Macrocyclic Antifungal Metabolites from the Spanish Dancer Nudibranch *Hexabranchus sanguineus* and Sponges of the Genus *Halichondria*

Michael R. Kernan,[1] Tadeusz F. Molinski,[2] and D. John Faulkner*

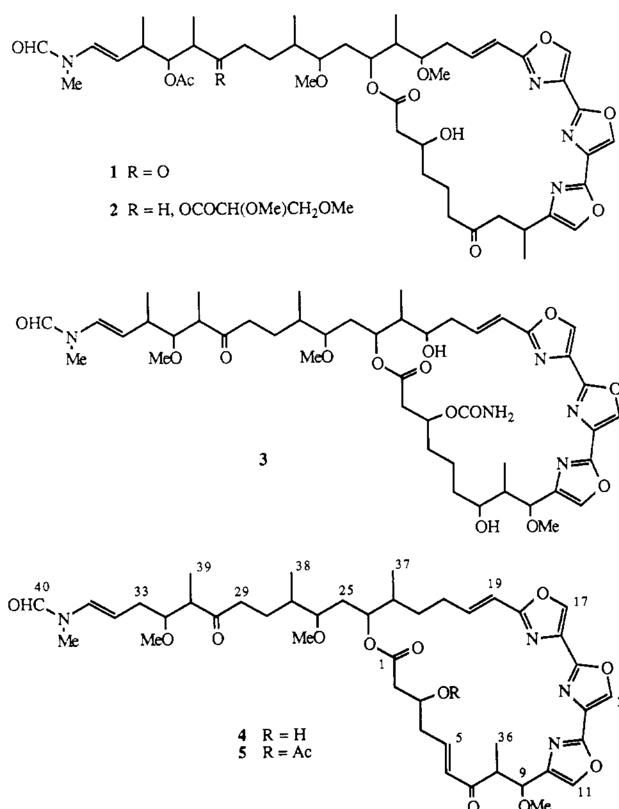
Scripps Institution of Oceanography and Department of Chemistry, University of California, San Diego (A-012F), La Jolla, California 92093

Received April 19, 1988

A marine sponge *Halichondria* sp. contained halichondramide (4) as the major constituent and dihydrohalichondramide (6), isohalichondramide (10), acid 11, imide 12, and ester 13 as minor metabolites. The compounds isolated from the nudibranch *Hexabranchus sanguineus* varied depending on collecting location but usually included dihydrohalichondramide (6) and tetrahydrohalichondramide (8). The macrolides 4, 6, 8, and 10 have antifungal activity and inhibit cell division in the fertilized sea urchin egg assay.

It has often been observed that the metabolites extracted from dorid nudibranchs are either identical with or closely related to compounds found in marine sponges.[3] Most dorid nudibranchs obtain these metabolites directly from their sponge diet and are believed to employ the chemicals to deter potential predators.[4] In 1986 it was reported that the cytotoxic and antifungal macrolides, ulapualides A (1) and B (2), were isolated from the eggmasses of the nudibranch *Hexabranchus sanguineus*,[5] and a related macrolide, kabiramide C (3), was found in unidentified nudibranch eggmasses.[6] During our studies of antifungal metabolites from marine sponges, we had isolated a number of related macrolides from sponges of the genus *Halichondria*, among which was halichondramide (4).[7] We now report the structural elucidation of halichondramide (4) and six related metabolites from a sponge of the genus *Halichondria* and specimens of the nudibranch *H. sanguineus* both collected at Kwajalein Atoll.

Two sponges of the genus *Halichondria* have been investigated. A species of *Halichondria* from Palau, Western Caroline Islands, contained kabiramide C (3) and related compounds[8] that had previously been isolated from the unidentified nudibranch eggmass collected in Kabira Bay, Ishigaki-jima Island (Ryukyus). A species of *Halichondria* from Kwajalein Atoll, Marshall Islands, gave a crude extract that strongly inhibited the growth of *Candida albicans*. Bioassay-directed chromatographic separation of the crude extract resulted in the isolation of halichondramide (4, 0.34% dry weight), dihydrohalichondramide (6, 0.03% dry weight), isohalichondramide (10, 0.007% dry weight), the acid 11 (0.002% dry weight), the imide 12 (0.008% dry weight), and the ester 13 (0.004% dry weight). Two typical specimens of the nudibranch *H. sanguineus* from Kwajalein contained dihydrohalichondramide (6, 0.265% dry weight), tetrahydrohalichondramide (8, 0.06% dry weight), and kabiramide C (3, 0.014% dry weight). A detailed account of the distribution of macrolide metabolites within *H. sanguineus* and its eggmasses will be presented elsewhere.[9]



Halichondramide (4) was obtained as a white powder, mp. 66–68 °C. The high-resolution, fast atom bombardment mass spectrum indicated a molecular formula of $C_{44}H_{60}N_4O_{12}$ although the ^{13}C NMR spectrum contained 52 signals. Since halichondramide (4) was clearly a single compound, as judged by HPLC, the duplication of signals was attributed to the two geometrical forms of an unsaturated formamide (IR 1660 cm^{-1}). The 360-MHz ^1H and 50-MHz ^{13}C NMR spectra were eventually assigned as shown in Table I (ref) as the result of a 2D C–H correlation experiment, a 2D long range C–H correlation experiment (COLOC), a COSY experiment, and exhaustive decoupling experiments. The structure of halichondramide (4) was elucidated by interpretation of ^1H and ^{13}C NMR experiments and by comparison of spectral data with those of kabiramide C (3).

(1) Present address: Department of Zoology, University of Auckland, Auckland, New Zealand.
(2) Present address: Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84112.
(3) Karuso, P. In *Bioorganic Marine Chemistry*; Scheuer, P. J., Ed.; Springer: Berlin, 1987; Vol. 1, pp 31–60.
(4) Faulkner, D. J.; Ghiselin, M. T. *Mar. Ecol. Prog. Ser.* **1983**, *13*, 295. Cartê, B.; Faulkner, D. J. *J. Chem. Ecol.* **1986**, *12*, 795. Thompson, J. E.; Walker, R. P.; Wratten, S. J.; Faulkner, D. J. *Tetrahedron* **1982**, *38*, 1865.
(5) Roesener, J. A.; Scheuer, P. J. *J. Am. Chem. Soc.* **1986**, *108*, 846.
(6) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Koseki, K.; Noma, M. *J. Am. Chem. Soc.* **1986**, *108*, 847.
(7) Preliminary communication; Kernan, M. R.; Faulkner, D. J. *Tetrahedron Lett.* **1987**, *28*, 2809.
(8) The metabolites from the Palau specimen of *Halichondria* are identical with those found in the unidentified nudibranch eggmasses from Kabira Bay. Fusetani, N., personal communication.
(9) Pawlik, J. R.; Kernan, M. R.; Molinski, T. F.; Harper, M. K.; Faulkner, D. J. *J. Exp. Mar. Biol. Ecol.*, in press.

