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Ehrenasterol and biemnic acid; new bioactive compounds from the Red Sea sponge *Biemna ehrenbergi*

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ABSTRACT

In continuation of our efforts to identify bioactive compounds from the Red Sea marine sponges, we have recently investigated the organic extract of the sponge *Biemna ehrenbergi*. This study resulted in the isolation of eight compounds including a new sterol, ehrenasterol (**1**), a new C_{24}-acetylenic acid, biemnic acid (**2**), together with six known compounds including a hopanoid, three steroids and two nucleosides. The isolated compounds were identified as (22*E*)-ergosta-22-ene-8,14-epoxy-3,7-dione (**1**), (*E*)-tetracos-8-en-5-ynoic acid (**2**), (22*E*)-ergosta-5,8,22-trien-7-one-3β-ol (**3**), 32,35-anhydrobacteriohopanetetrol (**4**), (24*R*)-ergosta-6,22-diene-5,8-epidioxy-3-ol (**5**), melithasterol B (**6**), thymidine (**7**) and 2′-deoxyuridine (**8**). The structures of the isolated compounds were assigned by different spectral data including 1D and 2D NMR (COSY, HSQC, and HMBC) and high-resolution mass spectrometry. Compound **1** displayed inhibition zone of 20 mm at 100 μg/disc against *Escherichia coli* in the disc diffusion assay. Similarly, compounds **2** and **4** displayed inhibition zones of 20 and 18 mm respectively against *Candida albicans* at the same concentration. Compounds **1**–**3** displayed weak cytotoxic activity against human colon adenocarcinoma (HCT-116) cancer cell line.

1. Introduction

Marine invertebrates provided significant bioactive compounds including the anticancer agents psammaplins (Quiñoà and Crews, 1987; Rodriguez et al., 1987), didemnin B (Rinehart et al., 1987; Nuijen et al., 2000), dolastatin-10 (Luesch et al., 2001), ecteinascidin-743 (Rinehart et al., 1990; Wright et al., 1990) and halichondrin B (Bai et al., 1991). Currently, 62.5% (10 out of 16) of the marine-derived marketed drugs or in the advanced phases of clinical trials (Phase I–Phase III) are produced by marine invertebrates, mostly sponges (Mayer et al., 2010). The major area of application of these drugs includes cancer, pain and viral infections (Mayer et al., 2010). This fact clearly demonstrates and reflects the importance of phylum Porifera (sponges) as a potential source for future drug discovery and development. The current international marine pharmaceutical pipeline include three FDA-approved drugs and one EU-registered drug: Cytarabine® and Trabectedin® – antitumorals, Vidarabine® –antiviral, and Ziconotide® – analgesic; two undergoing Phase III trials: eribulin mesylate and soblidotin – antitumorals; seven undergoing Phase II trials: DMXBA – antidementia, pseudopterosins – wound healing, and plinabulin, plitidepsin, elisidepsin, PM1004 and Tasidotin – antitumorals; and three undergoing Phase I trials: bryosatin, hemiasterlin and marizomib – antitumorals (Mayer et al., 2010). Members of the genus *Biemna* have proven to be a rich source of biologically active secondary metabolites including steroids (Xiaochun et al., 2008; Huang et al., 2006; Delseth et al., 1979; Bensemhoun et al., 2008) and alkaloids (Aoki et al., 2003; Sorek et al., 2006; Zeng et al., 1993). The pyridoacridine alkaloids isolated from the sponge *Biemna fortis* displayed neuronal differentiation in a neuroblastoma cell line (Aoki et al., 2003). In the course of our continuous effort to identify bioactive compounds from Red Sea marine sponges, we have studied the sponge *Biemna ehrenbergi*. This study resulted in the identification of two new compounds including one sterol,

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ehrenasterol (**1**), and an acetylenic acid, biemnic acid (**2**) together with the previously reported compounds; (22*E*)-ergosta-5,8,22-trien-7-one-3β-ol (**3**) (Luo et al., 2006), 32,35-anhydrobacteriohopanetetrol (**4**) (Bradley et al., 2010; Cooke et al., 2008; Costantino et al., 2001), (24*R*)-ergosta-6,22-diene-5,8-epidioxy-3-ol (**5**) (Xiaochun et al., 2008), melithasterol B (**6**) (Xiaochun et al., 2008), thymidine (**7**) and 2′-deoxyuridine (**8**) (Zhou et al., 2005; Ralifo et al., 2007; Hammoda et al., 2007; Abou-Hussein et al., 2007; Deng et al., 1998). The structures of the compounds were achieved based on different spectroscopic data including HRESIMS, 1D (^{1}H NMR and ^{13}C NMR) and 2D (COSY, HSQC, and HMBC) NMR spectroscopy.

