New fatty acids from the Red Sea sponge *Mycale euplectellioides*

Gamal A. Mohamed\(^{a,b}\), Ali E.E. Abd-Elrazek\(^b\), Hashim A. Hassanean\(^c\), Abdulrahman M. Alahdal\(^d\), Ameen Almohammad\(^d\) and Diaa T.A. Youssef\(^{a,c,*}\)

\(^{a}\)Department of Natural Products, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia; \(^{b}\)Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Assiut 71524, Egypt; \(^{c}\)Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt; \(^{d}\)Department of Clinical Pharmacy, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia

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Chemical investigation of the Red Sea sponge *Mycale euplectellioides* afforded two new compounds; hexacosa-(6\(^Z\),10\(^Z\))-dienoic acid methyl ester (1) and hexacosa-(6\(^Z\),10\(^Z\))-dienoic acid (2), along with two known compounds: icosa-(8\(^Z\),11\(^Z\))-dienoic acid methyl ester (3) and \(\beta\)-sitosterol (4). The structures were elucidated by the interpretation of their spectral data. The total methanol extract (TME) of the sponge exhibited potent antimicrobial activity against the different strains at a concentration of 100 \(\text{mg/mL}\). All tested fractions did not exhibit any activity against *Serratia marcescens* and tested fungal strains. The TME and different fractions displayed anti-inflammatory and antipyretic activities at doses of 100 and 200 \(\text{mg/kg}\) compared with indomethacin (8 \(\text{mg}\)). The TME exhibited a remarkable hepato-protective effect in CCl\(_4\)-induced liver damage compared with silymarin. Furthermore, compounds 1 and 2 displayed weak activity against A549 non-small cell lung cancer, the U373 glioblastoma and the PC-3 prostate cancer cell lines.

**Keywords:** Red Sea sponge; *Mycale euplectellioides*; fatty acids; anti-inflammatory; hepato-protective; cancer growth inhibitory activity

1. Introduction

The sea is an important source of new natural compounds (Skropeta 2008; Blunt et al. 2013). The Red Sea has high levels of marine biodiversity and great seasonal fluctuations of air and water temperatures. The marine environment is proven to be a rich source of natural products that have a wide variety of biological activities. During the last three decades, more than 15,000 natural products have been isolated from marine organisms.\(^1\) Sponges have evolved a vast number of bioactive secondary metabolites to protect themselves against predation, overgrowth by other organisms and bacterial and fungal infections (Proksch et al. 2003; Schupp et al. 2009). Sponges of the genus *Mycale* are a rich source of bioactive natural compounds with diverse chemical structures. The mycalamides (triisoxazole-containing macrolides) (Perry et al. 1990), mycalolides (Fusetani et al. 1989), diterpenoid rotalins (Corriero et al. 1989), mycalisines (Kato et al. 1985), polybrominated C-15 acetogenins (Giordano et al. 1990), brominated isocoumarins (Fusetani et al. 1991) and norterpene cyclic peroxides (Capon & MacLeod 1987) are examples of compounds isolated from the genus *Mycale*. In this study, we report the isolation and structural elucidation of two new compounds (1 and 2), together with two known compounds (3 and 4) from the Red Sea sponge *Mycale euplectellioides*, which are reported here for the first time.

\(*\)Corresponding author. Email: dyoussef@kau.edu.sa

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from the sponge (Figure 1). The total methanol extract (TME), EtOAc and aqueous fractions were evaluated for their antimicrobial, anti-inflammatory, antipyretic and hepato-protective activities. The in vitro growth inhibitory activity of the isolated compounds against non-small cell lung cancer (NSCLC), glioblastoma (GBM) and PC-3 prostate cancer cell lines was evaluated.

