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ICHTHYOTOXIC, CYTOTOXIC AND ANTIMICROBIAL ACTIVITY OF SOME SPONGES OF THE COLOMBIAN CARIBBEAN

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ABSTRACT

The icthyotoxic, cytotoxic and antimicrobial activities of aqueous, ethanol and chloroform extracts of 11 sponges from the Santa Marta region, Colombian Caribbean, were assayed. All studied sponges showed antimicrobial activity; 6 were ichthyotoxic and 7 were cytotoxic. **Ircinia campana, Verongula rigida, Agelas conifera** and **Didiscus oxeata,** were found to have activity in all three kinds of tests.

RESUMEN

Se determinó la actividad ictiotóxica, citotóxica y antimicrobiana de los extractos acuoso, etanólico y clorofórmico de 11 esponjas de la región de Santa Marta, Caribe colombiano. Todas las esponjas estudiadas presentaron actividad antimicrobiana, 6 resultaron ictiotóxicas y 7 citotóxicas. **Ircinia campana, Verongula rigida, Agelas conifera** y **Didiscus oxeata** mostraron actividad en los tres tipos de ensayos.

INTRODUCTION

Early in this century, Richet (1906) first observed that an aqueous extract of the marine sponge *Suberites domuncula* was toxic to some laboratory mammals. Since then, the biological activity of extracts of many marine sponges has been studied. To date, diverse toxicities have been found against fishes and other animals (e.g. Green, 1977a; Craft and Kellner, 1977; Bakus and Thun, 1979), against cultured mammal cells and tumors (Rinehart *et al.*, 1981), against fertilized sea urchin eggs (e.g. Ruggieri *et al.*, 1961; Stempien *et al.*, 1970), against dissociated sponge cells (Huysecom and van de Vyver, 1985)

and against microorganisms (e.g. Burkholder an Rützler, 1969; Burkholder, 1973; Green, 1977b; Bakus *et al.*, 1985; McCaffrey and Endean, 1985) and virus (Rinehart *et al.*, 1981).

During the last 10 to 15 years the active substances present in many of these sponge extracts have been isolated and chemically identified (see Hashimoto, 1979 for a general review). Compounds which are toxic to animals include halitoxin (Schmitz *et al.*, 1978), the latrunculins (Kasham et al., 1980), and the nor-sesterpenes sigmosceptrellin-A, -B and -C (Albericci *et al.*, 1982). Similar research with extracts showing cytotoxic activity has produced compounds which effectively inhibit cleavage of sea urchin eggs (Jacobs *et al.*, 1981; Fusetani *et al.*, 1983). Furthermore, some 50 antimicrobial compounds have been found, and at least one drug, cytosine arabinoside, a potent antimicrobial and antiviral agent, has been developed (Bérdy, 1982).

We have initiated a survey of the biological activity of marine sponges of the continental coast of Colombia in the Caribbean Sea, a previously unstudied region (The San Andrés and Providencia archipelago, Colombian insular territory in the western Caribbean, was surveyed for biological activity sponges and other animals by Rinehart *et al.*, 1981, but detailed results of the species of sponges assayed are not known). The ichthyotoxic and antimicrobial activities found in 11 common sponges of the Santa Marta region are reported here. Some biological implications of these activities are briefly discussed. The isolation and identification of the chemical compounds present in some of these active extracts is currently being carried out and will be described in a latter publication (Duque, in preparation).

MATERIALS AND METHODS

Sponges were collected by SCUBA in the vicinity of the city of Santa Marta, at depths of 6-30 m, in October 1983, and then frozen. Except for *Didiscus oxeata,* the sponges chosen for these assays were locally abundant. Several specimens of most species had to be pooled in order to obtain sufficient volumes of extract. In table 1, a list of the species used, depth of collection, color, and geographical location of any previous, comparable research is given.

Table 1. Classification, depth of collection color and references to previous research on the marine sponges used in this study. Sponges collected in the Santa Marta region, Colombian Caribbean, identified according to Zea (in press).

