A New Cytotoxic Brominated Acetylenic Hydrocarbon from the Marine Sponge *Haliclona* sp. with a Selective Effect against Human Breast Cancer

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Three acetylenic brominated derivatives were isolated from a Red Sea sponge, *Haliclona* sp. One of them, 18-bromooctadeca-9(*E*),17(*E*)-dien-7,15-diynoic acid (**3**), is a known metabolite, and the other two are new compounds, (1*E*,5*E*,12*E*,19*E*)-1,22-dibromodocosa-1,5,12,19-tetraen-3,14,21-triyne (**1**) and methyl 18-bromooctadeca-9(*E*),17(*E*)-dien-7,15-diynoate (**2**) which was isolated for the first time as a natural metabolite. Structures of all compounds were determined based on extensive spectroscopic measurements [1D (^{1}H, ^{13}C and DEPT) and 2D (HSQC, HMBC and NOESY) NMR, MS, UV, and IR]. All compounds, except **3**, were evaluated for their cytotoxicity employing four cancer cell lines, *i.e.* MCF-7 (human breast cancer), HepG2 (human hepatocellular carcinoma), WI-38 (skin carcinoma), and *Vero* (African green monkey kidney). Compounds **1** and **2** had potent selective anti-tumour activity towards MCF-7 cells with IC_{50} values of 32.5 and 50.8 μM, respectively.

Key words: Marine Sponge *Haliclona* sp., Acetylenic, Brominated Fatty Acid

Introduction

Cancer is one of the leading causes of death in the world (Li *et al*., 2009). According to the International Agency for Research on Cancer, more than 7 million people died from cancer in 2008, and this number is anticipated to triple by 2030 (Balachandran and Govindarajan, 2005). Cancer treatment is designed by two major approaches, *i.e.* chemical-biological and target-based approaches. The first has gained significant attention in the last decades (Chang, 1998). For instance, the Food and Drug Administration approved imatinib mesilate as a first-line treatment for chronic myelogenous leukemia (Parker *et al.*, 1997). The diversity of bioactive natural metabolites resulted in growing interest to return to natural remedies and to increase research in this area, finally leading to the discovery of taxol (Sporn and Suh, 2000).

Sponges of the genus *Haliclona* (Phylum Porifera; class, Demospongiae; order, Haplosclerida; family, Chalinidae) were proven to be the source of more than 200 compounds, belonging to different classes, such as haliclonacyclamines (Mudianta *et al*., 2009; Ayyad *et al*., 2009), haliclonadiamines, papuamine (Satoe *et al.*, 2009; Masayoshi *et al.*, 2009), halipeptins (Barker *et al.*, 2007), and halaminols A, B, and C (McDermott *et al*., 1996). The brominated fatty acids and the polyacetylenes are interesting due to their unique chemical structures as well as their biological activities (Gribble, 1996; Dembitsky and Srebink, 2002).

Results

In the course of our projects on the isolation of bioactive metabolites from marine organisms, we have collected a marine sponge, identified

as *Haliclona* sp., from the waters around Saudi Arabia, and isolated and identified three brominated unsaturated derivatives of which two are new, *viz.* (1*E*,5*E*,12*E*,19*E*)-1,22-dibromodocosa-1,5,12,19-tetraen-3,14,21-triyne (**1**) and methyl 18-bromooctadeca-9(*E*),17(*E*)-dien-7,15-diynoate (**2**), while the third one, 18-bromooctadeca-9(*E*),17(*E*)-dien-7,15-diynoic acid (**3**), is a known metabolite (Fig. 1). Compounds **1** and **2** were evaluated for their *in vitro* anticancer activities and found to be selective against the breast cancer cell line MCF-7 (human breast cancer) with IC_{50} values of 32.5 and 50.8 μM, respectively (Table I).

The molecular formula of **1** was established as $C_{22}H_{24}{}^{79}Br_2$ based on HREIMS determinations (*m/z* = 264.1024 [M^+]). The ^{13}C NMR spectrum of **1** showed 22 resonances attributable to eight methylene, eight methine groups, and six quaternary carbon atoms. An extensive interpretation of the ^1H and ^{13}C NMR spectral data identified ten elements of unsaturation (Table II) that could be attributed to four carbon-carbon double bonds and three carbon-carbon triple bonds which were disubstituted acetylenic based on the IR spectral data (ν = 2220 cm^{-1}), and conjugations are apparent from the UV spectrum (λ_{max} = 285, 272, 228 nm). The molecule thus has a straight chain and no hydroxy groups.

