1. Introduction

Soft coral of the genus Sarcophyton (subclass Octocorallia; order Alcyonacea; family Alcyoniidae) contain a diversity of cyclic diterpenes that usually contain ethers, lactones or furanes around a cembrane framework. These cembrane diterpenoids exhibit a wide range of structural diversity and biological activity. Cembranoids, the main metabolites identified in the genus Sarcophyton have been shown to serve as an effective chemical defense against natural predators of coral.

The leather coral Sarcophyton ehrenbergi (von Marenzeller, 1886) produces diverse metabolites with distinct chemical structures as well as promising biological activities. Additionally, prostaglandins (PGs) that regulate a broad range of physiological activities, have been isolated from S. ehrenbergi.

The Red Sea contains a high endemic biota including approximately 50 genera of hermatypic soft coral. While Red Sea marine invertebrates have been historically under-reported within the scientific literature, intensive investigation of Red Sea marine life has occurred over the past ten years. To continue efforts to identify new marine metabolites from Red Sea soft coral, herein we report three cembrene diterpenoids isolated from S. ehrenbergi (Fig. 1). Absolute stereochemistry of the newly reported compounds was determined by time-dependent density functional theory-electronic circular dichroism (TDDFT-ECD) calculations. All isolated metabolites were probed against three human cancer cell lines.

2. Results and discussion

Freshly collected S. ehrenbergi were rapidly frozen with an optical rotation of [α]D25 +10.1 (c 0.02, CHCl3). The molecular formula C21H32O2 was determined by high-resolution electron ionization mass spectrum (HREIMS) (m/z 346.2127 [M – H2O]+, calcd 346.2149).

The IR spectrum showed absorption bands at νmax 3450 cm⁻¹ and 1754 cm⁻¹ for hydroxyl and keto groups, respectively. The 13C NMR and distortion less enhancement by polarization transfer (DEPT) spectrum showed 21 carbon oxygenated proton signals at δH 5.45 (d; J = 10.0 Hz), δH 3.57 (brd; J = 5.0 Hz), four methyl singlets at δH 3.34 (brd, J = 5.0 Hz), δH 5.45 (d; J = 10.0 Hz); four methyl singlets at...
\( \delta_H \) 2.02 s, 1.83 s, 1.11 s and 1.03, as well as, one methyl of a methoxy group at \( \delta_H \) 3.20 s; olefinic signal at \( \delta_H \) 5.14 (d; \( J = 10.0 \) Hz) signed for a tri-substituted double bond (Table 1). 1D and 2D NMR spectroscopic data comparison (Table 1) closely corresponded to those of previously isolated metabolites from \textit{Sarcophyton} species as well as a previously isolated skeleton by Hegazy et al., 2017 (ref. 5-13, 22 and 23) (Fig. 2).

The signal at \( \delta_H \) 5.45 (d; \( J = 10.0 \) Hz) correlated with a proton signal at \( \delta_H \) 5.14 (d, \( J = 10.0 \) Hz) and quaternary olefinic carbons at \( \delta_C \) 147.0 and \( \delta_C \) 163.0 in DQF-COSY and HMBC (Fig. 2), respectively, allowed for the assignments of H-2, H-3, C-4 and C-1, respectively.\(^8-10,23-25\) Correlations in the HMBC spectrum showed several informative connections: H-3 to carbon signals at \( \delta_C \) 13.6 (q, olefinic) \( \delta_C \) 34.6 (t), allowed for the assignment of H-18 (\( \delta_H \) 2.02, s) and H-5 (\( \delta_H \) 2.37, brd, \( J = 14.0 \)), respectively; methyl signal \( \delta_H \) 1.83 (s) to C-1 and carbon signal at \( \delta_C \) 174.0 (C\text{O}) attributed to H-17 and C-16, respectively as well as supporting the location of C-1/C-2 lactone ring; methyl singlet at \( \delta_H \) 1.11 to \( \delta_C \) 13.6, \( \delta_C \) 37.0 and 78.5 allowed for the location of H-3-19 (\( \delta_C \) 13.6), C-9 and C-8, respectively; the oxygenated broad doublet at \( \delta_H \) 3.57 (\( \delta_C \) 79.0) to C-9 and C-20, was assigned to H-11. The assignment of H-7, H2-6 and C-5

### Table 1

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\( a \) \( J \) values (Hz) in parentheses, obtained at 500 and 125 MHz for \( ^1H \) and \( ^13C \) NMR, respectively. \( b \) Recorded in CDCl\(_3\). \( c \) Recorded in CDCl\(_3\)-CD\(_3\)OD (9 : 1).
was detected through the correlation of the oxygenated methine signal at $\delta_H$ 3.14 (brd, $J = 5.0$) to a methylene multiplet at $\delta_H$ 1.30/2.21 and a carbon signal at $\delta_C$ 34.6 in DQF-COSY and HMBC, respectively. Additionally, a correlation was detected in DQF-COSY between H-13 ($\delta_H$ 1.78, m) and H-14 ($\delta_H$ 2.43, brt, $J = 12.2$) as well as to C-20 in HMBC analyses (Fig. 2). An HMBC correlation established the site of a methoxy group ($\delta_H$ 3.20 s, $\delta_C$ 48.3 q) at C-8.

