9. Current and temperature measurements were made at several (up to seven) depths between 15 and 250 m with vector-averaging current meters (VACM) suspended beneath surface buoys tautly moored at 0°, 109°30'W from March 1980 until April 1982 and at 0°, 108°W from April to October 1982. The 108°W data are used to extend to 109°30'W time series beyond April 1982. Previous current measurements recorded simultaneously at 0°, 109°30'W and 0°, 110°30'W for 100 days in 1979 showed that there is little amplitude or phase difference in the currents for short zonal separations at these longitudes for frequencies less than 0.25 cycle per day. Vector-averaging wind recorder measurements at 3.5-m height were also recorded routinely. All data were recorded at 15-minute intervals and water depth was about 3.4 km. Deployment intervals were about 6 months. Previous studies [D. Halpern *et al.*, *J. Geophys. Res.* **86**, 419 (1981)] of upper ocean VACM measurements in deep water regions showed that at frequencies below 0.3 cycle per hour the amplitudes of current fluctuations recorded by VACM suspended within the upper 100 m beneath a surface-following buoy were only 5 to 10 percent larger than corresponding measurements made beneath a spar buoy or subsurface buoy.
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11. Contribution No. 618 from the NOAA/ERL Pacific Marine Environmental Laboratory.

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Siphonodictidine, a Metabolite of the Burrowing Sponge *Siphonodictyon* sp. That Inhibits Coral Growth

Abstract. *Siphonodictidine is the major secondary metabolite of an undescribed Indo-Pacific sponge* Siphonodictyon *sp. that burrows into living coral heads. The structure of siphonodictidine was determined from spectral data. Laboratory bioassays suggest that siphonodictidine and, by analogy, the siphonodictyals from* S. coralliphagum *are responsible for maintaining zones of dead coral polyps around the oscular chimneys of these sponges.*

Most burrowing sponges, like the relatively common *Cliona* species, burrow into shells, rocks, or dead corals. A few sponges, such as *Siphonodictyon coralliphagum* (*1*) from the Caribbean and the undescribed *Siphonodictyon* species (*2*) burrow deep into living coral heads, leaving only the oscular chimneys exposed. In order to survive, these sponges must be able to prevent overgrowth by the coral polyps and are observed to have a 1- to 2-cm zone of dead coral polyps around the base of each oscular chimney. Rützler (*1*) suggested that the dead zone was maintained by the production of mucus that flowed down the oscular chimney and spread around the base. We contend that the mucus acts as a carrier for secondary metabolites that are toxic to coral polyps. We now report the structure of a toxic secondary metabolite, siphonodictidine (**1**) from the Indo-Pacific *Siphonodictyon* species and present evidence to support the hypothesis that **1** is responsible for inhibiting coral growth around the base of the oscular chimney.

While collecting marine organisms at Palau, Western Caroline Islands (*3*), we encountered a sponge that was similar in appearance and habitat to the burrowing sponge *S. coralliphagum*, except that the protruding oscular chimneys were an off-white color in contrast to the familiar yellow oscular chimneys of *S. coralliphagum*. Only the oscular chimneys of the undescribed *Siphonodictyon* sp. could be collected, but even after collection the sponge material exuded a sticky mucus. The ethyl acetate soluble material from a methanol extract of the sponge was chromatographed on a Sephadex LH-20 column with methanol as eluant. Fractions that showed antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* were combined and chromatographed again on Sephadex LH-20 with a mixture of dichloromethane and methanol (1:1) as eluant, and obtained siphonodictidine (**1**, 1.06 percent, dry weight) as the major metabolite (*4*).

Siphonodictidine (**1**) had the molecular formula $C_{16}H_{25}N_3O$. A positive Sakaguchi test (*5*) and a signal at δ 157.4 in the ^{13}C nuclear magnetic resonance (NMR) spectrum indicated the presence of a guanidine group. Condensation of **1** with 2,4-pentanedione yielded the corresponding 4,6-dimethylpyrimidine derivative **2**, confirming the presence of the guanidine group (*6*).

The sesquiterpenoid portion of **1** was identified by interpretation of the spectral data. Comparison of the ^1H NMR data (Table 1) and ^{13}C NMR data (Table 2) of **1** with those of the model compounds marislin (**3**) from *Chromodoris marislae* (*7*) and the hydroquinone **4** from *Sinularia lochmodes* (*8*), indicated that the molecules were identical in the C-4 to C-14 region. In the ^1H NMR spectrum ($CDCl_3$) the signal at δ 3.76 (t, 1 H, J = 7 Hz), coupled to a NH proton signal at 7.80 (br t, 1 H) and an olefinic proton signal at 5.21 (br t, 1 H, J = 7 Hz), was assigned to the C-1 methylene group attached to the guanidine group. Addition of methanol-d_4 to the sample resulted in exchange of the -NH protons causing the signal at δ 3.78 to appear as a sharp doublet. The major mass spectral fragmentation peaks at m/z of 126 (100 percent, $C_6H_{12}N_3$), 148 (7.6 percent, $C_{10}H_{12}O$), and 149 (5 percent, $C_{10}H_{13}O$) result from cleavage of the bond between C-4 and C-5.