2. Results and discussion

Compound 1 was isolated as an oily substance. The gas chromatograph (GC)–mass spectrometer (MS) of 1 revealed a molecular ion peak at m/z 406 [M]+, which is consistent with a formula of C_{27}H_{50}O_{2}. The ^1H NMR spectrum exhibited four olefinic protons at δ_H 5.36 (1H, m, H-6), 5.35 (1H, m, H-7), 5.34 (1H, m, H-10) and 5.33 (1H, m, H-11) consistent with two olefinic double bonds in 1. It also revealed characteristic signals for terminal CH₃ protons of fatty acid at δ_H 0.87 (3H, t, J = 6.8 Hz), broad signals at δ_H 1.25–2.29 for methylene protons and one methoxy at δ_H 3.66 suggesting the fatty acid methyl ester nature of 1. The ^13C NMR and HSQC spectral data revealed the presence of 27 carbon signals consisting of an ester carbonyl at δ_C 174.3 (C-1), 4 olefinic carbons at δ_C 130.5 (C-6), 130.4 (C-7), 128.9 (C-10) and 128.8 (C-11), 1 methoxy at δ_C 51.4, 20 methylenes between 22.7 and 34.1 ppm and a methyl carbon at δ_C 14.1. The HMBC spectrum (Figure S11, see Supplementary materials) clearly pinpointed correlations of H-5 to C-6 and C-7, H-7 to C-5, H-9 to C-11, H-10 and H-11 to C-9, which established the position of the double bonds between C-5/C-6 and C-10/C-11 and further confirmed by the fragment ion peaks at m/z 291 (4%) and 141 (46%) for C-6/C-7 double bond and m/z 182 (5%) and 169 (6%) for C-10/C-11 double bond. The methyl ester nature was confirmed by the HMBC cross-peak between the methoxy group and C-1 (δ_C 178.3) (Pouchert & Behnke 1993). In addition, cross-peaks of H-2 to C-1 (δ_C 178.3) and C-3 (δ_C 24.7) were observed. The geometry of the double bonds was deduced to be Z based on coupling constant values and by comparing with the literature (Hsu & Turk 2008; Elkhayat et al. 2012). Accordingly, the
structure of 1 was assigned as hexacosa-(6Z,10Z)-dienoic acid methyl ester and considered as a new natural product.

Compound 2 was isolated as white crystals. Its GC–MS spectrum revealed a molecular ion peak at \( m/z \) 392 [M]\(^+\), that is compatible with a molecular formula of \( C_{26}H_{48}O_2 \), implying 3 degrees of unsaturation. Compound 2 is 14 mass units less than 1. The NMR spectral data of 2 were quite similar to those of 1, but the signals associated with the methoxy group (\( \delta_H 3.66/\delta_C 51.4 \)) were not present. The position of double bond was established by the COSY and HMBC correlations and confirmed by the fragmentation pattern in GC–MS spectrum (Pouchert & Behnke 1993; Hsu & Turk 2008). The fragment peak at \( m/z \) 127 and 168 (12%), 224 confirmed the position of double bonds between C-6/C-7 and C-10/C-11, respectively. On the basis of these findings, 2 was assigned as hexacosa-(6Z,10Z)-dienoic acid and considered as a new compound.

The known compounds (3 and 4) were identified through the analysis of the spectroscopic data and comparison of their data with those in the literature as icosa-(8Z,11Z)-dienoic acid methyl ester (3) (Pouchert & Behnke 1993) and \( \beta \)-sitosterol (4) (Sayed et al. 2007).

