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Research Article

SEARCH FOR NEW LEADS FROM MARINE MACROFAUNA: COLLECTION FROM KUWAITI ARABIAN GULF COAST

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ABSTRACT

Objective: This study aimed at collecting marine macroorganisms, followed by extracting the collected samples, purifying and identifying the pure compounds from the bioactive fractions those have been determined.

Materials and Methods: A collection trip was executed to collect sponges from near Qaruh Island located to the south east of Kuwait City. Several samples were collected, and two of these samples were processed, extracted and chromatographed to yield pure compounds. The identity of the compounds were determined spectrally. Antimicrobial activites were also evaluated.

Results: Out of the eight collected sponge samples only two, 04AG1 and 04AG2, were in good amounts. Sample 04AG1, identified as *Agelas* sp., gave IC_{50} values (µg/ml) of 100, 85, 70 and 80 against *Candida albicans* ATCC 90028, *Cryptococcus neoformans*, *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus*, respectively. This sponge gave, after purification, the known cholesterol and a carotenoid compound. Furthermore, Sample 04AG2 was identified as *Phakellia* sp. and showed no promising antibacterial activities. After purification, it afforded lanosterol, *β*-sitosterol, oroidin and a fatty acid with undetermined length.

Conclusion: No previous work on marine organisms from the Arabian Gulf has ever been published. The coral reefs located in the Gulf are attractive virgin spots yet to be investigated. Few sponge samples were collected and few compounds were isolated and identified from two samples. This work established a new marine natural products chemistry program at Kuwait University and the Gulf area. The collection of only few samples from the Gulf showed poor biodiversity that is attributed to the massive damage of the natural habitats there due to high contamination rate of the Gulf caused by the high oil spill during the Gulf war.

Keywords: Marine Macrofauna.

INTRODUCTION

The world's oceans cover more than 70% of the earth's surface and contain over 200,000 invertebrate and algal species [1]. Total global biodiversity is estimated at between 3 and $500x10^6$ species of prokaryote and eukaryote organisms spread across 70 or more phyla [2]. The marine macrofauna alone are estimated between 0.5 and $30x10^6$ species and represents a boarder range of taxonomic diversity than that found in the terrestrial environment. On the other hand, the global marine macrofauna are the source of $2.5x10^{10}$ to $1.5x10^{12}$ primary products and an associated extensive range of secondary products [2]. Interestingly, only a few thousand novel compounds from marine organisms have been described, and if the novelty and complexity were the only criteria, then the success of research in this area would be assured, for there are so many marine natural products that have no counterparts in the terrestrial world. For example, the structure assigned to maitotoxin represents, perhaps, the most complex secondary metabolite described to date. Although a surprisingly large portion of marine natural products have interesting pharmacological properties, a commercially successful "drug from the sea" remains a difficult, but possible, task to achieve. This means that further investigations are still needed in this direction. Moreover, marine natural products are frequently the subjects of biochemical investigations, synthesis programs and biosynthetic studies.

Lots of marine-derived bioactive compounds have made and continue to make significant contributions to biomedicine. These compounds may fall in one of two categories; bio-products in current use and promising leads. Examples of the first category are the antiviral agent ara-A from its sponge (*Cryptotethia crypta*) progenitor spongothymidine, and the antineoplastic ara-C from spongouridine [3]. On the other hand, examples of the promising leads category are the antitumor agent didemnin B that was isolated from the tunicate *Tridemnum solidum* [4], and the antiviral agents avarol and avarone that were isolated the sponge *Dysidea avara* [5].

Literatures abound with many more interesting marine molecules with fabulous biological activities. However, no work on marine organisms from the Arabian (Persian) Gulf has ever been published. There are several coral reefs near from the Kuwaiti Arabian Gulf coast located to the east and southeast regions of the gulf. These reefs are attractive virgin spots yet to be investigated.

The importance of this project is to establish a new marine natural products chemistry program, for the first time at Kuwait University, which should promote the research and graduate study at the Faculty of Pharmacy. Moreover, isolating several new chemical entities that could serve as drug leads is another added importance.

