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Two new polyacetylene derivatives from the Red Sea sponge *Xestospongia* sp.

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Abstract: Two new polyacetylenes (**1** and **2**), along with two known C-30 steroids (**3** and **4**) were identified from the Red Sea sponge, *Xestospongia* sp. The chemical structures were determined based on extensive spectroscopic measurements 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HSQC and HMBC) NMR, UV, IR and MS. The new compounds **1** and **2** were evaluated for their antimicrobial and antitumor activities. **1** and **2** were active against multidrug- resistant bacteria with MICs ranged from 2.2 to 4.5 μM. No toxicity was recorded for the two tested compounds up to 5 μM using *Artemia salina* as a test organism. Compound **2** showed excellent antifungal activity against some pathogenic fungi such as *Aspergillus niger* and *Candida albicans* (MIC 2.2–2.5 μM) and antitumor activity against both Ehrlich ascites carcinoma and lymphocytic leukemia (LD_{50} 5.0 μM).

Keywords: acylglycoacetylenes; antimicrobial; carcinoma; marine sponges.

1 Introduction

Barrel sponges, or the species of the genus *Xestospongia* (class Demospongia, order Haplosclerida, family Petrosiidae) might be considered as exclusive producers of some interesting secondary metabolites. These include the halogenated polyacetylenic lipids, which have been shown to possess a broad spectrum of biological activities such as antimicrobial, HIV-integrase inhibition, and Na^+/K^+ ATPase inhibition [1–4]. The genus *Xestospongia* contains long-chain polyacetylenic alcohols with chemotaxonomic markers [5], and polyacetylenes with antimicrobial, cytotoxic, antitumor, antiviral, and immunosuppressant bioactivities, and also as enzyme inhibitors [6, 7]. Search for bioactive metabolites from *Xestospongia* led to the identification of two new polyacetylenes, *viz*. acylglycerolipid and xestospongiamide. Similarly, the extract from *Xestospongia* sp., collected from Pohnpei, Federated States of Micronesia, exhibited antimicrobial activity against *Pseudomonas aeruginosa* and *Mycobacterium intracellulare* with IC_{50} values of 1.03–2.07 μg/mL [8]. Here we present the isolation and identification of two further new polyacetylenes from this sponge.

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2 Materials and methods

2.1 General

Silica gel GF 254 (Merck, Darmstadt, Germany) was used for analytical TLC. PTLC was performed on aluminum oxide plates (20 × 20 cm) of 250 μm thickness. Electron impact mass spectra were determined at 70 ev on a Kratos MS-25 instrument (Manchester, UK). 1D and 2D NMR spectra were recorded on Bruker AVANCE III WM 600 MHz spectrometers (Karlsruhe, Germany) and ^{13}C NMR at 150 MHz. Tetramethylsilane (TMS) was used as internal standard. Plates were sprayed with 50%-sulphuric acid in methanol and heated at 100 °C for 1–2 min.

2.2 Sponge sample

The sponge *Xestospongia* sp. was collected from deep water (15 m depth) of Sharm Obhur (21°29′31″N 39°11′24″E), Jeddah, Saudi Arabia, and was identified by Biologist Kamal Al-Dahoody (Faculty of Maritime Studies, King Abdulaziz University). A Voucher sample (JAD 02013) has been deposited at the Chemistry Department, Faculty of Science, King Abdulaziz University. The sponge was barrel shaped (25 cm length and 15 cm diameter) with a large cavity in the

center. It is characterized by the presence of finger-like structures on the outside surface. The color is maroon to pink. Out of the water, it appears brown or light pink. The skeleton is composed of a tangential layer of single spicules in the ectosomal skeleton. The choanosomal skeleton shows multispicular reticulation forming regular tracts.

