BRIAN W. SULLIVAN
D. JOHN FAULKNER*
Scripps Institution of Oceanography (A-012F)
University of California at San Diego
La Jolla, California 92093

Chemical Studies of the Burrowing Sponge *Siphonodictyon coralliphagum*

Abstract

Secondary metabolites together with the toxicity and calcium ion chelating ability of the major metabolites were examined in specimens of *Siphonodictyon coralliphagum* from Belize, the Bahamas, Ponape, and Kwajalein. The most noticeable differences in secondary metabolite composition occurred between specimens burrowing into living coral and those found in dead coral. These differences are thought to arise because *S. coralliphagum* forma *typica* and other forms burrowing in living coral need to maintain a "dead zone" around the oscular chimney of the sponge to prevent aggression by coral polyps. A mechanism by which secondary metabolites may assist in the excavation process is also proposed.

Marine sponges are a prolific source of secondary metabolites, most of which have been studied because of their unique chemical structures or useful pharmacological properties (for a recent review see Faulkner, 1984). Relatively few studies have defined natural roles for sponge metabolites although many functions have been proposed. Bakus and Green (1974) postulated that toxic sponge metabolites evolved in response to fish predation and showed that sponge toxicity was higher in tropical waters, paralleling the increased diversity in fishes. Jackson and Buss (1975) proposed that sponge metabolites inhibit the growth of spatial competitors in reef environments either by killing the competitor or by rendering the substrate around the sponge unsuitable for other organisms. Sponge metabolites may also protect the sponge from surface fouling by inhibiting the growth or development of the larvae of fouling organisms (Bergquist, 1978; Thompson et al., 1985) and might enhance the feeding efficiency of sponges by causing aggregation of bacteria (Bergquist and Bedford, 1978).

*Author to whom correspondence should be addressed.

In the majority of the studies cited above, sponge metabolites have been treated as if they were all similar molecules having similar functions. There is, however, ample evidence that the biological activity of each metabolite is highly dependent on molecular structure, even when the metabolites might appear closely related. It is preferable to study the biological activity of pure compounds rather than crude extracts since sponges probably exude pure compounds or mixtures of specific compounds, as has been demonstrated in the case of *Aplysina fistularis* (Walker et al., 1985). Bioassays performed using crude extracts can be extremely valuable to indicate the presence of biologically active compounds, but it must be recognized that they are of limited value when used in a quantitative manner to compare samples.

In our experience, it has been very difficult to ascribe a specific ecological function to specific sponge metabolites. For example, it is well documented that dorid nudibranchs store selected sponge metabolites that render the nudibranch distasteful to fish (Faulkner and Ghiselin, 1983). Although it is true that the same metabolites should protect the sponge from predation by fishes, is this really the most important function for the metabolites or are they equally useful in preventing fouling (Thompson, 1985)? In order to avoid some of these ambiguities, we have studied the chemistry of burrowing sponges since the effects attributed to chemicals are highly specific.

Sponges of the genus *Siphonodictyon* [now considered to be a junior synonym of *Aka*—Ed.] belong to a small group of sponges that burrow into limestone substrata. Some *Siphonodictyon* sponges have the unique ability to burrow into living coral heads, leaving only the oscular chimneys exposed (Rützler, 1971). The oscular chimney is ringed by a "dead zone" which is devoid of living coral polyps and thus protects the sponge from overgrowth (Figure 1). This is in sharp contrast with *Siphonodictyon* sponges that grow on dead coral and are heavily fouled by coralline and other algae, other sponges, bryozoans, and tunicates. We expected to observe significant differences in the secondary metabolites of the sponges depending on their growth form. Furthermore, we expected to find that the secondary metabolites of species of *Siphonodictyon* would chelate calcium ions if they were involved in the burrowing process.