Classification	Depth of collection (m)	Color	Previous comparable work			
			site	activity tested[a]	result	reference
Class Demospongiae						
Order Dictyoceratida						
Ircinia campana (Lamarck)	15	reddish brown	Cozumel-Belize	I	+	Bakus and Thun, 1979
Smenospongia aurea (Hyatt)	12-15	purplish gray	Cozumel-Panama	A	+	Rinehart et al.,1981
			Belize	A	+	Djura et al., 1980
Order Verongida						
Aplysina cauliformis (Carter)	25-30	yellow brown	Cozumel-Belize	I	-	Bakus and Thun, 1979
			Puerto Rico	A	+	Sharma and Burkholder, 1967[c]
Verongula rigida (Esper)	20	bright yellow + dark tinges				
Pseudoceratina crassa (Hyatt)	20	bright yellow + dark tinges	Veracruz	I	+	Green,1977a[d]
			Puerto Rico	A	+	Fulmor et al.,1970[d] Burkholder,1973[d]
Order Haplosclerida						
Xestospongia muta (Schmidt)	15	reddish brown	Cozumel-Belize	I	+	Bakus and Thun, 1979
Order Poecilosclerida						
Iotrochota birotulata (Higgin)	15	black + green tinges	Veracruz	I	-	Green,1977a
			Cozumel-Belize	I	+	Bakus and Thun, 1979
			Jamaica	I	+	Stempien et al.,1970
			"	C	+	" "
Neofibularia nolitangere (Duchassaing and Michelotti)	6	reddish brown	Cozumel-Belize	I	+	Bakus and Thun,1979
Orden Axinellida						
Agelas conifera (Schmidt)	12-20	dark orange				
Agelas clathrodes (Schmidt)	12-20	bright orange	Cozumel-Belize	I	-	Bakus and Thun, 1979
Uncertain order[b]						
Didiscus oxeata Hechtel	13	bright yellow (but fouled)				

a. A = Antimicrobial; C = cytotoxic against sea urchin eggs; I = ichthyotoxic.
b. cf. van Soest (1984).
c. As *Verongia c.*
d. As *Ianthella ardis* de Laubenfels.

Ichthtyotoxicity assays·

Bicolor damselfish, *Stegastes partitus,* 0.7-3.1 g wet weight, collected in Santa Marta Bay at 5-8 m depth and acclimated in an aqúarium for 2-3 days, and goldfish, *Carassius auratus,* 0.5-1.5 g wet weight, commercially acquired, were used for ichthyotoxicity assays.

Sponge extracts were prepared by the method of Green (1977a), homogenizing the sponge in solvent (20 ml of water or ethanol for each 5 g wet weight sponge) and centrifuging at 3000 rpm for 15 min. The supernatant was filtered to remove fine particles and cells and then lyophilized. The weight of extract corresponding to 5 g wet weight of the sponge was diluted in 300 ml sea-water (for *S. partitus*) or fresh-water (for *C. auratus*) in 600 ml beakers. One fish was placed in each beaker for 90 min, and its behavior recorded at 15 min interval. If the fish survived, it was then transferred to a jar with the appropiate uncontaminated water and its behavior observed for 24 hrs. The controls for aqueous extracts consisted of a fish in a beaker containing 300 ml of uncontaminated water. For ethanol extracts, the control was the same as above, but a beaker from which 20 ml of ethanol had been previously evaporated, was used instead. Aeration was provided throughout the experiments both for assays and controls. All experiments were done in duplicate.

Cytotoxicity assays:

Adults of the variegated urchin, *Lytechinus variegatus,* were collected in Santa Marta Bay at 0.5-6 m depth and used immediately for the experiments. Handling and care procedures were those of Hinnegardner (1975): eggs and sperm were obtained from the urchins by intracoelomic injection on the oral side with 0.5-1 ml of 0.5M KCl. Each egg spawn was washed with filtered sea-water through a 250 μm mesh net, and kept in a final volume of 100 ml. One or two drops of sperm were added to the egg suspension which then was stirred. A minute later, the eggs were checked under the inverted microscope. If some eggs were still unfertilized, more sperm was added until fertilization of the majority was achieved.