2. Results and discussion

2.1. Purification of compounds *1–8*

The frozen sponge was cut into very small pieces, followed by extraction with a mixture of MeOH:CH_2Cl_2 (1:1) at room temperature. The total extract was suspended in H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated under vacuum and the resulting residue was subjected to vacuum liquid chromatography on silica gel, followed by several steps of purification using different techniques including partition and size exclusion chromatography. Finally, eight pure compounds were isolated including two new compounds (**1–2**) together with six previously reported compounds (**3–8**).

2.2. Structure elucidation of compound *1*

The molecular formula of compound **1** (Fig. 1) was deduced as $C_{28}H_{42}O_3$ as deduced from the HRESIMS data, suggesting eight degrees of unsaturation. An extensive examination of ^{1}H NMR and ^{13}C NMR spectra data, together with the data of ^{1}H–^{1}H COSY, multiplicity-edited HSQC and HMBC experiments, allowed the establishment of a sterol skeleton with a 22(23)-double bond. Investigation of ^{13}C NMR spectrum and HSQC experiment allowed the assignment of six methyls, eight methylenes, eight methines including two protonated sp^2 carbons in addition to six quaternary carbons. The ^{1}H NMR, ^{13}C NMR and HSQC experiment confirmed the structure of a steroid possessing an ergostane skeleton. This was proved from the signals at $\delta_{H/C}$ 0.75/15.3 and 1.27/23.3, which were attributed to H_3-18/C-18 and H_3-19/C-19 respectively. In addition, four methyl doublets were resonating at $\delta_{H/C}$ 1.14/24.1, 0.81/19.6, 0.79/20.0 and 0.88/17.5 assignable for H_3-21/C-21, H_3-26/C-26, H_3-27/C-27 and H_3-28/C-28 respectively. The sp^2 carbons

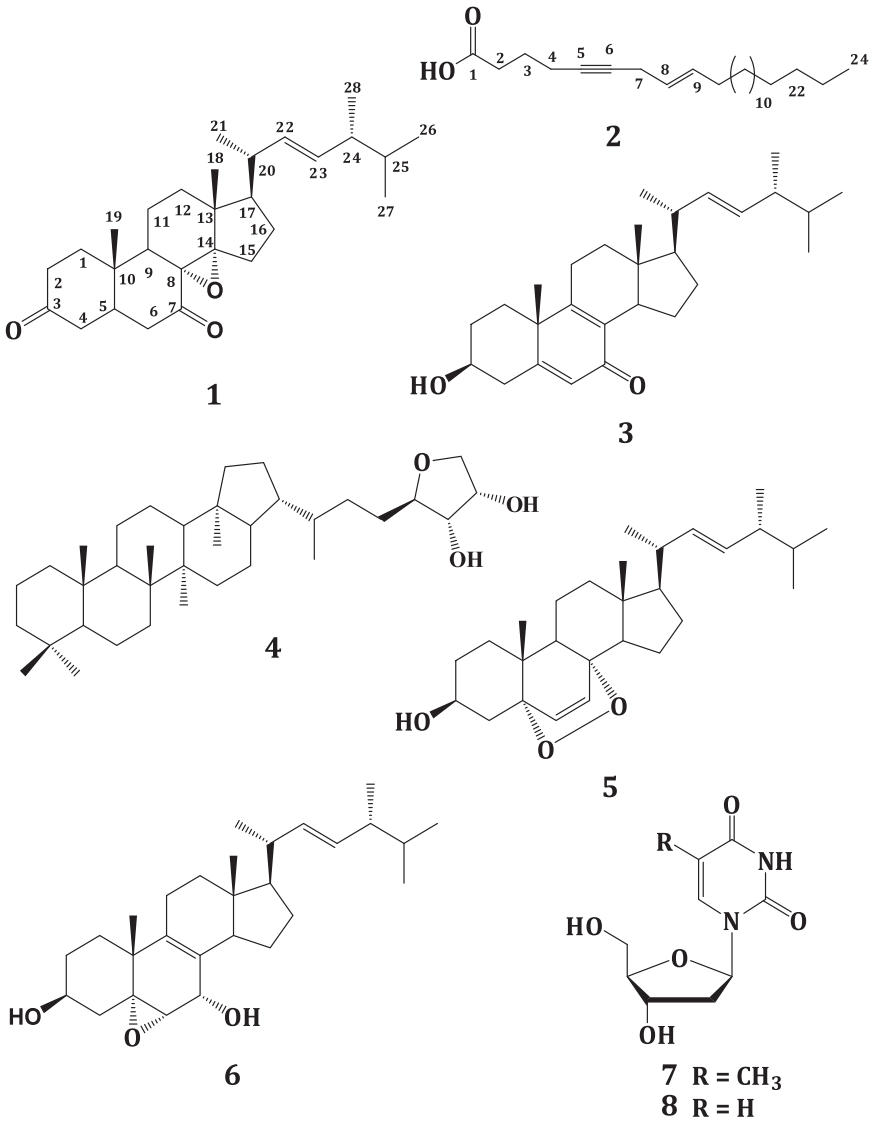

Fig. 1. Structures of compounds **1–8**.

Table 1
NMR spectroscopic data of **1** ($CDCl_3$, 600 and 150 MHz).