Methods and Results

Our first investigation of *Siphonodictyon coralliphagum* described the isolation of siphonodictyal-A (see structure *1* in Figure 3) and siphonodictyal-B *(2)* from oscular chimneys collected from the vicinity of Lighthouse and Glover reefs, Belize (Sullivan et al., 1981). The structure initially proposed for siphonodictyal-B was corrected using newer spectroscopic techniques (^{1}H nuclear Overhauser effect difference spectroscopy) that clearly established the substitution pattern about the aromatic ring in *2*. We later reported the isolation of siphonodictidine (Figure 2) from an undescribed species of *Siphonodictyon* from Palau (Sullivan et al., 1983). Siphonodictidine was shown to be toxic to a coral in laboratory experiments. The significant differences in the secondary metabolites of the two species of *Siphonodictyon* provided the impetus for a more detailed study of *Siphonodictyon* sp.

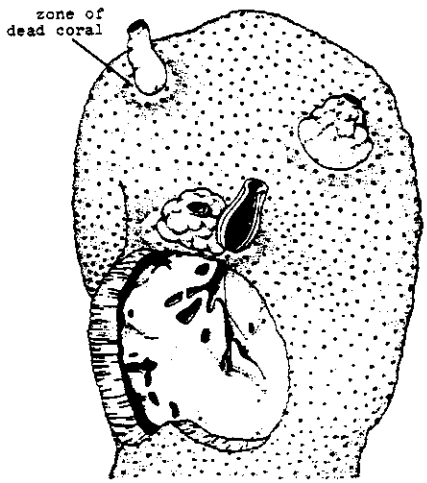

Figure 1. Cutaway view of *Siphonodictyon coralliphagum* growing out of *Stephanocoenia michelinii* (from Rützler, 1971).

Siphonodictyon coralliphagum from the Caribbean was described as having four distinct morphological forms, forma *typica*, forma *tubulosa*, forma *obruta*, and forma *incrustans*, all belonging to one population (Rützler, 1971). We examined the secondary metabolites of two of the forms: forma *typica*, which excavates in living coral; and forma *tubulosa*, found excavating in dead coral. Samples of each form were collected in Belize and the Bahamas. We also examined specimens of *Siphonodictyon coralliphagum* from Ponape and Kwajalein in the Pacific Ocean.

Eight samples of *Siphonodictyon coralliphagum* forma *typica* and six samples of *S. coralliphagum* forma *tubulosa* were collected at Carrie Bow Cay, Belize, in November 1983. Each sample was extracted separately, and the crude extracts were compared by thin-layer chromatography (tlc) and ^{1}H NMR spectroscopy. (It is important to use more

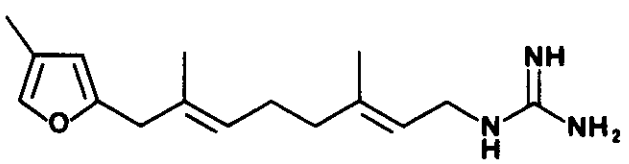

Figure 2. Structure of siphonodictidine from *Siphonodictyon* sp. from Palau.

than one technique for making comparisons since tlc only discriminates between compounds of dissimilar polarity: different compounds often provide identical tlc patterns.) The six samples of f. *tubulosa* were considered indistinguishable and were combined. Of the eight samples of f. *typica* one sample was too small to analyze and the remaining samples were combined into three groups: two (A and B) of three samples each and one (C) unique sample. Subsequent analysis indicated that the three groups differed mainly in the relative proportions of three major metabolites.

Table 1 lists the quantities of each metabolite obtained (Figure 3) from the different samples of *Siphonodictyon coralliphagum*. The collection location and the status of the

Table 1. Isolated yields (% dry weight) of metabolites (Figure 3) from *Siphonodictyon coralliphagum* samples

Sponge form	Collection location	Coral status	Compound 1	2	3	4	5	6	7
unknown	Belize	Live	0.12	0.90					
typica A	Belize	Live		0.11			0.03	0.12	
B		Live		0.21			0.03	0.04	
C		Live		0.20			0.04	0.09	
tubulosa	Belize	Dead						0.09	
typica	Bahamas	Live	3.41				0.25	0.51	
tubulosa	Bahamas	Dead					0.05	0.07	
unknown	Ponape	Live			0.21	0.51	0.10		0.08
unknown	Kwajalein	Dead			0.23		0.05		

