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Constituents of the Jamaican Sponge *Iotrochota birotulata*

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Abstract *Iotrochota birotulata*, an abundantly occurring sponge collected off the coast of Port Royal, Jamaica was investigated to identify its main constituents and evaluate the bioactivity associated with its crude extracts. The compounds renierapurpurin and the tyrosine derivative 1,3-dibromo-5-{2-[(*p*-hydroxyphenyl)-acetamido]ethyl}-2-[3-(-methyl-2-butenamido)-propoxy] benzene were isolated from the crude extract along with the common steroid β-sitosterol. The carotenoid derivative renierapurpurin was identified in this species for the first time. The structures of these compounds were elucidated using spectral analysis. When the crude extracts of the sponge were tested against the sweet potato weevil *Cylas formicarius elegantulus*, 100% mortality was observed at a concentration of 2 µg/mL after 72 hours.

Keywords: *Iotrochota birotulata, bioassay, zebrafish, sweet potato weevil*

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1. Introduction

The *Iotrochota* genus has been shown to possess a variety of secondary metabolites, including alkaloids, a toxin [1], ecdysteroids [2] sphingolipids [3], bromoindole and tyrosine derivatives. [4,5] A few of isolates exhibit remarkable biological activities such as cytotoxicity, anti-inflammatory activity, ATP-citrate lyase inhibitory activity and antibacterial activity. Records have shown that the methanol/water extracts significantly inhibited larval attachment at 0.1 $mgmL^{-1}$ and are considered as possessing anti-settlement activity [6].

As part of our ongoing research to find bioactive compounds from the tropical marine sponges for the treatment of diseases or for agricultural applications, the Jamaican *I. birotulata* was investigated. In search of biologically active secondary metabolites, we have isolated two known compounds from *I. birotulata* collected from the coast of Port Royal, Jamaica.

2. Materials and Methods

NMR data was recorded on a Bruker Vector 2000 – 200 MHz spectrometer and a Bruker Vector 2000 – 500 MHz spectrometer equipped with a 5 mm inverse detection probe with deuterochloroform ($CDCl_3$), deuterated methanol (CD_3OD) or deuterated dimethyl sulfoxide (DMSO-d_6) and tetramethylsilane (TMS) as the internal standard. IR spectra were recorded on a Tensor 37 spectrophotometer. Optical rotations were determined on an Anton Pear MCP polarimeter in methylene chloride solutions at 589 nm. HREIMS measurements were conducted by MEDAC limited on a Thermo Finnigan, MAT900XP. LCMS data was determined on a Bruker microTOF with an Agilent LC 1100 system and all samples were prepared in methanol (1 mg/mL). UV spectra were recorded on a Hewlett-Packard 8453A diode array spectrophotometer. Thin layer chromatography (TLC) was conducted using pre-coated silica gel plates viewed under UV light at 254 or 366 nm. Ninhydrin and Molybdenum sprays were used to visualize the compounds. All solvents were distilled before use. Solutions were concentrated *in vacuo* on a Büchii 011 Řotavap rotary evaporator.

2.1. Sponge Material

The sponge, *Iotrochota birotulata*, was collected in October 2009 from Port Royal, Jamaica. The sponge was identified by Marlon Hibbert from the Life Science Department at the University of the West Indies as *Iotrochota birotulata*. Commonly referred to as the green finger sponge, it forms ramose, bushy aggregates of thick branches (1.5 cm diameter) with a conulose surface. The sponge is purplish to black in colour and occasionally green in life, and is usually colonised by abundant golden zooanthids. When squeezed, a dark exudate emanated from the sponge.