Position	δ_C (m)[a]	δ_H (m, *J* in Hz)	HMBC[b]
1	35.8 (CH_2)	2.84 (m), 2.20 (m)	3, 9
2	34.0 (CH_2)	2.32 (m), 2.14 (m)	3
3	207.7 (qC)	–	–
4	36.7 (CH_2)	2.30 (m), 2.21 (dd, 16.2, 6)	3, 9
5	53.2 (CH)	3.05 (br t, 8.4)	6, 10
6	40.5 (CH_2)	2.95 (d, 13.2)	3, 5, 7, 8
		1.96 (d, 13.2)	9, 14
7	214.8 (qC)	–	–
8	60.0 (qC)	–	–
9	50.0 (CH)	2.90 (br d, 13.2)	
10	39.9 (qC)	–	–
11	27.2 (CH_2)	2.00 (m), 1.64 (m)	13
12	38.5 (CH_2)	2.81 (m), 2.37 (m)	11
13	45.3 (qC)	–	–
14	65.6 (qC)	–	–
15	29.7 (CH_2)	1.29 (m)	
16	25.5 (CH_2)	2.31 (m), 2.10 (m)	
17	45.4 (CH)	1.97 (m)	8, 14, 20
18	15.3 (CH_3)	0.75 (s)	8, 13,14, 17
19	23.3 (CH_3)	1.27 (s)	1, 5, 6, 9, 10
20	36.8 (CH)	2.42 (m)	
21	24.1 (CH_3)	1.14 (d, 6)	13, 20,22, 23
22	135.2 (CH)	5.20 (m)	20
23	131.9 (CH)	5.20 (m)	20
24	43.2 (CH)	1.84 (m)	22
25	33.0 (CH)	1.47 (m)	26, 27
26	19.6 (CH_3)	0.81 (d, 7.2)	24, 25, 27
27	20.0 (CH_3)	0.79 (d, 7.2)	24, 25, 26
28	17.5 (CH_3)	0.88 (d, 1.8)	22, 23, 24, 25

[a] Multiplicities were deduced from multiplicity-edited HSQC.
[b] HMBC correlations are from proton(s) stated to the indicated carbons.

of the olefinic moiety at C-22 and C-23 were detected at δ_C 135.2 and 131.9, respectively. The presence of two carbonyl functionalities was proved from the signals resonating at δ_C 207.7 and 214.8 in ^{13}C NMR spectrum. From HMBC, cross peaks were noticed from H_2-1, H-2a, H-6a to the carbonyl signal at δ_C 207.7 confirming its location at C-3 (Table 1 and Fig. 2). The cross peak observed from H-6b at δ_H 1.96 to the second carbonyl signal at δ_C 214.8 confirmed its location at C-7. The ^{13}C NMR spectrum and HSQC experiment declared the presence of two quaternary oxygenated carbons resonating at δ 60.0 and 65.6, suggesting

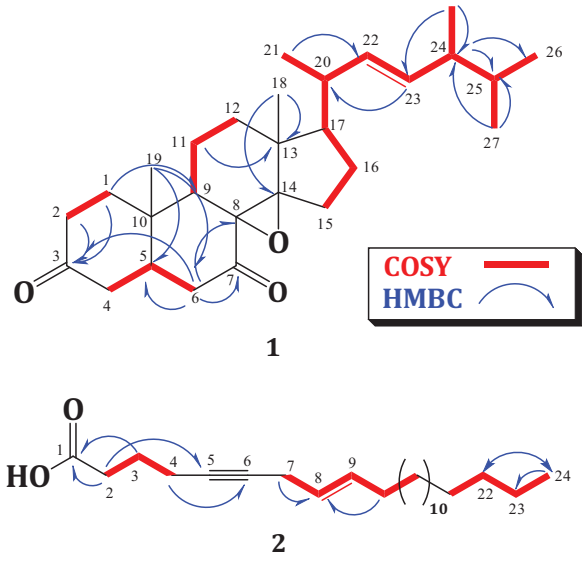

Fig. 2. Key ^1H–^1H COSY and HMBC correlations for **1** and **2**.

epoxy functionalities, completing the degrees of unsaturation (Xiaochun et al., 2008). Their situation was confirmed from HMBC through the cross peaks from each of H-6b (δ_H 1.96), H_3-18 (δ_H 0.75) to C-8 (δ_C 60.0) and also from H-6a (δ_H 2.95), H-6b (δ_H 1.96), H_3-18 (δ_H 0.75) to C-14 (δ_C 65.6). These correlations proved the placement of the epoxy functionality at C-8/C-14. The configuration at C-8 and C-14 was confirmed from comparison of NMR data with closely related compounds previously isolated from *B. fortis* (Xiaochun et al., 2008). From the previous discussion, compound **1** was assigned as (22*E*)-ergosta-22-ene-8,14-epoxy-3,7-dione and reported here as a new natural product named as ehrenasterol.