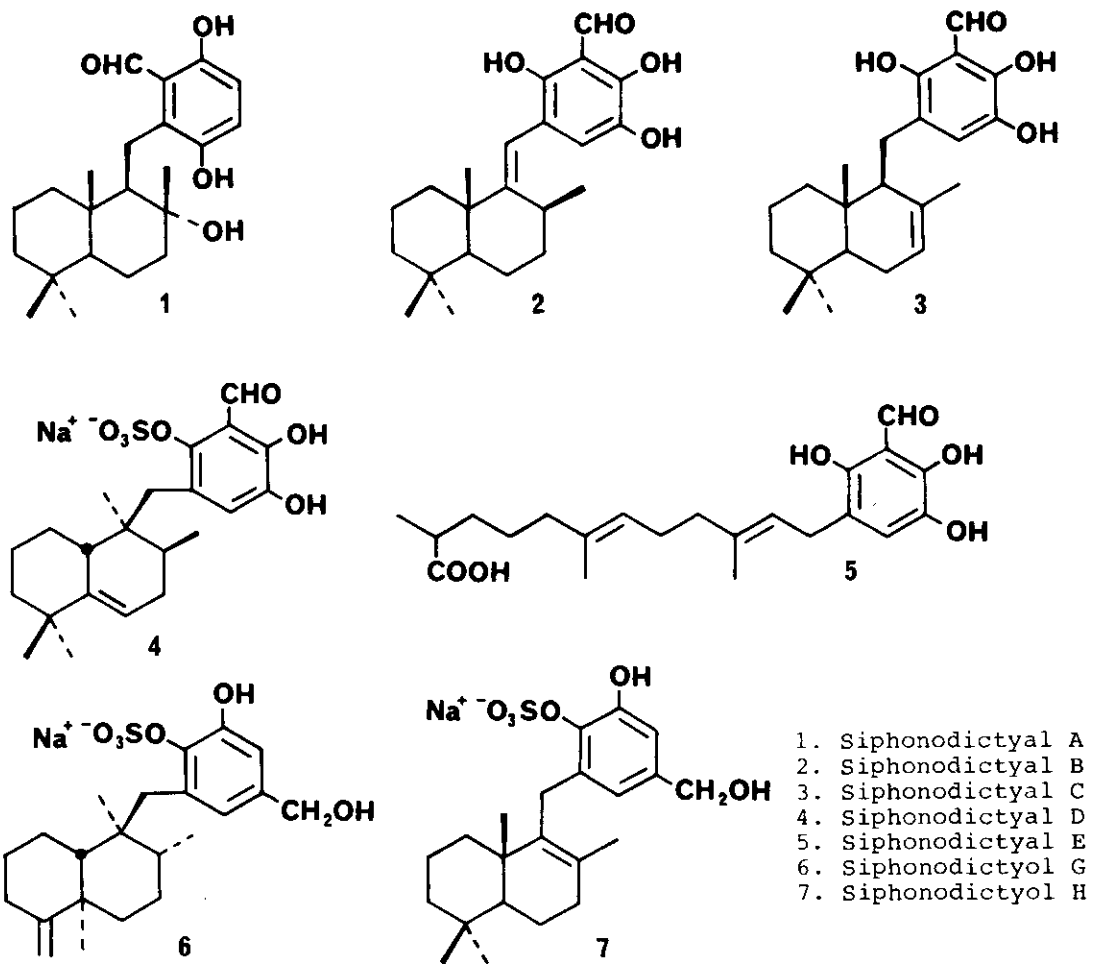

Figure 3. Structures of *Siphonodictyon coralliphagum* metabolites listed in Table 1.

coral substrate (living or dead) are recorded together with the yield of a metabolite expressed as % dry weight of sponge tissue. The structures of the metabolites were elucidated using physicochemical methods, except for that of siphonodictyal D *(4)*, which was determined by X-ray analysis (J. Clardy, pers. comm.). Details of the structural chemistry have been presented elsewhere (Sullivan et al., 1986). The metabolites isolated from *S. coralliphagum* samples comprised between 0.09% and 4.17% dry weight. The higher concentrations of metabolites were always associated with collections that comprised only the oscular chimneys of sponges from living coral heads. Smaller quantities of metabolites are associated with sponge samples living in dead coral. These differences may be due to the oscular chimneys containing less particulate inorganic material or they may represent a true concentration of the metabolites. Siphonodictyal E *(5)* and siphonodictyol G *(6)* were found in both forms of *S. coralliphagum* but siphonodictyal B *(2)* was found only in forma *typica* that grows in living corals. Similarly, siphonodictyal C *(3)* and siphonodictyal E *(5)* were found in both samples of *S. coralliphagum* from the Pacific but siphonodictyal D *(4)* and siphonodictyol H *(7)* were found only in the sample from living corals.