To evaluate the biological activities of the different extracts of the Red Sea M. euplectellioides, the extracts were evaluated in a panel of different biological screens including antimicrobial, anti-inflammatory, antipyretic and hepato-protective. In addition, the pure compounds were evaluated for their cancer growth inhibitory activity against three cell lines. In all screens, positive as well as negative controls were used. The antimicrobial results (Table 1) revealed that the TME was the most active fraction against all bacterial strains at a concentration of 100 mg/mL, while EtOAc fraction exhibited moderate activity against Bacillus cereus at all concentrations and Staphylococcus aureus at a concentration of 25 mg/mL. The aqueous fraction exhibited high activity against Pseudomonas aeruginosa (concentration 50 mg/mL). All tested fractions did not exhibit any activity against Serratia marcescens and all fungal strains. The TME and different fractions reduced the carrageenan-induced oedema with maximum effects being obtained after 4 h at concentrations of 100 and 200 mg/kg compared with indomethacin (Table 2). The tested extracts exhibited antipyretic activity at doses of 100 and 200 mg/kg for each. They control the hyperthermia for 4 h without decrease in activity compared with reference compound indomethacin (8 mg/kg) (Table 3). The decrease in CCl\(_4\)-induced elevated enzyme levels and serum bilirubin in animals after treatment with the TME revealed that it has a remarkable hepato-protective effect in CCl\(_4\)-induced liver damage compared with silymarin. This may be due to the maintenance of structural integrity of hepatocytic cell membrane or repair the damage of live cells (Table 4). The isolated compounds

| Inhibition zone diameter (mm/sample) | EtOAc fraction\(^a\) | Aqueous fraction\(^a\) | TME\(^a\) | Control\(^b\) | Control\(^c\) |
|-------------------------------------|----------------------|-----------------------|-----------|---------------|---------------|
| Organism                            | 25 50 100            | 25 50 100             | 25 50 100 | 25            | 25            |
| S. aureus                           | 9 0 8                | 8 11 0                | 0 0 14    | 22            | –             |
| B. cereus                           | 13 11 13             | 0 11 8                | 0 10 15 p.i. | 27            | –             |
| E. coli                             | 0 0 0                | 8 0 9                 | 9 0 13    | 25            | –             |
| P. aeruginosa                       | 0 0 0                | 8 10 0                | 0 0 13    | 15            | –             |
| S. marcescens                       | 0 0 0                | 0 0 0                 | 0 0 11    | 30            | –             |

Note: p.i., partial inhibition.
\(^a\) Concentration (mg/mL).
\(^b\) Chloramphenicol as antibacterial standard.
\(^c\) Clotrimazole as antifungal standard.
| Group          | Dose (mg/kg) | 1 h     | 2 h     | 3 h     | 4 h     |
|---------------|-------------|---------|---------|---------|---------|
| Control       | –           | 8.2 ± 0.04 | 7.9 ± 0.01 | 7.8 ± 0.03 | 8.2 ± 0.04 |
| Indomethacin  | 8           | 5.9 ± 0.09 (28.1)** | 5.7 ± 0.08 (27.9)** | 5.3 ± 0.08 (32.1)** | 4.4 ± 0.14 (45.1)** |
| EtOAc fraction| 200         | 7.7 ± 0.11 (6.1) | 7.0 ± 0.12 (11.4)** | 6.7 ± 0.07 (14.1)** | 6.2 ± 0.09 (28.1)** |
|               | 100         | 7.9 ± 0.14 (3.7) | 7.2 ± 0.14 (8.9)** | 6.9 ± 0.08 (12.8)** | 6.4 ± 0.06 (22.0)** |
| Aqueous fraction | 200       | 6.5 ± 0.15 (20.7)** | 6.3 ± 0.08 (20.3)** | 6.2 ± 0.15 (20.5)** | 5.5 ± 0.15 (32.9)** |
|               | 100         | 6.7 ± 0.15 (18.3)** | 6.5 ± 0.07 (16.5)** | 6.3 ± 0.14 (19.2)** | 5.8 ± 0.12 (30.5)** |
| TME           | 200         | 6.3 ± 0.15 (23.2)** | 6.0 ± 0.05 (24.1)** | 5.9 ± 0.04 (24.4)** | 5.8 ± 0.08 (29.3)** |
|               | 100         | 6.6 ± 0.13 (19.5)** | 6.2 ± 0.07 (21.5)** | 6.1 ± 0.05 (21.8)** | 6.1 ± 0.08 (25.6)** |

Notes: SE, standard error. n = 6. Differences with respect to the control group were evaluated using the student’s t-test (*P < 0.05, **P < 0.01, ***P < 0.001).
from the *M. euplectellioides* were tested for in vitro growth inhibitory activity. Compounds 1 and 2 displayed weak activity against all cancer cell lines (Table 5).