2.2. Extraction and Isolation

The sponge was carefully cleaned of all extraneous debris, cut into small pieces and stored frozen prior to being lyophilized. The sponge (1.2 kg, dry weight) was extracted at room temperature using the following solvents: *n*-hexane (2 x 8 L), methylene chloride (2 x 8 L) and methanol (2 x 8 L). After the methanol extraction, 250 g of the sponge was extracted with water (2 x 800 mL). The extracted solvents were filtered and each was

concentrated using a rotary evaporator to yield crude residues. A portion of the hexane extract (20 g) was placed on a silica gel column and eluted with solvents of increasing polarity ranging from hexane to methylene chloride and then methanol. Fourteen fractions were collected. The fraction eluting in 10% methylene chloride: hexane contained a bright red-orange solid. TLC analysis revealed that the compound present was highly conjugated. The compound was purified using 5% methylene chloride: hexane to obtain 17 mg of compound 1.

The fraction eluting in 50% methylene chloride: hexane comprised 8.1 g of a white solid. NMR studies suggested that the fraction constituted a mixture of steroids. The major component of the mixture however, was β-sitosterol.

The methylene chloride extract (10 g) was placed on a flash silica gel column and eluted with solvents of increasing polarity starting from hexane through to methanol. Fraction 10, which eluted in 100% ethyl acetate, was placed on a second silica gel column and eluted with a solvent mixture ranging from 80% ethyl acetate to 100% methanol. Sub-fraction 4 (118 mg), which eluted in 100% ethyl acetate, was further purified over silica to obtain 19 mg of compound **2**.

2.3. Insecticidal Assay

The insecticidal assays were determined using a modified version of that reported by Facey and coworkers [7]. Two-week-old adult *Cylas formicarius elegantulus* were cultured on sweet potato tubers (*Ipomoea* species) in glass aquaria in the laboratory at 25± 2°C and 65 to 68 % relative humidity. Extracts and fractions weighing 2.0 mg were dissolved in 50 µL of acetone or methanol before the final stock of 0.1% (w/v) was made up using a mixture of acetone-water (8:2; v/v). From the stock solution, aliquots of 2.0 µL, 4.0 µL and 6 µL were applied to the insects at the junction between the thorax and abdomen and the percentage mortality recorded at 48 hours. For the initial screening, 10 insects in two replicates of five were used. If the samples were found to be active (i.e. inflicting 40% or greater mortality at 48 hours) the experiment would be repeated with another set of 10 insects. Thus, N = 20, in two replicates of 10 for the active compounds. As the control, twenty insects were treated with 6 L of acetone-water (8:2; v/v) solution.

3. Results and Discussion

Chemical analysis of the organic extracts of the sponge led to the isolation of two known compounds. These were a tyrosine derivative (1) and renierapurpurin (2). β-Sitosterol was also isolated. The structures of the compounds were determined by spectroscopic techniques and comparison of spectroscopic data with those of reported compounds [2,8].

The structure of the compound 1 was determined to be 1,3-dibromo-5-{2-[(*p*-hydroxyphenyl)-acetamido]ethyl}-2-[3-(-methyl-2-butenamido)-propoxy] benzene based on comparisons with the data reported in the literature. It was previously isolated from the *I. birotulata* sponge collected along the coast of Little San Salvador Island, Bahamas [2].

A pseudomolecular ion peak $[M-H]^+$ was displayed at m/z 565, 567 and 569 in a 1:2:1 ratio for compound 1. This corroborated the presence of two bromine atoms in the molecule. The molecular ion peak displayed at *m/z* 565.0325 (calculated 565.0338, Δ 2.3 ppm) corresponded to $C_{24}H_{28}Br_2N_2O_4$ as the molecular formula for the compound.

The presence of two carbonyl functionalities was confirmed by the two carbon atoms resonating at δ_C 172.09 and 167.80. Evidence of a four-proton AB system at δ_H 6.79 and 6.98 in the aromatic region was revealed in the ^{1}H NMR spectrum. This strongly suggested a *para* disubstituted benzene ring; the carbon atoms for these protons resonated at δ_C 115.79 and 129.53 respectively. The presence of two broad methyl singlets at δ_H 2.16 and 1.87 confirmed the isobutenyl portion of the structure and these were both long-range coupled to an olefinic proton resonating at δ_H 5.62.