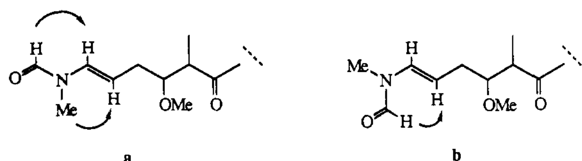
0022-3263/88/1953-5014$01.50/0 © 1988 American Chemical Society

Table I. 360-MHz ^1H and 50-MHz ^{13}C NMR Data for Halichondramide (4)[a]

carbon no.	^1H NMR	^{13}C NMR	carbon no.	^1H NMR	^{13}C NMR
1		172.6	24	5.10 (m, 1 H)	74.0
2	2.50 (dd, 1 H, *J* = 15, 10.5 Hz),	42.2	25	1.60 (m, 2 H)	32.0
	2.50 (dd, 1 H, *J* = 15, 4.5 Hz)		26	2.97 (br d, 1 H)	81.6
3	4.47 (m, 1 H)	67.3	27	1.75 (m, 1 H)	34.5
4	2.52 (m, 2 H)	40.3	28	1.74 (m, 1 H), 1.36 (m, 1 H)	24.8
5	7.27 (dt, 1 H, *J* = 16, 7.7 Hz)	146.1	29	2.53 (m, 2 H)	41.1 [41.0]
6	6.22 (bd, 1 H, *J* = 16 Hz)	133.0	30		213.5
7		202.8	31	2.74 (m, 1 H)	48.6
8	4.00 (dq, 1 H, *J* = 8.6, 7 Hz)	44.0	32	3.48 (m, 1 H)	82.1
9	4.36 (d, 1 H, *J* = 8.6 Hz)	77.1	33	2.46 (m, 1 H), 2.13 (m, 1 H)	30.1 [30.0]
10		139.1	34	5.10 (dt, 0.7 H, *J* = 14.4, 8.2 Hz) [5.12	105.1 [106.8]
11	7.66 (s, 1 H)	136.8		(dt, 0.3 H, *J* = 15.4, 6.2 Hz)]	
12		155.2	35	6.52 (d, 0.7 H, *J* = 14.4 Hz) [7.13	130.1 [126.1]
13		130.7		(d, 0.3 H, *J* = 15.4 Hz)]	
14	8.10 (s, 1 H)	137.1	36	0.94 (d, 3 H, *J* = 7 Hz)	13.2
15		156.2	37	0.92 (d, 3 H, *J* = 7 Hz)	14.5
16		129.6	38	0.85 (d, 3 H, *J* = 7 Hz)	15.4
17	8.06 (s, 1 H)	137.1	39	0.98 (d, 3 H, *J* = 7 Hz)	12.7 [12.8]
18		162.9	10	8.29 (s, 0.7 H) [8.06 (s, 0.3 H)]	162.1 [160.8]
19	6.28 (br d, 1 H, *J* = 16 Hz)	114.5	NMe	3.04 (s, 2 H) [3.08 (s, 1 H)]	27.5 [32.8]
20	7.15 (ddd, 1 H, *J* = 16, 8.2, 6.7 Hz)	143.7	OMe (9)	3.18 (s, 3 H)	56.8
21	2.49 (m, 1 H), 2.23 (m, 1 H)	27.5	OMe (32)	3.30 (s, 3 H)	57.4 [57.2]
22	1.67 (m, 1 H), 1.29 (m, 1 H)	31.3	OMe (26)	3.32 (s, 3 H)	57.8
23	1.92 (m, 1 H)	35.6			

[a] Data for minor geometrical isomer are within square brackets.

The structure of the C31(C39)–C35(C40) portion of halichondramide (4) was established by interpretation of the ^1H NMR COSY experiment and was confirmed by selective decoupling experiments. Both the major and minor geometrical forms of the unsaturated formamide were clearly observed. The coupling constants observed for the olefinic protons indicated a trans geometry for both the major and minor isomers. In the major isomer, nuclear Overhauser enhancements were observed between H40 and H35 and between NCH_3 and H34 while in the minor isomer, irradiation of H40 caused an enhancement of H34. These data indicated restricted rotation about the N–C35 bond, resulting in partial structures a and b, rather than the usual restricted rotation about the N–C40 bond. Long-range couplings between the methoxyl signal at δ 3.30 and the C32 signal at δ 82.1, the C32 signal and the H39 signal at δ 0.98 (d, 3 H, *J* = 7 Hz) and the H39 signal and the saturated carbonyl signal at δ 213.5 allowed the carbonyl group to be placed at C30. Unfortunately, no other long-range couplings to C30 could be observed.



The tris(oxazole) portion of the molecule from C10 to C20 was most easily established by comparison of the spectral data of halichondramide (4) with those of kabiramide C (3).[6] The contiguous nature of the olefin and oxazole rings was confirmed by the observation of the following three-bond long-range couplings: δ 7.66 (H11)–155.2 (C12)–8.10 (H14)–156.2 (C15)–8.06 (H17)–160.6 (C18)–7.15 (H20). The 16-Hz coupling constant between H19 and H20 is consistent with a trans olefinic bond.

The C20–C27 (C37, C38) portion of the molecule could be traced by interpretation of the COSY spectrum. The position of the methoxy group at C26 was established by observation of three-bond couplings between the methoxyl signal at δ 3.32 (s, 3 H) and C26 (δ 81.6) and between C26 and the methyl (38) signal at δ 0.85 (d, 3 H, *J* = 7 Hz). There was a three-bond coupling between the methyl (37) signal at δ 0.92 (d, 3 H, *J* = 7 Hz) and C24 (δ 74.0) and a direct coupling between C24 and the H24 signal at δ 5.10 (m, 1 H), but no three-bond coupling between H24 and C1 was observed. However, the chemical shift value for H24 strongly suggested that the ester group was attached at C24.

The C4–C9 portion of halichondramide (4) was elucidated predominantly from three-bond couplings. The H5 proton was long-range coupled to the C7-carbonyl signal at δ 202.8, which was in turn coupled to the methyl (36) signal at δ 0.94 (d, 3 H, *J* = 7 Hz). The methyl (36) signal was three-bond coupled to the C9 signal at δ 77.1 that was in turn coupled to a methoxyl signal at δ 3.18 (s, 3 H). The COSY spectrum also clearly showed the H9–H8–H36 couplings. Although neither the three-bond coupling between C9 and H11 nor the allylic coupling between H9 and H11 were observed, the nuclear Overhauser enhancement of H9 caused by irradiation of H11 established the presence of the C9–C10 bond. The 16-Hz coupling constant between the H5 and H6 signals requires a trans α,β-unsaturated enone. The H5 proton was coupled to a methylene group: unfortunately the signals for the methylene protons were among a group of seven overlapping signals in the region δ 2.49–2.53 that contained five of the nine proton signals remaining unassigned. It was tempting to assume a ring size and oxidation pattern analogous to that of kabiramide C (3) but the COSY experiment could not be unambiguously interpreted. We performed a series of 2D *J*-resolved experiments in which the proton signals in the δ 2.49–2.53 region were resolved and compared with the signals obtained by decoupling the H5 signal at δ 7.27 and the C*H*OH signal at δ 4.47 (m, 1 H). These experiments were consistent with the proposed structure and resulted in the assignments in Table I.[10]

The proposed structure was further supported by analysis of ^1H NMR spectra of the corresponding monoacetate 5, obtained by treatment of halichondramide (4) with acetic anhydride in pyridine. Analysis of the COSY spectrum of acetate 5 showed that the olefinic proton signal at δ 7.03 (ddd, 1 H, *J* = 16, 8, 7 Hz) was coupled to

(10) We thank Dr. James N. Shoolery, Varian Associates, for suggesting this experiment.