Fifteen g wet weight of sponge were homogenized overnight in 100 ml of each solvent (water, ethanol, chloroform), and centrifuged at 3000 rpm for 15 min. The supernatant was filtered to remove fine particles and cells and then lyophilized. Assays were carried out according to the methodology used at Scripps Institution of Oceanography (J. Faulkner, pers. comm., 1982). Depending on the solubility,

the lyophilized extracts were diluted in water, ethanol, propilene-glycol or mixtures of these solvents, to a concentration of 0.048 mg/µl. 25 µl of each dilution were poured into a separate 6 cm diameter petri dish containing 3 ml of sea water. 3 ml of the fertilized egg suspension were added to each dish, which was then covered. After 90 min of frequent stirring (when approximately 100% of the control eggs would have reached the 8-cell stage), the eggs were fixed with 10% formalin. Counts of various fields, haphazardously chosen under the inverted microscope, were made to estimate the percentage of each cell stage (undivided, 2, 4 and 8 cells). Controls consisted of the fertilized eggs suspension in petri dishes exposed to 25 ul of the appropiate solvent. Both assays and controls were made in duplicate.

Active extracts, i.e. those which inhibited $>$ 80% of the eggs, were further tested for their time-dependent effect on the first cleavage of fertilized eggs using the method of Jacobs *et al.* (1981). Duplicate fertilized egg suspensions were placed in petri dishes and a series of sponge extracts (prepared as above) were added at 5, 15, 25, 35, 45 and 55 min after fertilization. These experiments were terminated about 65 min after fertilization by fixation with 10% formalin when approximately 100% the control eggs had reached the 2-cell stage. The eggs were counted, and the relative proportions of divided and undivided eggs were calculated. For comparisons, colchicine (100 ug/ml), a known inhibitor of sea urchin egg cleavage (Young *et al.*, 1960), was added to other fertilized egg suspensions in the same fashion as above. A second set of controls, fixed in 10% formalin at each of the above-menioned times, recorded the actual percentage of undivided cells present in the suspensions at these addition times.

Antimicrobial assays:

The bacteria *Bacillus subtilis* (gram +), *Staphylococcus aureus* (gram +), *Streptococcus pyogenes* (gram +), *Escherichia coli* (gram-), *Pseudomonas aeruginosa* (gram-), *Salmonella typhi* (gram-), and the fungus *Candida albicans* (gram +); were used for the antimicrobial assays.

Sponge extracts were prepared as for the cytotoxicity assays. Standard bacterial and fungal growth inhibition tests were performed as follows: 6 mm diameter, sterile, filter paper disks were prepared with inoculation of 500 µg of each extract dissolved in 20 µl of water or water-ethanol (for the aqueous and ethanol extracts), or in petroleum ether (for the chloroform extracts). The disks were left to dry at room temperature and then stored in sterile conditions in a freezer until

used. Each microbe culture was incubated for 24 hrs in brain-heart infusion broth (37 g/l, Oxoid Ltd.). 0.2 ml aliquots of each of the bacteria cultures were inoculated in 10 ml isosensitest agar (31.4 g/l, Oxoid Ltd.), maintained for 2 hrs at 45°C and poured into 10 cm diameter petri dishes. 0.5 ml aliquots of the *C. albicans* culture was inoculated in 20 ml agar enriched with 1% malt, incubated for 36 hrs and also poured into 10 cm diameter petri dishes. After solidification, the disks containing the extracts were placed on the agar containing the microbes, and allowed to diffuse at room temperature for 2 hrs. Then the dishes were incubated for 24 hrs at 27°C, at which time diameter of any inhibition zones were measured. Paired disks for each sponge extract were employed. Paired commercial antibiotic disks (chloramphenicol, tetracycline, decacine) of known concetration were used as standards for the quantification of the inhibition zones.