After association of all protons with directly attached carbon atoms via 2D NMR (HMQC) spectral measurements, it was possible to deduce the structure of **1** by interpretation of the ^1H-^1H COSY and ^1H-^{13}C HMBC spectra. From the ^1H and ^{13}C NMR spectral data, four olefinic signals with δ_H 6.66 ppm (d, *J* = 13.8 Hz, H-1; δ_C 117.8 ppm, C-1), 6.31 ppm (d, *J* = 13.8, 2.4 Hz, H-2; δ_C 117.6 ppm, C-2), 5.58 ppm (d, *J* = 16.0 Hz, H-5; δ_C 110.0 ppm, C-5), and 6.17 ppm (dt, *J* = 16.0, 6.6 Hz, H-6; δ_C 144.3 ppm, C-6), respectively, were identified. From the ^1H-^1H COSY spectrum of **1**, a ^1H-^1H spin system between H-1 and H-2 and between H-5 and H-6 was derived. Further investigation of the HMBC NMR spectral data indicated correlations between H-1 and C-2 and C-3; between H-2 and C-1, C-3, and C-4; between H-5 and C-3, C-4, and C-6; and between H-6 and C-4 and C-5, respectively. On these bases, fragment 1 was established (Fig. 2).

The ^1H-^1H spin system was observed between H-12 (δ_H 6.17 ppm, dt, *J* = 16.0, 6.6 Hz) and H-13 (δ_H 5.58 ppm, d, *J* = 16.0 Hz). Long-range C-H correlations were observed between the resonances of H-12 and those of C-13 and C-14; between H-13 and those of C-12 and C-14 and C-15, which led to the definition of fragment 2 (Fig. 2).

Further investigation of the COSY spectrum identified a ^1H-^1H spin system between H-19 (δ_H 6.02 ppm, dt, *J* = 15.6, 7.2 Hz) and H-20 (δ_H 5.49 ppm, br d, *J* = 15.6, 3.6 Hz). Long-range C-H correlations were observed between the resonances of H-19 and those of C-20 and C-21, as

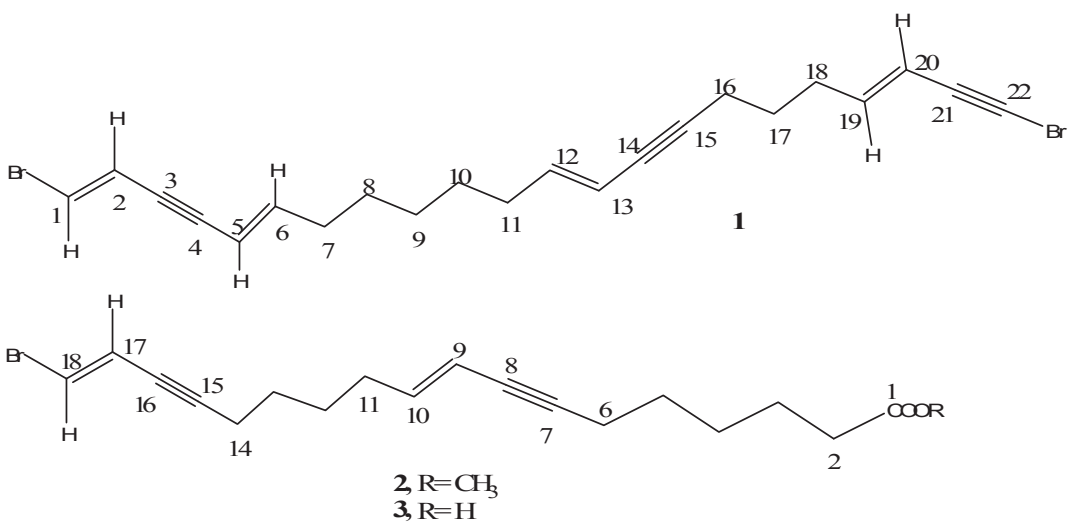

Fig. 1. Chemical structures of (1*E*,5*E*,12*E*,19*E*)-1,22-dibromodocosa-1,5,12,19-tetraen-3,14,21-triyne (**1**), methyl-18-bromooctadeca-9(*E*),17(*E*)-dien-7,15-diynoate (**2**), and 18-bromooctadeca-9(*E*),17(*E*)-dien-7,15-diynoic acid (**3**).