Table II. ^{13}C NMR Spectral Data of Halichondramide (4), Dihydrohalichondramide (6), Tetrahydrohalichondramide (8), Isohalichondramide (10), and Acid 11[a]

carbon no.	4	6	8	10	11
1	172.6	172.8	172.6	172.8	172.8
2	42.2	42.0	42.2	43.0	42.5
3	67.3	68.8	68.6	68.8	67.2
4	40.3	37.1	36.9	38.3	40.5
5	146.1	20.0	22.4	145.2	146.2
6	133.0	42.6	33.8	128.0	133.0
7	202.8	211.2	70.2	200.3	202.8
8	44.0	48.4	38.9	50.2	44.0
9	77.1	78.2	82.6	77.7	77.1
10	139.1	139.9	141.8	139.6	139.5
11	136.8	136.0	135.9	136.0	136.9
12	155.2	154.8	155.2	154.9	155.5
13	130.7	131.3	131.4	131.2	131.0
14	137.1	137.5	137.2	137.6	137.1
15	156.2	156.1	156.4	156.3	156.5
16	129.6	130.0	130.2	130.0	129.9
17	137.1	137.5	137.2	137.6	137.2
18	162.9	162.9	162.8	163.2	163.1
19	114.5	115.5	116.6	115.1	114.8
20	143.7	143.5	142.7	144.8	144.2
21	28.9	28.8	28.9	29.3	29.2
22	31.3	31.2	31.1	31.6	31.7
23	35.6	35.7	35.3	36.4	35.4
24	74.0	74.1	73.2	74.7	74.4
25	32.0	32.1	31.5	32.8	31.7
26	81.6	81.7	81.8	81.8	81.7
27	34.5	34.5	34.6	34.5	35.0
28	24.8	24.8	24.9	24.9	25.3
29	41.1 (41.0)	41.3	41.5	41.5	41.0
30	213.5	213.4	213.6	213.5	213.4
31	48.6	48.8	48.8	48.9	49.0
32	82.1	82.3	82.4	82.4	77.6
33	30.1 (30.0)	30.3 (30.2)	30.4 (29.7)	30.5	42.5
34	105.1 (106.8)	105.3 (106.9)	105.3 (107.0)	105.5 (107.1)	42.6
35	130.1 (126.1)	130.3 (126.2)	130.3 (126.3)	130.4 (126.4)	172.1
36	13.2	9.8	9.0	7.7	13.1
37	14.5	15.2	15.0	15.6	14.5
38	15.4	15.4	15.4	16.0	15.3
39	12.7 (12.8)	12.6 (12.7)	12.8	12.7 (12.8)	12.3
40	162.1 (160.8)	162.0 (160.7)	162.1 (160.7)	162.1 (160.7)	
OMe (9)	56.8	57.1	59.4	57.5	56.8
OMe (26)	57.8	58.0	58.1	58.0	58.3
OMe (32)	57.4 (57.2)	57.6 (57.4)	57.7 (57.5)	57.8	57.7
NMe	27.5 (32.8)	27.4 (32.9)	27.5 (33.0)	27.6 (32.8)	

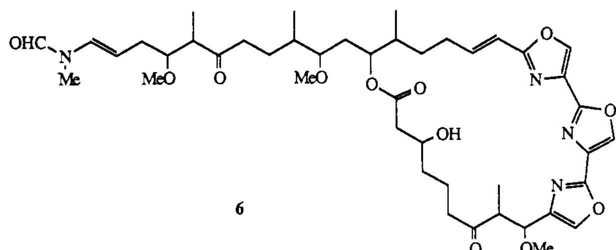
[a] The data for the minor geometrical isomers are in parentheses.

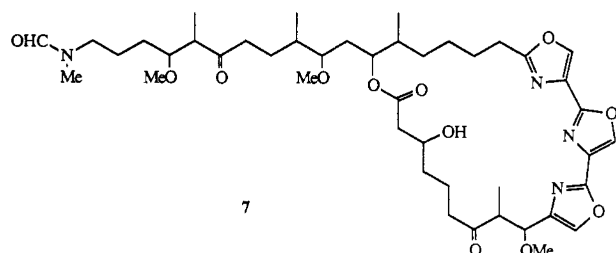
two methylene proton signals at δ 2.83 (m, 1 H) and 2.64 (m, 1 H) that were in turn coupled to a signal at δ 5.46 (m, 1 H) assigned to the *CH*(OAc) proton. The signal at δ 5.46 (m, 1 H) was also coupled to two methylene proton signals at δ 2.91 (dd, 1 H, J = 14, 9 Hz) and 2.71 (dd, 1 H, J = 14, 7 Hz) that must be adjacent to the ester carbonyl group. The remaining two methylene groups in halichondramide (4) must therefore be at C2 and C29. The mass spectrum of halichondramide (4) contained fragment ions at m/z 312 and 474 (524 − MeOH − H_2O) that resulted from cleavage of the 24–25 bond. The data are all compatible with the proposed structure of halichondramide (4). As is the case for all other compounds in this group, the stereochemistry of halichondramide (4) remains to be determined.

Dihydrohalichondramide (6) has the molecular formula $C_{44}H_{62}N_4O_{12}$, which differs from that of halichondramide (4) by the addition of two hydrogens. Examination of the ^{1}H NMR COSY spectrum revealed that the two compounds were identical in the C7–C40 region. The H5 and H6 olefinic protons of halichondramide (4) were conspicuously absent. The ^{13}C NMR spectrum (Table II) of dihydrohalichondramide (6) contained two new aliphatic methylene carbons in place of two of the olefinic carbons of halichondramide (4). The stereochemistry of dihydrohalichondramide (6), though unknown, appears identical with that of halichondramide (4) as judged by the decoupling data, NOEDS, and COLOC experiments. Although it was not possible to selectively reduce halichondramide (4) to dihydrohalichondramide (6), hydrogenation of both compounds gave the same reduction product 7, indicating that they had the same stereochemistry at the nine undefined chiral centers.

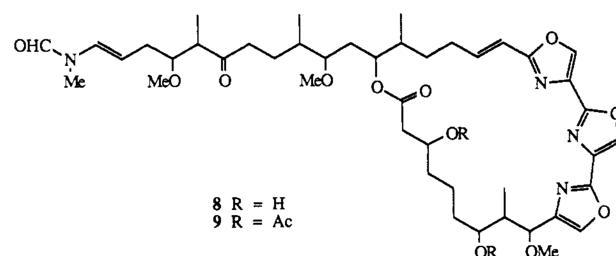
Tetrahydrohalichondramide (8) has the molecular formula $C_{44}H_{64}N_4O_{12}$. The ^{13}C NMR spectrum (Table II) contained only one ketone carbonyl signal and six signals for aliphatic carbons bearing oxygen. Since the ^{1}H COSY NMR spectrum indicated that the C19–C40 portion of the molecule was identical with that of halichondramide (4), the structure 8 was proposed for tetrahydrohalichondramide. The COSY experiment showed allylic coupling between the H11 signal at δ 7.63 (d, 1 H, J = 1.3 Hz) and the H9 signal at δ 4.38 (dd, 1 H, J = 4.5, 1.3 Hz), but further correlations to H9 could not be interpreted because many signals overlap in the δ 2.4–2.6 region of the spectrum. However, a 2D homonuclear relayed coherence transfer (RCT) experiment[11] clearly revealed coupling between the H9 signal at δ 4.38 and the H8 signal at δ 2.46

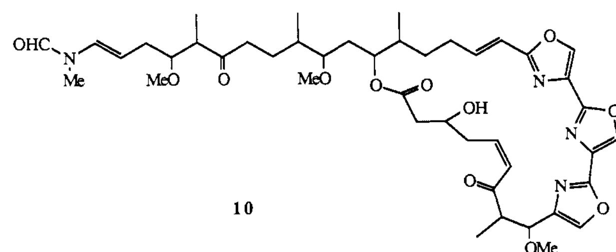
(11) Wagner, G. *J. Magn. Reson.* **1983**, *55*, 151.

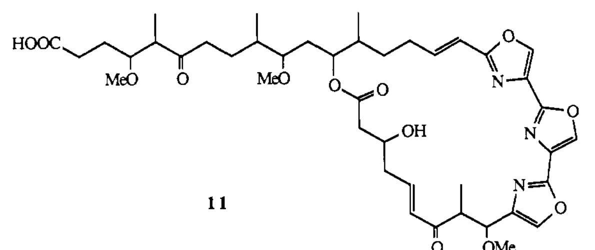




(br qd, 1 H, J = 7, 4.5 Hz) that was in turn coupled to the methyl signal at δ 1.03 (d, 3 H, J = 7 Hz). Decoupling difference spectra indicated a small coupling between the H7 signal at δ 3.93 (m, 1 H) and the H8 signal at δ 2.46 and confirmed the H7-H8 coupling. Furthermore, irradiation of the H7 signal at δ 3.93 caused significant nuclear Overhauser enhancements of the signals at δ 4.38 (H9), 2.46 (H8), and 7.63 (H11). These data established the position of the secondary alcohol at C7. Acetylation of tetrahydrohalichondramide (**8**) produced the expected diacetate **9**. Treatment of dihydrohalichondramide (**6**) with lithium tri-*tert*-butoxyaluminum hydride reduced both carbonyl groups to give a mixture of products, but if the reaction was stopped after 25% consumption of starting material, the major product was the desired tetrahydrohalichondramide (**8**).