This study culminated in the collection of eight macrofuna followed by the isolation of cholesterol (1), lanosterol (2), *β*-sitosterol (3), and the nitrogen-containing oroidin (4), whose isolation, structure elucidation and antimalarial and antimicrobial activities are the subject of this manuscript. Additionally, tentatively identified carotenoid compound and a fatty acid with undetermined length were isolated, as well.

MATERIALS AND METHODS

General Experimental Procedure

The IR spectra were recorded as neat solids using an FT/IR-4100 JASCO spectrophotometer. The ^1H and ^{13}C NMR were obtained on a Bruker Avance II-600 spectrometer operating at 600 and 125 MHz, respectively. Both ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, except oroidin was in DMSO-d_6, and the chemical shift values were expressed in δ (ppm) relative to the internal standard TMS. For the ^{13}C NMR spectra, the number of attached protons was determined by DEPT 135°. 2D-NMR data were obtained using the standard pulse sequences of the Bruker Avance II-600 for COSY, HSQC, and HMBC. HRMS was carried out using a Bruker Bioapex FTMS with Electrospray Ionization Spectrometer.

Sample Material

Eight sponges were collected from the sandy bottom at a depth of -10 m near Qaruh Island, 30 miles south east of Kuwait City, on the Kuwaiti Arab Gulf coast, October 2004. About half a kilogram of the two most abundant sponges, 04AG1 and 04AG2, and only 100 g of

each of the remaining six samples were collected. Representative voucher specimens were cut and stored in 70% aqueous ethanol.

Extraction

Sample 04AG1 (410 g) was lyophilized, cut into small pieces and macerated in a high-speed blinder with 95% ethanol (0.5 L x 3). The resulting extracts were filtered and the residual tissue was percolated at room temperature using 95% ethanol. This process was done successively until no further color was extracted. The extract was concentrated down to 50 ml *in vacuo*. Finally, it was completely evaporated using *Savant*™ concentration unit to afford 16.9 g of black solid residue. Likewise, sample 04AG2 (120 g) was processed to afford 9.1 g of black solid residue.

Fractionation and Chromatographic Purification of 04AG1 Extract

6.5 g of the dried residue was successively partitioned between *n*-hexane and 15% aqueous acetonitrile pre-saturated with each other. Evaporation yielded 1.84 g and 0.66 g of acetonitrile and hexane fractions, respectively. Acetonitrile fraction was chromatographed over flash silica (200 g, 12.5 x 5 cm, silica gel G, 230-400 mesh) using methylene chloride: methanol starting with 97.5: 2.5 and ending with 75: 25. Similar fractions, based on thin layer chromatography analysis, were collected and pooled together to give seven main fractions; fraction 1 (19.2 mg), fraction 2 (7.4 mg), fraction 3 (2.1 mg), fraction 4 (28.3 mg), fraction 5 (13.8 mg), fraction 6 (108.8 mg) and fraction 7 (100 mg).

TLC analysis of fractions 1 and 2, using methylene chloride: methanol; 9.5: 0.5 as a developing solvent and visualized using *p*-anisaldehyde/sulphuric acid followed by heating, revealed the presence of several sterols in these fractions. These types of compounds are very abundant in marine organisms.

The least polar one of these compounds has been purified over a silica gel column using methylene chloride to afford 10 mg of colorless powder. IR and 1D and 2D-NMR analyses of this compound suggested its identity as cholesterol (**1**).

Fraction 4 showed mainly an orange-colored compound suggesting its carotenoid nature. The presence of carotenoids in marine macrofauna has been reported before in sponges, shellfishes and the phylum rhodophyta [6-11]. This compound was purified to give 5 mg and its identity was tentatively established as carotenoid. Fractions 3, 5, 6 and 7 showed several UV active compounds. These fractions were subjected to several purification processes including C-18 chromatography. These processes afforded several minute fractions that were too impure and small to be unambiguously identified. Taxonomic identification of sample 04AG1 revealed its identity as *Agelas* sp.

Fractionation and Chromatographic Purification of 04AG2 Extract

04AG2 dried residue was successively partitioned between *n*-hexane and 15% aqueous acetonitrile pre-saturated with each other. Evaporation of both fractions yielded 5.33 g of acetonitrile extract and 1.87 g of hexane extract. Both extracts were considered for further purification processes.