The significance of these results will depend on the toxicity of the metabolites. Leith Webb, James Cook University, has assayed siphonodictyal B *(2)*, siphonodictyal C *(3)*, and siphonodictyol G *(6)* against the coral *Acropora formosa* using the method previously employed for siphonodictidine (Sullivan et al., 1983). She reported that "at high doses, siphonodicytal B *(2)* and siphonodictyol G *(6)* were toxic. At moderate doses, only siphonodictyal B was toxic. Siphonodictyal C *(3)* was not toxic although it gave a response (in a respirometer) that was significantly different from controls. *A. formosa* recovered from the acute shock of siphonodictyal C." The assays suggest that siphonodictyal B is the most toxic of the metabolites, as predicted from the distribution data, but we need more quantitative toxicity data before we can be certain. Rützler (1971) suggested that the "dead zone" around the base of the oscular chimneys was maintained by a flow of mucus down the outside of the oscular chimney and over the surrounding coral tissue. We have shown that the mucus contains the secondary metabolites described above and may therefore serve as a carrier to hold toxic secondary metabolites in contact with the coral tissues.

The burrowing mechanism of *S. coralliphagum* is not completely understood (for a review see Pomponi, 1980). It is generally accepted that the majority of the limestone substrate is removed in the form of particles but a small quantity of calcium carbonate must be dissolved. The dissolution of calcium carbonate into seawater that is saturated or supersaturated with calcium is thermodynamically unfavorable and must involve the expenditure of energy. The siphonodictyals could theoretically serve to transport calcium ions against the thermodynamic gradient using the hydroquinone-quinone oxidation potential as the energy source. The 2-hydroxy benzaldehyde (salicaldehyde) or 2-hydroxy sulfate groups can chelate calcium ions that would be released by oxidation of the hydroquinone to a quinone (Figure 4). Since we were unable to duplicate the hydroquinone-quinone redox system, we decided to determine the ability of the siphonodictyals to remove calcium ions from an aqueous solution into an organic phase, *n*-butanol. These experiments are by no means quantitative. Equilibration of an aqueous calcium chloride solution with water causes an increase in the concentration of calcium ion in the aqueous phase due to the fact that some water dissolves in the n-butanol. To emphasize the nonquantitative nature of the experiments we have simply reported the readings obtained from the atomic absorption spectrometer, rather than attempting to convert these readings into concentrations (Table 2, Figure 5). These experiments clearly demonstrate that *n*-butanol solutions of siphonodictyals B *(2)*, C *(3)*, D *(4)* and siphonodictyol G *(6)* all removed a significant amount of calcium ions from the aqueous solution. These data support the hypothesis that the secondary metabolites are involved in the transport of calcium ions from the interior of the sponge into seawater. We cannot, however, provide any evidence for or against the involvement of secondary