2.3 Extraction and isolation of compounds

The freeze-dried sponge (90 g) was extracted two times with 6 L of a mixture of CH_2Cl_2-MeOH (1:1, v/v) for 24 h at 22°, and a viscous dark reddish oil was obtained (7.8 g). This extract was fractionated on NP-Silica (2.5 × 75 cm, 500 g, Merck 7739), employing a gradient technique with four solvent systems: Pet. ether: diethyl ether (9:1, v/v); Pet. ether: $CHCl_3$ (9:1); Pet. ether: EtOAC (7:3) and $CHCl_3$: MeOH (9:1), each of 500 mL, giving four fractions F-A, F-B, F-C and F-D of 90, 14, 88 and 30 mg, respectively. The fraction (F-A) that was purified by RP-18 HPLC (eluent, MeOH:H_2O, 65:35), and re-purified on PTLC-silica gel using $CHCl_3$:MeOH (9.5:0.5), yielded compound **4**. The fraction (F-B) which was purified by PTLC-silica gel using $CHCl_3$: ETOAc (6:4), yielded compounds **3**, **2** and **1**, respectively.

2.4 Acid Hydrolysis of 1.

A solution of **1** (2 mg in 2 mL of MeOH) and 10 mL of 7% HCl/MeOH (3:7) was left for 10 h (23 °C), monitored by TLC. At the end of this period, the mixture was extracted with EtOAc, and the aqueous layer neutralized with 7% KOH. After evaporation of the solvent, the residue was extracted with C_6H_5N. HPLC purification of the C_6H_5N extract (Lichrosorb100 NH; Knauer, Berlin, Germany) using MeCN/H_2O (8:2) as eluent yielded glucose (0.5 mg).

Compound **1** was isolated as a white amorphous powder, R_f = 0.29 $CHCl_3$: EtOAc (6:4, 2.5 mg). IR (film) cm^{-1}: 3371, 2921, 2851, 2206, 1736, 1459; ^1H and ^{13}C NMR spectral data are shown in Table 1. Compound **2** was isolated as pale yellow oil, with R_f = 0.25 ($CHCl_3$: EtOAc (6:4, 1.5 mg). IR (film) cm^{-1}: 3434, 2852, 2215, 1741, 1459, 1200; ^1H and ^{13}C NMR spectral data are shown in Table 1.

2.5 Antimicrobial activity and toxicity

2.5.1 Tested microbes: The tested bacteria *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, P. aeruginosa, Staphylococcus aureus*, and *Streptococcus pneumoniae* were obtained from King Fahd General Hospital. The tested fungi were *Aspergillus niger, Candida albicans* ATCC10231, *Candida tropicalis, Cryptococcus neoformans, Epidermophyton* sp., *Microsporum gypseum, Trichophyton mentagrophytes and Trichophyton rubrum* were from the culture collection of Dr. Roger Bonaly, Biochimie Microbienne, Faculté de Pharmacie, Nancy, France.

The sensitivity of the pathogenic bacteria and fungi to compounds **1** and **2** was tested using the agar well diffusion method [9]. The two compounds were dissolved in DMSO, which was also

Table 1: ^1H (600 MHz), ^{13}C (150MHz) NMR data of **1** and **2**.