In conclusion, this study resulted in the identification of four compounds from the Red Sea marine sponge *M. euplectellioides*. Compounds 1 and 2 are new natural compounds, while 3 and 4 were reported here for the first time from the sponge. The TME, EtOAc and aqueous fractions exhibited antimicrobial, anti-inflammatory, antipyretic and hepatoprotective activities. Compounds 1 and 2 exhibited weak cytotoxic activity.

### 3. Experimental

#### 3.1. General experimental procedures

Melting points were obtained in an Electrothermal 9100 Digital Melting Point (Electrothermal Engineering Ltd, Essex, England). IR was measured with a Shimadzu Infrared-400
spectrophotometer (Shimadzu, Kyoto, Japan). Optical rotation was recorded on a Perkin-Elmer Model 341 LC Polarimeter (Perkin-Elmer, Waltham, MA, USA). GC–MS was recorded on GC–MS, Clarus 500 GC–MS (PerkinElmer, Shelton, CT, USA) was used. The software controller/integrator was Turbo Mass, version 4.5.0.007 (PerkinElmer). An Elite 5 GC–MS capillary column (30 mm × 0.25 mm × 0.5 μm, PerkinElmer) was used. The carrier gas was helium (purity 99.9999%) at a flow rate of 2 mL/min (32 psi, flow initial 55.8 cm/s, split; 1:40). Temperature conditions were as follows: inlet line temperature, 200°C; source temperature, 150°C; trap emission, 100°C and electron energy, 70 eV. The column temperature program was: 50°C for 5 min, increased to 220°C (rate, 20°C/min), and held for 5 min. The injector temperature was 220°C. MS scan was from 50 to 650 m/z. NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on Jeol Oxford NMR YH-400 using CDCl3 as solvent. NMR spectra were referenced to the solvent signal (CDCl3: 7.26 ppm for 1H and 77.0 ppm for 13C). Column chromatographic separation was performed on silica gel 60 (0.04–0.063 mm). TLC was performed on pre-coated TLC plates with silica gel 60 F254 (0.2 mm, Merck, Darmstadt, Germany). The compounds were detected by spraying with anisaldehyde/H2SO4 reagent and heating at 110°C for 1–2 min. Authentic sterols were obtained from the Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University and the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut.

3.2. Sponge material
The sponge, M. euplectellioides (Keller, 1889), was collected at depth 7–10 m off Hurghada. It forms upright hollow lobes. The voucher specimen measures 10 cm high, 6–8 cm in diameter, with a spinose macerated surface. Live sponge is red in colour and fades on preservation in 70% EtOH. The skeleton consists of a wide-meshed, loosely anastomosing, open network of spongin fibres, which are quite variable in thickness (50–500 μm) and enclose a variable core of 10–70 spicules in cross-section. Spicules are thin subtylostyles with wide axial lumen measuring, 180–230 μm×1–2 μm. Microseleres were not found, but presumably these were washed out with the copious mucous produced by this sponge when collected (as known for this species). The voucher specimen is registered in the collections of the Netherlands Centre of Biodiversity Naturalis under number ZMA Por. 16626. Another voucher specimen has been deposited at the Red Sea Invertebrates Collection at the Department of Pharmacognosy of Suez Canal University under the code no. DY-13.

3.3. Extraction and isolation
The freeze-dried sponge (280 g) was extracted with EtOH (4 × 2 L). The combined extracts were concentrated under reduced pressure to afford a dark brown residue (4.5 g). The total EtOH extract was subjected to vacuum liquid chromatography using 50% n-hexane:CHCl3 (4 × 500 mL) and EtOAc (4 × 500 mL), which were separately concentrated to give 1.2 and 2.5 g, respectively. The 50% n-hexane:CHCl3 fraction (1.2 g) was subjected to repeated silica gel columns (100 g × 50 cm × 5 cm) using n-hexane:EtOAc in order of increasing polarity to afford compounds 1 (17 mg, colourless oil), 2 (8 mg, white crystals), 3 (22 mg, colourless oil) and 4 (41 mg, white crystals).