RESULTS

Ichthyotoxicity:

Results for duplicate toxicity tests of 10 sponges on *Stegastes partitus* are given in table 2. The relative degree of toxicity is expressed in the arbitrary scale adopted by Green (1977a), and the behavioral categories are similar to those of Bakus and Thun (1979). Only the aqueous and ethanol extracts of *Didiscus oxeata* were highly toxic, producing death within 5-8 min, whereas the aqueous extract of *Ircinia campana* and the ethanol extract of *Pseudoceratina crassa* were moderately toxic (death in 60 and 65 min respectively). Of the remainder, only the aqueous extracts of *P. crassa, Verongula rigida* and *Iotrochota birotulata* were very mildly toxic (death in 780, 1230 and 1440 min respectively). None of the other extracts killed *S. partitus*. In contrast, only the ethanol and aqueous extracts of *D oxeata* (both strongly toxic, producing death at 9 and 12 min respectively), and the ethanol extract of *Agelas conifera* (moderately toxic, death at 86 min), were active against *Carassius auratus* (Table 2). Note that the extracts of the latter were non-toxic to *S. partitus*.

Of the 10 studied sponges, 7 have been previously tested for ichthyotoxicity in other areas of the Caribbean Sea (see table 1). Their extracts were tested against *Fundulus heteroclitus* by Stempien *et al.* (1970), against *C. auratus* by Green (1977a), and against both *C. auratus* and the marine fish *Abudefduf saxatilis* by Bakus and Thun

Tabla 2. Ichthyotoxic activity on the bicolor damselfish, **Stegastes partitus**, by sponge extracts.

Sponge	Extract[a]	Death time[b] (min)	Toxicity[c]	Fish behavior[d]
Ircinia campana	A	60	+++	
	E	--	NT	N
Aplysina cauliformis	A	--	NT	F,R
	E	--	NT	F,R
Verongula rigida	A	1230	+	
	E	--	NT	N
Pseudoceratina crassa	A	780	+	
	E	65	+++	
Xestospongia muta	A	--	NT	F,R
	E	--	NT	L,R
Iotrochota birotulata	A	1440	+	
	E	--	NT	L,R
Neofibularia nolitangere	A	--	NT	N
	E	--	NT	N
Agelas conifera	A	--	NT	L,R
	E	--	NT[e]	N
Agelas clathrodes	A	--	NT	N
	E	--	NT	N
Didiscus oxeata	A	8	++++[f]	
	E	5	++++[g]	

a. A = aqueous; E = ethanol.
b. Mean death time based on two replicates; -- = no death.
c. Categories of mean toxicity: + + + + = dead before 60 min, highly toxic; + + + = dead within 60-120 min, moderately toxic; + + = dead within 120-720 min, mildly toxic; + = dead within 720 - 1440 min, very mildly toxic; NT = survived 24 hrs, non-toxic. All controls were non-toxic (Fishes showed normal behavior and survived throughout the assay).
d. F = Fish turned on its side even when forced to normal position; L = fish movement much slower than normal; N = normal behavior; R = fish recovers when placed in clean water.
e. Also moderately toxic to *Carassius auratus*, mean death at 86 min.
f. Also highly toxic against *Carassius auratus*, mean death at 9 min.
g. Also highly toxic against *Carassius auratus*, mean death at 12 min.

(1979). Of these sponges, *I. campana* and *P. crassa* (as *Ianthella ardis* in Green, 1977a) were also found to be toxic; similarly, *Aplysina cauliformis* and *Agelas clathrodes* were both non-toxic. Results on *Iotrochota birotulata,* however, range from non-toxic (Green, 1977a), to varying degrees of toxicity under different experimental conditions (Stempien *et al.*, 1970; Bakus and Thun; 1979, this study). Moreover, both *Neofibularia nolitangere,* which is well known for it irritating properties on human skin, and *Xestospongia muta* were found to be toxic by Bakus and Thun (1979), whereas they were non-toxic in our assays.

Cytotoxicity:

The effects of our sponge extracts on the cleavage of fertilized eggs of *Lytechinus variegatus* are shown in table 3. The scale of activity adopted here is arbitrary. Extracts that inhibit first cleavage in 80-100% of the eggs are usually regarded as highly active (J. Faulkner, pers. comm., 1982).

Table 3. Inhibition of cleavage on variegated urchin, Lytechinus variegatus, fertilized eggs by sponge extracts.