1						2		
P.	δ_C [a,b]	δ_H [c]	P.	δ_C	δ_H	P.	δ_C	δ_H
1	104.7 (d)	4.28 (d, 7.8)	13″	29.6 (t)	1.50 (m)	1	173.1 (s)	–
2	75.0 (d)	3.17 (t, 8.4)	14″	19.8 (t)	2.27 (td, 6.6, 1.8)	2	33.9 (t)	2.33 (t, 7.2)
3	77.9 (d)	3.33 (t, 9.0)	15″	93.7 (s)	–	3	24.4 (t)	1.63 (p, 7.2)
4	71.9 (d)	3.32 (t, 9.0)	16″	78.3 (s)	–	4	28.3 (t)	1.41 (p, 7.2)
5	78.0 (d)	3.27 (m)	17″	118.0 (d)	6.23 (dt, 13.8, 1.8)	5	27.9 (t)	1.52 (p, 7.2)
6	62.7 (t)	3.67 (dd, 12.0, 5.4)	18″	119.1 (d)	6.69 (d, 13.8)	6	19.1 (t)	2.29 (2H, td, 7.2, 1.2)
		3.87 (dd, 12.0, 2.0)	1‴	174.7 (s)	–	7	88.5 (s)	–
1′	68.7 (t)	3.75 (dd, 10.8, 6.0)	2‴	34.8 (t)	2.34 (td, 7.2, 1.8)	8	79.2 (s)	–
		3.99 (dd, 10.8, 6.0)	3‴	25.5 (t)	1.62 (m)	9	110.2 (d)	5.47 (1H, dd, 15.6, 1.2)
2′	71.6 (d)	5.27 (m)	4‴	28.9 (t)	1.43 (m)	10	142.6 (d)	6.02 (1H, dt, 15.6, 6.6)
3′	63.9 (t)	4.22 (dd, 12.0, 6.0)	5‴	29.1 (t)	1.51(m)	11	32.3 (t)	2.10 (2H, q, 6.6)
		4.44 (dd, 12.0, 6.0)	6‴	19.8 (t)	2.27 (td, 6.6, 1.8)	12	27.7 (t)	1.48 (2H, m)
1″	175.0 (s)	–	7‴	88.5 (s)	–	13	28.4 (t)	1.50 (2H, m)
2″	34.0 (t)	2.45 (t, 7.2)	8‴	80.8 (s)	–	14	19.2 (t)	2.25 (2H, td, 6.6, 18)
3″	25.2 (t)	1.80 (quin, 7.2)	9‴	112.0 (d)	5.46 (dt, 15.6, 1.8)	15	92.7 (s)	–
4″	19.3 (t)	2.34 (td, 7.2, 1.8)	10‴	143.5 (d)	5.98 (dt, 15.6., 6.6)	16	77.5 (s)	–
5″	89.1 (s)		11‴	33.0 (t)	2.20 (q, 6.6)	17	118.0 (d)	6.17 (1H, dt, 13.8, 1.8)
6″	80.3 (s)		12‴	33.3 (t)	2.20 (q, 6.6)	18	117.1 (d)	6.57 (1H, d, 13.8)
7″	111.6 (d)	5.46 (dt, 15.6, 1.8)	13‴	145.7 (d)	6.13 (dt, 15.6, 6.6)	1′	68.9 (d)	5.26 (1H, dd, 6.6, 4.2)
8″	142.8 (d)	5.98 (dt, 15.6, 6.6)	14‴	111.1 (d)	5.62 (dd, 15, 1.8)	2′	62.1 (t)	4.28 (1H, dd, 12.6, 4.2)
9″	33.5 (t)	2.10 (q, 6.6)	15‴	91.3 (s)	–			4.13 (1H, dd, 12.6, 6.6)
10″	28.9 (t)	1.43 (m)	16‴	85.6 (s)	–			
11″	28.9 (t)	1.43 (m)	17‴	118.7 (d)	6.37 (dd, 13.8, 1.8)			
12″	29.4 (t)	1.50 (m)	18‴	118.9 (d)	6.79 (d, 13.8)			

[a]All assignments are based on 1D and 2D measurements (HMBC, HSQC, COESY). [b]Implied multiplicities were determined by DEPT (C = s, CH = d, CH_2 = t). [c]J in Hz. Compound **2** was measured in $CDCl_3$, while compound **1** was measured in MeOH-d.

employed as negative control, and 100 µL were used to fill each well. Minimal inhibitory concentration (MIC) was determined using the microdilution method [10]. Toxicity of 1 and 2 was determined using the brine shrimp lethality test [11]. The antitumor activity against Ehrlich carcinoma and lymphoma cell lines was determined and the percentage of cell viability (LD_{50}) was calculated [12]. Each reading is the mean value of three replicates ± SD.

3 Results and conclusion

In continuation of our research program on compounds from marine organisms, the sponge material was collected from Saudi territorial waters. Extensive fractionation of the organic extract employing NP-Silica gel, preparative thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) yielded four metabolites (**1–4**). Two were new acetylenic derivatives: an acylglycerolipid,2′-O-(7*E*,17*E*-18-bromo-octaoctadeca-7,17-dien-5,15-diynoyl)-3′-*O*-(9*E*,13*E*,17*E*)-18-bromo-octaoctadeca-9,13,17-dien-7,15-diynoyl-1′-*O*-*β*-*D*-galactopyranosyl glycerol (**1**), and xestospongiamide (**2**). The other two metabolites had been previously identified: known 26,27-Dimethylergosta-5,24(28)-dien-3β-ol (xestosterol) (**3**) and the xestosterol ester of 18-bromooctadeca-(9*E*,17*E*)-diene- 7,15-diynoic acid (**4**; Figure 1).