3.4. Spectral data
3.4.1. Hexacosa-(6E,10E)-dienoic acid methyl ester (1)
Colourless oil (17 mg, MeOH); [α]D +18.9 (c = 0.5, CHCl3); IR (KBr) νmax 2925, 1725, 1643, 1469, 1389, 1180, 723 cm−1; 1H NMR (400 MHz, CDCl3): δH 2.29 (2H, t, J = 7.6 Hz, H-2), 1.57 (2H, m, H-3), 1.63 (2H, m, H-4), 2.07 (2H, m, H-5), 5.36 (1H, m, H-6), 5.35 (1H, m, H-7), 2.01 (2H, m, H-9), 5.34 (1H, m, H-10), 5.33 (1H, m, H-11), 1.35–1.25 (30 H, m, H-8, H-12 to H-25), Natural Product Research 1087
0.87 (3H, t, J = 6.8 Hz, H-26), 3.66 (3H, s, OCH3); 13C NMR (100 MHz, CDCl3): δC 174.3 (s, C-1), 34.1 (t, C-2), 25.0 (t, C-3), 24.8 (t, C-4), 27.3 (t, C-5), 130.5 (d, C-6), 130.4 (d, C-7), 27.4 (t, C-9), 128.9 (d, C-10), 128.8 (d, C-11), 31.9-22.8 (t, C-8, C-12 to C-25), 14.1 (q, C-26), 51.4 (q, OCH3); GC–MS: m/z 406 (9%) [M]+, 291 (4%), 169 (6%), 141 (46%), 81 (100%), 57 (29%).

3.4.2. Hexacosa-(6E,10E)-dienoic acid (2)
White crystals (8 mg, MeOH); m.p. 142–143°C; [α]D + 19.1 (c = 0.5, CHCl3); IR (KBr) νmax 2922, 1742, 1635, 1121, 1028, 1104, 792 cm⁻¹; 1H NMR (400 MHz, CDCl3): δH 2.36 (2H, t, J = 7.5 Hz, H-2), 1.65 (2H, m, H-3), 1.74 (2H, m, H-4), 2.11 (2H, m, H-5), 5.37 (1H, m, H-6), 5.39 (1H, m, H-7), 2.04 (2H, m, H-9), 5.37 (1H, m, H-10), 5.39 (1H, m, H-11), 1.36-1.27 (30 H, m, H-8, H-12 to H-25), 0.91 (3H, d, J = 6.5 Hz, H-26); 13C NMR (100 MHz, CDCl3): δC 178.3 (s, C-1), 33.8 (t, C-2), 24.7 (t, C-3), 24.6 (t, C-4), 27.3 (t, C-5), 128.9 (d, C-6), 130.6 (d, C-7), 27.4 (t, C-9), 128.6 (d, C-10), 129.9 (d, C-11), 31.9-22.7 (t, C-8, C-12 to C-25), 14.1 (q, C-26); GC–MS: m/z 392 (23%) [M]+, 307 (4%), 168 (12%), 127 (54%), 81 (100%), 57 (72%).

3.5. Antimicrobial assay
The organisms used in this study were S. aureus (AUMC No. B-54) and B. cereus (AUMC No. B-5) as Gram-positive bacteria, Escherichia coli (AUMC No. B-53), P. aeruginosa (AUMC No. B-73) and S. marcescens (AUMC No. B-55) as Gram-negative bacteria, Candida albicans (AUMC No. 418), Geotrichum candidum (AUMC No. 226), Fusarium oxysporum (AUMC No. 5119), Aspergillus flavus (AUMC No. 1276), Scopulariopsis brevicaulis (AUMC No. 729) and Trichophyton rubrum (AUMC No. 1804) as fungi. They were obtained from the Assiut University Mycology Center, Assiut (http://www.aun.edu.eg/aumc/Catalog.htm). The procedure was carried out as previously described (Bonev et al. 2008; Ibrahim, Mohamed & Al-Musayeib 2012).