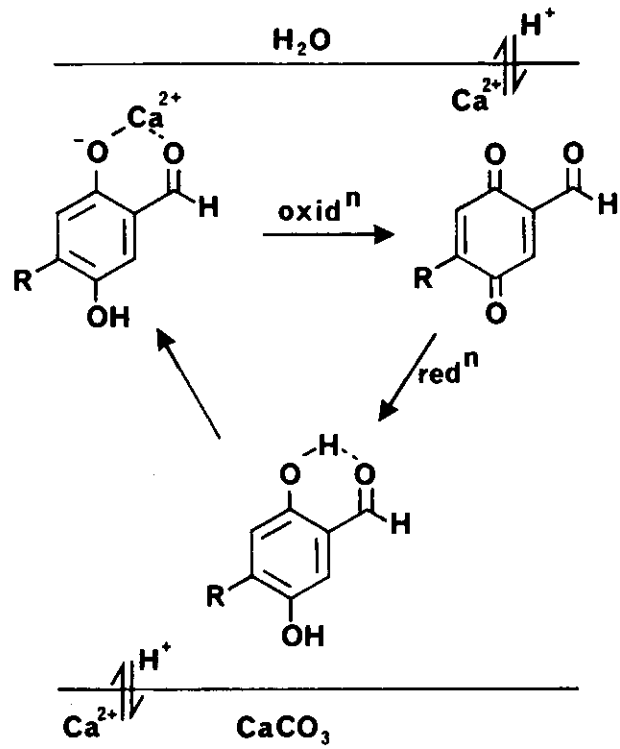

Figure 4. A proposed mechanism for the transport of calcium ions by 2,5-dihydroxybenzaldehydes.

Table 2. Results of calcium chelation experiments

Parameters	Compound				
	2	3	4	6	isozonarol[a]
Concentration of n–BuOH solution (mM)	4.8	5.0	4.6	3.6	11.3
Original Ca^{2+} concentration (ppm)	2	5	5	5	5
Atomic absorption values for Ca^{2+} in aqueous solution:					
(1) after extraction with n–butanol alone	0.103	0.51	0.51	0.51	0.52
(2) after extraction with n–butanol solution of compound	0.027	0.33	0.33	0.36	0.45
Ca^{2+} removal (%)	74	35	35	29	14

[a]Isozonarol is a phenol that can form a calcium salt but lacks the ability to form a chelate

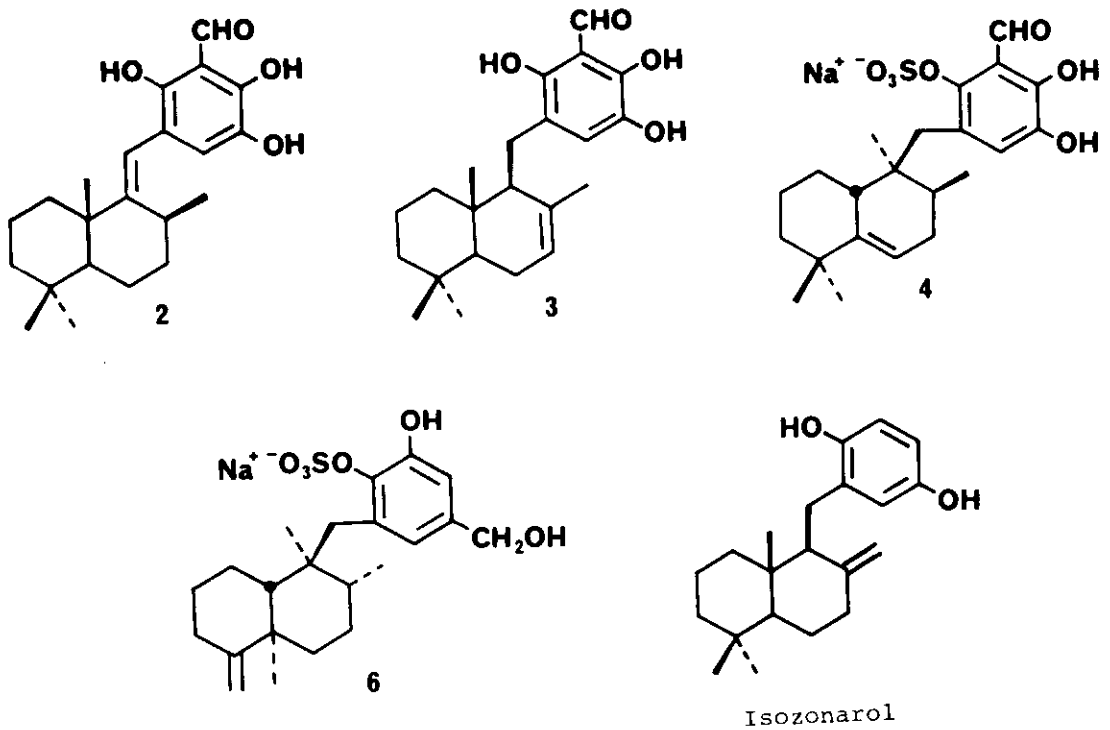

Figure 5. Structure of *Siphonodictyon coralliphagum* metabolites and of isozonarol (control) used in calcium chelation experiments, Table 2.

metabolites in the actual process of dissolution of the calcium carbonate substrate.