The planar structure assignment of 1 and the C-7/C-12 ether linkage were proposed by 1D, 2D NMR and HREIMS data. The data comparison with those of sarcoehrenbergilid A, as previously reported,23 suggested that 1 and sarcoehrenbergilid A23 differ only in stereochemistry.

The NOESY spectrum revealed that a $\gamma$-lactone at H-2 ($\delta_H$ 5.45, d, $J = 10.0$ Hz) correlated with CH$_3$-18 ($\delta_H$ 2.02, s); a vicinal coupling with H-3 established a trans configuration and a $\beta$-orientation for H-2. NOSEY correlations were observed between three methyl groups with alpha protons (e.g., CH$_3$-20 with H-10a, CH$_3$-19 with H-6a/H-10a, and CH$_3$-17 with H-14a) (Fig. 3). H-7 and H-11 was assigned to a $\beta$-configuration based on NOSEY correlations with H-5b and H-14b, respectively. Absolute configuration was established by experimental and TDDFT-simulated ECD spectra. All possible conformations of 1 within energy window of 10 kcal mol$^{-1}$ were generated and optimized at B3LYP/6-31G* level of theory. The first 50 excitation states were then computed based on time-dependent density-functional theory (TDDFT) at B3LYP/6-31G* level in methanol by the PCM model. The generated TDDFT-ECD spectra were Boltzmann-weighted and compared to the experimental spectrum (Fig. 4). The TDDFT-simulated ECD spectrum was in a good agreement with the corresponding experimental ECD spectra (Fig. 4). This comparison revealed the absolute configuration and therefore 1 was assigned as 2S,16S,12S-diepoxy-11R-hydroxy-8R-methoxy-16-keto-cembr-12Z,3E-diene (sarcoehrenbergilid D).
Compound 2 was isolated as a white powder with a negative optical rotation of $[\alpha]_{D}^{23} = -5.4$ (c 0.03, CHCl$_3$). The molecular formula (C$_{20}$H$_{30}$O$_{5}$) was detected by high resolution electron ionization (HREIMS) spectrum (m/z 350.2094 [M$^+$], calcd 350.2093). HREIMS analysis exhibited a molecular ion peak at m/z 350.2094 [M$^+$] (calcd) The IR spectrum showed characteristic bands at $\nu_{\text{max}}$ 3445 cm$^{-1}$ and 1747 cm$^{-1}$ for hydroxyl and keto groups, respectively. The $^{13}$C NMR spectrum revealed twenty carbon signals (Table 1) classified by DEPT as six quaternary, four methines, six methylenes and four methyl carbons. 1D and 2D NMR spectroscopic data were quite close to saroehrenbergilid A, a formerly isolated diterpenoid from $S$. ehrenbergi except for an absence of methoxyl groups. For 2 there is an upfield carbon signal at $\delta_{C}$ 74.5 and a downfield methyl signal at $\delta_{C}$ 20.7 for C-8 and CH$_3$-19, respectively.

Stereochemistry was established based on coupling constants and NOESY experiments (Fig. 3). NOESY correlation indicated that 2 has the same relative stereochemistry as saroehrenbergilid A. To determine absolute configuration, TDDFT-ECO calculations were performed on the $2R,7S,8R,11R,12S$- and $2S,7R,8S,11S,12R$-enantiomers. The final Boltzmann-weighted TDDFT-ECO spectra were then compared to the corresponding experimental ECD curve (Fig. 4). According to the data depicted in Fig. 4, the $2R,7S,8R,11R,12S$-enantiomer reproduced all the transitions of the experimental ECD spectrum. Therefore, 2 was assigned as $2R,16:7S,12S$-diol-11R-hydroxy-8R-methoxy-16-keto-cembra-12,3E-diene (saroehrenbergilid E). Compound 3 was isolated as a colorless oil with a negative optical rotation of $[\alpha]_{D}^{23} = -10.8$ (c 0.01, CHCl$_3$). The molecular formula of C$_{20}$H$_{30}$O$_{5}$ was detected by high resolution electron ionization (HREIMS) analysis (m/z 332.1993 [M$-$H$_2$O$^+$], calcd 332.1998).