Hexane extract was fractionated over flash silica (200 g, 30 x 3 cm, silica gel G, 230-400 mesh) using gradient elution procedure starting with 100% chloroform and gradually doubling the methanol fraction every 500 ml (1%, 2%, 4%, 8%, 16% and finally 20% methanol in chloroform). This afforded two main fractions; fraction1 (160 mg) and fraction 2 (793 mg). Fraction 1 was further purified over flash silica gel (20 g, 10 x 1 cm) using 1% ethyl acetate in petroleum ether to give 35 mg of lanosterol (**2**) [12].

Fraction 2, which upon evaporation showed possibly pure crystals, was purified via crystallization from petroleum ether to give 146 mg of *β*-sitosterol (**3**) as pure colorless needles. The presence of β-sitosterol in marine organisms, particularly sponges, has been documented before [13,14]. The mother liquor was chromatographed over a silica gel column (100 g, 22 x 2 cm) using ethyl acetate: petroleum ether: acetic acid; 2: 98: 0.1 to afford six subfractions. Subfractions 1 and 3 were similar, and hence added, to compounds 2 and 3, respectively. This afforded 181 mg of 2 and 188 mg of 3. Other subfractions were impure and too little to be fully identified.

On the other hand, part of the acetonitrile fraction (3.0 g) was chromatographed over flash silica (400 g, 36 x 5 cm, silica gel G, 230-400 mesh) using chloroform: methanol; 95: 5, as an eluting solvent. Similar fractions, based on TLC analysis, were collected and pooled together to give ten main fractions; fraction 1 (388 mg), fraction 2 (166 mg), fraction 3 (35 mg), fraction 4 (46 mg), fraction 5 (7.2 mg), fraction 6 (16 mg), fraction 7 (20 mg), fraction 8 (213 mg), fraction 9 (50 mg) and fraction 10 (469 mg).

TLC analysis of these fractions using chloroform: methanol; 9.5: 0.5 as a developing solvent and visualized using *p*-anisaldehyde/sulphuric acid followed by heating, revealed that, all fractions, except fraction 8, are mixtures of highly overlapped compounds. Several chromatographic techniques were applied in an attempt to purify these fractions. However, chronological TLC analysis of these fractions, showed the high possibility of fast deterioration of the compounds in these fractions.

Having thermo-, pressure- or light-labile compounds is common in marine organisms. One possible explanation would be the different environmental conditions, e.g., high pressure, low temperature, high salinity, and low/no light intensities, under which these compounds were biosynthesized and stored in their bio-sources. These conditions are certainly different from those under which the extraction, isolation and purification were conducted.

On the other hand, fraction 8 showed some relatively stable entities that can be purified. Chromatographic purification of this fraction over a silica gel column (50 g, 14 x 2 cm) using gradient elution procedure starting with 100% chloroform and gradually doubling the methanol fraction every 500 ml (1%, 2%, 4%, 8%, 16% and finally 20% methanol in chloroform) afforded 15.5 mg of a pale yellow powder. This compound was found to be oroidin alkaloid (**4**) [15,16]. Taxonomic identification of sample 04AG2 revealed its identity as *Phakellia* sp.

Cholesterol (1)

colorless tiny needles; IR neat υ_{max} 3373 (OH), 2927 (C-H) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; High resolution ESIMS *m/z* 386.3522 $[M]^+$ (calcd for $C_{27}H_{46}O$ 386.3549).

Lanosterol (2)

white powder; IR neat υ_{max} 3420 (OH), 2930 (C-H), 1645 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; High resolution ESIMS *m/z* 426.3881 $[M]^+$ (calcd for $C_{30}H_{50}O$ 426.3862).

Sitosterol (3)

colorless tiny needles; IR neat υ_{max} 3422 (OH), 2937 (C-H), 1645 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; High resolution ESIMS *m/z* 414.3821 $[M]^+$ (calcd for $C_{29}H_{50}O$ 414.3861).

Oroidin (4)

pale yellow powder; IR neat υ_{max} 3130 (NH), 1665 (C=O) cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1. NMR data were identical to those previously reported [15,16]. High resolution ESIMS *m/z* 387.9373 $[M+H]^+$ (calcd for $C_{11}H_{12}Br_2N_5O$ 387.9408).