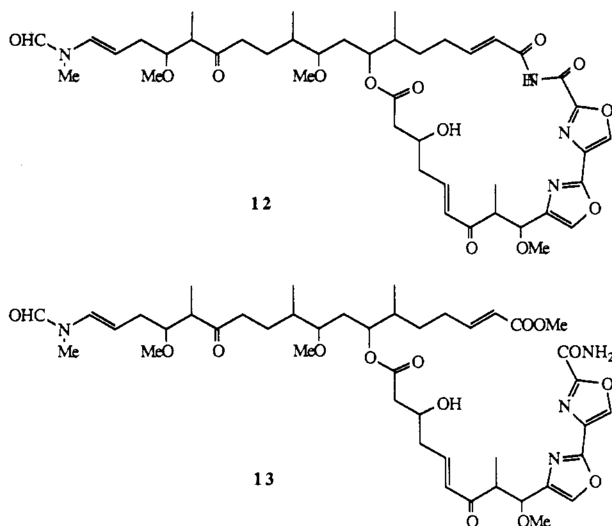
Isohalichondramide (**10**) is an unstable compound that slowly isomerized to halichondramide (**4**) when allowed to stand in $CDCl_3$ solution. Both the ^1H and ^{13}C NMR spectra of isohalichondramide (**10**) were almost identical with those of halichondramide (**4**) except in the C1–C9 region. The ^1H NMR spectrum contained signals at δ 6.40 (dt, 1 H, J = 11, 6 Hz, H5) and 6.52 (d, 1 H, J = 11 Hz, H6) that were assigned to the hydrogens on a cis olefin conjugated to a ketone (^{13}C NMR δ 200.3). Some indication of the geometry of the C6–C11 region of isohalichondramide (**10**) was obtained from an NOEDS experiment: irradiation of the H9 signal at δ 4.97 (dd, 1 H, J = 4, 1 Hz) caused a 4% enhancement of the H11 signal at δ 7.53 (d, 1 H, J = 1 H) and a 7% enhancement of the H6 signal at δ 6.52. These and all other spectral data are completely consistent with a 5,6-cis geometrical isomer of halichondramide.

Three compounds that appear to have arisen by oxidation of halichondramide have been isolated in small quantities. The acid **11** has the molecular formula $C_{42}H_{57}N_3O_{13}$. Both the ^1H and ^{13}C NMR spectra clearly indicated a single compound with no evidence of geometrical isomers. We therefore proposed that the acid **11** had resulted from oxidation of halichondramide (**4**) at C35 with concomitant loss of the *N*-methylformamide group. The spectral data were completely in accord with the proposed structure. The acid **11** was synthesized in 37% yield by oxidation of halichondramide (**4**) with Jones' reagent, thereby confirming the proposed structure.

The imide **12** is a relatively unstable compound of molecular formula $C_{43}H_{60}N_4O_{13}$ that reacts with methanol to form the methyl ester **13**. The infrared spectrum contained an imide band at 1760 cm^{-1}. Comparison of the ^1H NMR spectrum of the imide **12** with that of halichondramide (**4**) indicated that one of the oxazole proton signals in halichondramide (**4**) was replaced by a signal at δ 9.86 (br s, 1 H) that was assigned to the N*H* proton of an imide. The chemical shifts of the ^1H NMR signals for H19 and H20 in the imide **12** and particularly the trans-disubstituted α,β-unsaturated ester **13**[12] suggested that the C16–18 oxazole ring has been replaced by an imide. The observation of a nuclear Overhauser enhancement of the H19 signal at δ 6.11 (d, 1 H, J = 16 Hz) on irradiation of the N*H* signal also supported this assignment. Methanolysis of imide **12** occurs at C18, resulting in the opening of the macrocyclic ring to obtain an acrylate ester. There is no evidence that any other functionality on these molecules has been altered, but without extensive 2D NMR data the possibility cannot be completely excluded.

Halichondramide (**4**), dihydrohalichondramide (**6**), tetrahydrohalichondramide (**8**), isohalichondramide (**10**), and the acid **11** all showed significant activity against *C. albicans* at 0.5 μg/disk in the standard disk assay but did not inhibit a range of Gram positive or Gram negative bacteria. The imide (**12**) and ester (**13**) showed only marginal activity against *C. albicans*. Further studies showed that halichondramide (**4**) still produced a zone of inhibition against *C. albicans* at 0.01 μg/disk and in both dilution assays it inhibited *C. albicans* at 0.2 μg/mL and *Trichophyton mentagrophytes* at 12.5 μg/mL. Halichondramide (**4**) and isohalichondramide (**10**) both inhibited cell division in fertilized sea urchin egg at 4 μg/mL, while dihydrohalichondramide (**6**) and tetrahydrohalichondramide (**8**) were both active at 1 μg/mL, and the acid **11** and imide **12** were much less active. The trends observed in antimicrobial and cytotoxicity data roughly parallel those of fish-feeding deterrence.[9] Unfortunately,

(12) Observed values: δ 5.89 (H19), 6.85 (H20); calculated values: δ 5.86 (H19), 6.87 (H20). Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd Ed.; Pergamon: Oxford, 1969; p 185.



halichondramide (4) and related compounds are probably too toxic for medical use: when injected subcutaneously in mice, halichondramide caused lethargy, edema, paralysis, and mortality at doses as low as 1.4 mg/kg.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer 783 spectrophotometer and workstation. Ultraviolet spectra were obtained in methanol solution on a Perkin-Elmer Lambda 3B UV/vis spectrophotometer. Proton NMR spectra were recorded in $CDCl_3$ solution, unless otherwise stated, at 360 MHz on a spectrometer constructed from an Oxford narrow-bore magnet and a Nicolet 1180E FT data system by Dr. John Wright for the UCSD NMR facility; all chemical shifts are reported with respect to Me_4Si (δ = 0). Carbon-13 NMR spectra were recorded at 50 MHz on an IBM WP-200 SY spectrometer with an Aspect 2000 data system; all chemical shifts are reported with respect to Me_4Si (δ = 0). Mass spectra were obtained from the regional facilities at UC Riverside or the University of Minnesota. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a 10-cm microcell. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. All solvents used were distilled from glass prior to use.

Collection and Extraction of *Halichondria* sp. The sponge was collected from Kwajalein Atoll in September 1986 and was immediately frozen. A portion of this sponge was freeze-dried, covered with 20% methanol in dichloromethane, sonicated for 1 h, and stored overnight. The solvent was filtered and evaporated, and the sponge was extracted two more times with 20% methanol in dichloromethane in the same manner. The solvent was removed in vacuo from the combined extracts. The resulting green slurry was purified by counter-current chromatography using hexane–ethyl acetate–methanol–water (3:7:5:5) as the solvent system. The upper organic phase was used as the mobile phase, and the flow rate was kept at 10 mL/min. Three antifungal fractions were isolated. The first two fractions contained halichondramide (4) in addition to some related metabolites, fats, and sterols, while the third fraction contained the acid 11 (0.002% dry weight). The two halichondramide containing fractions were each purified by HPLC on Dynamax C-18 (75% acetonitrile–water followed by 80% methanol–water) to give halichondramide (4, 0.34% dry weight), dihydrohalichondramide (6, 0.03% dry weight), isohalichondramide (10, 0.007% dry weight), the imide 12 (0.008% dry weight), and the ester 13 (0.004% dry weight).

