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New Cembranoid Diterpenes from *Sarcophyton trocheliophorum*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KAS and MS performed solvent extraction of the soft coral Sarcophyton trocheliophorum, fractional and compounds purifications using different chromatographic means, structural elucidation of the pure compounds using intensive spectroscopic studies (NMR & MS), and wrote the first draft of the manuscript. Author MAG collected the soft coral from the Red Sea and identified it on the bases of morphological taxonomy. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To isolate the unpolar bioactive diterpenes in pure forms from the soft coral *Sarcophyton trocheliophorum*, collected from Red Sea at the Egyptian coasts, and to identify their structures using diverse spectroscopic means (NMR [1D&2D] and MS). Additionally, to study the antimicrobial and cytotoxicity of the isolated compounds compared with the main crude extract of the soft coral.
Study Design: Collection of the soft coral, *Sarcophyton trocheliophorum* from Red Sea at coast of Hurgada, east Egypt, its homogenization, maceration in suitable organic solvent for extraction of the desired bioactive compounds and their purification by a series of different chromatographic

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techniques, identification of the compounds by mass spectrometry and NMR (1D&2D) spectroscopy, and then determination of the antimicrobial and cytotoxicity for them compared with their original crude extract.

Place and Duration of Study: Institute of Organic and Biomolecular Chemistry, University of Göttingen, Germany; and Chemistry of Natural Compounds Department, National Research Centre, Egypt, between August, 2008 and December, 2010.

Methodology: The soft coral *Sarcophyton trocheliophorum* was homogenized in a blender, macerated with chloroform-methanol, and the chloroform layer was concentrated *in vacuo*; while aqueous methanol solution was re-extracted with *n*-butanol, and the latter was concentrated to dryness. The resultant whole crude extract was fractionated using several subsequent column chromatographies on silica gel, and the fast fractions containing the unpolar components were purified by different column chromatographies (silica gel, Sephadex column and preparative TLC) to deliver the desired diterpenes in pure form. Structures of the afforded diterpenes were identified by mass spectrometry (ESI and HRESI-MS), NMR analysis (^{1}H,^{13}C NMR, and 2D NMR) and by comparison with reference data. The antimicrobial activity was determined by disc diffusion assay, while the cytotoxicity was determined using brine shrimp assay.

Results: Two new unpolar diterpenes, *cis*-cembrene C (1) and *cis*-cembrenene C (2) were isolated along with (+)-sarcophine (3), (+)-sarcophytoxide (4) and (-)-sarcophytoxide (5), from the chloroform-soluble extract of soft coral *Sarcophyton trocheliophorum*, collected from Red Sea. Four additional unpolar sesquiterpenes; β-elemene (6), caryophyllene (7), alloaromadendrene (8) and 1-methyl-4-(5-methyl-1-methylene-hex-4-enyl)-cyclohexene; bisabolene (9) were identifed by GC-MS analysis. Structures of the new diterpenes 1-2 were identified by spectroscopic data (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, HMQC, HMBC, HREI-MS and HRESI-MS) interpretation and comparison with related structures. The antimicrobial and cytotoxic activities of compounds 1-5 were evaluated in comparison with the original soft coral crude extract.

Conclusion: The soft coral *Sarcophyton trocheliophorum* is a prolific source for production of numerous bioactive compounds with diverse structures and biological activities which can be exploited for their commercial production.

Keywords: Cembrane diterpenes; Sarcophyton trocheliophorum; biological activities.

1. INTRODUCTION

Corals are symbiotic associations of coral animals with their algal partners (Zooxanthellae) [1,2]. They are rich sources of secondary metabolites used as chemical defense compounds to deter predators [3]. Soft corals belonging to the genus of Sarcophyton has been characterized by the production of cembranoid diterpenes, furanoditerpenes; in which the isopropyl group is functionalized as a γ-lactone [4-7] among with norditerpenes and sesquiterpenes [8,9]. A lot of biological activities were reported for these metabolites, including cytotxicity [10-14], antimicrobial activity [15] and inhibition of the lipopolysaccharide-induced production of the proinflammatory cytokine TNF-α [16].