Sponge/ Extract[a]-conc. (µg/ml)	% of eggs[b]				Activity
	undivided (% inhibition)[c]	2 cells	4 cells	8 cells	
Ircinia campana					
A-200	87.2	12.8	0	0	++++
E-200	0	0	0	100.0	NA
C-200	40.1	9.1	2.7	48.1	+++
Smenospongia aurea					
A-200	3.8	2.6	25.9	68.0	++
E-200	100.0	0	0	0	++++
Aplysina cauliformis					
A-200	0.4	0	0.7	98.9	NA
E-200	1.1	0	3.5	95.4	NA
C-200	2.1	0	0.4	97.5	NA
Verongula rigida					
A-200	0.5	0.4	0	99.1	NA
E-200	3.0	3.0	39.0	55.0	++
E-400	0	4.5	29.5	66.0	++
C-200	7.5	0	0	92.5	NA
Pseudoceratina crassa					
A-200	0.6	0	0	99.4	NA
E-200	0	0	0	100.0	NA
C-200	1.4	0	0.4	98.2	NA
Xestospongia muta					
A-200	0	0	3.5	96.5	NA
E-200	5.2	4.2	0	90.6	NA
C-200	69.4	17.8	12.8	0	+++
Iotrochota birotulata					
A-200	0	0	0	100.0	NA
E-200	0	0	6.4	93.6	NA
C-200	2.7	0.8	3.2	93.3	NA
Neofibularia nolitangere					
A-200	0	1.7	0.1	98.2	NA
E-200	0	0	3.2	96.8	NA
C-200	11.4	1.2	1.6	85.8	+
Agelas conifera					
A-200	0	0.7	2.4	96.9	NA
E-200	0	4.8	40.7	54.5	++
E-400	29.2	27.3	39.5	4.0	+++
C-200	1.4	1.5	3.3	93.8	NA

Tabla 3. Continuación.

Agelas clathrodes					
A-200	0	0.7	0	99.3	NA
E-200	0	1.8	1.7	96.5	NA
Didiscus oxeata					
A-200	100.0	0	0	0	++++
E-200	100.0	0	0	0	++++
C-200	100.0	0	0	0	++++

a. A = aqueous; E = ethanol; C = chloroform.
b. Mean values based on duplicates, subtracting the control values of any particular stage if the latter were different from 0% for undivided, 2 and 4 cells, and 100% for 8 cells.
c. % inhibition = percentage of undivided eggs.
d. Categories of mean activity; + + + + = inhibition 80-100%, highly active; + + + = inhibition 20-80%, moderately active; + + = inhibition 0-20%, but the percentaje of eggs with 8 cells was less than 80%, mildly active; + = inhibition 0-20%, but the percentage of eggs with 8 cells was 80-90%, very mildly active; NA = more than 90% of the eggs reached 8 cells, non-active. Controls were always non-active.

Of the 11 sponges studied, only *Ircinia campana* (aqueous extract), *Smenospongia aurea* (ethanol extract), and *Didiscus oxeata* (aqueous, ethanol and chloroform extracts) showed high cytotoxic activity (>80% inhibition), while *Xestospongia muta* (chloroform extract) and *Ircinia campana* (chloroform extract) were moderately active (20-80% inhibition). The concentrations of the ethanol extracts of *Verongula rigida* and *Agelas conifera* were doubled (E-400, see table 3) to determine if their mild activity (3 and 0% inhibition respectively, but <80% of the eggs reached the 8-cell stage) was a result of low concentration and/or impurity of the active substance. Although the activity of *A. conifera* was increased by this procedure to moderately active (20-80% inhibition range), that of *V. rigida* was virtually unchanged.

The curves of first cleavage inhibition of the extracts of the four most active sponges are shown in figure 1. Although their overall inhibitory behavior resembled that of colchicine, their effects were less intense and lasted for shorter periods of time. It can be seen in figure 1 that the inhibitory effect of the active extracts and of colchicine are clearly centered on cell division processes ocurring before about 40 min after fertilization.