In laboratory assays measuring rates of photosynthesis and respiration in the hard coral *Acropora formosa* (*9*), respiration was stimulated by **1** at concentrations of 10^{-2} to 10^{-1} ppm in seawater. The rate of photosynthesis was unaffected at these concentrations but was slightly depressed at concentrations over 10 ppm. At a concentration of 100 ppm acute toxicity was observed with cell lysis occurring as the tissue was stripped from the skeleton. At the higher concentration, **1** was a quick-acting toxin, showing an effect in 5 to 30 minutes. However, its effects on photosynthesis and particularly respiration in *A. formo-*

Table 1. Hydrogen nuclear magnetic resonance (NMR) data for siphonodictidine (**1**), marislin (**3**) and hydroquinone **4**; recorded in $CDCl_3$ solution at 360 MHz (except at 100 MHz for **4**) with internal tetramethylsilane as standard (δ = 0). Abbreviations: s, singlet; t, triplet; m, multiplet; and J, coupling constant.

H at	1	3	4
C-1	3.78 (t, 2 H, J = 7 Hz)		3.35
C-2	5.21 (t, 1 H, J = 7 Hz)	5.73	5.3
C-4	2.05 (m, 2 H)	~ 2.2	~ 2.15
C-5	2.12 (m, 2 H)	~ 2.2	~ 2.15
C-6	5.18 (t, 1 H, J = 7 Hz)	5.18	5.3
C-8	3.22 (s, 2 H)	3.22	3.3
C-10	5.86 (s, 1 H)	5.86	5.95
C-12	7.06 (s, 1 H)	7.06	7.1
C-13	1.97 (s, 3 H)	1.98	2.0
C-14	1.58 (s, 3 H)	1.60	1.65
C-15	1.68 (s, 3 H)	2.20	1.75

Table 2. Carbon-13 nuclear magnetic resonance data for siphonodictidine (**1**), marislin (**3**), and hydroquinone (**4**); recorded in $CDCl_3$ solution (C_6D_6 for **3**) at 20 MHz with internal tetramethylsilane as standard (δ = 0).

Atom	1	3	4
C-1	39.6*	188.8	28.8
C-2	117.8	115.4	122.9†
C-3	141.2	162.3	137.6†
C-4	39.0*	40.7	39.6
C-5	26.2	26.0	26.5
C-6	125.5	125.4	126.2†
C-7	132.2	133.0	132.3†
C-8	38.2*	38.7	38.4
C-9	153.9	154.5	154.3
C-10	108.6	109.2	108.8
C-11	120.3	120.7	120.5
C-12	137.4	138.2	137.6
C-13	9.6	9.8	9.7
C-14	15.9*	15.9	16.0
C-15	16.3*	19.0	16.0

*Signals may be interchanged. †We have reassigned these signals.

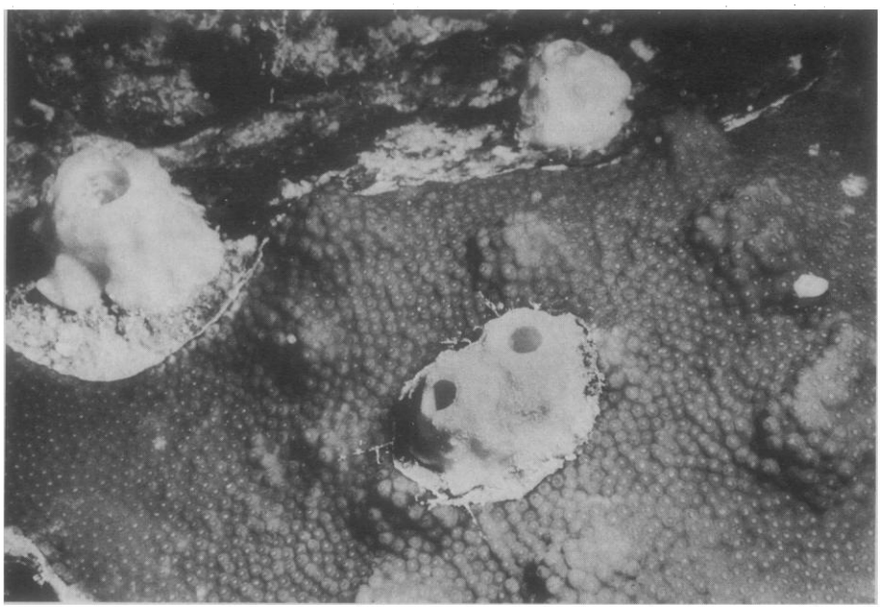

Fig. 1. A photograph of *Siphonodictyon coralliphagum* infesting *Montastrea cavernosa* showing the dead zone between the oscular chimney of the sponge and the coral polyps. [Photograph by Leo Buss]

sa suggest that lower concentrations may be toxic over a long period (*10*).