2.3. Structure elucidation of compound **2**

Compound **2** was isolated as a white residue. Combined spectral data including HRESIMS, HSQC, ^1H NMR and ^{13}C NMR established its molecular formula as $C_{24}H_{42}O_2$. The ^{13}C NMR spectrum together with HSQC experiment revealed the presence of two acetylenic carbons (δ_C 77.9 and 80.9), two sp^2 carbons (δ_C 127.7 and 131.3), one carbonyl functionality (δ_C 176.7), one methyl (δ_C 14.1), in addition to eighteen methylene carbons. ^1H–^1H COSY and HSQC experiments allowed the assembly of the C-2/C-4 and C-7/C-24 units. HMBC experiment confirmed the terminal carboxylic moiety. HMBC revealed that H-2 (δ_H 2.56) is correlated with C-1 (δ_C 176.7), C-5 (δ_C 77.9) and H-3 (δ_H 2.47) is correlated with C-1 (δ_C 176.7), C-5 (δ_C 77.9), C-6 (δ_C 80.9), also H-4 (δ_H 2.16) is correlated with C-5 (δ_C 77.9), C-6 (δ_C 80.9); which is a further confirmation of the C-2/C-4 unit. Similarly, HMBC correlations illustrated in Table 2, confirmed the second unit (C-7/C-24). The connection of the two units through C-5/C-6 was confirmed from HMBC where cross peaks were noticed from each of H-2 (δ_H 2.56), H-3 (δ_H 2.47), H-4 (δ_H 2.16) to C-5 (δ_C 77.9) and from each of H-3 (δ_H 2.47), H-4 (δ_H 2.16), H-7 (δ_H 2.21) to C-6 (δ_C 80.9). The configuration at the double bond between C-8 and C-9 was determined to be *E* based on the large coupling constant between H-8 and H-9 ($J_{8,9}$ = 15.5 Hz) (Shaala et al., 2011). Therefore, compound **2** was assigned as (*E*)-tetracos-8-en-5-ynoic acid and was given the generic name biemnic acid.

Table 2
NMR spectroscopic data of **2** ($CDCl_3$, 600 and 150 MHz).

Position	δ_C (m)[a]	δ_H (m, *J* in Hz)	HMBC[b]
1	176.7 (qC)	–	–
2	33.6 (CH_2)	2.56 (t, 7.2)	1, 5
3	14.5 (CH_2)	2.47 (brt, 7.2)	1, 5, 6
4	19.1 (CH_2)	2.16 (t, 6.6)	5, 6
5	77.9 (qC)	–	–
6	80.9 (qC)	–	–
7	26.8 (CH_2)	2.21 (t, 7.2)	6, 8, 9
8	127.7 (CH)	5.37 (dt, 15.5, 7.2)	7, 10
9	131.3 (CH)	5.43 (dt, 15.5, 7.2)	7, 10
10	27.3 (CH_2)	2.02 (q, 7.2)	8, 9, 11, 12
11	22.6–31.9 (CH_2)	1.26–1.33 (m)	8, 9
12	22.6–31.9 (CH_2)	1.26–1.33 (m)	
13	22.6–31.9 (CH_2)	1.26–1.33 (m)	
14	22.6–31.9 (CH_2)	1.26–1.33 (m)	
15	22.6–31.9 (CH_2)	1.26–1.33 (m)	
16	22.6–31.9 (CH_2)	1.26–1.33 (m)	
17	22.6–31.9 (CH_2)	1.26–1.33 (m)	
18	22.6–31.9 (CH_2)	1.26–1.33 (m)	
19	22.6–31.9 (CH_2)	1.26–1.33 (m)	
20	22.6–31.9 (CH_2)	1.26–1.33 (m)	
21	22.6–31.9 (CH_2)	1.26–1.33 (m)	
22	22.6–31.9 (CH_2)	1.26–1.33 (m)	
23	22.6–31.9 (CH_2)	1.26–1.33 (m)	24
24	14.1 (CH_3)	0.88 (t, 7.2)	23

[a] Multiplicities were deduced from DEPT and multiplicity-edited HSQC.
[b] HMBC correlations are from proton(s) stated to the indicated carbons.

2.4. Structure elucidation of compounds **3–8**

Interpretation of NMR data of compounds **3**,**5** and **6** confirmed their identity as the known steroids (22*E*)-ergosta-5,8,22-trien-7-one-3β-ol (**3**) (Luo et al., 2006), (24*R*)-ergosta-6,22-diene-5,8-epidioxy-3-ol and melithasterol B respectively (Xiaochun et al., 2008). The structure of **4** was proved as 32,35-anhydrobacteriohopanetetrol (Bradley et al., 2010; Cooke et al., 2008). It is worthy to mention that hopanoid derivatives have been isolated mainly from bacterial sources in addition to sponges (Neunlist et al., 1988; Neunlist and Rohmer, 1985; Inatomi et al., 2000; Costantino et al., 2001). Similarly, after investigation of the different spectral data of compounds **7** and **8**, they were identified as the known nucleosides thymidine and 2′-deoxyuridine respectively, which were previously isolated from different marine organisms (Zhou et al., 2005; Ralifo et al., 2007; Hammoda et al., 2007; Abou-Hussein et al., 2007; Deng et al., 1998).

