetradecanoate. A mixture of the *threo* and *erythro* diols of isopropyl 2,3-dihydroxytetradecanoate were obtained using the prodecure described directly above except that a 1:1 mixture of the (*E*) and (*Z*) isomers of isopropyl tetradec-2-enoate (9 mg, 0.034 mmol) was used to yield a mixture of *threo* and *erythro* diols (8 mg, 79%). This mixture was purified and separated by flash chromatography on silica using ethyl acetate/hexane (25:75, $R_f = 0.36$, 0.27 respectively). Isopropyl (*erythro*)-2,3-dihydroxytetradecanoate was isolated as a white solid: mp 53−54 °C; IR ($CHCl_3$) 2930, 2855, 1724 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, $J = 7$ Hz), 1.25 (18H, br s), 1.30 (6H, d, $J = 6$ Hz), 1.45 (2H, m), 2.12 (1H, d, $J = 7$ Hz, OH), 3.02 (1H, d, $J = 6$ Hz, OH), 3.83 (1H, m), 4.16 (1H, dd, $J = 6$, 4 Hz), 5.13 (1H, m, $J = 6$Hz); EIMS *m/z* = 215 (2), 118 (100).

(2*S*,2′*S*,3*R*)-*sec*-Butyl 2,3-Dihydroxytetradecanoate and (2*R*,2′*S*,3*S*)-*sec*-Butyl 2,3-Dihydroxytetradecanoate. The same procedure used for the synthesis of racemic isopropyl (*threo*)-2,3-dihydroxytetradecanoate was used to prepare the *threo* enantiomers of *sec*-butyl 2,3-dihydroxytetradecanoates from (*S*)-*sec*-butyl (*E*)-tetradec-2-enoate (3 mg, 0.011 mmol). An inseparable diastereomeric mixture of *threo* diols was formed and purified by silica flash chromatography using ethyl acetate/hexane (20:80, R_f 0.32, 2.5 mg, 74%): IR ($CHCl_3$) 2930, 2855, 1725 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, $J = 7$ Hz), 0.92 (3H, t, $J = 7$ Hz), 1.25 (18H, br s), 1.27 (3H, d, $J = 6$ Hz), 1.59 (4H, m), 1.83 (1H, br d), 3.04 (1H, br t), 3.84 (1H, m), 4.03 (1H, m), 4.98 (1H, br q, $J = 6$ Hz); EIMS *m/z* = 215 (21), 118 (100).

(2*R*,2′*S*,3*S*)-*sec*-Butyl 2,3-Dihydroxytetradecanoate. Methylsulfonamide (1 mg, 1 equiv) was added to a stirred mixture of *tert*-butyl alcohol/water (1:1, 0.2 mL) contaning AD-mix-α (15 mg).[20] The mixture was stirred at room temperature for 30 min and then cooled to 0 °C. (*S*)-*sec*-Butyl (*E*)-tetradec-2-enoate (3.0 mg, 0.011 mmol) in *tert*-butyl alcohol (50 μL) was added, and the slurry was stirred until TLC revealed the absence of starting material (24 h). Sodium sulfite (18 mg, 10 equiv) was added to the reaction mixture which was stirred for an additional hour, after which it was diluted with ethyl acetate (50 mL), washed with aqueous sodium hydroxide (5 mL, 2 N), water (10 mL), and brine (10 mL), dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography with ethyl acetate/hexane as eluent (35:65) to yield (2*R*,2′*S*,3*S*)-*sec*-butyl 2,3-dihydroxytetradecanoate (2.5 mg, 74%): IR ($CHCl_3$) 2930, 2855, 1725 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, $J = 7$ Hz), 0.91 (3H, t, $J = 7$Hz) 1.25 (18H, br s), 1.27 (3H, d, $J = 6$ Hz), 1.59 (4H, m), 1.83 (1H, br d), 3.02 (1H, br d), 3.83 (1H, m), 4.03 (1H, m), 4.97 (1H, br q, $J = 6$ Hz); MS (EI) *m/z* = 215 (25), 132 (100); HRFABMS (NH_3) obsd *m/z* = 317.2692 ($M + H)^+$, $C_{18}H_{37}O_4$ requires *m/z* = 317.2692.