Collection and Extraction of *H. sanguineus*. Two specimens of *H. sanguineus* (48 g dry weight) were collected in September 1986 from subtidal reefs at Kwajalein Atoll and were immediately frozen. The thawed animals were exhaustively extracted with acetone (3 × 500 mL), and the combined extracts were concentrated to an aqueous suspension (~200 mL) that was extracted with ethyl acetate (3 × 200 mL). The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and evaporated to obtain a deep red oil. The oil was chromatographed on TLC grade silica gel with a gradient of 1–5% methanol in dichloromethane to obtain two macrolide-containing fractions. The red pigments were removed from each fraction by passage of a 75% aqueous methanol solution through C-18 SepPaks (Waters). The less polar fraction was subjected to LC on a Dynamax C-18 column with 85% aqueous methanol as eluant to obtain dihydrohalichondramide (6, 127 mg, 0.265% dry weight) while, under similar conditions, the more polar fraction gave tetrahydrohalichondramide (8, 28 mg, 0.058% dry weight) and kabiramide C (3, 13 mg, 0.027% dry weight).

Halichondramide (4): fine white needles; mp 66–68 °C; $[\alpha]_D$ −100.7° (*c* 0.42, MeOH); UV (MeOH) 231 nm (ϵ 23 400); IR ($CHCl_3$) 3600–3200 (br), 1720, 1700, 1680, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) see Table I; ^{13}C NMR ($CDCl_3$) see Table II; FABMS obsd *m/z* 837.4360, $C_{44}H_{61}N_4O_{12}$ $(M + H)^+$ requires 837.4286.

Dihydrohalichondramide (6): colorless glass; $[\alpha]_D$ −69.7° (*c* 1.68, MeOH); UV (MeOH) 247 nm (ϵ 32 000); IR ($CHCl_3$) 3600–3300 (br), 1720–1680 (br), 1658 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.28 (s, 0.7 H, H40), 8.08 (s, 1 H, H17), 8.06 (s, 0.3 H, H40), 8.05 (s, 1 H, H14), 7.76 (s, 1 H, H11), 7.19 (d, 0.3 H, J = 15 Hz, H35), 7.03 (dt, 1 H, J = 16, 7 Hz, H20), 6.52 (d, 0.7 H, J = 14 Hz, H35), 6.38 (d, 1 H, J = 16 Hz, H19), 5.13 (br dd, 1 H, J = 11, 7 Hz, H24), 5.08 (ddd, 1 H, J = 14, 8, 5 Hz, H34), 4.71 (d, 1 H, J = 6 Hz, H9), 4.58 (d, 1 H, J = 6 Hz, OH), 4.32 (m, 1 H, H3), 3.46 (m, 1 H, H32), 3.38 (s, 3 H, OMe9), 3.36 (qd, 1 H, J = 7, 6 Hz, H8), 3.33 (s, 3 H, OMe26), 3.29 (s, 2 H, OMe32), 3.28 (s, 1 H, OMe32), 3.07 (s, 1 H, NMe), 3.03 (s, 2 H, NMe), 2.97 (br d, 1 H, J = 10 Hz, H26), 2.72 (m, 1 H, H31), 2.50 (m, 1 H, H21), 2.45 (m, 1 H, H33), 2.43 (m, 1 H, H2), 2.23 (m, 1 H, H21), 2.18 (m, 1 H, H2), 2.13 (ddd, 1 H, J = 16, 8, 5 Hz, H33), 1.73 (m, 2 H, H27), 1.59 (m, 1 H, H25), 1.48 (m, 1 H, H25), 0.98 (d, 2 H, J = 7 Hz, H39), 0.975 (d, 3 H, J = 7 Hz, H36), 0.96 (d, 1 H, J = 7 Hz, H39), 0.90 (d, 3 H, J = 7 Hz, H37), 0.83 (d, 3 H, J = 7 Hz, H38); ^{13}C NMR (see Table II); FABMS obsd *m/z* 861.4229, $C_{44}H_{62}N_4O_{12}Na$ $(M + Na)^+$ requires 861.4262.

Tetrahydrohalichondramide (8): colorless glass; UV (MeOH) 247 nm (ϵ 34 000); IR ($CHCl_3$) 3600–3400 (br), 1715, 1690, 1658 cm^{-1}; $[\alpha]_D$ −27° (*c* 0.54, MeOH); ^1H NMR ($CDCl_3$) δ 8.28 (s, 0.7 H, H40), 8.09 (s, 1 H, H17), 8.07 (s, 0.3 H, H40), 8.04 (s, 1 H, H14), 7.63 (d, 1 H, J = 1 Hz, H11), 7.18 (d, 0.3 H, J = 14 Hz, H35), 6.93 (dt, 1 H, J = 16, 8 Hz, H20), 6.52 (d, 0.7 H, J = 14 Hz, H35), 6.34 (d, 1 H, J = 16 Hz, H19), 5.16 (m, 1 H, H24), 5.13 (m, 0.3 H, H34), 5.10 (ddd, 0.7 H, J = 11, 9, 7 Hz, H34), 4.61 (br s, 1 H, OH), 4.38 (dd, 1 H, J = 5, 1 Hz, H9), 4.22 (m, 1 H, H3), 3.93 (m, 1 H, H7), 3.53 (s, 3 H, OMe9), 3.45 (m, 1 H, H32), 3.34 (s, 3 H, OMe26), 3.293 (s, 2 H, OMe32), 3.286 (s, 1 H, OMe32), 3.07 (s, 1 H, NMe), 3.04 (s, 2 H, NMe), 2.95 (br d, 1 H, J = 10 Hz, H26), 2.75 (dq, 1 H, J = 9, 7 Hz, H31), 2.53 (m, 2 H, H2), 2.50 (m, 1 H, H33), 2.48 (m, 1 H, H21), 2.46 (br qd, 1 H, J = 7, 4.5 Hz, H8), 2.25 (m, 1 H, H21), 2.17 (m, 1 H, H33), 1.73 (m, 2 H, H23, H27), 1.55 (m, 1 H, H25), 1.50 (m, 1 H, H25), 1.03 (d, 3 H, J = 7 Hz, H36), 0.98 (d, 2 H, J = 7 Hz, H39), 0.97 (d, 1 H, J = 7 Hz, H39), 0.89 (d, 3 H, J = 7 Hz, H37), 0.83 (d, 3 H, J = 7 Hz, H38); ^{13}C NMR ($CDCl_3$) see Table II; FABMS obsd *m/z* 863.4418, $C_{44}H_{64}N_4O_{12}Na$ $(M + Na)^+$ requires 863.4418.

Isohalichondramide (10): oil; ^1H NMR ($CDCl_3$) δ 8.28 (s, 0.7 Hz, H40), 8.08 (s, 1 H, H17), 8.05 (s, 1 H, H14), 7.53 (d, 1 H, J = 1 Hz, H11), 7.16 (d, 0.3 H, J = 14 Hz, H40), 7.04 (ddd, 1 H, J = 16, 9, 5 Hz, H20), 6.52 (d, 1 H, J = 11 Hz, H6), 6.52 (d, 0.7 H, J = 14 Hz, H35), 6.40 (dt, 1 H, J = 11, 6 Hz, H5), 6.28 (d, 1 H, J = 16 Hz, H19), 5.09 (m, 2 H, H24, H34), 4.97 (dd, 1H, J = 4, 1 Hz, H9), 4.47 (m, 1 H, H3), 3.48 (s, 3 H, OMe9), 3.45 (m, 1 H, H32), 3.31 (s, 3 H, OMe26), 3.28 (s, 2 H, OMe32), 3.27 (s, 1 H, OMe32), 3.07 (m, 1 H, H8), 3.06 (s, 1 H, NMe), 3.03 (s, 2 H, NMe), 2.94 (br d, 1 H, J = 10 Hz, H26), 2.71 (m, 1 H, H31), 2.70 (br d, 1 H, J = 14 Hz, H2), 2.64 (dd, 1 H, J = 14, 4 Hz, H2), 2.50 (m, 3 H), 2.44 (m, 1 H), 2.15 (m, 2 H), 1.78 (m, 1 H), 1.73 (m, 1 H), 1.64 (m, 1 H), 1.60 (m, 1 H), 1.44 (m, 2 H), 1.28 (m, 2 H), 0.97 (d, 2 H, J = 7 Hz, H39), 0.96 (d, 1 H, J = 7 Hz, H39), 0.95 (d, 3 H, J = 7 Hz, H39), 0.91 (d, 3 H, J = 7 Hz, H37), 0.80 (d, 3 H, J = 7 Hz, H38); ^{13}C NMR ($CDCl_3$) see Table II; FABMS *m/z* 859.4132, $C_{44}H_{60}N_4O_{12}Na$ $(M + Na)^+$ requires 859.4105.