2.5. Biological activities of the isolated compounds

The compounds were evaluated for their antimicrobial activity against three pathogens. Compound **1** displayed significant inhibition zone of 20 mm at a concentration of 100 μg/disc against *Escherichia coli* ATCC 25922 in the disc diffusion assay. Similarly, compounds **2** and **4** displayed inhibition zones of 20 and 18 mm respectively against *Candida albicans* ATCC 14053 at the same concentration (100 μg/disc). Other compounds were inactive against these pathogens. Nystatin was used as a positive antifungal control in this assay and showed inhibition zone of 22 mm against *C. albicans* ATCC 14053 at a concentration of 25 μg/disc, while ciprofloxacin was used as a positive control against *E. coli* and showed inhibition zone of 20 mm at 25 μg/disc. In addition, the compounds were evaluated for their cytotoxicity against human colon adenocarcinoma (HCT-16) cells. Compounds **1**–**3** showed weak cytotoxicity with IC_{50} of 45, 60 and 40 μM respectively. Other compounds were inactive against this cell line.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO digital polarimeter. HRESIMS was recorded on a LTQ Orbitrap (ThermoFinnigan, Bremen, Germany). The IR spectra were measured on a Shimadzu Infrared-400 spectrophotometer (Kyoto, Japan). UV spectra were measured on a Hitachi 300 Spectrophotometer. 1D and 2D NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on Bruker Avance DRX 600 MHz spectrometers using $CDCl_3$ and CD_3OD as solvents. NMR spectra were referenced to the residual protonated solvent signals ($CHCl_3$: 7.26 ppm for ^1H and 77.0 ppm for ^{13}C; CH_3OD: 3.30 ppm for ^1H and 49.0 ppm for ^{13}C). Column chromatographic separations were performed on silica gel 60 (0.04–0.063 mm) and Sephadex LH-20 (0.25–0.1 mm, Pharmacia). The HPLC separation was performed on a RP_{18}, 250 mm × 10 mm, 5 μm Phenomenex Luna column using CH_3CN:H_2O as mobile phase at 220 nm and at a flow rate of 2.0 mL/min. TLC was performed on precoated TLC plates with silica gel 60 F254 (0.2 mm, Merck). Spots were visualized by UV absorption at λ_{max} 255 and 366 nm followed by spraying with *P*-anisaldehyde/H_2SO_4.

3.2. Animal materials

The Sponge was collected at a depth of 9 meters off Jeddah in May 2012. The sponge forms a basal mass with broad grooved digitations and hard consistency. The sizes of the voucher fragments are up to 8 cm height with individual digitations measuring 5 cm × 2 cm. The sponge is dark brown color in live. The skeleton is dense, course, vaguely plumose but largely confused with loose bundles of 10 or more style without visible spongin. The spicules robust styles measure up to 810 μm × 20 μm, and three sizes of sigmas, 48–51 μm, 30–36 μm and 15–18 μm were recorded. The microzeas measure 50 μm and the trichodragmas measure 120–225 μm × 12–24 μm. These characters comply with the specimen and subsequent descriptions of this common species. The vouchers are registered in the collection of Netherlands Center for Biodiversity Naturalis under number ZMA Pro. 16622. Another specimen was kept at the Department of Natural Products at Faculty of Pharmacy, King Abdulaziz University under number DY-9.

3.3. Extraction and purifications of compounds **1–8**

The frozen sponge was cut into very small pieces (640 g) followed by extraction with a mixture of MeOH:CH_2Cl_2 (1:1) (3× 2000 mL) at room temperature. The total extract was suspended in H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated under vacuum and the resulting residue (5.50 g) was subjected to vacuum liquid chromatography on silica gel using n-hexane: EtOAc:MeOH gradients to give seven main fractions. Fraction 3 (350 mg) was chromatographed on silica gel column using pet. ether:$CHCl_3$:MeOH gradient to give 8 main subfractions. Subfraction 4 (69 mg) was purified on Sephadex LH-20 using 20% $CHCl_3$ in MeOH to obtain 25 mg of impure **2**. The compound was purified on RP HPLC column using 70% ACN in H_2O to afford pure **2** (7 mg). Similarly, subfraction 7 (120 mg) was purified on Sephadex LH-20 using 20% $CHCl_3$ in MeOH to obtain impure **4** (45 mg) and **5** (33 mg). These compounds were subjected to final purification on ODS HPLC column (250 mm × 10 mm) using 80% ACN in H_2O to give the pure compounds **4** (11 mg) and **5** (8.5 mg). Fraction 5 (500 mg) was fractionated on silica gel column and eluted with pet. ether: $CHCl_3$:MeOH gradient to give five subfractions. Subfraction 3 (73 mg) was subjected to Sephadex LH-20 column eluted with 20% $CHCl_3$ in MeOH. The fraction contained compounds **1**, **3** and **6** was purified on ODS HPLC column (250 mm × 10 mm) using 70% ACN in H_2O to give compound **1** (5.3 mg), **3** (7 mg) and **6** (13 mg). Fraction 7 (610 mg) was purified on silica gel column and eluted with $CHCl_3$: MeOH gradient to afford five main subfractions. Subfractions 3 (51 mg) and 5 (44 mg) were purified separately on Sephadex LH-20 using MeOH. Final purification was performed on C18 Sep-Pak cartridges (1.0 g) eluted with H_2O:ACN gradient to give compound **7** (11 mg) and **8** (9 mg).