Table I. *In vitro* cytotoxic activities of **1** and **2** against four cell lines.

Compound	IC_{50} [μM] [a]			
	HepG2	WI-38	*Vero*	MCF-7
1	336.8 ± 1.07	257.0 ± 0.02	274.4 ± 2.04	32.5 ± 0.16
2	305.5 ± 3.67	240.1 ± 1.98	220.6 ± 2.47	50.8 ± 0.03
5-Fu[b]	60.8 ± 0.14	31.5 ± 0.09	45.4 ± 0.05	26.2 ± 0.01

[a] Mean ± standard deviation, n = 5. HepG2, human hepatocellular carcinoma; WI-38, skin carcinoma; *Vero*, African green monkey kidney cells; MCF-7, human breast cancer.
[b] Fluorouracil (5-Fu) is used as a positive control.

Table II. ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR spectral data of compounds **1** and **2**[a].

C	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	117.8 (d)	6.66 (1H, d, 13.8)[b]	178.5 (s)	-
2	117.6 (d)	6.31 (1H, br d, 13.8, 2.4)	33.7 (t)	2.34 (2H, t, 7.8)
3	82.2 (s)	-	24.2 (t)	1.63 (2H, m)
4	87.9 (s)	-	27.2 (t)	1.53 (2H, m)
5	110.0 (d)	5.58 (1H, d, 16.0)	27.7 (t)	1.53 (2H, m)
6	144.3 (d)	6.17 (1H, dt, 16.0, 6.6)	19.2 (t)	2.29 (2H, m)
7	32.0 (t)	2.11 (2H, m)	88.5 (s)	-
8	28.4 (t)	1.40 (2H, m)	79.2 (s)	-
9	28.8 (t)	1.30 (2H, m)	110.0 (d)	5.47 (1H, br dt, 15.6, 1.8)
10	28.4 (t)	1.40 (2H, m)	141.4 (d)	6.01 (1H, dt, 15.6, 7.2)
11	32.0 (t)	2.11 (2H, m)	32.4 (t)	2.10 (2H, m)
12	144.3 (d)	6.17 (1H, dt, 16.0, 6.6)	28.3 (t)	1.52 (2H, m)
13	110.0 (d)	5.58 (1H, d, 16.0)	28.3 (t)	1.52 (2H, m)
14	79.7 (s)	-	19.2 (t)	2.30 (2H, m)
15	90.3 (s)	-	92.2 (s)	-
16	18.8 (t)	2.39 (2H, m)	77.4 (s)	-
17	23.9 (t)	1.85 (2H, m)	117.8 (d)	6.17 (1H, dt, 14, 2.4)
18	33.2 (t)	2.45 (2H, m)	117.2 (d)	6.58 (1H, d, 14)
19	141.7 (d)	6.02 (1H, dt, 15.6, 7.2)	-	-
20	116.5 (d)	5.49 (1H, br d, 15.6, 3.6)	-	-
21	88.6 (s)	-	-	-
22	84.9 (s)	-	-	-
OMe	-	-		3.67 (3H, s)

[a] All assignments are based on 1D and 2D NMR measurements (HSQC, HMBC, and COSY).
[b] Implied multiplicities as determined by DEPT (C, s; CH, d; CH_2, t; CH_4, q). J in Hz.

well as between H-20 and those of C-19, C-21, and C-22, allowing to define fragment 3 (Fig. 2).

Upon further investigation of the ^{1}H NMR spectral data, three multiplets assigned at δ_H 2.45, 1.85, and 2.39 ppm were found to integrate two protons each. In the ^{13}C NMR spectral data, three methylene carbon atoms (δ_C 18.8, 23.9, and 33.2 ppm) were identified. From the ^{1}H-^{1}H COSY spectrum of **1**, ^{1}H-^{1}H spin systems between H_2-16 and H_2-17, and between H_2-17 and H-18 were derived. This deduction was supported by HMBC correlations, leading to the definition of fragment 4 (Fig. 2).