Acid 11: oil; UV (MeOH) 231 nm (ϵ 23 400); IR ($CHCl_3$) 3600–3200 (br), 1720, 1700, 1680, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) 8.11 (s, 1 H, H14), 8.06 (s, 1 H, H17) 7.66 (s, 1 H, H11), 7.19 (dt, 1 H, J = 16, 7 Hz, H5), 7.14 (dt, 1 H, J = 16, 7 Hz, H20), 6.27

(d, 1 H, J = 16 Hz, H19), 6.22 (d, 1 H, J = 16 Hz, H6), 5.13 (br t, 1 H, J = 7 Hz, H24), 4.47 (m, 1 H, H3), 4.36 (d, 1 H, J = 9 Hz, H9), 4.00 (dq, 1 H, J = 9, 7 Hz, H8), 3.51 (m, 1 H, H32), 3.34 (s, 3 H, OMe26), 3.27 (s, 3 H, OMe32), 3.18 (s, 3 H, OMe9), 0.99 (d, 3 H, J = 7 Hz, H39), 0.91 (d, 3 H, J = 7 Hz, H36), 0.90 (d, 3 H, J = 7 Hz, H37), 0.87 (d, 3 H, J = 7 Hz, H38); ^{13}C NMR ($CDCl_3$) see Table II; FABMS obsd m/z 834.4083, $C_{42}H_{57}N_3O_{13}Na$ (M + Na)$^+$ requires 834.3789.

Imide 12: oil; UV (MeOH) 230 nm (ϵ 17000); IR ($CHCl_3$) 1762, 1710, 1690, 1660 cm^{-1}, ^{1}H NMR δ 9.86 (br s, 1 H, NH), 8.39 (s, 1 H, H14), 8.28 (s, 0.7 H, H40), 8.06 (s, 0.3 H, H40), 7.75 (s, 1 H, H-11), 7.29 (dt, 1 H, J = 15, 5 Hz, H5), 7.16 (d, 0.3 H, J = 14 Hz, H35), 6.96 (dt, 1 H, J = 16, 8 Hz, H20), 6.52 (d, 0.7 H, J = 14 Hz, H35), 6.19 (d, 1 H, J = 16 Hz, H6), 6.11 (d, 1 H, J = 16 Hz, H19), 5.21 (br dt, 1 H, J = 9, 3 Hz, H-24), 5.08 (ddd, 1 H, J = 14, 8, 7 Hz, H-34), 4.44 (d, 1 H, J = 9 Hz, H9), 4.29 (m, 1 H, H3), 3.92 (dq, 1 H, J = 9, 7 Hz, H8), 3.45 (m, 1 H, H32), 3.36 (s, 3 H, OMe26), 3.28 (s, 2 H, OMe32), 3.275 (s, 1 H, OMe32), 3.18 (s, 3 H, OMe9), 3.07 (s, 1 H, NMe), 3.03 (s, 2 H, NMe), 3.00 (br d, 1 H, J = 10 Hz, H26), 2.72 (m, 1 H, H31), 2.70 (dd, 1 H, J = 16, 3 Hz, H2), 2.57 (dd, 1 H, J = 16, 10 Hz, H2), 2.50 (m, 3 H), 2.45 (m, 1 H), 2.23 (m, 1 H), 2.14 (m, 1 H), 1.85 (m, 1 H), 1.77 (m, 1 H), 1.75 (m, 1 H), 1.65 (m, 1 H), 1.55 (m, 1 H), 1.40 (m, 1 H), 1.30 (m, 2 H), 0.98 (d, 3 H, J = 7 Hz), 0.97 (d, 3 H, J = 7 Hz), 0.88 (d, 3 H, J = 7 Hz), 0.87 (d, 3 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 213.5 (s), 202.7 (s), 172.3 (s), 162.4 (s), 162.1 (d), 160.8 (d), 154.9 (s), 154.7 (s), 151.1 (s), 150.9 (d), 142.4 (d), 141.8 (?), 139.4 (s), 137.9 (d), 134.9 (d), 131.2 (s), 130.4 (d), 126.3 (d), 122.4 (d), 107.0 (d), 105.4 (d), 82.4 (d), 82.0 (d), 77.2 (d), 66.8 (d), 57.8 (d), 57.7 (d), 57.0 (d), 56.8 (d), 48.8 (d), 43.8 (d), 41.3 (t), 41.2 (t), 41.0 (t), 40.2 (t), 37.0 (d), 34.3 (d), 33.8 (t), 32.9 (q), 30.4 (t), 30.3 (t), 29.8 (t), 27.6 (q), 27.5 (t), 24.9 (t), 18.9 (q), 15.4 (q), 14.8 (q), 12.8 (q); FABMS m/z 863 (M + Na)$^+$, 841 (M + H)$^+$.

Ester 13: oil; IR ($CHCl_3$) 3600–3200 (br), 1715, 1680, 1657 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.49 (s, 1 H, H14), 8.29 (s, 0.7 Hz, H40), 8.06 (s, 0.3 H, H40), 7.75 (s, 1 H, H11), 7.17 (d, 0.3 H, J = 15 Hz, H35), 7.00 (dt, 1 H, J = 16, 8 Hz, H5), 6.85 (d, 1 H, J = 16, 7 Hz, H20), 6.52 (d, 0.7 H, J = 15 Hz, H35), 6.28 (d, 1 H, J = 16 Hz, H6), 5.89 (d, 1 H, J = 16 Hz, H19), 5.10 (m, 2 H, H24, H34), 4.41 (d, 1 H, J = 10 Hz, H9), 4.24 (m, 1 H, H3), 4.05 (s, 3 H, COOMe), 3.58 (dq, 1 H, J = 9, 7 Hz, H8), 3.46 (m, 1 H, H32), 3.33 (s, 3 H, OMe26), 3.293 (s, 2 H, OMe32), 3.287 (s, 1 H, OMe32), 3.18 (s, 3 H, OMe9), 3.07 (s, 1 H, NMe), 3.04 (s, 2 H, NMe), 2.98 (m, 1 H, H26), 2.74 (m, 1 H, H31), 2.57 (dd, 1 H, J = 16, 10 Hz, H2), 2.50 (m, 3 H), 2.45 (m, 1 H), 2.29 (m, 1 H), 2.18 (m, 1 H), 2.14 (m, 1 H), 1.76 (m, 2 H), 1.65 (m, 1 H), 1.56 (m, 2 H), 1.28 (m, 2 H), 0.98 (d, 2 H, J = 7 Hz, H39), 0.97 (d, 1 H, J = 7 Hz, H39), 0.90 (d, 3 H, J = 7 Hz, H36), 0.88 (d, 3 H, J = 7 Hz, H37), 0.84 (d, 3 H, J = 7 Hz, H38); ^{13}C NMR ($CDCl_3$) δ 213.4 (s), 201.7 (s), 171.9 (s), 168.1 (s), 162.1 (d), 160.8 (d), 155.5 (s), 154.7 (s), 145.4 (d), 142.8 (d), 141.2 (d), 140.3 (s), 137.7 (d), 132.9 (d), 132.5 (s), 130.6 (d), 126.6 (d), 123.4 (d), 107.3 (d), 105.7 (d), 82.7 (d), 82.1 (d), 77.1 (d), 75.5 (d), 67.1 (d), 58.0 (d), 57.8 (d), 57.6 (d), 57.1 (d), 53.4 (d), 49.2 (d), 47.0 (d), 41.6 (t), 41.3 (t), 39.8 (t), 36.4 (d), 34.7 (d), 33.0 (q), 31.5 (t), 30.9 (t), 30.8 (t), 29.7 (t), 29.4 (t), 27.7 (q), 25.2 (d), 15.5 (q), 15.0 (q), 14.1 (q), 12.8 (q).