Determination of Absolute Configuration of (4*E*,6*E*)-2,3-Dihydroxytetradecadienoic Acid of Aciculitin B (2). (a) Reduction of Aciculitin B (2). A solution of aciculitin B (**2**, 10 mg) and 10% Pd/C in MeOH was stirred overnight at ambient temperature under H_2 (1 atm). The solution was centrifuged, and the supernatant was filtered through a 0.5 μm filter and lyophilized overnight to yield 9.6 mg of one reduction product.

(b) Analysis for 2,3-Dihydroxytetradecanoic Acid. The reduction product of **2** (1 mg) was hydrolyzed for 12 h at 100 °C in 5 N HCl. The hydrolysate was derivatized and analyzed by GCMS as described in (b) above. The diastereotopic *erythro* and *threo* dihydroxy acids were separable as the isopropyl esters (R_f 18 and 19 min, 100−200 °C at 4 °C/min) and revealed that the diol had the *threo* configuration. The isopropyl esters of the *threo* enantiomers were inseparable under these conditions. The hydrolysate of the reduction product and the *threo* enantiomers, (2*R*,3*S*) and (2*S*,3*R*), of 2,3-dihydroxytetradecanoic acid were therefore reacted with (*S*)-*sec*-butanol as described in (b) above to form the chiral esters, and the alcohols were acylated with pentafluoropropionic anyhydride. Chiral GC-MS analysis showed aciculitin B to contain (2*S*,3*R*)-2,3-dihydroxytetradecadienoic acid. Retention times (100−200 °C at 4 °C/min): (2*R*,3*S*)-2,3-dihydroxy-tetradecanoic acid, 18.22 min; (2*S*,3*R*)-2,3-dihydroxy-tetradecanoic acid, 18.45 min.

Isolation of Aciculitamides A (4) and B (5). The sponge *Aciculites orientalis* (NCI-013) was collected in 1990 at Antolang on Negros Island, the Philippines, and immediately frozen at −20 °C. The frozen specimen was transported to Scripps, lyophilized, and extracted successively with hexanes (1200 mL) and 50% MeOH/CH_2Cl_2 (1200 mL). The methanolic extracts (800 mg) were chromatographed on a Sephadex LH-20 column using methanol as eluent. Fractions containing the peptides were combined and further purified by RP-HPLC on a Dynamax C-8 column (30% aqueous CH_3CN) to obtain aciculitamide A (**4**, 160 mg, 0.04% dry wt) as the major compound and aciculitamide B (**5**, 12 mg, 0.003% dry wt) as a minor component.