Bioactivity Evaluation

10 mg of samples 04AG1 and 04AG2 were submitted to the National Center for the Development of Natural Products, School of Pharmacy, the University of Mississippi, USA, to evaluate their *in vitro* antimalarial and antimicrobial activities.

RESULTS AND DISCUSSION

The frozen coarsely minced *Agelas* sp. and *Phakellia* sp. sponges were percolated several times using 95% ethanol. The dried ethanol

extracts were partitioned between aqueous acetonitrile and hexane. The acetonitrile fraction of *Agelas* sp. was chromatographed several times using flash silica gel to afford the known cholesterol (**1**). The tentatively identified carotenoid was also isolated. On the other hand, the hexane fraction of *Phakellia* sp. was chromatographed and purified several times to culminate lanosterol (**2**) and *β*-sitosterol (**3**). Likewise, the acetonitrile fraction afforded the alkaloid oroidin (**5**) after several chromatographic processes. The identity of these compounds was established from their spectral data. Full NMR data are unambiguously reported in Table 1. These data were identical to those previously reported for cholesterol [17], lanosterol [18,19], -sitosterol [20,21] and oroidin [15,16].

Table 1: It shows NMR Data for compounds 1-4[a]

	Cholesterol (1)		**Lanosterol (2)**		***β*-Sitosterol (3)**		**Oroidin (4)**	
#	δ_C, m[b]	δ_H (m, *J* in Hz)	δ_C, m	δ_H (m, *J* in Hz)	δ_C, m	δ_H (m, *J* in Hz)	δ_C, m	δ_H (m, *J* in Hz)
1	37.4, t	1.13 (m), 1.80 (m)	36.0, t	1.54 (m), 1.79 (m)	37.3, t	1.13 (m), 1.80 (m)	-	8.52 (br s)
2	31.8, t	1.55 (m), 1.80 (m)	28.3, t	1.47 (m), 1.72 (m)	31.6, t	1.55 (m), 1.80 (m)	104.6, s	-
3	71.7, d	3.25 (m)	79.4, d	3.23 (dd, 4.4, 4.4)	71.8, d	3.52 (m)	97.9, s	-
4	42.5, t	2.25 (m), 2.30 (m)	39.3, s	-	42.2, t	2.25 (m), 2.30 (m)	111.0, d	6.89 (s)
5	140.8, s	-	50.8, d	1.05 (m)	140.8, s	-	128.0, s	-
6	121.8, d	5.36 (m)	18.7, t	1.74 (m), 1.49 (m)	121.7, d	5.36 (m)	158.7, s	-
7	32.0, t	1.50 (m), 1.95 (m)	26.9, t	2.01 (m), 1.91 (m)	31.9, t	1.50 (m), 1.95 (m)	-	12.50 (br s)
8	31.8, d	1.55 (m)	134.8, s	-	31.9, d	1.55 (m)	39.8, t	3.94 (t, 4.9)
9	50.3, d	0.95 (m)	134.8, s	-	51.2, d	0.95 (m)	124.7, d	6.13 (dt, 16.2, 4.9)
10	36.6, s	-	37.4, s	-	36.5, s	-	116.2, d	6.20 (d, 16.2)
11	21.2, t	0.95 (m), 1.50 (m)	21.4, t	1.91 (m), 2.01 (m)	21.1, t	0.95 (m), 1.50 (m)	126.8, s	-
12	39.9, t	1.15 (m)	31.4, t	1.45 (m), 1.70 (m)	39.8, t	1.16 (m)	113.0, d	6.97 (s)
13	42.5, s	-	44.9, s	-	42.3, s	-	-	12.76 (br s)
14	56.9, d	1.05 (m)	50.2, s	-	56.8, d	1.05 (m)	147.5, s	NH_2: 7.45 (s)
15	24.3, t	1.60 (m)	31.3, t	1.35 (m), 1.60 (m)	24.3, t	1.60 (m)	-	-
16	28.3, t	1.55 (m)	28.6, t	1.36 (m), 1.59 (m)	28.3, t	1.55 (m)	-	-
17	56.4, d	1.10 (m)	50.8, d	1.48 (m)	56.0, d	1.10 (m)	-	-
18	11.9, q	0.68 (s)	15.8, q	0.69 (s)	11.9, q	0.68 (s)	-	-
19	19.4, q	1.01 (s)	19.5, q	0.98 (s)	19.4, q	1.01 (s)	-	-
20	35.9, d	1.40 (m)	36.7, d	1.49 (m)	36.2, d	1.40 (m)	-	-
21	18.8, q	0.92 (d, 6.4)	19.0, q	0.91 (s)	18.8, q	0.92 (d, 6.4)	-	-
22	36.3, t	1.01 (m)	36.7, t	1.54 (m)	33.9, t	1.25 (m)	-	-
23	23.9, t	1.35 (m)	25.3, t	1.96 (m)	26.1, t	1.25 (m)	-	-
24	39.6, t	2.05 (m)	125.7, d	5.10 (t)	45.9, t	2.25 (m)	-	-
25	28.1, d	1.55 (m)	131.3, s	-	29.2, d	1.58 (m)	-	-
26	22.6, q[c]	0.82 (d, 6.5)	26.1, q	1.68 (s)	19.8, q[c]	0.82 (d, 6.5)	-	-
27	22.8, q[c]	0.83 (d, 6.5)	17.9, q	1.60 (s)	19.3, q[c]	0.83 (d, 6.5)	-	-
28	-	-	28.4, q	1.00 (S)	23.1, t	1.55 (m)	-	-
29	-	-	16.2, q	0.81 (s)	12.2, q	0.85 (t, 7.5)	-	-
30	-	-	24.6, q	0.88 (s)	-	-	-	-