Siphonodictidine (**1**) constitutes more than 1 percent (dry weight) of *Siphonodictyon* sp. Although we have not measured the concentration of siphonodictidine in the mucus produced by the sponge, it is reasonable to assume that the mucus can hold a lethal concentration of siphonodictidine in contact with the coral tissue and maintain a defensive perimeter for the sponge.

In addition to the above study, we have also examined *S. coralliphagum* (*11*), a sponge that burrows into coral heads, and *S. mucosa* (*12*), a sponge that grows partially buried in coral debris. All three sponges produced copious quantities of mucus but only the two species that burrow into living corals contained biologically active compounds. Two antimicrobial compounds, siphonodictyal-A (**5**) and siphonodictyal-B (**6**) were isolated from the oscular chimneys of *S. coralliphagum*. The yellow color of the mucus of *S. coralliphagum* suggested that **5** and **6**, the major yellow metabolites of the sponge, were present in the mucus. We suspect that the siphonodictyals are also toxic to corals since Webb and Coll (*9*) have demonstrated that **4** but not the corresponding quinone was toxic to *A. formosa*. The siphonodictyals **5** and **6** in the mucus of *S. coralliphagum* and **1**, which is either exuded in or absorbed by the mucus of the Indo-Pacific *Siphonodictyon* sp., appear to serve the same role of inhibiting the growth of coral polyps around the base of the oscular chimneys of the sponges.

Studies (*13*, *14*) with *Aplysina fistularis* have demonstrated that sponges are capable of performing de novo biosynthesis of natural products that are then localized within the spherules of spherulous cells. Spherulous cells are present in *Siphonodictyon* spp. (*15*) but their function is not known. It seems remarkable that two sponges of the same genus can synthesize such different metabolites to perform the same ecological function. The majority of biologically active sponge metabolites have been assigned a passive role, such as inhibiting the settling and growth of epiphytes (*16*) or detering predation. Siphonodictidine (**1**) appears to be used in an aggressive manner to kill coral polyps in the vicinity of the sponge.

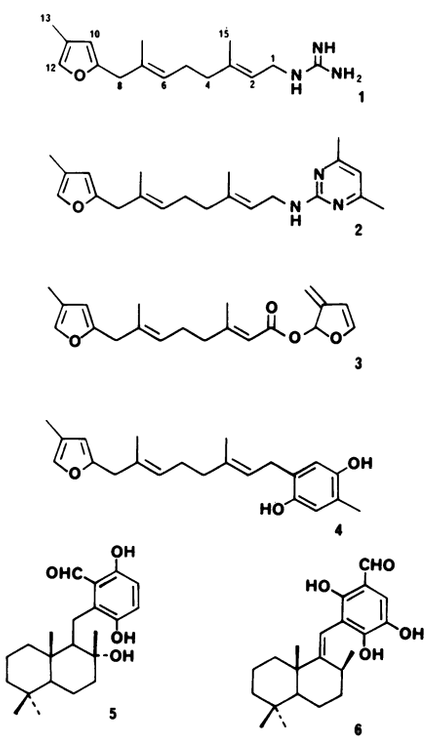

Fig. 2. Structures of siphonodictidine (**1**) from *Siphonodictyon* sp., the corresponding 4,6-dimethylpyrimidine derivative **2**, marislin (**3**) from *Chromodoris marislae*, the hydroquinone **4** from *Sinularia lochmodes*, and siphonodictyals A and B (**5** and **6**) from *Siphonodictyon coralliphagum*.

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3. Oscular chimneys of *Siphonodictyon* sp. were collected by hand with scuba equipment (-5 m), from the fringing reef west of Koror, Palau. The coral into which the sponge had burrowed was not collected or identified.
4. Siphonodictidine inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Pseudomonas aeruginosa* at 50 μg per disk and of *Bacillus subtilis* at 10 μg per disk. It did not inhibit the growth of two marine bacteria at 100 μg per disk nor did it inhibit cell division in fertilized sea urchin eggs at the standard test concentration of 16 μg per milliliter of seawater.
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