3.4. Biological evaluation of the compounds

3.4.1. Evaluation of cytotoxic activities

Cytotoxicity of the compounds was evaluated against human colon adenocarcinoma (HCT-116) cancer cell line by using the MTT (Soria-Mercado et al., 2005; Youssef et al., 2014). The cells were incubated overnight at 37 °C in 5% CO_2/air in microtiter plates. Tested compound, etoposide (positive control), and DMSO (negative control) were added to the top row of a 96-well microtiter plate and serially diluted (1:4) downward. After a 72 h incubation, cell viability was determined colorimetrically using a Molecular Devices Emax microplate reader (490 nm), recording the amount of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) reduced to formazan using the Cell Titer 96 Aqueous nonradioactive cell proliferation protocol (Promega). Minimum inhibitory concentration (IC_{50}, μM) values were calculated using the program SOFTmax PRO (Molecular Devices). Etoposide was used as a positive cytotoxicity control in HCT-16 assay and displayed cytotoxicity with IC_{50} of 2.0 μM.

3.4.2. Evaluation of the antimicrobial activity

The *in vitro* antimicrobial activity was evaluated using disc diffusion method as previously described (Kiehlbauch et al., 2000). Varieties of test microorganisms were used including a Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923), a Gram-negative bacteria (*Escherichia coli* ATCC 25922) and yeast (*C. albicans* ATCC 14053). Adjusted inoculum of each microorganism, equivalent to a turbidity of 0.5 McFarland standards, were streaked separately using sterile swabs over the surface of Muller Hinton agar plates. Sterile discs (6 mm diameter) were impregnated with 100 μg of each compound and applied to the inoculated plates. The plates were incubated at 37 °C for 24 h. Solvent control discs were used to determine any solvent effect. Gentamycin and ciprofloxacin were used as antibacterial standards, while nystatin used as an antifungal standard. The activity of each compound was determined by measuring the diameter of the inhibition zone in mm. The technique was performed in duplicate and the mean diameter of each inhibition zone was recorded.

3.5. Spectroscopic data of the compounds

*3.5.1. Ehrenasterol [(22E)-Ergosta-22-ene-8,14-epoxy-3,7-dione] (**1**)*

White residue; $[\alpha]_D = -10.6^\circ$ (*C* 0.8, $CHCl_3$); UV (MeOH) λ_{max} ($\log \varepsilon$) = 221 (4.05) nm; IR (KBr) γ_{max} 2986, 2870, 1720 (br), 1450, 1233, 1172, 1136 cm^{-1}; HRESIMS *m/z* 427.3212 (calcd for $C_{28}H_{43}O_3$, $[M+H]^+$, 427.3221); NMR data, see Table 1.

*3.5.2. Biemnic acid (**2**)*

White residue; IR (KBr) γ_{max} 3200 (br, weak), 3026, 2918, 2848, 2110, 1711, 1635, 1408, 1212, 1072 cm^{-1}; HRESIMS *m/z* 363.3260 (calcd for $C_{24}H_{43}O_2$, $[M+H]^+$, 363.3263); NMR data, see Table 2.