Compound **1** was isolated as a white powder. The molecular formula, $C_{45}H_{58}O_{10}Br_2$, was determined by HRESIMS at *m/z* 916.2384 $[M]^+$. Liquid chromatography mass spectrometry (LCMS; positive-ion-mode) at *m/z* 916 $[M]^+$ indicated 16 degrees of unsaturation. The 1:2:1 isotopic distribution at *m/z* 916, 918 and 920 indicated the presence of two bromine atoms. Moreover, the peak at *m/z* 179 could be assigned to a hexose sugar unit (Figure 2). The ^{13}C NMR spectrum indicated the presence of 45 signals, categorized by an DEPT NMR experiment into two carbonyl δ_C (174.3 and 175.0 ppm), eight quaternary (93.7, 91.3, 89.1, 88.5, 85.6, 80.8, 80.3, and 78.3), 10 olefinic (145.7, 143.5, 142.8, 112.0, 119.1, 118.9, 118.7, 118.0, 111.6 and 111.1), 16 methylene (34.8, 34.0, 33.5, 33.3, 33.0, 29.7, 29.4, 29.1, 28.9, 28.9, 28.9, 25.5, 25.2, 19.8, 19.8 and 19.3), an acetal (104.7) and three oxygenated methylene (68.7, 63.9 and 62.7) carbons, respectively. The ^{1}H NMR spectrum comprised signals due to a sugar structure, such as oxymethines (δ_H 4.28, 3.33, 3.32, 3.27 and 3.17), and ^{13}C NMR revealed the signal of a typical acetal carbon δ_C 104.7. ^{13}C, ^{1}H and H-H COESY NMR spectral data indicated the presence of a glycerol moiety, based on the assignment of the three vicinal oxygenated carbons δ_C 68.7, 71.6 and 63.9. The downfield chemical shift indicated a substituted glycerol. Two esterified carbonyl functions (δ_C 174.3 and 175.0) and

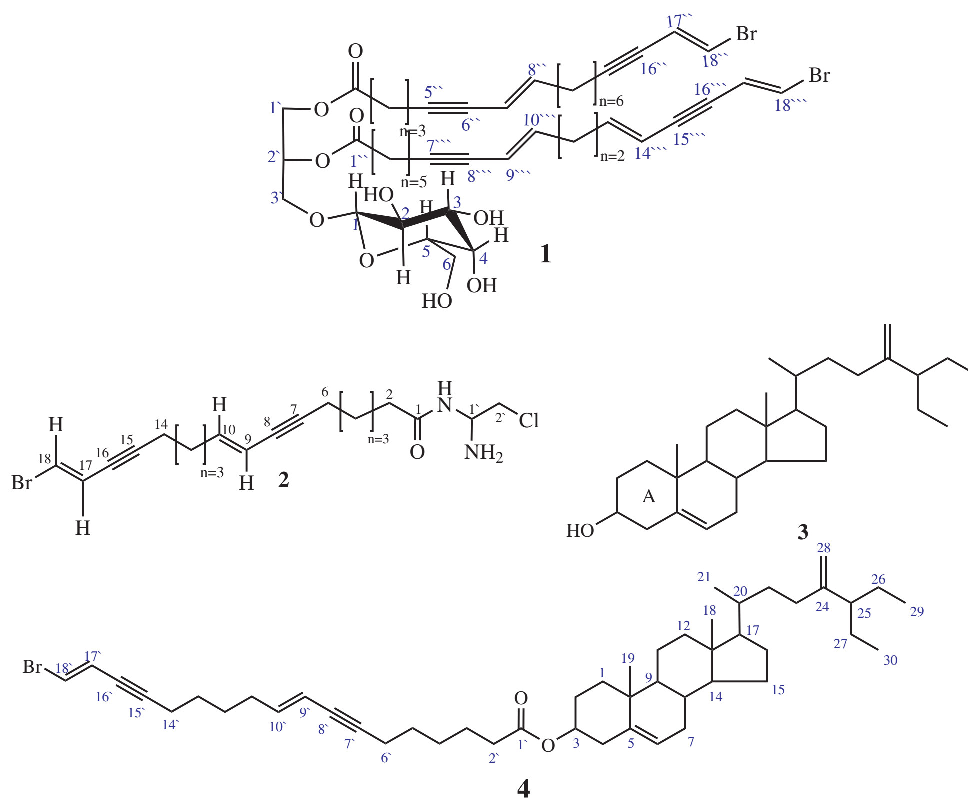

Figure 1: Structures of compounds **1–4**.