The HMBC correlations of δ_H 6.79 (H-2) with δ_C 156.53 (C-1) and 125.51 (C-4); and δ_H 6.98 (H-3 and H-5) with δ_C 156.53 (C-1) allowed the structural determination of the atoms in the phenolic benzene ring. the link between the phenolic benzene ring and one of the secondary amide functionalities present in the molecule was established by the correlation of δ_H 3.49 (H-7) with δ_C 125.51 (C-4) and 172.09 (C-8) established. The correlations of δ_H 3.43 (H-10) with δ_C 172.09 (C-8), 33.85 (C-11) and 137.90 (C-12) revealed that the amide group was also linked to a tetra-substituted benzene ring. The ether linkage between C-15 and C-18 was confirmed by the correlation peak of δ_H 4.06 (H-18) with δ_C 151.79 (C-15). Additionally, the linkage of the isobutenyl group with the second amide group was also established by the HMBC correlation of δ_H 5.62 (H-23) with δ_C 167.80 (C-22), 27.13 (C-25) and 19.86 (C-26).

Table 1. NMR Data for Compound 1

Position	^{13}C	^{1}H	HMBC	COSY
1	156.5	-		
2, 6	115.8	6.79, m	C-1, C-4	H-3,5
3, 5	129.5	6.98, m	C-1	H-2,6
4	125.5	-		
7	42.9	3.49, br s	C-4, C-8	
8	172.1	-		
9	-	3.43, m		
10	40.1	3.57, m	C-8, C-11, C-12	H-11
11	33.9	2.69, m	C-10,12, 13, 17	H-10
12	137.9	-		
13, 17	132.9	7.33, s	C-11, 15	
14, 16	125.5	-		
15	151.8	-		
18	71.8	4.06	C-15, 19, 20	H-19
19	30.7	2.16, m	C-15, 18	H-18, 20
20	36.3	3.61, m	C-18, 19, 22	
21	-	6.00, br s	C-22	
22	167.8	-		
23	118.4	5.62, br s	C-22, 25, 26	H-25, 26
24	151.7	-		
25	27.1	1.87, s	C-23, 24, 26	
26	19.9	2.16, s	C-23, 24	
1 – OH	-	8.24, br s		