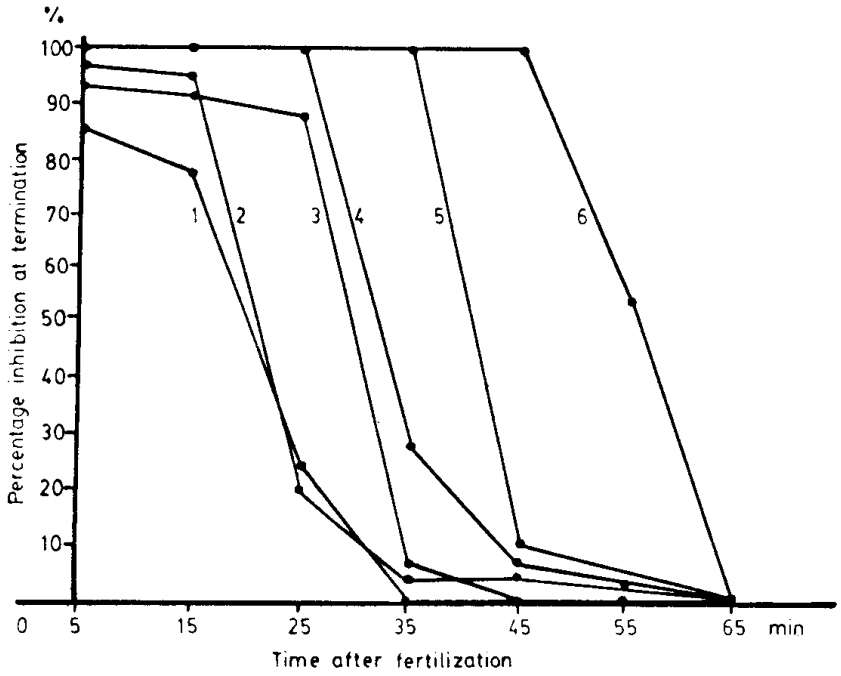

Figure 1. Mean pecentage inhibition of the first cleavage of *Lytechinus variegatus* duplicate fertilized egg suspensions by four sponge extracts and colchicine, each added at 6 times after fertilization (black dots). Percentage of undivided eggs calculated about 65 min after fertilization when approximately 100% of the control eggs had reached the 2-cell stage. 1: *Ircinia campana* aqueous extract 200 µg/ml; 2: *Didiscus oxeata* aqueous extract 200 µg/ml; 3: *Smenospongia aurea* ethanol extract 200 µg/ml; 4: *D. oxeata* ethanol extract 200 µg/ml; 5: colchicine control 100 µg/ml; 6: actual percentage of undivided eggs at each addition time (from controls lixed in 10% formalin at these times).

Of the 11 sponges examined here, only *Iotrochota birotulata* has previously been tested for cytotoxicic activity (see table 1). Whereas Stempien *et al.* (1970) found that extracts of Jamaican specimens caused loss of bilaterality in developing larvae of the echinoid *Arbacia punctulata,* our samples had no effect on early cleavage of the eggs of *L. variegatus.*

Antimicrobial activity:

Results of the tests for antimicrobial activity are given in table 4. Relative activity is not assessed here, although the diameter of inhibition of bacterial growth can be regarded as an estimate of the strength of the extract.

All 11 sponges inhibited the growth of at least two microorganisms, and six of them actually inhibited five or more. Of a total of 284 tests, 39 were positive against gram-positive bacteria, 31 against gram-negative bacteria, and 7 against the gram-positive yeast, *Candida albicans.* In our tests, antimicrobial activity was more

Table 4. Antimicrobial activity of sponge extracts.

