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The Investigation of the Ball Sponge *Cinachyrella kuekenthali* from Two Different Environments on Jamaica's North Coast

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Abstract

The purpose of this study was to examine specimens of the Jamaican Ball sponge *Cinachyrella kuekenthali*, collected from two proximal locations approximately 10 km apart on the North Coast of the island at comparable depths of 10 - 17 m. The locations represented two distinctly different environments—a silty bay area in proximity to a shipping channel (Columbus Park, Discovery Bay) in contrast to a pristine, clear reef wall (Rio Bueno, Trelawny). Two individuals were collected in a preliminary study in which cholesterol was found to be the main constituent of both extracts. Five individuals were collected from Columbus Park while six individuals were sourced from Rio Bueno. The specimens were extracted separately with dichloromethane to afford gum-like substances. From the results of the study, the masses of the Columbus Park specimens were higher (average: 97.22 g) than that of the Rio Bueno-sourced organisms (42.57 g) but the quantities of the dichloromethane extracts were lower (2.06% vs. 3.81%), suggesting that the Columbus Park sponges were more focused on survival than metabolite production.

Keywords

Cinachyrella kuekenthali, Discovery Bay, Dichloromethane Extract, Steroids

1. Introduction

The marine environment has been a potent source of compounds, exhibiting new molecular frameworks and outstanding biological activity. Jamaica is an island in the Caribbean with a reef area of over 1200 km^2, much of which is under threat due to environmental factors including nutrient pollution, sedimentation and overfishing. Jamaica's shallow marine environment is very diverse with over 2700 animals and over 750 algal plants being documented. Extensive stu-

dies of the Discovery Bay area have been conducted for many decades to identify marine organisms [1] as well as to examine climate effects, facilitate coral reef restoration projects, fisheries research and maintaining nurseries.

Cinachyrella kuekenthali is an orange sponge belonging to the family Tetillidae. Known commonly as the orange ball, it is found in tropical and sub-tropical habitats of the world and grows up to 15 cm in size. Also referred to as *Cinachyra*, chemical investigations have been conducted on several species to identify the presence of a range of compounds. Of the *Cinachyra* species examined, *C. tarentina* yielded cholestane steroids including cholest-4-ene-3,6-dione [2] while the water-soluble extracts of *C. alloclada* displayed antitumour activity and its dichloromethane extract produced 17-*Z*-tetracosenyl-1-glycerol ether [3]. Cholest-4-en-3-one and two 6-hydroximino-4-en-3-one steroids were found in an assemblage of *C. alloclada* and *C. apion* [4]. In a study of Senegalese collections of *C. alloclada* and *C. kuekenthali* at different depths, a range of cholestane steroids was also identified [5].

Shimogawa and coworkers examined an Okinawan *Cinachyrella* sp. from which the alkaloid cinachyramine was isolated [6]. Pyrazole alkaolids and 2-methoxy acetylenic acids were identified in Indonesian populations of *Cinachyrella* sp. [7] while Venezuelan populations of *C. kuekenthali* were found to contain 2-*N*-acetylglucosamine-O-threonine, a derivative of an amino acid [8]. The study of *C. cavernosa*, another member of the *Cinachyra* genus, led to the identification of ceramides and a tetillapyrone [9].

Cytotoxic compounds have been identified in *Cinachyrella* sp. including the macrolide cinachyrolide A, found in small quantities in a Japanese *Cinachyra* sp [10] while *C. enigmata* from Papau New Guinea yielded an unusual 18-membered phosphomacrolide which exhibited significant cytotoxicity [11].

Nineteen compounds, including fatty acids, steroids, purines and benzene derivatives were confirmed by extensive NMR data of the purified compounds from *Cinachyrella australiensis* [12] while eighteen typical and six new fatty acids, including 6-bromo-Δ^5,9-nonacosadienoic acid, were found in the Saudi Red Sea collection of a *Cinachyrella* sponge extract [13].