Aciculitamide A (4): pale yellow powder; $[\alpha]_D = +44.6$ (*c* 3.27, MeOH); UV (MeOH) 226 nm (ϵ 47 180), 273 nm (ϵ 9120), 364 nm (ϵ 3440), (MeOH + NaOH) 231 nm (ϵ 35 960), 280 nm (ϵ 4880), 430 nm (ϵ 3100); IR (neat, AgCl) 3390, 3310, 2927, 1744, 1666, 1659, 1650, 1642, 1547, 1536, 1530 cm^{-1}; ^{1}H NMR (500 MHz, 2:1 acetone-d_6/DMSO-d_6) δ **Thr** 1.21 (3H, d, $J = 5.5$ Hz, H-4), 4.18 (1H, m, H-2), 4.17 (1H, m, H-3), 5.66 (1H, br d, $J = 10.5$ Hz, OH), 8.01 (1H, d, $J = 9$ Hz, NH), ***m*-Tyr** 2.98 (1H, br t, $J = 14$ Hz, H-3), 3.87 (1H, m, H-3), 5.14 (1H, br dd, $J = 12$, 9.5 Hz, H-2), 7.06 (1H, d, $J = 8.5$ Hz, H-5′), 7.52 (1H, dd, $J = 8.5$, 2 Hz, H-6′), 8.00 (1H, br s, H-2′), 8.54 (1H, d, $J = 9.5$ Hz, NH), 12.10 (1H, s, OH), **dAbu-1** 1.87 (3H, d, $J = 7$ Hz, H-4), 5.77 (1H, q, $J = 7$ Hz, H-3), 10.28 (1H, br s, NH), **Hgln** 2.55 (1H, dd, $J = 16$, 7 Hz, H-4), 2.65 (1H, dd, $J = 16$, 7 Hz, H-4), 4.17 (1H, m, H-2), 4.56 (1H, m, H-3), 5.11 (1H, d, $J = 7$ Hz, OH), 6.91 (1H, br s, NH-5), 7.44 (1H, br s, NH-5), 9.11 (1H, d, $J = 5$ Hz, NH-2), **Gly** 3.44 (1H, dd, $J = 17$, 6 Hz, H-2), 3.76 (1H, dd, $J = 17$, 6 Hz, H-2), 7.88 (1H, t, $J = 6$ Hz, NH), **Gln** 2.04 (1H, m, H-3), 2.19 (1H, m, H-3), 2.37 (1H, m, H-4), 2.46 (1H, m, H-4), 3.88 (1H, m, H-2), 6.65 (1H, br s, NH-5), 7.05 (1H, br s, NH-5), 7.44 (1H, br s, NH-2), **MeO-His** 1.98 (1H, d, $J = 14.5$ Hz, H-3), 2.08 (1H, dd, $J = 14.5$, 9 Hz, H-3), 3.14 (3H, s, OMe), 4.92 (1H, br t, $J = 9$ Hz, H-2), 8.16 (1H, d, $J = 8.5$ Hz, NH), 9.67 (1H, s, NH-3′), **Dab** 1.16 (3H, d, $J = 7$ Hz, H-4), 4.63 (1H, dd, $J = 9.5$, 4.5 Hz, H-2), 4.88 (1H, m, H-3), 7.45 (1H, d, $J = 9$ Hz, NH-3), 9.01 (1H, d, $J = 9.5$ Hz, NH-2), **dAbu-2** 1.85 (3H, d, $J = 7$ Hz, H-4), 5.87 (1H, q, $J = 7$ Hz, H-3), 9.90 (1H, br s, NH), **Dhtda** 0.90 (3H, t, $J = 7$ Hz, H-14), 1.27−1.29 (8H, m, H-10 to H-13), 1.38 (2H, m, H-9), 2.09 (2H, q, $J = 7.5$ Hz, H-8), 3.94 (1H, dd, $J = 6$, 2 Hz, H-2), 4.31 (1H, dd, $J = 9$, 2 Hz, H-3), 5.65 (1H, dd, $J = 15$, 9 Hz, H-4), 5.84 (1H, dt, $J = 15.5$, 7.5 Hz, H-7), 6.14 (1H, dd, $J = 15.5$, 11 Hz, H-6), 6.26 (1H, dd, $J = 15$, 11

Hz, H-5), 6.30 (1H, br d, J = 6 Hz, OH), **Lyx** 3.27 (1H, dd, J = 11, 6 Hz, H-5), 3.29 (1H, t, J = 10.5 Hz, H-5), 3.51 (1H, m, H-4), 3.60 (1H, m, H-2), 3.64 (1H, m, H-3), 3.70 (1H, d, J = 11 Hz, OH-4), 4.33 (1H, d, J = 5.5 Hz, OH-3), 4.38 (1H, d, J = 4 Hz, OH-2), 4.59 (1H, d, J = 2 Hz, H-1); ^{13}C NMR (125 MHz, 2:1 acetone-d_6/DMSO-d_6) δ **Thr** 173.1 (C-1), 66.5 (C-3), 56.7 (C-2), 18.4 (C-4), ***m*-Tyr** 170.8 (C-1), 160.8 (C-4′), 138.0 (C-6′), 128.7 (C-1′), 126.5 (C-2′), 118.1 (C-5′), 112.6 (C-3′), 52.5 (C-2), 33.5 (C-3), **dAbu-1** 169.0 (C-1), 131.2 (C-2), 123.1 (C-3), 13.1 (C-4), **Hgln** 173.3 (C-5), 170.0 (C-1), 66.4 (C-3), 60.6 (C-2), 39.7 (C-4), **Gly** 175.7 (C-1), 44.3 (C-2), **Gln** 174.2 (C-5), 168.8 (C-1), 56.7 (C-2), 31.4 (C-4), 28.0 (C-3), **MeO-His** 186.3 (C-5′), 170.6 (C-1), 162.3 (C-2′), 97.2 (C-4′), 51.2 (OMe), 50.3 (C-2), 42.2 (C-3), **Dab** 169.0 (C-1), 57.8 (C-2), 45.4 (C-3), 21.9 (C-4), **dAbu-1** 165.4 (C-1), 132.4 (C-2), 116.1 (C-3), 13.3 (C-4), **Dhtda** 171.3 (C-1), 136.7 (C-7), 136.0 (C-5), 129.4 (C-6), 126.3 (C-4), 75.2 (C-3), 74.8 (C-2), 32.4 (C-8), 31.6 (C-12), 29.1 (C-9), 28.9 (C-10, C-11), 22.4 (C-13), 13.7 (C-14), **Lyx** 95.7 (C-1), 71.5 (C-3), 70.9 (C-2), 67.8 (C-4), 63.1 (C-5); HRFABMS, obsd m/z = 1433.6223, $C_{63}H_{90}N_{14}O_{23}Na$ requires m/z = 1433.6201.