From the ^{1}H NMR spectral data, three multiplets assigned at δ_H 2.11, 1.40, and 1.30 ppm were found to integrate four, four, and two protons, respectively. In the ^{13}C NMR spectral data, three chemical shifts appearing at δ_C 32.0, 28.4, and 28.8 ppm indicated five methylene carbon atoms based on the DEPT spectral data. From the ^{1}H-^{1}H COSY spectrum of **1**, ^{1}H-^{1}H spin systems between H_2-7 and H_2-8, and between H_2-8 and H_2-9 were derived. From the ^{1}H-^{1}H COSY spectrum of **1**, ^{1}H-^{1}H spin systems between H_2-7 and H_2-8, between H_2-8 and H_2-9, between H_2-9 and H_2-10, and between H_2-10 and H_2-11 were supported by

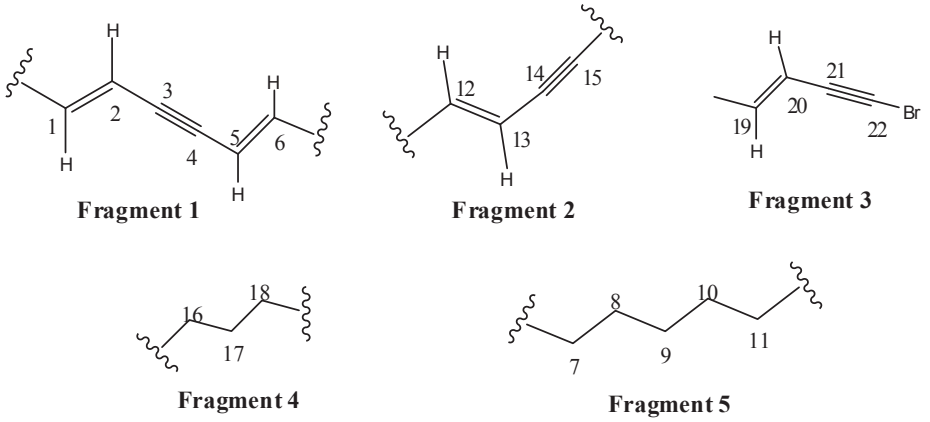

Fig. 2. Fragments of **1** deduced from NMR spectroscopic data.

HMBC correlations and allowed the definition of fragment 5 (Fig. 2).

The connections between the five fragments were established through the interpretation of the ^{1}H-^{1}H COSY and HMBC NMR spectral data. The remaining two positions at C-1 and C-22 were suspected to be occupied by two hydroxy groups or halogen atoms. An extensive investigation of the mass and ^{13}C NMR spectra, respectively, indicated that they are indeed brominated, based on the chemical shifts δ_C 117.8 and 84.9 ppm. This conclusion was supported by comparison of the observed with published data. The relative stereochemistry of **1** was derived from the coupling constants. A computer survey employing different data bases indicated that compound **1** is a new acetylenic derivative, *i.e.* (1*E*,5*E*,12*E*,19*E*)-1,22-dibromodocosa-1,5,12,19-tetraen-3,14,21-triyne.

The structure of **2** was elucidated based on the molecular formula of $C_{19}H_{25}BrO_2$ which was derived from the HREIMS measurements (*m/z* = 364.1024 [M$^+$]). After extensive study of the ^{1}H and ^{13}C NMR spectral data (Table II), we realized that the molecular mass of **2** is higher than that of **3** by 14 mass units, indicating that it is the methyl ester of **3**. Thus compound **2** was identified as methyl 18-bromooctadeca-9(*E*),17(*E*)-dien-7,15-diynoate (Fig. 1), which has been isolated here for the first time as a natural compound, while it was previously prepared by semi-synthesis of **3** (Hirsh *et al.*, 1987). As methanol was not employed in the extraction and purification of **2**, an artifact can be excluded.

The cytotoxicity of the new compounds **1** and **2** was assessed in four cancer cell lines, *i.e.* MCF-7 (human breast cancer), HepG2 (human hepatocellular carcinoma), WI-38 (skin carcinoma), and *Vero* (African green monkey kidney) (Table I). 5-Fluorouracil, a known anticancer drug, was used as a positive control. Compounds **1** and **2** had significant selective antitumour activity towards the breast cancer cell line MCF-7 with IC_{50} values of 32.5 and 50.8 μM, respectively.