*3.5.3. (22E)-Ergosta-5,8,22-trien-7-one-3β-ol (**3**)*

White powder; $[\alpha]_D = -18.5^\circ$ (*C* 0.8, $CHCl_3$); UV (MeOH) λ_{max} ($\log \varepsilon$) = 248 (4.5) nm; IR (KBr) γ_{max} 3420, 2960, 2932, 2844, 1676, 1625, 1456, 1370, 1268, 1180, 1050 cm^{-1}; ESIMS *m/z* 411.3 $[M+H]^+$. Molecular formula: $C_{28}H_{42}O_2$. ^1H NMR (600 MHz, $CDCl_3$): δ_H 2.06 (m, H-1a), 1.28 (dd, 13.8, 4.2, H-1b), 1.97 (m, H-2a), 1.71 (m, H-2b), 3.67 (m, H-3), 2.60 (dd, 4.8, 1.8, H-4a), 2.53 (m, H-4b), 6.04 (d, *J* = 1.2, H-6), 2.47 (m, H-11a), 2.42 (m, H-11b), 2.07 (dd, 7.2, 3.6, H-12a), 1.49 (m, H-12b), 2.23 (m, H-14), 2.55 (m, H-15a), 1.24 (m, H-15b), 1.84 (m, H-16a), 1.27 (m, 16b), 1.23 (brd, 13.2, H-17), 0.65 (s, H-18), 1.34 (s, H-19), 2.07 (m, H-20), 1.05 (d, 6.6, H-21), 5.18 (dd, 15.2, 8.0, H-22), 5.24 (dd, 15.2, 8.5, H-23), 1.85 (m, H-24), 1.48 (m, H-25), 0.84 (d, 6.6, H-26), 0.82 (d, 6.6, H-27), 0.92 (d, 7.2, H-28). ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 34.8 (C-1), 30.8 (C-2), 72.1 (C-3), 42.0 (C-4), 161.2 (C-5), 126.9 (C-6), 186.5 (C-7), 134.2 (C-8), 161.8 (C-9), 43.0 (C-10), 24.8 (C-11), 35.7 (C-12), 42.1 (C-13), 51.4 (C-14), 24.9 (C-15), 29.6 (C-16), 53.6 (C-17), 12.1 (C-18), 23.9 (C-19), 40.5 (C-20), 21.1 (C-21), 135.6 (C-22), 132.3 (C-23), 42.5 (C-24), 33.3 (C-25), 19.8 (C-26), 19.1 (C-27), 17.8 (C-28).

*3.5.4. 32,35-Anhydrobacteriohopanetetrol (**4**)*

White needles; ESIMS *m/z* 529.4 $[M+H]^+$. Molecular formula: $C_{35}H_{60}O_3$. ^1H NMR (600 MHz, $CDCl_3$): δ_H 1.34 (m, H-1a), 1.12 (m, H-1b), 1.35 (m, H-2a), 1.34 (m, H-2b), 1.52 (m, H-3), 0.71 (brd, *J* = 2.7 Hz, H-5), 1.52 (m, H-6a), 1.30 (m, H-6b), 1.46 (m, H-7a), 1.23 (m, H-7b), 1.23 (m, H-9), 1.72 (m, H-11a), 1.52 (m, H-11b), 1.45 (m, H-12a), 1.38 (m, H-12b), 1.31(m, H-13), 1.33 (m, H-15a), 1.24 (m, H-15b), 1.25 (m, H-16), 1.27 (m, H-17), 1.65 (m, H-19a), 0.77 (m, H-19b), 1.78 (m, H-20a), 1.52 (m, H-20b), 1.75 (m, H-21), 1.50 (m, H-22), 0.84 (s, H_3-23), 0.79 (s, H_3-24), 0.82 (s, H_3-25), 0.94 (s, H_3-26), 0.93 (s, H_3-27), 0.70 (s, H_3-28), 0.95 (brs, H_3-29), 1.51 (m, H-30a), 1.17 (m, H-30b), 1.58 (m, H-31a), 1.52 (m, H-31b), 3.60 (m, H-32), 3.75 (brd, H-33), 4.24 (m, H-34), 4.12 (dd, *J* = 10.2, 5.4 Hz, H-35a), 3.68 (dd, *J* = 9.6, 3.6 Hz, H-35b); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 41.8 (C-1), 18.6 (C-2), 41.6 (C-3), 33.2 (C-4), 56.1 (C-5), 20.9 (C-6), 33.1 (C-7), 42.1 (C-8), 50.4 (C-9), 37.3 (C-10), 22.7 (C-11), 23.9 (C-12), 49.3 (C-13), 41.7 (C-14), 33.6 (C-15), 29.7 (C-16), 54.4 (C-17), 44.3 (C-18), 40.3 (C-19), 27.6 (C-20), 46.0 (C-21), 36.7 (C-22), 33.4 (C-23), 21.5 (C-24), 15.9 (C-25), 16.5 (C-26), 16.6 (C-27), 15.8 (C-28), 20.1 (C-29), 31.6 (C-30), 29.9 (C-31), 83.0 (C-32), 75.8 (C-33), 71.1 (C-34), 72.6 (C-35).