Studies on the effects which the geographic locations of marine plants and animals have on the production of metabolites have shown that differences tend to exist in the metabolite concentrations. For example, research conducted by Wessels and coworkers on three sea hares, two from the same general area and another from a different location, demonstrated that the presence and concentrations of the main metabolites were variable [14]. In an interesting study of colonies of the soft coral *Briaereum asbestinum* in the Bahamas and St. Croix, US Virgin Islands, extensive variations in the production of feeding deterrents of the briarane and asbestinane skeletal types were observed [15]. While the briarane to asbestinane diterpene ratio was found to be higher in soft corals collected from shallow water than at deeper depths in two locations in the Bahamas, the shallow water extracts from St. Croix had greater proportions of the asbestinanes.

Examples of studies with sponges include research work on the *Jaspis* sponge which demonstrated that variations existed in the concentration and occurrence of several of the metabolites [16]. It should be noted that bioactive compounds found in sponge extracts are often thought to be produced by the symbiotic bacteria and this has been shown in research on the *Theonella swinhoei* sponge from which cytotoxic macrolide swinholide A was obtained. In this study, the sponge cells were found to be devoid of the compound while a mixed population of unicellular heterotrophic bacteria contained the compound. This has served to promote research in the area with a view to investigating the sponge symbionts [17]. Alternatively, variation in chemical profiles of marine organisms could be due to genetic variability or site-specific factors including sedimentation levels, predation pressures, nutrient levels, light and algal overgrowth which also influence the concentration of compounds [18].

High intercolony variability in the presence of defensive chemicals was observed with the sponge *Chondrilla nucula*, but the extent of feeding deterrency was consistent between reef and mangrove dwellers from both the Bahamas and Florida [19]. Extract masses from the Bahamas, however, were found to be higher.

In a study of congeneric species from Hong Kong and the Bahamas it was found that there were differences in the types of bacterial isolates obtained [20]. Hydrogels prepared incorporating extracts from both sponges resulted in the inhibition of larval settlement in the Hong Kong sponge, *Mycale adhaerens*, while the Bahamas sourced sponge extract (*M. laxissima*) had no effect on the larvae of *Hydroides elegans*, a polychaete. The chemistry of the two sponges also differed with respect to their fatty acid profiles. Extracts of the Hong Kong sponge were found to inhibit the microbial growth of a wide range of bacteria isolated from its own sponge surface.

An example of a study of a sponge species which did not differ in metabolite concentration is that of the sponge *Crambe crambe* which was collected from different geographical regions in the Mediterranean (Liguarian Sea and Sardinia Sea) and examined for the occurrence of crambescidins and crambescins [21]. Principal Component Analysis (PCA) plots showed that there was no statistical difference in the concentration of those metabolites with respect to the geographical location or depth at which the specimens were collected.

Studies of different marine organisms have therefore largely shown that the geographical location can impact metabolite production. This present study of *C. kuekenthali* was conducted to ascertain the impact, if any, which the environment in which the sponges are grown has on the metabolite concentrations.

2. Materials and Methods

Cinachyrella sp. samples were collected from two locations (**Figure 1**), the Rio Bueno area (Code: RB1, **Figure 2**) and the Columbus Park (Code: CP1, **Figure 3**) in the Discovery Bay area in June 2008 in a preliminary collection at a depth of 13 - 17 m. The samples were frozen upon collection and cut up into small

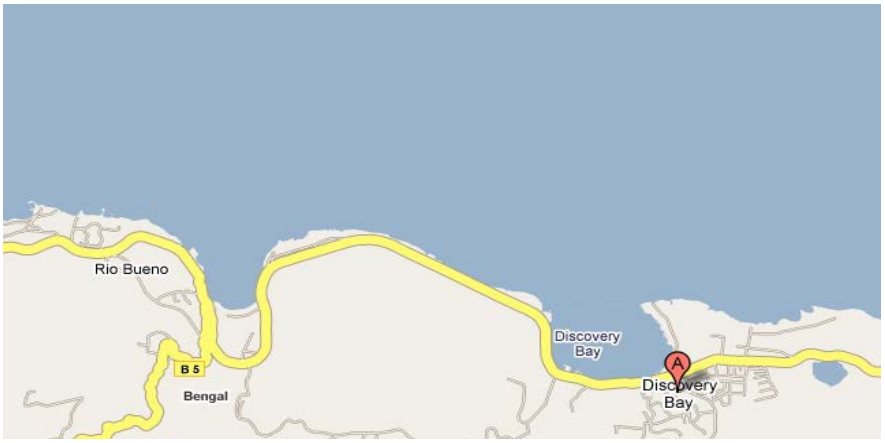

Figure 1. Map showing the collection sites of Discovery Bay and Rio Bueno (Courtesy of Google maps).