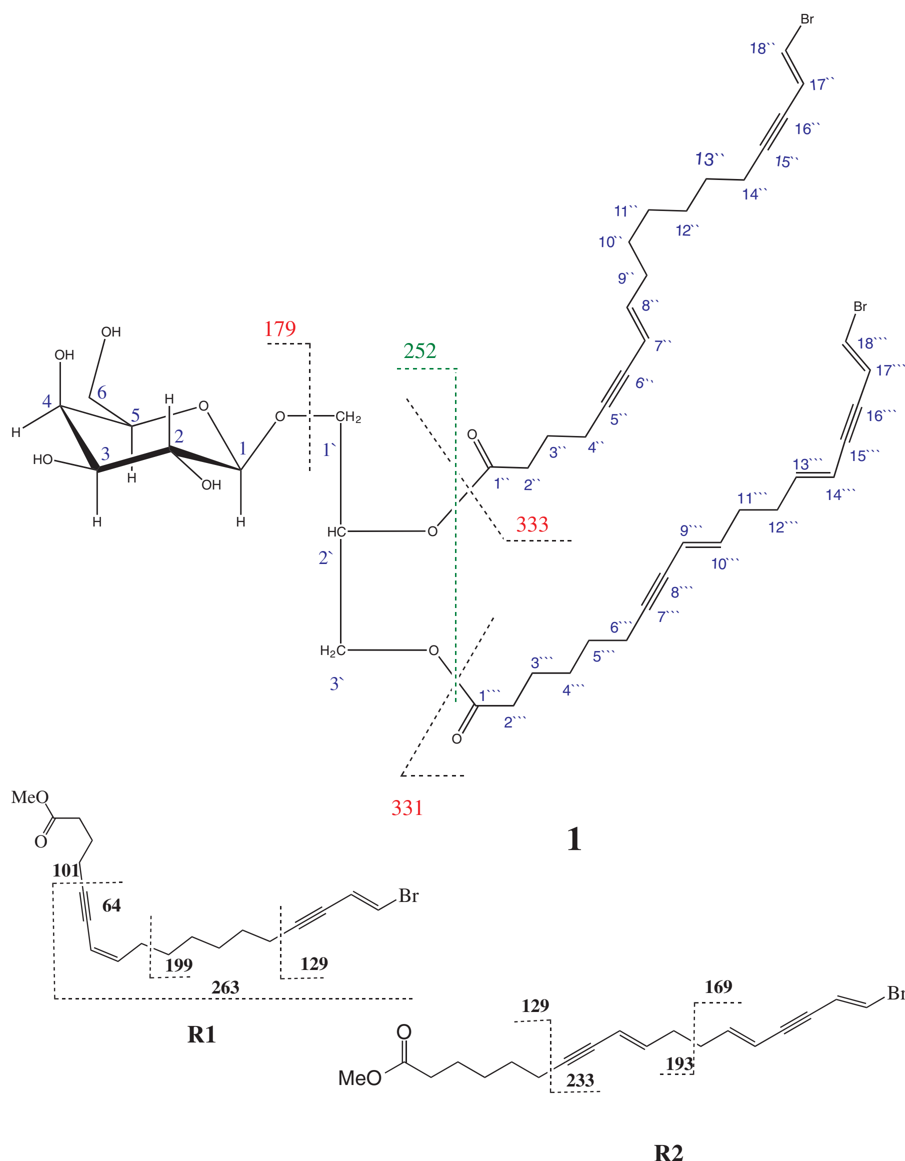

Figure 2: Fragmentation of LCMS spectrum of compound **1** and prominent mass spectral fragment peaks (*m/z*) of **R1** and **R2**.

the IR absorption band at 1736 cm^{-1} assigned a diglyceride skeleton. The stereochemistry of the sugar moiety was revealed to be that of a glucose derivative based on the large coupling constants (9 Hz) between H_2-H_3, H_3-H_4, H_4-H_5. The β-anomeric structure was assigned from the coupling constant (J_{1-2} = 7.8 Hz) and NOESY correlations of H_1/H_3 and H_1/H_5. Hence, **1** is a glucopyranosyl diacylglycerol. The acid hydrolysis and methanolysis method of Hirsh et al. [13] yielded two UV_{254} absorbing substances after purification employing column and PTL chromatography (*cf. exp.*). The two substances were assigned as R_1 and R_2 methyl esters (**R1** and **R2**, respectively).