Acetylation of Halichondramide. A solution of halichondramide (4, 4.4 mg) in pyridine (2 mL) and acetic anhydride (2 mL) was stirred overnight. The solvents were removed in vacuo, and the resulting oil was purified by HPLC on ODS-Partisil (80% methanol–water) to obtain halichondramide acetate (5, 4.5 mg, 99% yield).

Halichondramide acetate (5): oil; UV (MeOH) 235 nm (ϵ 26000); IR ($CHCl_3$) 1735, 1710, 1690, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 0.7 H, H40), 8.10 (s, 1 H, H14), 8.06 (s, 0.3 H, H40), 8.04 (s, 1 H, H17), 7.66 (s, 1 H, H11), 7.26 (dt, 1 H, J = 16, 7, Hz, H20), 7.24 (d, 0.3 H, J = 14 Hz, H35), 7.03 (ddd, 1 H, J = 16, 8, 7 Hz, H5), 6.53 (d, 0.7 H, J = 14 Hz, H35), 6.27 (d, 1 H, J = 16 Hz, H14), 6.16 (d, 1 H, J = 16 Hz, H6), 5.46 (m, 1 H, H3), 5.07 (m, 2 H, H24, H34), 4.32 (d, 1 H, J = 8 Hz, H9), 4.09 (m, 1 H, H8), 3.46 (m, 1 H, H32), 3.31 (s, 3 H, OMe), 3.28 (s, 3 H, OMe), 3.11 (s, 3 H, OMe9), 3.07 (s, 1 H, NMe), 3.04 (s, 2 H, NMe), 2.97 (br d, 1 H, J = 10 Hz, H26), 2.91 (dd, 1 H, J = 14, 9 Hz, H2), 2.83 (m, 1 H, H4), 2.71 (dd, 1 H, J = 14, 7 Hz, H2), 2.64 (m, 1 H, H4), 2.52 (m, 2 H), 2.45 (m, 2 H), 2.32 (m, 1 H), 2.17 (s, 3 H, OAc), 2.15 (m, 1 H), 1.93 (m, 1 H), 1.75 (m, 1 H), 1.60 (m, 1 H), 1.53 (m, 2 H), 1.30 (m, 2 H), 1.22 (m, 1 H), 0.98 (d, 3 H, J = 7 Hz, H39), 0.93 (d, 3 H, J = 7 Hz, H36), 0.88 (d, 3 H, J = 7 Hz, H37), 0.84 (d, 3 H, J = 7 Hz, H38); FABMS obsd m/z 879.4429, $C_{46}H_{63}N_4O_{12}$ (M + H)$^+$ requires 879.4391.

Hydrogenation of Halichondramide (4) or Dihydrohalichondramide (6). A solution of halichondramide (4, 25.8 mg) in ethyl acetate (5 mL) containing 10% palladium on carbon catalyst (1 mg) was stirred under an atmosphere of hydrogen for 12 h. The catalyst was removed by filtration, the solvent was evaporated, and the residue was purified by LC on ODS-Partisil to obtain the formamide 7 (26 mg, 98% yield). Hydrogenation of dihydrohalichondramide (6, 6.5 mg) under similar conditions gave a quantitative yield of the same formamide 7, as indicated by TLC and ^{1}H NMR spectroscopy.

Formamide 7: oil; IR ($CHCl_3$) 3550–3300 (br), 1710, 1660, 1580 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.43 (s, 0.7 H), 8.37 (s, 0.3 H), 8.15 (s, 1 H), 8.13 (s, 1 H), 7.60 (s, 1 H), 4.99 (br t, 1 H, J = 7 Hz), 4.67 (d, 1 H, J = 7 Hz), 4.64 (br, OH), 4.15 (m, 1 H), 3.44 (m, 1 H), 3.36 (s, 3 H), 3.30 (s, 3 H), 3.27 (s, 3 H), 2.89 (s, 1 H), 2.86 (s, 2 H), 0.98 (d, 3 H, J = 7 Hz), 0.96 (d, 3 H, J = 7 Hz), 0.86 (d, 3 H, J = 7 Hz), 0.81 (d, 3 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 213.2 (s), 211.1 (s), 172.8 (s), 166.8 (d), 162.6 (s), 140.0 (s), 138.7 (d), 137.6 (d), 136.2 (d), 131.5 (s), 129.6 (s), 82.0 (d), 81.9 (d), 78.3 (d), 75.3 (d), 68.5 (d), 57.9 (q), 57.8 (2 C, q), 57.4 (q), 49.7 (t), 48.9 (t), 48.7 (t), 44.2 (t), 42.3 (t), 42.1 (t), 41.3 (t), 37.1 (d), 36.5 (t), 34.7 (q), 33.2 (t), 31.0 (t), 29.7 (q), 29.4 (t), 27.3 (t), 27.1 (t), 26.1 (t), 25.4 (t), 25.2 (t), 23.2 (t), 19.8 (t), 17.5 (q), 15.4 (q), 12.6 (q), 12.4 (q), 10.1 (q).

Acetylation of Tetrahydrohalichondramide (8). A solution of tetrahydrohalichondramide (8, 5 mg) in pyridine (0.5 mL) and acetic anhydride (0.5 mL) was allowed to stand at room temperature overnight, and the solvents were then removed under high vacuum. The residue was purified by LC on a C-18 support with use of 70% aqueous acetonitrile as eluant to obtain the diacetate 9 (5 mg): glass; ^{1}H NMR ($CDCl_3$) δ 8.28 (s, 0.7 H, H40), 8.10 (s, 1 H, H14), 8.06 (s, 0.3 H, H40), 8.05 (s, 1 H, H17), 7.58 (s, 1 H, H11), 7.18 (dt, 1 H, J = 16, 8 Hz, H20), 7.18 (d, 0.3 H, J = 14 Hz, H35), 6.52 (d, 0.7 H, J = 14 Hz, H35), 6.26 (d, 1 H, J = 16 Hz, H19), 5.28 (m, 2 H, H3, H7), 5.08 (m, 1 H, H34), 5.00 (br t, 1 H, J = 9 Hz, H24), 3.97 (d, 1 H, J = 8.5 Hz, H9), 3.45 (m, 1 H, H32), 3.31 (s, 3 H, OMe26), 3.29 (s, 2 H, OMe32), 3.28 (s, 1 H, OMe32), 3.19 (s, 3 H, OMe9), 3.07 (s, 1 H, NMe), 3.03 (s, 2 H, NMe), 2.93 (br d, 1 H, J = 10 Hz, H26), 2.74 (dd, 1 H, J = 16, 11 Hz, H2), 2.74 (dq, 1 H, J = 9, 7 Hz, H31), 2.63 (dd, 1 H, J = 16, 3 Hz, H2), 2.62 (m, 1 H, H8), 2.07 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 0.99 (d, 2 H, J = 7 Hz, H39), 0.97 (d, 1 H, J = 7 Hz, H39), 0.92 (d, 3 H, J = 7 Hz, H37), 0.83 (d, 3 H, J = 7 Hz, H38), 0.71 (d, 3 H, J = 7 Hz, H36). FABMS, m/z 925 (M + H)$^+$, 865 (M − AcOH + H)$^+$.