Figure 2. Sponge species growing at the site in Rio Bueno (RB).

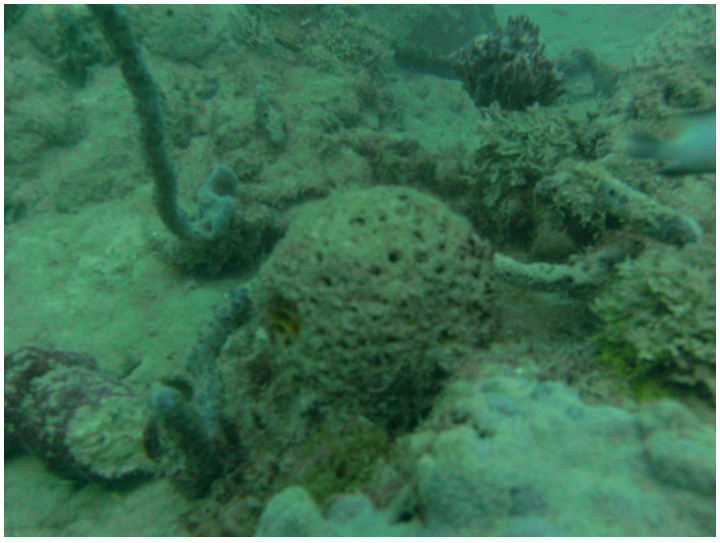

Figure 3. Sponge species growing at the site in Columbus Park (CP), Discovery Bay.

pieces prior to extraction with CH_2Cl_2 (1.2 L × 2). The sponge samples were weighed upon drying. The solvents were removed in vacuo to afford brown gums which were subsequently weighed.

The second collection of the specimens was made in July 2009 in which five sponges were collected from Columbus Park by SCUBA diving at a depth of 10 - 13 m and six individuals were collected from Rio Bueno (depth: 13 - 17 m). Samples were cut up, frozen and then extracted using CH_2Cl_2 to afford gums upon the removal of the solvent in vacuo.

Column chromatography was performed on a gravity or flash column packed with SiO_2. The silica was presoaked with the eluting solvent. The CH_2Cl_2 extracts of the preliminary 2008 samples were chromatographed by gravity column chromatography on a silica column (height: 35.4 cm, diameter: 2.9 cm) using eluent solvent of increasing polarity from 100% hexane to EtOAc/hexane mixtures with final elution in EtOAc:MeOH and 100% MeOH. For the column on the Rio Bueno (RB1) CH_2Cl_2 extract, eighty (80) fractions were collected while sixty-one (61) fractions were collected from the column on the Columbus Park (CP1) CH_2Cl_2 extract. Combinations were effected on the basis of the TLC profiles of the fractions. ^{1}H-NMR analyses were carried out on eight selected fractions of the seventeen combination fractions of CP (CP1-A-Q) and ten selected combined fractions of the fifteen total combined fractions of RB1 (RB1-A-O). ^{13}C-NMR analyses were done on combinations CP1-K and RB1-H while RB1-M was purified by carrying out a flash column chromatography using eluent solvent of increasing polarity from 100% EtOAc to 100% MeOH to afford twenty-four (24) fractions which were combined (based on the TLC profiles) and subjected to ^{1}H-NMR analysis.

^{1}H-NMR and ^{13}C-NMR spectra of the fractions were recorded on a 200 MHz NMR spectrometer in $CDCl_3$.

3. Results and Discussion

It was hypothesized that the sponge extracts from the *Cinachyrella* sponge from the different environments may vary quantitatively and qualitatively. In the preliminary work with the sponge, the single specimen collected from the silty area of Columbus Park was significantly greater in mass (263.3 g) than the sample collected from the pristine location at Rio Bueno (148.3 g). The quantity of the resultant dichloromethane extract was, however, a quarter of the yield (by percentage) of the Rio Bueno sponge (**Table 1**). The ^{1}H-NMR spectra of the crude CH_2Cl_2 extracts were comparable as were the ^{13}C-NMR spectra except for the presence of an additional signal attributable to a carboxylic acid functional group of a fatty acid in RB1.