The IR spectrum showed characteristic bands at $\nu_{\text{max}}$ 3445 cm$^{-1}$ and 1747 cm$^{-1}$ for hydroxyl and keto groups, respectively. The $^{13}$C NMR spectrum (Table 1) showed 20 carbon resonances classified by DEPT analysis as four methylenes, six methylenes and six quaternary carbons. The 1D ($^{1}$H, $^{13}$C) as well as 2D NMR ($^{1}$H-$^{1}$H COSY, HSQC, and HMBC) (Fig. 2) spectroscopic data closely matches a previously reported cembrene compound. The NOESY correlation (Fig. 3) as well as the $^{1}$H and $^{13}$C NMR analyses indicated that 3 is a C-2 epimer of the previously isolated sarcophylolide E through the clear difference in downfield shift of H-3 ($\delta_{H}$ 5.10, d, $J = 10.0$). Additionally, several carbon signals showed downfield chemical shift in comparison of sarcophylolide E: $\delta_{C}$ 37.0/36.2 (C-5), 73.1/71.8 (C-12), 123.1/121.7 (C-15), and 175.5/174.9 (C-17), respectively. The carbon signals at $\delta_{C}$ 163.9 (C-1) and 36.3 (C-13) showed upfield chemical shift in comparison with sarcophylolide E ($\delta_{C}$ 165.8 (C-1) and 37.3 (C-13)).

The relative configuration for 3 was established based on coupling constants and NOESY experiments (Fig. 3). A NOE correlation between H-7 ($\delta_{H}$ 3.09 dd, $J = 7.5, 2.5$) and H-11 ($\delta_{H}$ 3.29 brd, $J = 10.0$) established an alpha linkage for the ether bridge between C-7 and C-11. The NOE correlations between H-3 and the $\gamma$-lactone-(H-2) as well as vicinal coupling constant indicated a trans-geometry for H-2 and H-3 of the olefinic bond (Fig. 3). As expected, the experimental ECD for 3 and published compound, sarcophylolide E, showed inverted direction for positive and negative cotton effect as well as optical rotation (Fig. 4). These data indicated that 3 is the C-2 epimer of sarcophylolides E. Thus, 3 was confirmed to be $2S,16:7R,11R$-diol-12S-hydroxy-8S-methoxy-16-keto-cembra-12,3E-diene (saroehrenbergilid F).

Isolated metabolites 1–3 were tested for cytotoxic activity toward lung (A549), colon (Caco-2) and liver (HepG2) human cancer cell lines based on an MTT reduction assay (Fig. 5). Compounds 1–3 showed most potent activity toward A549 cells with IC$_{25}$ values of 23.3, 27.3, and 25.4 $\mu$M, respectively. Compound 2 and 3 showed weaker activity toward liver (HepG2) human cancer cell lines with IC$_{25}$ values of 22.6 and 31.8 $\mu$M, respectively. The treated human colon cancer cells (Caco-2) cell viability was over 100% for all the isolated compounds (IC$_{25}$ >...
100 µM). Since primary necrosis is not easily differentiated from secondary necrosis that occurs with apoptosis, the mode of action will not be considered. To differentiate these distinct biological events requires apoptotic assays accompanying necrosis measurements. A combined necrosis/apoptotic time-course will be presented in a subsequent study to elaborate on mode of action.

3. Experimental section

3.1. General experimental procedures

Circular dichroism was measured on JASCO 810 spectropolarimeter. HREIMS data were collected on a JEOL JMS-700 instrument (Tokyo, Japan). NMR spectra were recorded on a Bruker 500 NMR spectrometer (Japan). JASCO P-2200 polarimeter and JASCO FT/IR-6300 spectrometer was used for optical rotation and infrared measurements, respectively.

Normal-phase silica gel 60 (230–400) column chromatography (CC) as well as aluminum TLC plates (silica gel 60 F<sub>254</sub> (Merck, Darmstadt, Germany) were used for purification and monitoring spotting, respectively. A H<sub>2</sub>SO<sub>4</sub> : MeOH (1 : 9) spraying reagent was used for spot visualization after heating. HPLC purification was performed using Shimadzu HPLC-RID-10A with YMC-Pack ODS-A analytical (250 × 4.6 mm i.d.) and preparative (250 × 20 mm i.d.) columns (YMC, Tokyo, Japan) for separation.

3.2. Animal material

Sarcophyton ehrenbergi coral was collected from the Red Sea on the Egyptian coast at Hurghada, in March 2016 and identified by Dr M. Al-Hammady. A voucher specimen (03RS27/1) was deposited in the National Institute of Oceanography and Fisheries, marine biological station, Hurghada, Egypt.

3.3. Extraction and isolation

Sliced frozen soft coral (2 kg, total wet weight) were extracted with CH<sub>2</sub>Cl<sub>2</sub> : MeOH (1 : 1, v/v) at room temperature (3 L × 4 times). Isolation protocol was performed as described previously by Hegazy <i>et al.</i>, 2017 (ref. 23) to afford 1 (5.5 mg), 2 (4 mg) and 3 (6 mg).