Sponge/ Extract[a]/ Standards	Inhibition zones (in mm)[b]						
	Microorganisms[c]						
	B.s. (gram+)	S.a. (gram+)	S.p. (gram+)	E.c. (gram-)	P.a. (gram-)	S.t. (gram-)	C.a. (gram+)
Ircinia campana							
A	-	-	7.2	8.0*	7.0*	-	-
E	8.0*	9.0	9.0	9.0	-	-	-
C	7.0*	-	7.2	-	-	-	7.0
Smenospongia aurea							
A	-	-	-	-	7.0*	-	-
E	8.0	8.0	7.2	7.0	7.0*	-	7.0
Aplysina cauliformis							
A	-	-	-	-	-	-	-
E	-	-	7.5	7.0	-	-	-
C	-	-	-	-	-	-	-
Verongula rigida							
A	7.7	-	-	-	7.0*	-	-
E	-	7.0	7.0	-	7.0*	-	-
C	-	-	-	-	-	-	-
Pseudoceratina crassa							
A	-	-	-	-	-	-	-
E	7.0	-	-	-	-	9.3	-
C	7.0	-	-	-	-	-	-
Xestospongia muta							
A	7.0*	-	7.2	-	7.0*	7.0*	-
E	7.5*	-	8.0	7.5	7.0*	-	-
C	7.0	9.2	14.5	9.8	-	-	7.5
Iotrochota birotulata							
A	-	-	7.0	-	-	-	-
E	-	-	7.0	7.0	-	-	-
C	-	-	-	-	-	-	-
Neofibularia nolitangere							
A	-	-	7.0	7.0*	7.0*	-	-
E	-	-	7.0	7.0	7.0*	-	-
C	7.0	-	7.5	8.0	-	7.0	-
Agelas conifera							
A	-	-	-	-	7.0*	-	-
E	9.5	12.0	10.2*	7.6	8.2*	-	-
C	-		-	-	-	-	-
Agelas clathrodes							
A	-	-	-	-	-	-	-
E	9.0	-	-	-	-	-	-
C	-	-	-	-	-	-	7.0
Didiscus oxeata							
A	8.0*	-	-	-	7.0*	-	7.0
E	9.2	8.0	9.0*	8.2*	7.0*	7.5	8.2
C	9.0	11.2	15.0	12.8	-	7.0	9.5
Chloramphenicol-30 ug	23.0*	28.0*	30.0	9.0*	8.0*	27.0*	-
Tetracycline-30 ug	16.0	26.0*	35.0	10.0	12.0	16.0*	-
Decacine-30 ug	-	-	8.0*	-	27.0*	-	-
Mistatine-100 ug	-	-	-	-	-	-	28.0*

a. A = aqueous; E = ethanol; C = chloroform.

b. Diameter of zone showing complete inhibition, including disk (6 mm), mean of duplicates; when asterisk is given, there is an additional zone of reduced density in the periphery; — = absence of inhibition.

c. *B.s. = Bacillus subtilis; S.a. = Staphylococcus aureus; S.p. = Streptococcus pyogenes; E.c. = Escherichia coli; P.a. = Pseudomonas aeruginosa; S.t. = Salmonella thyphi; C.a. = Candida albicans.*

frequent in the ethanol extracts, although the strongest activity was found in chloroform extracts. Indeed, the greatest diameters of inhibition were shown by the chloroform extract of *Didiscus oxeata* (15.0 and 12.8 mm against *Streptococcus pyogenes* and *Escherichia coli* respectively) by the chloroform extract of *Xestospongia muta* (14.5 mm against *S. pyogenes*), and by the ethanol extract of *Agelas conifera* (12.5 mm against *Staphylococcus aureus*). *D. oxeata* also inhibited all the other microorganisms tested. Not surprisingly, most of the antibiotic standards showed far more inhibitory activity than the sponge extracts. Exceptions were *D. oxeata* which inhibited *E. coli* more than chloramphenicol and tetracycline; and decacine which showed less inhibition against *S. pyogenes* than did 5 of the 17 active extracts.

Antimicrobial activity has been previously reported for 3 of these sponges (see table 1). From Puerto Rican specimens of *Pseudoceratina crassa,* an isomer of the antibiotic aeroplysinin-1 was isolated (Fulmor *et al.,* 1970; Burkholder, 1973, both as *Ianthella ardis*). *Smenospongia aurea* collected between Cozumel and Panamá inhibited *Bacillus subtilis* (Rinehart *et al.,* 1981). The same species from Belize inhibited *S. aureus* and *C. albicans* (Djura *et al.,* 1980). *Aplysina cauliformis* from Puerto Rico showed antimicrobial activity in assays made by Sharma and Burkholder (1967, as *Verongia c.*), Gjessing and Ruskin (1977) also discovered antibiotic activity in bacteria isolated from Virgin Islands specimens of the latter species (as *Verongia longissima*).