Aciculitamide B (4): pale yellow powder; $[\alpha]_D$ = +50.5 (*c* 0.26, MeOH); UV (MeOH) 226 nm (ϵ 47620), 274 nm (ϵ 9170), 363 nm (ϵ 3350); IR (neat, AgCl) 3400, 3310, 2930, 1743, 1667, 1660, 1651, 1644, 1547, 1537, 1531 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ **Thr** 1.11 (3H, d, J = 5.5 Hz, H-4), 4.04 (1H, m, H-2), 4.04 (1H, m, H-3), 5.71 (1H, br, OH) 7.81 (1H, d, J = 8.5 Hz, NH), ***m*-Tyr** 3.03 (1H, m, H-3), 3.72 (1H, m, H-3), 5.02 (1H, m, H-2), 7.10 (1H, d, J = 8 Hz, H-5′), 7.53 (1H, br d, J = 8 Hz, H-6′), 7.87 (1H, br s, H-2′), 8.41 (1H, d, J = 8.5 Hz, NH), 12.01 (1H, s, OH), **dAbu-1** 1.71 (3H, d, J = 7 Hz, H-4), 6.00 (1H, q, J = 7 Hz, H-3), 10.09 (1H, br s, NH), **Hgln** 2.47 (2H, m, H-4), 4.18 (1H, br d, J = 9 Hz, H-2), 4.44 (1H, m, H-3), 6.95 (1H, br s, NH-5), 7.41 (1H, br s, NH-5), 8.96 (1H, br s, NH-2), **Gly** 3.30 (1H, dd, J = 17, 6 Hz, H-2), 3.60 (1H, dd, J = 17, 6 Hz, H-2), 7.65 (1H, br, NH), **Gln** 1.93 (1H, m, H-3), 1.98 (1H, m, H-3), 2.27 (2H, m, H-4), 3.84 (1H, m, H-2), 6.79 (1H, br s, NH-5), 7.01 (1H, br s, NH-5), 7.45 (1H, br s, NH-2), **MeO-His** 1.83 (1H, d, J = 14.5 Hz, H-3), 1.92 (1H, m, H-3), 3.03 (3H, s, OMe), 4.77 (1H, m, H-2), 8.02 (1H, br, NH), 9.78 (1H, s, NH-3′), **Dab** 1.09 (3H, d, J = 7 Hz, H-4), 4.55 (1H, m, H-2), 4.74 (1H, m, H-3), 7.31 (1H, d, J = 8 Hz, NH-3), 8.72 (1H, d, J = 8 Hz, NH-2), **dAbu-2** 1.78 (3H, d, J = 7.5 Hz, H-4), 5.76 (1H, q, J = 7.5 Hz, H-3), 9.91 (1H, br s, NH), **Dhtda** 0.85 (3H, t, J = 7 Hz, H-14), 1.24−1.27 (8H, m, H-10 to H-13), 1.33 (2H, m, H-9), 2.04 (2H, q, J = 7.5 Hz, H-8), 3.70 (1H, br d, J = 4.5 Hz, H-2), 4.18 (1H, br d, J = 9 Hz, H-3), 5.54 (1H, dd, J = 15, 9 Hz, H-4), 5.77 (1H, dt, J = 15.5, 7.5 Hz, H-7), 6.07 (1H, dd, J = 15.5, 10 Hz, H-6), 6.15 (1H, dd, J = 15, 10 Hz, H-5), 6.13 (1H, br d, J = 4.5 Hz, OH), **Lyx** 3.18 (2H, m, H-5), 3.46 (1H, m, H-2), 3.50 (1H, m, H-3), 3.55 (1H, m, H-4), 3.78 (1H, d, J = 9.5 Hz, OH-4), 4.45 (1H, br s, H-1), 4.54 (1H, d, J = 5.5 Hz, OH-3), 4.64 (1H, d, J = 4 Hz, OH-2); ^{13}C NMR (125 MHz, 2:1 acetone-d_6/DMSO-d_6) δ **Thr** 172.4 (C-1), 65.9 (C-3), 56.3 (C-2), 18.2 (C-4), ***m*-Tyr** 170.2 (C-1), 160.2 (C-4′), 138.0 (C-6′), 128.4 (C-1′), 126.2 (C-2′), 118.0 (C-5′), 112.4 (C-3′), 52.3 (C-2), 32.4 (C-3), **dAbu-1** 167.1 (C-1), 131.3 (C-2), 124.7 (C-3), 12.1 (C-4), **Hgln** 173.3 (C-5), 170.1 (C-1), 66.4 (C-3), 60.5 (C-2), 39.5 (C-4), **Gly** 175.1 (C-1), 44.9 (C-2), **Gln** 173.5 (C-5), 169.7 (C-1), 57.1 (C-2), 30.9 (C-4), 27.3 (C-3), **MeO-His** 185.8 (C-5′), 170.2 (C-1), 162.0 (C-2′), 96.7 (C-4′), 51.1 (OMe), 49.9 (C-2), 41.7 (C-3), **Dab** 168.7 (C-1), 56.4 (C-2), 44.0 (C-3), 21.6 (C-4), **dAbu-1** 165.1 (C-1), 131.8 (C-2), 116.4 (C-3), 13.2 (C-4), **Dhtda** 170.9 (C-1), 136.4 (C-7), 135.4 (C-5), 129.1 (C-6), 126.2 (C-4), 75.1 (C-3), 74.3 (C-2), 32.0 (C-8), 31.2 (C-12), 28.6 (C-9), 28.5 (C-10, C-11), 22.0 (C-13), 13.9 (C-14), **Lyx** 95.7 (C-1), 70.8 (C-2), 70.3 (C-3), 67.1 (C-4), 62.9 (C-5). FABMS, obsd m/z = 1544, $C_{63}H_{90}N_{14}O_{23}Cs$ requires m/z = 1543.53.