[a]compounds **1-3** were run in $CDCl_3$, while **4** in DMSO-d_6. [b]carbon multiplicities were determined by DEPT 135°.

[c]assignments within the same column are interchangeable.

Compound **1** was isolated as colorless needles, in a 0.006% yield, from the acetonitrile fraction of sample 04AG1. It was shown to possess the molecular formula $C_{27}H_{46}O$ as derived from the presence of the molecular ion peak at *m/z* 386.3511 [M^+] and NMR data. The IR spectrum showed an absorption band at 3373 cm^{-1}, indicating the presence of a hydroxyl group, in addition to a strong absorption at 2927 cm^{-1} due to saturated C-H stretch. Therefore, the identity of **1** was concluded to be cholesterol. ^{13}C NMR spectrum showed twenty seven carbon resonances as five quartets, eleven triplets, eight doublets and three singlets. Two of these resonances were in the aromatic/aliphatic region and one was in the oxygenated aliphatic region (Table 1). C-3 resonated at δc 71.7 as a doublet, and showed a cross peak in the HSQC spectrum with a multiplet proton resonated at δ_H 3.25 and assigned to H-3. This proton showed a ^3J correlation, in the HMBC spectrum, with a singlet carbon resonated at δ_C 140.8 and assigned to C-5. On the other hand, HMBC spectrum showed a ^4J cross peak between the other olefinic carbon (C-6, δc 121.8, d) and a singlet proton resonated at δ_H 1.01 and integrated for three protons (CH_3-19). These assignments have placed this double bond between C-5 and C-6. Other ^1H and ^{13}C NMR data were identical to those of cholesterol. Therefore, the identity of **1** was established to be cholesterol (Figure 1). Cholesterol has been reported before to exist in several marine organisms [22].

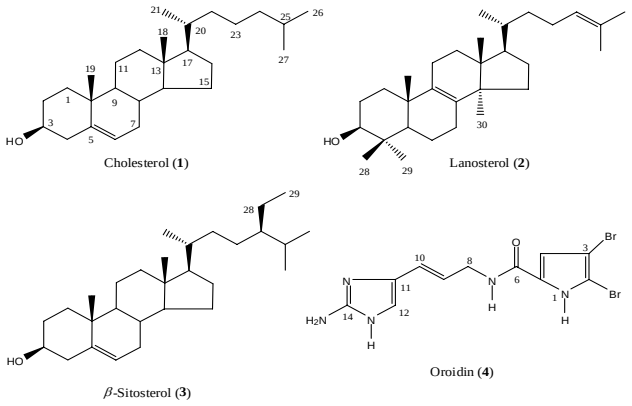

Fig. 1: It shows chemical structures of compounds 1-4

Moreover, the acetonitrile fraction of this sponge culminated into another compound that was isolated as orange amorphous powder in a 0.003% yield. It possesses a molecular weight of *m/z* 581.41, and an IR spectrum consistent with the presence of a hydroxyl group (3422 cm^{-1}), alkenyl (1645 cm^{-1}), and alkynyl (2124 cm^{-1}) functionalities. This compound was too scarce to be unambiguously identified. The identity of this compound was tentatively identified as carotenoid.