*3.5.5. (24R)-Ergosta-6,22-diene-5,8-epidioxy-3-ol (**5**)*

Silvery white needles; ESIMS *m/z* 431.3 $[M+H]^+$. Molecular formula: $C_{28}H_{46}O_3$. ^1H NMR (600 MHz, $CDCl_3$): δ_H 2.11 (dd, *J* = 13.8, 3.6 Hz, H-1a), 1.92 (dd, *J* = 13.8, 1.8, H-1b), 1.59 (m, H-2a), 1.25 (m, H-2b), 3.97 (m, H-3), 1.95 (m, H-4a), 1.23 (m, H-4b), 6.5 (d, *J* = 9.0 Hz, H-6), 6.23 (d, *J* = 9.0 Hz, H-7), 1.54 (m, H-9), 1.59 (m, H-11a), 1.40 (m, H-11b), 1.96 (m, H-12a), 1.70 (m, H-12b), 3.62 (m, H-14), 1. 5 (m, H-15a), 1.23 (m, H-15b), 1.77 (m, H-16a), 1.38 (m, H-16b), 0.70 (brd, H-17), 0.81 (s, H_3-18), 0.88 (s, H_3-19), 2.02 (m, H-20), 1.00 (d, *J* = 6.6 Hz, H_3-21), 5.14 (dd, *J* = 15.0, 7.8 Hz, H-22), 5.22 (dd, *J* = 15.0, 7.8 Hz, H-23), 1.84 (brt, *J* = 7.2, H-24), 1.46 (m, H-25), 0.82 (d, *J* = 6.0 Hz, H_3-26), 0.81 (d, *J* = 6.6 Hz, H_3-27), 0.90 (d, *J* = 6.6 Hz, H_3-28); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 36.9 (C-1), 30.1 (C-2), 66.4 (C-3), 39.3 (C-4), 79.4 (C-5), 130.7 (C-6), 135.4 (C-7), 82.1 (C-8), 51.0 (C-9), 36.9 (C-10), 20.6 (C-11), 34.6 (C-12), 44.5 (C-13), 51.6 (C-14), 23.3 (C-15), 28.6 (C-16), 56.1 (C-17), 12.8 (C-18), 18.1 (C-19), 39.7 (C-20), 20.8 (C-21), 135.1 (C-22), 132.2 (C-23), 42.7 (C-24), 33.0 (C-25), 19.6 (C-26), 19.9 (C-27), 17.5 (C-28).

*3.5.6. Melithasterol B (**6**)*

White powder; ESIMS *m/z* 429.3 $[M+H]^+$. Molecular formula: $C_{28}H_{44}O_3$. ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 30.1 (C-1), 31.9 (C-2), 68.1 (C-3), 39.3 (C-4), 66.4 (C-5), 63.0 (C-6), 68.7 (C-7), 130.7 (C-8), 135.1 (C-9), 36.8 (C-10), 23.3 (C-11), 34.6 (C-12), 42.7 (C-13), 50.9 (C-14), 24.7 (C-15), 29.7 (C-16), 51.6 (C-17), 12.8 (C-18), 22.7(C-19), 39.7 (C-20), 18.1 (C-21), 135.3 (C-22), 132.2 (C-23), 43.3 (C-24), 33.0 (C-25), 19.9 (C-26), 19.6 (C-27), 17.6 (C-28).

*3.5.7. Thymidine (**7**)*

White powder; ESIMS *m/z* 243.1 $[M+H]^+$. Molecular formula: $C_{10}H_{14}N_2O_5$. ^1H NMR (600 MHz, CD_3OD): δ_H 7.80 (s, H-4), 1.93 (s, CH_3), 6.27 (t, *J* = 6.6 Hz, H-1′), 2.23 (m, H-2′), 4.39 (m, H-3′), 3.90 (brd, *J* = 3.6 Hz, H-4′), 3.73 (dd, *J* = 12.0, 3.6 Hz, H-5a′), 3.77 (dd, *J* = 12.0, 3.0 Hz, H-5b′); ^{13}C NMR (150 MHz, CD_3OD): δ_C 166.4 (C-2), 111.5 (C-3), 138.2 (C-4), 152.4 (C-6), 12.2 (CH_3), 86.2 (C-1′), 41.2 (C-2′), 72.2 (C-3′), 88.8 (C-4′), 62.8 (C-5′).

3.5.8. 2′-Deoxyuridine (8)

White powder; ESIMS *m/z* 229.0 $[M+H]^+$. Molecular formula: $C_9H_{12}N_2O_5$. ^1H NMR (600 MHz, CD_3OD): δ_H 5.69 (d, *J* = 7.8 Hz, H-3), 7.98 (d, *J* = 7.8 Hz, H-4), 6.27 (t, *J* = 6.6 Hz, H-1′), 2.24 (m, H-2a′), 2.20 (m, H-2b′), 4.38 (m, H-3′), 3.91 (m, H-4′), 3.76 (m, H-5a′), 3.71 (m, H-5b′). ^{13}C NMR (150 MHz, CD_3OD): δ_C 152.28 (C-2), 102.6 (C-3), 142.5 (C-4), 166.3 (C-6), 86.6 (C-1′), 41.3 (C-2′), 72.3 (C-3′), 89.0 (C-4′), 62.8 (C-5′).

4. Conclusion

One new sterol, ehrenasterol and a new C_{24}-acetylenic acid; biemnic acid, together with six known compounds were isolated and identified from the Red Sea sponge *B. ehrenbergi*. The compounds were identified by interpretation of their spectral data including 1D, 2D NMR and HRESIMS. Compound **1** showed antimicrobial activity against *E. coli* ATCC 25922 while compounds **2** and **4** displayed activity against *C. albicans* ATCC 14053. Compounds **1**–**3** displayed weak cytotoxic activity against human colon adenocarcinoma (HCT-16) cells.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2015.04.024.

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