NMR analysis of combination CP1-K which eluted in 20% ethyl acetate: hexane confirmed the presence of the steroid cholesterol while combination RB1-H eluted from the column in 30% ethyl acetate: hexane and was found to contain mostly cholesterol ($C_{27}H_{46}O$, **Figure 4**) along with a fatty acid. Cholesterol is the principal sterol synthesized by animals.

Table 1. Results from collection of single specimens of the Ball Sponge from Columbus Park (CP1) and Rio Bueno (RB1).

Sample Code	Specimen mass/g (Dry weight)	CH_2Cl_2 Extract mass/g	Percentage yield	Main class of compounds present
CP1	263.3	0.604	0.23	Steroids (cholesterol)
RB1	148.3	1.612	1.1	Steroids (cholesterol)

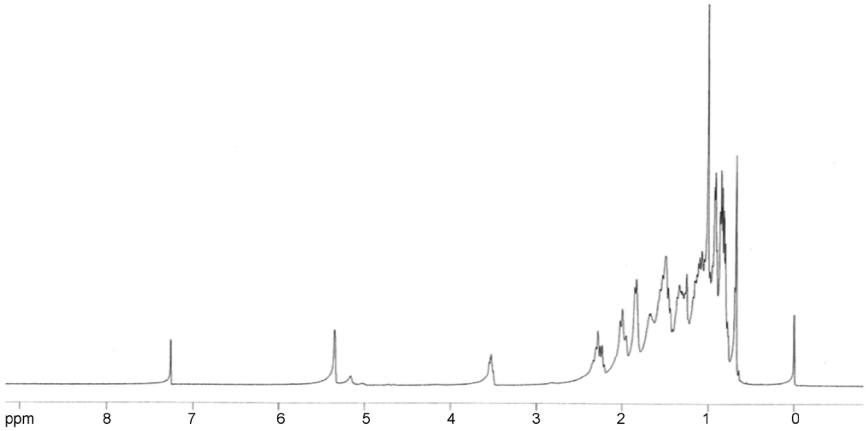

Figure 4. ^{1}H NMR spectrum of cholesterol obtained from the sponge extract.

The second collection of specimens of *C. kuekenthali* was evaluated for the dry masses of the sponges and the quantity of the dichloromethane extract obtained. As was observed with the preliminary collection, while the masses of the Columbus Park (CP2) ball sponges were generally higher than the Rio Bueno specimens, the yields of the extracts were higher for Rio Bueno (RB2) samples (**Table 2**).

The average mass of the CP2 sponges is 97.22 g while the average mass of the RB2 sponges is less than half of the CP2 mass at 42.57 g. The average percentage yield of the dichloromethane extract for the CP2 sponges was calculated at 2.06% of the dry weight of the organisms while the average percentage yield for the RB2 extract was determined to be 3.81%, almost twice that of the CP2 specimens.

It has been reported that *Cinachyrella sp.* can live at highly variable water depths (5 - 116 m) and at temperatures ranging between 20.4˚C and 26˚C but the concentration of nitrates (0.5 - 4.1 μmol/L), silicates (0.8 - 2.69 μmol/L) and phosphates (0.094 - 0.4 μmol/L) in the water column is essential to their survival and growth. Optimal dissolved oxygen concentrations should be between 4.07 and 4.89 μmol/L with oxygen saturation levels between 79.93% and 103.83% [22].

The silty bay area of Discovery Bay was teeming with life in spite of the significant degree of suspended solids in the water column as well as the solids which had settled on the sponges and the reef. It would not have been expected that such a wide variety of apparently healthy sponges would have survived under the

Table 2. Results from collection of Ball sponge specimens collected from Columbus Park (CP2) and Rio Bueno (RB2) sites.