Conclusions

This study provides interesting data regarding chemotaxonomy in sponges. The secondary metabolites *1–7* of *Siphonodictyon coralliphagum* are all of similar chemical structure but they clearly differ from siphonodictidine, isolated from an undescribed species of *Siphonodictyon* from Palau. The two *Siphonodictyon* species appear to have evolved different secondary metabolites to perform the essential task of killing coral polyps that threaten to overgrow the exhalant oscular tube of the sponge.

Acknowledgments

This research was supported by the California Sea Grant College Program (NA85AA-D-S9140, R/mp 30) and the National Institutes of Health (AI 11969). This is contribution number 211, Caribbean Coral Reef Ecosystems (CCRE) Program, National Museum of Natural History, Smithsonian Institution, partly supported by the EXXON Corporation.

Literature Cited

Bakus, G. J., and G. Green. 1974. Toxicity in Sponges and Holothurians: A Geographical Pattern. *Science*, 185:951–953.

Bergquist, P. R. 1978. *Sponges*. Berkeley, California: University of California Press. 268 pp.

Bergquist, P. R., and J. J. Bedford. 1978. The Incidence of Antibacterial Activity in Marine Demospongiae; Systematic and Geographic Considerations. *Marine Biology*, 46:215–221.

Faulkner, D. J. 1984. Marine Natural Products: Metabolites of Marine Invertebrates. *Natural Product Reports*, 1:551–598.

Faulkner, D. J., and M. T. Ghiselin. 1983. Chemical Defense and Evolutionary Ecology of Dorid Nudibranchs and Some Other Opisthobranch Gastropods. *Marine Ecology Progress Series*, 13:295–301.

Jackson, J. B. C., and L. Buss 1975. Allelopathy and Spatial Competition among Coral Reef Invertebrates. *Proceedings, National Academy of Sciences, U.S.A.*, 72:5160–5163.

Pomponi, S. A. 1980. Cytological Mechanisms of Calcium Carbonate Excavation by Boring Sponges. *International Review of Cytology*, 65:301–319.

Rützler, K. 1971. Bredin-Archbold-Smithsonian Biological Survey of Dominica: Burrowing Sponges, *Siphonodictyon* Bergquist, from the Caribbean. *Smithsonian Contributions to Zoology*, 77:1–37.

Sullivan, B., P. Djura, D. E. McIntyre, and D. J. Faulkner. 1981. Antimicrobial Constituents of the Sponge *Siphonodictyon coralliphagum*. *Tetrahedron Letters*, 37:979–982.

Sullivan, B. W., D. J. Faulkner, G. K. Matsumoto, H. Cun-heng, and J. Clardy. 1986. Metabolites of the Burrowing Sponge *Siphonodictyon coralliphagum*. *Journal of Organic Chemistry*, 51: 4568–4573.

Sullivan, B., D. J. Faulkner, and L. Webb. 1983. Siphonodictidine, a Metabolite of the Burrowing Sponge *Siphonodictyon* sp. that Inhibits Coral Growth. *Science*, 221:1175–1176.

Thompson, J. E. 1985. Exudation of Biologically-Active Metabolites in the Sponge *Aplysina fistularis*. I. Biological evidence. *Marine Biology*, 88:23–26.

Thompson, J. E., R. P. Walker, and D. J. Faulkner. 1985. Screening and Bioassays for Biologically-Active Substances from Forty Marine Sponge Species from San Diego, California, USA. *Marine Biology*, 88:11–21.

Walker, R. P., J. E. Thompson, and D. J. Faulkner 1985. Exudation of Biologically-Active Metabolites in the Sponge *Aplysina fistularis*. II. Chemical Evidence. *Marine Biology*, 88:27–32.