Material and Methods

General

Silica gel GF 254 (Merck, Darmstadt, Germany) was used for analytical thin-layer chromatorgraphy (TLC). Preparative thin-layer chromatography (PTLC) was performed on aluminum oxide plates (20 cm x 20 cm) of 250 μm thickness. Electron impact (EI) mass spectra were determined at 70 eV on a Kratos (Manchester, UK) MS-25 instrument. 1D and 2D NMR spectra were recorded in $CDCl_3$ on Bruker (Karlsruhe, Germany) AVANCE III WM 600 MHz spectrometers, ^{13}C NMR spectra at 150 MHz and ^{1}H NMR spectra at 600 MHz. Tetramethylsilane (TMS) was used as internal standard. Plates were sprayed with 50% sulfuric acid in methanol and heated at 100 °C for 1–2 min.

Sponge sample

The sponge *Haliclona* sp. was collected from Sharm Obhur, Jeddah, Saudi Arabia, and was identified by Dr. Yahia Folos (Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia). A voucher sample (JAD 04050) has been deposited at the Marine Chemistry Depart-

ment, King Abdulaziz University, Jeddah, Saudi Arabia.

Extraction and isolation of compounds

The freeze-dried sponge (40 g) was extracted two times with 6 L of a mixture of *n*-hexane/chloroform (1:1, v/v) for 24 h at 22 °C, and a viscous dark reddish oil was obtained (1.3 g). This extract was fractionated on NP-silica (5 x 25 cm, 50 g, Merck 7739), employing gradient elution from *n*-hexane to EtOAc; the fraction eluted with *n*-hexane/EtOAc (8:2, v/v, 300 mg) was further fractionated by vacuum liquid chromatography (VLC). The fraction eluted with *n*-hexane/EtOAc (9:1, 40 mg) was re-purified by PTLC employing *n*-hexane/EtOAc (9:1) and yielded **1**–**3**. All compounds were purified on Sephadex LH 20 with $CHCl_3$/MeOH (9:1) as solvent.

(1E,5E,12E,19E)-1,22-Dibromodocosa-1,5,12,19-tetraen-3,14,21-triyne (**1**): White residue. – Yield 4 mg (0.003%). – M.p. 35 °C. – R_f 0.83 (*n*-hexane/EtOAc, 9:1). – IR: ν = 2925, 2853, 2220 cm^{-1}. – UV/Vis (*n*-hexane): λ_{max} = 285, 272, 228 nm. – EIMS (70 eV): *m/z* (rel. int.) = 366, 368. – HREIMS: *m/z* = 446.0230 $[M]^+$, calcd. for $C_{22}H_{24}Br_2$ *m/z* = 446.0245. – ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$): see Table II.

Methyl-18-bromooctadeca-9(E),17(E)-dien-7,15-diynoate (**2**); Semi-solid white material. – Yield 4 mg (0.003%). – M.p. 42 °C. – R_f 0.74 (*n*-hexane/EtOAc, 9:1). – IR: ν = 3000, 2400, 2220, 1731, 1605, 1100 cm^{-1}. – UV/Vis (*n*-hexane): λ_{max} = 266, 252, 248 nm. – EIMS (70 eV): *m/z* (rel. int.): = 366/364, 285, 178, 172, 134. – HREIMS: *m/z* = 364.1024 $[M]^+$, calcd. for $C_{19}H_{25}BrO_2$ *m/z* = 364.1038. – ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$): see Table II.

18-Bromooctadeca-9(E),17(E)-dien-7,15-diynoic acid (**3**): White material. – Yield 6 mg (0.005%). – M.p. 65 °C. – R_f 0.20 (*n*-hexane/EtOAc, 9:1). – IR: ν = 3100, 2496, 2220, 1714, 1605 cm^{-1}. – UV/Vis (*n*-hexane): λ_{max} = 235 nm. – EIMS (70 eV): *m/z* (rel. int.) = 352/350, 271, 178, 172, 134. – HREIMS: *m/z* = 350.0869 $[M]^+$, calcd. for $C_{18}H_{23}BrO_2$ *m/z* = 350.0881. – ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$): see Table II.

Bioassays for cytotoxicity

Cytotoxic activities of compounds **1** and **2** were tested against the human hepatocellular carcinoma cell line HepG2, the skin carcinoma cell line WI-38, the normal adult African green monkey kidney cell line *Vero*, and the human breast cancer cell line MCF-7. The percentage of viable cells was estimated by using 5-fluorouracil as a positive standard anticancer drug. The assays were performed according to published protocols (Abdel-Wahab *et al.*, 2011).

Statistical analysis

All experiments were conducted five times and data are expressed as the mean with the standard error of the mean (S.E.M.).

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