Sample Code	Specimen mass/g (Dry weight)	CH_2Cl_2 Extract mass/g	Percentage yield (%)	Major class of compounds detected
CP2 1	135.02	4.36	3.23	Steroids
CP2 2	127.85	1.37	1.07	Steroids
CP2 3	138.99	3.07	2.21	Steroids
CP2 4	60.47	1.75	2.89	Steroids
CP2 5	23.78	0.21	0.88	Steroids
RB2 1	27.83	0.85	3.05	Not reported
RB2 2	30.35	1.28	4.22	Steroids
RB2 3	34.92	1.80	5.15	Steroids
RB2 4	82.26	3.60	4.38	Steroids
RB2 5	26.24	1.05	4.00	Not reported
RB2 6	53.84	1.09	2.03	Not reported

prevailing conditions at Columbus Park. One postulate regarding the reason for the larger organisms existing in this environment is that these sponges have to increase in size in order to facilitate more efficient flow through of water containing the nutrients required obtain sufficient food to survive. Morphological changes in sponges have been reported in the literature and are theorized to sometimes be due to environmental differences [18]. The larger the size and number of incurrent pores are, the more bacteria and tiny particles will enter the sponge cells prior to the water removal from the osculum. The increased availability of food in the form of picoplankton (including prochlorophytes and heterotrophic bacteria) at depth for the tube sponge *Callyspongia vaginalis* was deemed to be the main reason why specimens transplanted at the deeper site (25 m vs. 12 m) were larger in size [23].

The difference in the concentration of secondary metabolites in the sponges grown at the two sites was extremely marked. The preliminary collection saw a four-fold difference in the extract mass (1.1% vs. 0.23%), while variation in the second collection was approximately two-fold (3.81% vs. 2.06%). This significant difference could be attributed to the fact that the Columbus Park sponges are expending their energy to survive while the pristine environment of the Rio Bueno reef walls allow those sponges to utilize their energy for the production of secondary metabolites in abundance.

It would be expected that there would be a difference in the types of predators existing at each location since predators like fishes are not sessile and are therefore capable of changing their location if the environment is unfavourable to their survival. It should be noted, though, that a variety of fishes were fairly abundant at both collection sites. In a 2013 study, Pawlik and coworkers concluded that sponges with chemical defenses grew more slowly than undefended species,

demonstrating a resources trade-off between growth and the production of the bioactive secondary metabolites [24]. The CP sponges may not be susceptible to the levels of attack that the RB sponges would be experiencing. Maida and coworkers studied the soft coral *Sinularia flexibilis* from sites with differences in competition levels and found that, as would be expected, the concentration of one of the bioactive terpenes, flexibilide, was highest at the most competitive site [25]. The main constituents of the sponge extracts are steroids and fatty acids, both of which have been found to play various roles in the environment [26].

While it should be noted that sponge maturity levels may be correlated to the size of the organism and the larger sponges may be deemed to be at a greater level of maturity than the smaller sponges, this factor could have an impact on the nature and volume of secondary metabolites produced by the sponge. In our study, the sponges denoted CP2-5, RB2-1 and RB2-5 are comparable in their dry weights, at 23.78, 27.83 and 26.24 g respectively. The percentage yields of the extracts were found to be 0.88%, 3.05% and 4.00% respectively, making it clear that the differences in the metabolite concentration could not be soley as a consequence of the perceived maturity level of the sponges.

While our study did not evaluate the bacterial count of each of the sites, further investigations into the bacterial content of the sponges may also provide clues regarding factors which contribute to the differences in metabolite concentrations. This has been observed with the bryozoan *Bugula neritina* in a study by McGovern and coworkers where several species of *B. neritina* were examined from two different geographical locations (Western Atlantic and South of Cape Halteras) and it was concluded that the variation in palatability of their larvae to predators was due largely to the presence or absence of a bacterium within the collected bryozoans [27].

4. Conclusion

In conclusion, the significant differences in sponge size and the inverse proportion with respect to the metabolite concentration suggest that the environment in which the organisms are growing severely impacts the *C. kuenkenthali* species. The need therefore for continued protection of our reefs from negative impacts such as sedimentation remains a very important consideration in the face of industrialization and the need to gain a livelihood from our oceans.

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Conflicts of Interest

The author declares no conflict of interest.

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