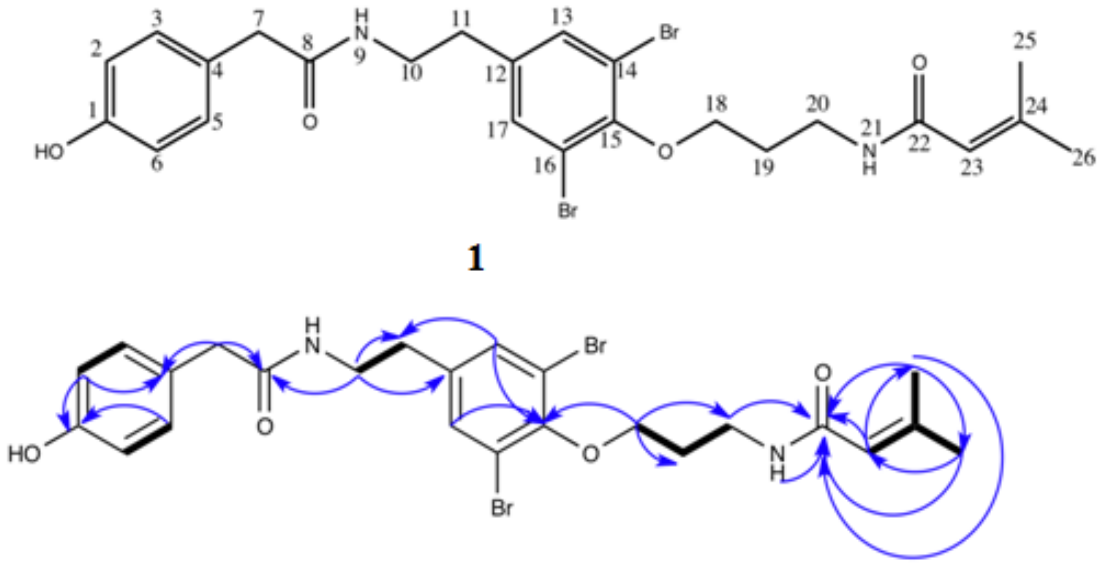

Figure 1. $^{1}H - {}^{1}H$ and $^{1}H - {}^{13}C$ couplings detected by the COSY and HMBC experiments in $CDCl_3$ for compound **1**

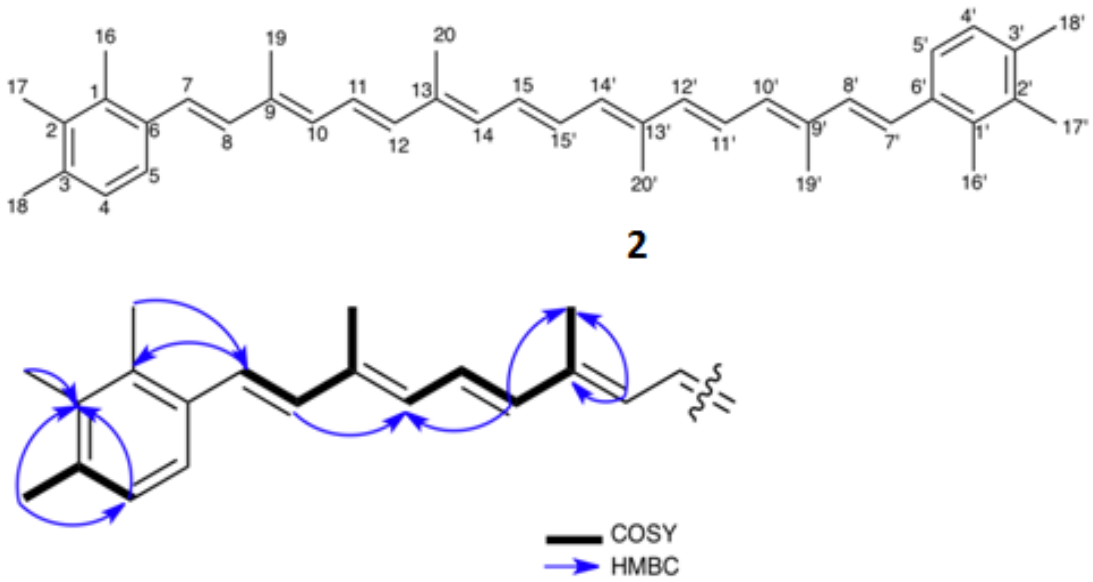

Figure 2. $^{1}H - {}^{1}H$ and $^{1}H - {}^{13}C$ couplings detected by the COSY and HMBC experiments in $CDCl_3$ for compound **2**

The structure of compound **2** was determined to be an aryl carotenoid, renierapurpurin. Renierapurpurin was isolated from the Japanese sea sponge *Reniera japonica* [8].

Compound **2** was obtained from the hexane extract as a red-orange powder displaying a molecular ion peak (HRCIMS) at *m/z* 528.3751 (calculated 528.3749, Δ 0.28ppm) corresponding to $C_{40}H_{48}$. The spectrum displayed molecular ion peaks characteristic of the polyene chain of typical carotenoids [(M), (M-92), M-106), 106 and 91)]. In addition, the diagnostic peak at m/z 133 which clearly indicated the presence of a trimethylphenyl end-group was observed. The NMR spectral study further corroborated the presence of the trimethylphenyl group. The proton atoms resonating at δ_H 2.23 (3H) and 2.28 (6H) are as a result of the three methyl groups attached to the aromatic nucleus [8,9].