Preparation of Tetrahydrohalichondramide (8) from Dihydrohalichondramide (6). A solution of dihydrohalichondramide (6, 20 mg) and lithium tris(*tert*-butoxyalumino)hydride (5 mg) in dry THF (5 mL) was stirred at 0 °C under an atmosphere of nitrogen for 30 min. Acetone (100 μL) was added to quench the reaction. Dichloromethane (25 mL) and water (25 mL) were added, the dichloromethane soluble material was dried over Na_2SO_4, the solvent was removed in vacuo, and the resulting oil was purified by HPLC on ODS-Silica (Dynamax C-18, 80% methanol–water) to obtain dihydrohalichondramide (6, 15 mg) and tetrahydrohalichondramide (8, 3.5 mg, 70% yield based on material consumed) that was identical with the natural product by ^{1}H NMR spectroscopy.

Conversion of Halichondramide (4) to the Acid 11. Halichondramide (4, 100 mg) was dissolved in dry acetone (10 mL) and cooled to −78 °C, and Jones' reagent (10 drops) was added. After being stirred for 30 min under argon, the reaction was quenched with 2-propanol (1 mL). Chloroform (20 mL) and water (20 mL) were added. The organic soluble portion was removed, and the aqueous layer was washed with additional chloroform (2 × 20 mL). The combined chloroform extracts were dried over anhydrous sodium sulfate, and the solvent was removed in vacuo to give a green oil that was purified by flash chromatography on silica (230–400 mesh, 1 cm × 4 cm, 0–20% methanol in dichloromethane) to obtain the acid 11 (35 mg, 37% yield) that was identical by ^{1}H and ^{13}C NMR spectroscopy with the natural product.

Acknowledgment. We thank C. Arneson and K. Jordan for assistance in collecting sponges and nudibranchs, Dr. Klaus Rützler for identifying the sponges, Mary Kay Harper for performing antimicrobial assays, and Virginia Scott and Dr. Thomas Matthews, Syntex Corporation, for antifungal and toxicity data. This research was supported by grants from the National Science Foundation (Grants CHE86-03091 and CHE84-14054) and the National Institutes of Health (Grant AI-11969).

Registry No. **4**, 113275-14-2; **5**, 113275-20-0; **6**, 116302-29-5; **7**, 116324-40-4; **8**, 116302-33-1; **9**, 116302-34-2; **10**, 116302-30-8; **11**, 116302-31-9; **12**, 116302-32-0; **13**, 116324-39-1.

Cacospongionolide: A New Antitumoral Sesterterpene, from the Marine Sponge *Cacospongia mollior*

Salvatore De Rosa* and Salvatore De Stefano

Istituto per la Chimica di Molecole di Interesse Biologico del CNR, Via Toiano n.2, 80072 Arco Felice, Napoli, Italy

Nevenka Zavodnik

Center for Marine Research "R. Bošković" Institute, Rovinj, Yugoslavia

Received March 29, 1988

A sesterterpene with a new carbon skeleton, cacospongionolide (**4a**), has been isolated from the marine sponge *Cacospongia mollior*. The structure of cacospongionolide was elucidated by spectral data, using 2D-NMR spectroscopy, and by chemical transformations. Cacospongionolide is a potent antitumor agent.

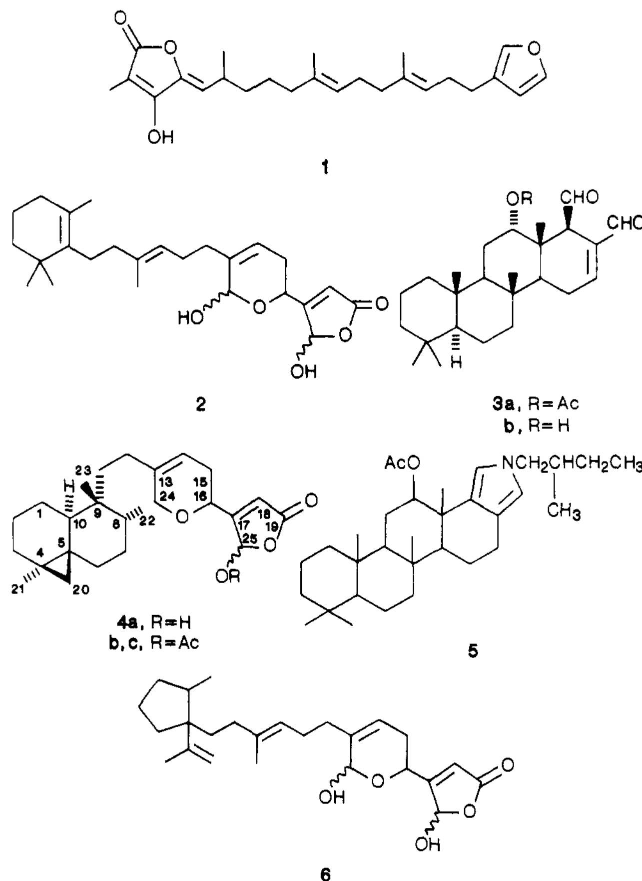
Marine organisms and in particular sponges have provided a large number of sesterterpenoids, several of which have shown a wide variety of biological activities. Some containing a tetronic acid moiety showed strong antibiotic activity against *Staphylococcus aureus*, e.g., variabilin[1] (**1**); others which were γ-hydroxybutenolides such as manoalide[2] (**2**) had analgesic and antiinflammatory properties[3]; still others were dialdehydes such as scalaradial[4] (**3a**), and its desacetyl derivative[5] **3b** had cytotoxic and/or antifeedant activity.[5]

In the course of our search for marine natural compounds that have biological activities, we have studied the marine sponge *Cacospongia mollior* (Schmidt), collected in the northern Adriatic, whose extract showed high cytotoxic activity (LD_{50} = 10 μg/mL) in the brine shrimp assay.[6] By fractionating the extract, we isolated a new sesterterpene (**4a**), named cacospongionolide, which is responsible for the biological activity.

The structure determination and some biological activities of this substance are reported in this paper.

From the same sponge, collected in the Tyrrhenian Sea, other authors have reported the isolation of sesterterpenoids with the scalarane skeleton, such as scalaradial[4] (**3a**) and the molliorins,[7] e.g., molliorin A (**5**).

Cacospongionolide, which did not give crystals suitable for X-ray analysis, had $[\alpha]_D$ 27° ($CHCl_3$, *c* 1.4) and the molecular formula $C_{25}H_{36}O_4$ from high-resolution mass measurement of the parent ion. The ultraviolet absorption at 222 nm (ϵ 4000) and infrared bands at 3330, 1780, and 1760 cm^{-1} were characteristic of a γ-hydroxybutenolide moiety. Its 500-MHz ^{1}H NMR spectrum was highly solvent dependent, and interpretation of the signals emanating from the polar moiety was difficult. In C_6D_6 solution, due to the presence of the lactol function, two sets of signals for H-16, H-18, and H-25 signals were observed, while in $CDCl_3$, a single set of broad signals was obtained. The same was true for the ^{13}C NMR spectrum. The NMR



(1) Faulkner, D. J. *Tetrahedron Lett.* 1985, *26*, 3821.
(2) De Silva, E. D.; Scheuer, P. J. *Tetrahedron Lett.* 1980, *21*, 1611.
(3) Jacobs, R. S.; Culver, P.; Langdon, R.; O'Brien, T.; White, S. *Tetrahedron* 1985, *41*, 981.
(4) Cimino, G.; De Stefano, S.; Minale, L. *Experientia* 1974, *30*, 846.
(5) Yasuda, F.; Tada, H. *Experientia* 1981, *37*, 110.
(6) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* 1982, *45*, 31.
(7) Cafieri, F.; De Napoli, L.; Iengo, A.; Santacroce, C. *Experientia* 1979, *35*, 157.

0022-3263/88/1953-5020$01.50/0 © 1988 American Chemical Society