3.3.1 Sarcoehrenbergilid D (1). White powder; [α]<sub>D</sub><sup>25</sup> +10.8 (c 0.02, CHCl<sub>3</sub>); FT-IR (KBr) <i>v</i>: 3435, 2941, 1748, 1462, and 1224 cm<sup>−1</sup>; 1H and 13C NMR data, see Table 1; HREIMS <i>m/z</i> 346.2127 [M – H<sub>2</sub>O]<sup>+</sup> (calcd 346.2149).

3.3.2 Sarcoehrenbergilid E (2). White powder; [α]<sub>D</sub><sup>25</sup> −5.4 (c 0.03, CHCl<sub>3</sub>); FT-IR (KBr) <i>v</i>: 3433, 2938, 1743, 1446, and 1218 cm<sup>−1</sup>; 1H and 13C NMR data, see Table 1; HREIMS <i>m/z</i> 350.2094 [M]<sup>+</sup> (calcd 350.2093).

3.3.3 Sarcoehrenbergilid F (3). White amorphous powder; [α]<sub>D</sub><sup>25</sup> −10.8 (c 0.01, CHCl<sub>3</sub>); FT-IR (KBr) <i>v</i>: 3441, 2932, 1742, 1448, and 1229 cm<sup>−1</sup>; 1H and 13C NMR data, see Table 1; HREIMS <i>m/z</i> 332.1993 [(M – H<sub>2</sub>O)]<sup>+</sup> (calcd 332.1998).

3.4. Biological activity

3.4.1 Cell lines. Three human cancer cell lines, A549 (non-small cell lung adenocarcinoma), Caco-2 (colon adenocarcinoma) and HepG2 (hepatocellular carcinoma) (ATCC®) were assayed with the purified compounds. All cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (FBS fetal bovine serum), 1% penicillin and incubated in 5% CO<sub>2</sub> at 37 °C.

3.4.2 MTT cytotoxicity assay. The cytotoxicity of tested compounds was investigated by a MTT assay. Cell lines were seeded and incubated overnight allowing cell adhesion to the plate well (5 × 10<sup>3</sup> cells per well; 96-well plate in a volume of 100 µL). To generate concentration-dependent curves, sample concentration was varied (100, 50, 25, 12.5, 6.25 µM) for a total well volume of 200 µL for 48 h. MTT solution (5 mg mL<sup>−1</sup>) was added (100 µL per well) for 90 min before measurements. After medium removal, dark blue formazan crystals formed in viable cells were dissolved in 100 µL of DMSO, followed by shaking for 10 min (200 rpm). The absorbance was recorded at 492 nm using a microplate reader (Sunrise™ microplate reader, Tecan Austria Gmbh, Grödig, Austria) for cell viability measurement. IC<sub>25</sub> values were expressed as a concentration of tested compound that inhibits 50% cell growth in comparison with a vehicle control (quadrat to octuplet treatment) by nonlinear regression model analyses using GraphPad Prism® v 6.0 software.
3.5. Computational functional theory calculations

Conformational analysis was performed using Omega2 software\textsuperscript{30} to obtain the possible conformers for 1–3 within energy window value of 10 kcal mol\textsuperscript{-1}. All resulting conformers were optimized at B3LYP/6-31G* level of theory using Gaussian09 software.\textsuperscript{30} Frequency calculations were then performed on the optimized structures to ensure the nature of the local minima as well as to estimate the Gibbs free energies. Time-dependent density functional theory (TDDFT) calculations with incorporating a polarizable continuum model (PCM) using methanol as a solvent were carried out at the B3LYP/6-31G* level of theory to calculate the first fifty excitation states. Electronic circular dichroism (ECD) spectra were finally generated using SpecDis 1.71 (SpecDis 2017 (ref. 31 and 32)) by applying Gaussian band shapes with $\sigma = 0.20–30$ eV. The generated ECD spectra were Boltzmann-averaged.

4. Conclusions

Cembrane diterpenoids (1–3) were isolated and identified from the S. ehrenbergi soft coral. The isolated compounds were tested against three human cancer cell lines, which resulted in 2 being the most potent compound against lung A549 cancer cell. The absolute stereochemistry of 1–3 were confirmed by comparing experimental and TDDFT-simulated ECD spectra.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by Alexander von Humboldt Foundation (Georg Förster Research Fellowship) to MEFH, National Research Centre, Egypt and the Welch Foundation (D-1478). Dr Elshamy gratefully acknowledges the Takeda Science Foundation, Japan for the financial support. Computational work was completed in part with resources supported by the Science and Technology Development Fund, STDF, Egypt, Grants No. 5480 & 7972.

Notes and references


30 OpenEye Scientific Software, *OMEGA2.5.1.4*, Santa Fe, NM, USA.