The HMBC correlations of δ_C 134.42 (C-2) with δ_H 2.23 (H-17), 2.28 (H-18) and 6.97 (H-4 and H-5) supported the structural arrangement of the atoms in the tetrasubstituted benzene ring. The conjugated double bond system was linked to the highly substituted ring through the correlation of δ_H 6.58 (H-7) with δ_C 125.49 (C-1). HMBC correlations for the conjugated double bond system included δ_H 6.30 (H-10) with δ_C 132.12 (C-8) and 138.00 (C-12); and δ_H 6.28 (H-14) with δ_C 137.68 (C-13) and 12.79 (C-20).

The UV-visible spectrum of compound **2** in Et_2O showed absorption maxima at 450 and 477 nm. The IR spectrum showed an absorption band at 3054 cm^{-1} that confirmed the presence of the =CH functionality. A band at 2987 cm^{-1} representing the C-H stretch was also present. The optical rotation on compound 2 was obtained in dichloromethane ($[\alpha]_D^{26}$: +40° (c. 0.0001)).

It should be noted that carotenoids have been shown to perform photoprotective functions in sponges and field studies with the sponge *Clathria prolifera* have shown that, when transplanted to a shaded environment, the production of carotenoids by the organism decreased markedly [10]. The production of carotenoids including renierapurpurin are thought to actually be derived from bacterial sponge symbionts or by the modification of dietary carotenoids [11].

Table 2. NMR data for Compound 2 in $CDCl_3$

Position	^{13}C	^{1}H (J/Hz)	COSY	HMBC ($^{1}H - ^{13}C$)
1, 1′	135.49	-		
2, 2′	134.42	-		
3, 3′	136.51	-		
4, 4′	128.06	6.97, d (6.5)	2.28	134.42
5, 5′	127.15	6.97, d (6.5)		
6, 6′	133.56	-		
7, 7′	126.48	6.58, d (11.0)	6.25	135.49
8, 8′	132.12	6.25, d (11.0)	6.58	132.81
9, 9′	134.24	-		
10, 10′	132.81	6.30, d (11.5)	6.68	
11, 11′	124.84	6.68, dd (15.0,11.5)	6.40, 6.30	
12, 12′	138.00	6.40, d (15.0)	6.68	12.79, 132.81
13, 13′	137.68	-		
14, 14′	138.99	6.28, d (11.5)		12.79, 137.68
15, 15′	130.18	6.65, m		
16, 16′	20.99	2.28, s	6.97	126.48
17, 17′	17.04	2.23, s		134.42
18, 18′	20.50	2.28, s	6.97	128.06, 134.42
19, 19′	12.79	2.00, s	6.25	
20, 20′	12.79	2.09, s	6.40	138.99

Preliminary bioassay of the crude organic extracts showed strong activity in the insecticidal assay at a dosage of 2 µg/insect after 72 hours (sweet potato weevil, *Cylas formicarius elegantulus*). No mortality was observed after a 48 hour period.

Table 3. Biological activities of the crude organic extracts of *I. birotulata* against *Cylas formicarius elegantulus*

Extract	Dosage/ µg per insect		
	6	4	2
	% Mortality after 72 h		
Hexane extract	100	100	100
Dichloromethane extract	100	100	100
Methanol extract	Inactive	Inactive	Inactive
Control (acetone (6µL))	Inactive	Inactive	15

The efficacy of the hexane and dichloromethane extracts against the economically important sweet potato weevil presents an opportunity to conduct further evaluation of these extracts with a view to identifying the bioactive components of the extracts. Through possible synergistic effects of the components of the extracts, discrete compounds could be less potent than the extract, however. Further studies will be undertaken to ascertain more data, and, the possible formulation of an agricultural product with an extract from this abundantly occurring sponge would serve to promote the sustainable exploitation of our marine resources.

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