Compound 2 was isolated as colorless powder, in a 0.18% yield, from the hexane fraction of sponge sample 04AG2. Compound **2** possesses a molecular formula of $C_{30}H_{50}O$ as concluded from its NMR data and mass spectrum due to the presence of a molecular ion peak at *m/z* 426.3881. This implied that **2** is a triterpene. ^{13}C NMR spectra showed thirty carbons resonated as eight quartets, ten triplets, five doublets, and seven singlets (Table 1). Four of these carbons resonated in the aromatic/olefinic region; at δc 134.8 (s, C-8), δc 134.8 (s, C-9), δc 125.7 (d, C-24), and δc 131.3 (s, C-25). C-24 showed ^1J correlation, in HSQC spectrum, with an olefinic proton resonating at δ_H 5.10 as a triplet. This proton was assigned to H-24. This proton showed ^3J correlations, in the HMBC spectra, with two quartet carbons resonating at δc 17.9 (C-27) and δc 26.1 (C-26). Consequently, H-26 and H-27 were assigned to two singlets resonating at δ_H 1.68 and 1.60, respectively. Moreover, other methyl groups protons were assigned due to the presence of correlations between them and the corresponding quartets. C-3 resonated at δc 79.4 and was connected to a proton resonated at δ_H 3.23 as double of doublet with two equal *J* values (4.4 Hz). The hydroxyl group at C-3has a β-disposition, since its corresponding α-epimer derivatives resonate about 3 ppm upfield than do the β-epimers [23]. Moreover, the small $J_{3,2}$ and $J_{3,2'}$ values (4.4, 4.4 Hz) indicated that they should be $J_{e,e}$ and $J_{e,a}$, a situation that can only be fulfilled when H-3 adopts the α-orientation leaving the β one for OH. H-3 showed three-bond correlations, in the HMBC spectra, with two quartet carbons resonating at δc 16.2 (C-29) and δc 28.4 (C-28). Other ^1H and ^{13}C NMR data were identical to those of lanosterol [18,19]. Therefore, the identity of **2** was established to be lanosterol (Fig 1). Lanosterol has been reported before to exist in several marine organisms [12].

Compound **3** was isolated as colorless needles, in a 0.28% yield, from the hexane fraction produced form the partitioning of sponge 04AG2 ethanol extract between *n*-hexane and 15% aqueous acetonitrile. The mass spectrum of **3** indicated its molecular weight as *m/z* 414.3821 [M^+], which was consistent with the molecular formula $C_{29}H_{50}O$. The IR spectrum showed a broad absorption at 3422 cm^{-1} suggesting the presence of a hydroxyl group, a strong absorption at 2937 cm^{-1} indicating the presence of saturated C-H skeleton, and a weak absorption at 1645 cm^{-1} indicating the possible presence of alkene functionality. NMR data of this compound were very similar to those of compound **1** (cholesterol) with the exception of having an additional ethyl group. ^{13}C NMR spectra of this compound showed twenty nine carbon resonances distributed as six quartets, eleven triplets, nine doublets and three singlets. Two of these carbons resonated in the aromatic/olefinic region at δc 140.8 (C-5, s) and δc 121.7 (C-6, d). This indicated the presence of a double bond. One carbon resonance was in the oxygenated aliphatic region (δc 71.8, C-3, d) (Table 1). Other carbon resonances were in non-oxygenated aliphatic region and were identical to those of sterols. H-3, resonating as a multiple at δ_H 3.52, was unambiguously assigned from the HSQC spectra. HSQC spectra were instrumental in connecting other protons to the assigned carbons. HMBC spectra showed ^3J coloration between H-3 and C-5. This carbon, in turn, showed another correlation with three protons resonating as a singlet at δ_H 1.01. COSY spectra showed that two methyl groups protons (δ_H 0.82, d, *J*=6.5 Hz, H_3-26 and δ_H 0.83, d, *J*=6.5 Hz, H_3-27) coupled to a common proton resonated as a multiplet at δ_H 1.58, H-25. C-25 was shown to resonate at δ_C 29.2 as a doublet. This established the presence of an isopropyl group, a common structural feature between many sterols. The presence of ^1J cross peak between H-25 and a quartet carbon resonated at δ_C 12.2, C-29, made it possible to rule out other sterols but β-sitosterol. Other NMR data were in a full agreement with those reported for β-sitosterol [20]. Compound **3** identity was established as β-sitosterol (Fig 1).