R1 was obtained as yellow oil with a molecular formula of $C_{19}H_{25}O_2Br$, as established by high resolution fast-atom bombardment mass spectrometry (HRFABMS).

Gas chromatography-mass spectrometry (GCMS) revealed the presence of one bromide atom (two mass unit isotopic peaks in 1:1 ratio). Combination of ^{13}C NMR spectrometry with a heteronuclear single quantum correlation (HSQC) experiment revealed the presence of four singlet acetylenic carbon atoms in **R1**, four protonated olefinic carbon atoms, along with signals for nine paraffinic methylene carbon atoms (Table 1).

The ^{1}H NMR spectrum featured the following: a brominated vinyl group [δ_H 6.23 (dt, 13.8, 1.8 Hz, H-17″ and 6.69 (d, 13.8, Hz, H-18″)] and a *trans*-disubstituted double bond [δ_H 5.46 (dt, 15.6, 1.8 Hz, H-7″ and 5.98 (dt, 15.6, 6.6 Hz, H-8″)], i.e. H-7″. The COESY and HSQC experiments allowed the assignment of the subunits C-2″/C-4″, C-7″/C-8″ and C-17″/C-18″ within **R1**, which were

substantiated by heteronuclear multiple bond correlation (HMBC) data. HMBC correlations of H_2-4″/C-5″, H-7″/C-5″, and H-17″/C-16 established the unambiguous placement of the conjugated diyne moieties at C-8″/C-5″ and C-15″/C-18″. The structure elucidation of **R1** based on NMR data was further confirmed by mass spectral analysis. Electron impact mass spectrometry (IMS) data of **R1** exhibited several conspicuous fragments containing linear alkyl chains [14]. Fragments observed at *m/z* 263, 199, 129, 101, and 64 supported the structure assigned for **R1** (Figure 2). Accordingly, the moiety $\mathbf{R_1}$ was assigned the structure 7*E*,17*E*-18-bromo-octaoctadeca-7,17-dien-5,15-diynoyl.

R2 was obtained as yellow oil with a molecular formula of $C_{19}H_{23}O_2Br$, as established by HRFABMS. GCMS revealed the presence of one bromide atom (two mass units' isotopic peaks in 1:1 ratio). By a similar treatment of both NMR and EIMS data, the connectivities, the geometry, and positions of double and triple bonds were indicated (Table 1 and Figure 2). Accordingly, the moiety $\mathbf{R_2}$ was assigned the structure 9*E*,13*E*,17*E*-18-bromo-octaoctadeca-9,13,17-dien-7,15-diynoyl. Taking into consideration that $\mathbf{R_1}$ and $\mathbf{R_2}$ may be interchangeable, compound **1** is a new acylglycerolipid; 2′-O-(7*E*,17*E*-18-bromo-octaoctadeca-7,17-dien-5,15-diynoyl)-3′-O-(9*E*,13*E*,17*E*)-18-bromo-octaoctadeca-9,13,17-dien-7,15-diynoyl-1′-*O*-*β*-*D*-glucopyranosyl glycerol (Figure 2).