General results:

The aqueous extract of *Ircinia campana,* the ethanol extract of *Agelas conifera,* and the aqueous and ethanol extracts of *Didiscus oxeata* each were toxic to fishes, and inhibited the cleavage of sea urchin eggs and the growth of bacteria. *Verongula rigida,* on the other hand, showed activity in the three kinds of tests, but its aqueous and ethanol extracts were each active in only two of the three tests ichthyotoxic and antimicrobial and cytotoxic and antimicrobial respectively).

DISCUSSION

We are unsure of the reasons for the different ichthyotoxic responses of the two species of fish. Whereas *S. partitus* was killed by the extracts of 5 of the 10 sponges tested, *C. auratus* was killed by

only 2 sponges, one of which was non-toxic to *S. partitus*. According to Bakus and Green (1974), fresh-water fishes are more resistant to saponins from holothurians than are marine fishes. The biological activity of the toxins may, on the other hand, depend on water salinity.

Toxicity in sponges has been widely assumed to function partly as an antipredator mechanism (Randall and Hartman, 1968; Burkholder, 1973; Bakus and Thun, 1979; Bakus, 1981). Toxicity tests on marine fish provide circumstancial evidence in support of this hypothesis (Bakus and Green, 1974). It is important to note that 5 out of 7 of our toxic extracts were aqueous. Thus, their compounds are potentially active in *in-situ,* possibly being released as chemical "warning signals" in the surrounding sea-water, as suggested by Green (1977a). Although aposematic colorations have been found in some sponges associated with toxic zoanthids (West, 1976), the color of our sponges, some which are very bright, is not related to their ichthyotoxic activity (Tables 1 and 2, see also Bakus and Thun, 1979).

To our knowledge, the biological significance, if any, of the inhibitory effect of marine sponge extracts on the cleavage of sea urchin eggs is not known. Although Walker and Thompson (in Walker, 1982) found evidence of a chemical mechanism for preventing epibiosis in the Californian sponge *Aplysina fistularis*, no clear relationship between bare sponge surface and positive cytotoxicity was found in our sponges. For example, even though *D. oxeata* was one of the most cytotoxic of our sponges, at Santa Marta it is heavily fouled by algae and invertebrates.

Many antimicrobial agents have been found in sponges (Bérdy, 1982), but their biological or ecological significance remains largely unknown (see Burkholder 1973; Green, 1977b; Bergquist and Bedford, 1978; McCaffrey and Endean, 1985).

It is well known that the same sponge extract may show more than one type of biological activity (e. g. Jakowska and Nigrelli, 1960; Stempien *et al.,* 1970; Green, 1977b; Jacobs *et al.,* 1981). Similarly, 4 of our extracts were toxic against fish, sea urchin eggs and bacteria. Such overlap may suggest that only one chemical compound is responsible for these diverse biological effects.

Our data, in conjunction with earlier studies (see results, especially ichthyotoxicity), suggest that considerable intraspecific geographical variation in the biological activity of some Caribbean sponges may exist. Green *et al.* (1985) have, however, recently reported that the levels of antimicrobial and ichtyotoxic activities

change seasonally in some Mexican sponges. Individual variation in chemical composition has also been noted for Belizean specimens of *S. aurea* by Djura *et al.* (1980). Hence, until more is known about any seasonal and within-populations variation, geographical comparisons need to be made with great care.

Screenings for biological activity in marine sponges and other organisms are mainly searches for reliable sources of chemical compounds with possible pharmaceutical or industrial uses. An understanding of the biological role of these activities, on the other hand, requires the use of chemically pure substances and controlled experimental manipulations. Both goals require the finding of an experimental system (e.g. a sponge and its associates) that is abundant and for which individual, seasonal and perhaps geographical variation in a given biological activity is known. Our preliminary survey constitutes the first step towards finding such a system.

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