The presence of sterols in marine organisms identical to those found in terrestrial plants shows the closeness in biosynthetic pathways in both organisms that should have come from, at least, similarity in enzymatic machineries.

Compound 4 was isolated as pale yellow powder, in a 0.023% yield, from the acetonitrile fraction of the sponge 04AG2. The mass spectrum of 4 indicated its molecular weight as *m/z* 387.9373 $[M+H]^+$, which was consistent with the molecular formula $C_{11}H_{12}Br_2N_2O$. The IR spectrum showed absorption bands at 3130 (N-H) and 1665 (C=O) cm^{-1}. Moreover, the IR spectrum was crucial to prove that this compound contains bromine atom(s). Additionally, 4 was shown to have nitrogen atom(s) since it gave a positive Dragendorff's test. ^{13}C NMR data showed eleven carbon resonances distributed as a triplets, four doublets and six singlets. One singlet carbon resonated in the carbonyl group region, δ_C 158.7, and was assigned to the amide carbonyl carbon C-6. This carbon has three-bond correlation, in the HMBC spectrum, to a singlet proton resonated at δ_H 6.89 (H-4). Nine carbons resonated in the olefinic/aromatic region and the last carbon resonated in the aliphatic region at δ_C 39.8 as a triplet and was assigned to C-8. The most downfield olefinic singlet carbon resonated at δ_C 147.5 and was concluded to be attached to more than one nitrogen atom. This carbon was assigned to C-14, i.e., C-2 in the 2-aminoimidazole moiety of 4. The olefinic proton, H-10, resonated at δ_H 6.20 as a doublet (*J*=16.2 Hz) showed ^3J cross peak with an olefinic doublet carbon appeared at δ_C 113.0. This resonance was assigned to C-12. On the other hand, H-9 resonated at δ_H 6.13 as double of triplet (*J*=16.2, 4.9 Hz). Consequently, all protons and carbons were unambiguously assigned from the HSQC and HMBC spectra. Therefore, compound 4 was identified as the bromopyrrole oroidin (Fig 1). ^1H and ^{13}C NMR data (Table 1) were identical to those reported before [15,16].

After bioactivity evaluation of sponge samples, it was shown that 04AG1 ethanol extract is inactive against the protozoa *Plasmodium falciparum*, however, it showed 100 % growth inhibition of *Candida albicans* ATCC 90028, *Cryptococcus neoformans*, *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* and *Aspergillus fumigatus* at a concentration of 500 µg/ml. Since the sample showed promising activity is this assay, it was promoted to the next assay. IC_{50} (µg/ml) values were found to be 100, 85, 70 and 80 against *Candida albicans* ATCC 90028, *Cryptococcus neoformans*, *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus*, respectively. Moreover, it has an MIC =500 µg/ml against *Aspergillus fumigatus*. On the other hand, sample 04AG2 extract showed no promising activities and, hence, it was not evaluated further.

CONCLUSION

No previous work on marine organisms from the Arabian Gulf has ever been published. This work established a new marine natural products chemistry program at Kuwait University and the Gulf area. Eight sponge samples were collected from the Arabian Gulf Kuwaiti coast and two samples were identified and studied in details. *Agelas* sp. exhibited promising antimicrobial activity against several bacteria and fungi, however, *Phakellia* sp. showed no promising antibacterial activities. Both samples yielded several compounds known to be found in terrestrial plants, in addition to the marine-based aminoimidazole alkaloid, oroidin. The collection of only few samples from the Gulf showed poor biodiversity that is attributed to the massive damage of the natural habitats there due to high contamination rate of the Gulf caused by the high oil spill during the Gulf war.

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