Compound **2** was isolated as pale yellow oil. The molecular formula, $C_{20}H_{28}BrClN_2O$, was deduced by LCMS at *m/z* 426:428:430 (2:3:1) $[M]^+$, indicating that **2** has two halogen atoms (one Br and one Cl), with seven degrees of unsaturation. The infrared (IR) spectrum showed the presence of a carbon–carbon triple bond (2215 cm^{-1}), a carbonyl ester group C=O (1741 cm^{-1}), amide and amine function bands (3120–3434 cm^{-1}). The ^{13}C and DEPT NMR measurements indicated 20 signals, categorized into 1) a quaternary carbonyl δ_C (173.1 ppm), 2) four quaternary acetylenic carbons (92.7, 88.5, 79.2 and 77.5), 3) four olefinic methine, (142.6, 118.0, 117.1 and 110.2), 4) a methine (68.9), 5) a halogenated methylene (62.1), and 6) nine methylene δc (33.9, 32.3, 28.4, 28.3, 27.9, 27.7, 24.4, 19.2 and 19.1) carbons, respectivekly (Table 1). So, compound **2** must be acyclic.

The ^1H and ^{13}C NMR together with ^1H-^1H COSY spectral data indicated a disubstituted carbon-carbon double bond [δ_H 6.17 ppm (dt, 13.8, 1.8 Hz, H-17; δ_C 118.0, C-17) and 6.57 (d, 13.8 Hz, H-18; δ_C 117.1, C-18)], which was coupled on one side only to a two proton signal at δ_H 2.25 (td, 6.6, 1.8 Hz, H-14). The small coupling (1.8 Hz) originated from a long range effect between H-17 and H-14 through the acetylenic group. The large coupling constants between H-17 and H-18 (13.8 Hz) indicated the *E* geometry of the olefinic moiety. Investigation of the HMBC spectral data indicated correlations between H-18 and C-17, C-16 and C-15 and between H-17 and C-18 and C-15 (Figure 3). The presence of an ion peak at *m/z* 129$[C_4H_2Br]^+$ in the LCMS supported this fragment (Figure 4), led to establishing fragment 1 (Figure 4).

The ^1H-^1H COSY spectrum showed that chlorinated methylene protons (H_2-2′) at δ_H 4.28 (dd, 12.6, 4.2 Hz) and 4.13 (dd, 12.6, 6.6 Hz), are coupled to a low field methine proton H-1′ at δ_H 5.26 (dd, 6.6, 4.2 Hz) of an ABX system. The attachment of a primary amine and amide to C-1′ shifted the absorption of H-1′ to a more down field value (δ_H 5.26). Further investigation of the HMBC spectrum indicated a correlation between H-1′ and C-2′ with the amidic carbonyl between H-2′ and C-1′ and C-1 (Figure 3) establishing fragment 3 (Figure 4).

With the aid of the data in Table 1 and Figure 4, the structures of fragments 2, 4, and 5 can be deduced. The connections between the fragments were performed through interpretation of ^1H-^1H COSY and HMBC spectral data. A computer survey of different data bases, including SciFinder, indicates that **2** is a new xestospongia amide. Compounds **3** and **4** were identified as xestosterol and xestosterol (9*E*,17*E*)-18-bromooctadeca-9,17-dien-7,15-diynoic acid, respectively, by comparing their spectroscopic data with those in the literature [15, 16]. The antimicrobial activities of **1** and **2** were determined against

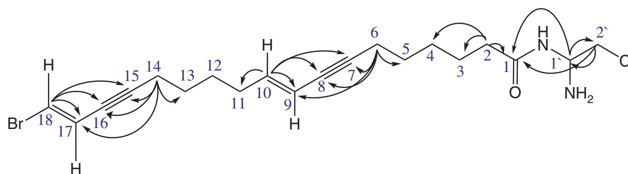

Figure 3: Selected HMBC of compound **2**.

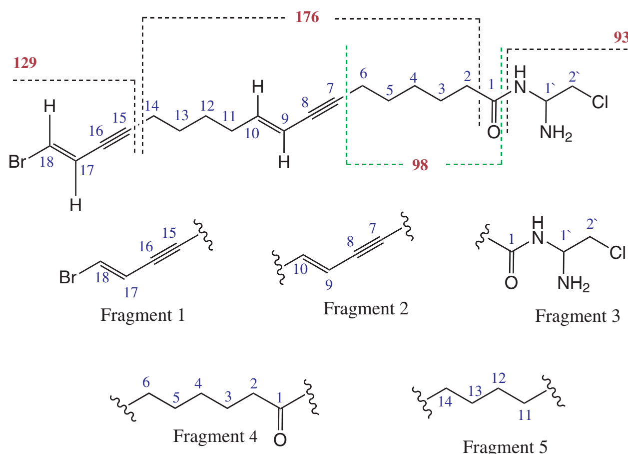

Figure 4: Fragmentation of LCMS spectrum of compound **2** and its main fragments.