3.6. Pharmacological studies
3.6.1. Animals
Adult male albino rats (120–150 g body weight) were used. All animal procedures were conducted in accordance with the internationally accepted principles for laboratory animals’ use and care as found in the European Community Guidelines and Institutional Ethical Committee Approval was obtained. The study protocol was approved by the Animal Ethical Committee of Assiut University. The animals were housed under standardised environmental conditions in the pre-clinical Animal House, Pharmacology Department, Faculty of Medicine, Assuit University. The animals were fed with standard diet and had free access to water. They were maintained at 24–28°C, 60–70% relative humidity, 12 h day and night cycle for 1 week to acclimatise to the environmental conditions.

3.6.2. Anti-inflammatory activity
The anti-inflammatory activity was evaluated in adult albino rats by carrageenan-induced rat hind paw oedema method according to the published procedures (Winter et al. 1962; Adams et al. 1968; Ali et al. 2013). The percentage of oedema inhibition (% of change) was calculated (Table 2).

3.6.3. Antipyretic activity
The antipyretic activity was screened in adult albino rats by using yeast-induced hyperpyrexia as previously described (Ibrahim, Mohamed & Al-Musayeib 2012; Ali et al. 2013). Rectal temperature of each rat was recorded after 1, 2, 3 and 4 h from administration of tested fractions.
3.6.4. *Hepato-protective activity*

The hepato-protective activity was determined as previously outlined (Bergmeyer & Bernt 1974; Bosma 1988; Mohamed et al. 2009). The animals were divided into four groups each of six animals.

**Group I:**
Normal control received distilled water (1 mL/kg) daily for 5 days and olive oil (1 mL/kg, intraperitoneal) on days 2 and 3.

**Group II:**
CCl₄ control received distilled water (1 mL/kg) daily for 5 days and CCl₄:olive oil (1:1, 1 mL/kg, intraperitoneal) on days 2 and 3.

**Group III:**
Treated with silymarin orally through intragastric feeding tube at dose of 50 mg/kg.

**Groups IV:**
Treated with TME orally through intragastric feeding tube at dose of 100 mg/kg.

3.6.4.1. *Biochemical estimations.* The rats were sacrificed on the sixth day and blood was collected from orbital sinus in plain tubes. The serum was obtained by centrifugation and serum samples were taken for biochemical assays; namely glutamate oxaloacetate transaminase and glutamate pyruvate transaminase.

The data were expressed as mean ± SE (n = 6). Results were analysed statistically by one-way ANOVA followed by comparison using Prism software (Graph Pab. Ver. 3.0).

3.6.5. *Cytotoxicity study*

Cancer growth inhibitory activity was examined against the A549 NSCLC, U373 GBM and PC-3 (prostate cancer) cell lines as described earlier (Mijatovic et al. 2008; Ibrahim, Mohamed, Shaala, et al. 2012).

4. Conclusion

Two new and two known compounds were isolated from the Red Sea sponge *Mycale euplectellioides*. The structures were elucidated by the different spectral data. The antimicrobial, anti-inflammatory, antipyretic, and hepato-protective activities of the total methanolic extract and fractions were evaluated. Furthermore, compounds 1 and 2 displayed weak cytotoxic activity.

**Supplementary material**

Supplementary material relating to this article is available online, alongside Figures S1–S11.

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**Conflict of interest**

There is no conflict of interest associated with the authors of this paper.
Note
1. MarinLit is a marine literature database produced and maintained by the Department of Chemistry, University of Canterbury, New Zealand, Version 2008.

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