Photooxidation of Aciculitin B (2). A solution containing aciculitin B (**2**, 9 mg) and a catalytic amount of rose bengal in DMSO (500 μL) was diluted with CH_2Cl_2 (9.5 mL) and stirred under an atmosphere of oxygen for 20 min. The solution was cooled to −70 °C with stirring and irradiated with a 400 W tungsten lamp for 6 h during which time the solution was kept at −70 °C with stirring. A solution of 5% diisopropylethylamine in MeOH was added to the reaction mixture, and UV irradiation was stopped. Methanolysis was continued for 1 h after which the solvents were removed under a stream of dry N_2. The reaction mixture was purified by HPLC to yield 1.2 mg (13.3% yield) of a product identical to aciculitamide A (**4**) by RP-HPLC, ^{1}H NMR [(500 MHz, CD_3OD) δ 3.04 (s 3H)], and FABMS [m/z 1412 (M + H)$^+$].

Acknowledgment. We thank Mr. Charlie Arneson of the Coral Reef Research Foundation for collecting the specimen of *Aciculites orientalis*, Dr. Rob Van Soest for identifying the specimen of *A. orientalis* collected in 1990 (NCI-013), Mary Kay Harper for bioassays, and Professors Ulrich Schmidt and Gilles Lajoie for providing samples of diaminobutyric acid and β-hydroxyglutamine, respectively. This research was generously supported by the National Science Foundation (Grant CHE 92-04647) and the National Institutes of Health (Grant CA 49084).

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **1**−**5** and TOCSY, ROESY, HMQC, and HMBC spectra for aciculitin B (**2**) (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA953628W