Table 2: The antimicrobial activities (diameter of inhibition zone, mm) of **1** and **2** and positive control compounds (5 μg/mL).

Tested bacteria	Diameter of inhibition zone[a]±SD (mm)			Tested fungi	Diameter of inhibition zone±SD (mm)		
	1	2	Ampicillin		1	2	Amph. B
Acinetobacter baumannii	14±3.2	15±3.2	NF	*Candida albicans*	NF	24±1.2	14±1.2
Escherichia coli	23±1. 7	12±3.2	9±1.2	*Candida tropicalis*	NF	17.4±1.7	NF
Klebsiella pneumoniae	15±1.2	19±3.2	7±2.2	*Cryptococcus neoformans*	NF	15.8±0.7	9±2.1
Pseudomonas aeruginosa	24±2.2	19±3.2	9±3.0	*Aspergillus niger*	NF	13±2.1	13±1.1
Staphylococcus aureus	12±1.4	13±1.8	19±1.4	*Epidermophyton* sp.	NF	14.0±1.8	10±1.2
MRSA	12±1.3	14±3.2	NF	*Microsporum gypseum*	NF	17.2±1.9	11±2.1
S. epidermidis	16±1.4	14±3.2	NF	*Trichophyton rubrum*	NF	16.0±1.0	9±1.7
Streptococcus pneumoniae	13±1.5	14±3.2	10±1.2	*Trichophyton mentagrophytes*	NF	19.4±1. 4	11±2.7

[a]Mean diameter of inhibition ± standard deviation; NF, no inhibition zone; Amph. B, amphotericin B; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 3: Minimal inhibitory concentration (μM) of **1** and **2**.

Tested bacteria	MIC (μM)			Tested fungi	MIC (μM)		
	1	2	Ampicillin		1	2	Amph. B
A. baumannii	4.2	2.5	≥2.6	*C. albicans*	2.2	≥5	0.9
K. pneumoniae	4.5	2.5	2.6	*A. niger*	2.5	≥5	1.1
P. aeruginosa	≥5	≥5	2.6	*Epidermophyton* sp.	2.5	≥5	0.9

Table 4: Toxicity and antitumor activity of **1** and **2**.

Test used	1	2	Bleomycin
Toxicity against *Artemia salina* (LD_{50}, μM)	≥5	≥5	≥5
Antitumor (LD_{50}, μM)			
Ehrlich Ascites carcinoma	≥5	5.0	0.02
Lymphocytic Leukemia	≥5	5.0	0.02

some multidrug-resistant bacteria using an agar well diffusion assay. DMSO was used as negative control while ampicillin and amphotericin B were used as positive antibacterial and antifungal agents, respectively. The mean diameter of the inhibition zones was in the range of 12–23 mm (Table 2). The antifungal activity was recorded against the dermatophytes *A. niger, C. albicans* ATCC1023, *C. tropicalis, C. neoformans*. Product **1** showed no antifungal activity, while compound **2** exhibited excellent antifungal activity against *A. niger, C. albicans, C. tropicalis, C. neoformans, Epidermophyton* sp., *M. gypseum, T. mentagrophytes,* and *T. rubrum* (diameters of inhibition zones ranged from 14–22 mm). MICs were calculated for each product and ranged from 2.2 to 4.5 μM for *A. baumannii, K. pneumonia* and *P. aeruginosa*; those for *C. albicans, A. niger,* and *Epidermophyton* sp. were in the range of 2.2–2.5 μM (Table 3). Compounds **1** and **2** were not cytotoxic against *Artemia salina*, while compound **2** exhibited antitumor activity against Ehrlich ascites carcinoma and lymphocytic leukemia at LD_{50} 5 μM (Table 4).

In conclusion, the results of this study confirm that product **2**, obtained from the *Xestospongia* extract, can be considered a potential material for designing effective antibacterial, antifungal and antitumor agents for treatment of multidrug resistant bacteria, tumors, dermatophytosis, and other fungal infections with no cell toxicity.

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