As follow up to our previous searching for novel bioactive metabolites from *Sarcophyton trocheliophorum*, collected from Red Sea [17], we described here the isolation and structural determination of two new cembrane diterpenes; *cis*-cembrene C (1) and *cis*-cembrenene C (2) along with the well known (+)-sarcophine (3), (+)-sarcophytoxide (4), and (-)-sarcophytoxide (5). This was together with the detection of four sesquiterpenes; *β*-elemene (6), *trans*-caryophyllene (7), alloaromadendrene (8) and cyclohexene,1-methyl-4-(5-methyl-1-methylene); bisabolene (9) by GC-MS analysis (Fig.1). The isolated compounds 1-5 were identified using intensive studies of NMR spectrosopy (^{1}H, ^{13}C, ^{1}H,^{1}H COSY, HMQC and HMBC) and mass spectrometry (EI, HRESI-MS), and by comparison with related structures as well. The antimicrobial and cytotoxic activities of the reported compounds were investigated.

2. MATERIALS AND METHODS

The NMR spectra were measured on Varian (Palo Alto, CA, USA) Unity 300 (300.145 MHz) and Varian Inova 500 (125.7 MHz) spectrometers. EI-MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosine as reference substance for HR-EIMS. HR-ESIMS were recorded by ESI MS on an Apex IV 7 Tesla Fourier-Transform Ion

Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). GC-MS was carried out using a Trace GC-MS Thermo Finnigan, EI ionization mode at 70 eV, instrument equipped with a capillary column CP-Sil 8 CB for amines (length: 30 m; inside diameter: 0.25 mm; outside diameter: 0.35 mm; film thickness: 0.25 *μ*m). The analysis was carried out at a programmed temperature: initial temperature 40°C (kept for 1 min), then increasing at a rate of 10°C/min until the final temperature of 280°C (kept for 10 min); injector temperature was at 250°C and detector temp. was set to 250°C; Helium (He) was used as carrier gas at a flow rate 1 mL/min; total run time 27 min, injection volume 0.2 *μ*L. Flash chromatography was carried out on silica gel (230-400 mesh). R_f values were measured on Polygram SIL G/UV_{254} TLC cards (Macherey-Nagel). Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex, Amersham Biosciences Ltd; purchased from Sigma-Aldrich Chemie, Steinheim, Germany).

2.1 Extraction, Isolation and Purification

Details of extraction and chromatographic purification of the soft coral *Sarcophyton trocheliophorum* were reported recently [17], where 10 fractions were obtained. GC-MS analysis of the unpolar fraction I afforded the listed compounds in Table 1. An application of the fast fraction I (1.7 g) to PTLC and elution with n-pentane resulted in a colourless oil of 4-isopropyl-1,7,11-trimethyl-cyclotetradeca-1,3,7,11-tetraene; *cis*-cembrene C (1, 150 mg). Purification of the second fast fraction II (1.2 g) using Sephadex LH-20 (DCM/MeOH, 60:40) delivered a major unpolar colourless oil (130 mg) of 4-isopropenyl-1,7,11-trimethyl-cyclotetradeca-1,3,7,11-tetraene-oid; *cis*-cembrenene C (2). A purification of the middle polar fraction VI (21.2 g) using silica gel column and elution with DCM-MeOH gradient afforded 8.2 g colourless needles of (+)-sarcophine (3). Further two related colourless solids of higher polarity than 3 were obtained; which on extensive purification using PTLC (DCM/MeOH, 95:5) followed by Sephadex LH-20 (DCM/MeOH, 60:40), obtained colourless solids of (+)-sarcophytoxide (4, 15.2 mg) and (-)-sarcophytoxide (5, 9.3 mg). The remaining fractions and sub-fractions obtained from the soft coral *Sarcophyton trocheliophorum* extract lacked the diterpenes analogues based on TLC analyses. These fractions and sub-fractions are still under investigations and will be reported in a fellow research work.

2.2 Antimicrobial Activity and Brine Shrimp Microwell Cytotoxic Assays

Details of our general antimicrobial activity testing were reported recently [17]. The cytotoxicity assay was performed according to Takahashi et al. [18] and Sajid et al. [19] screening.

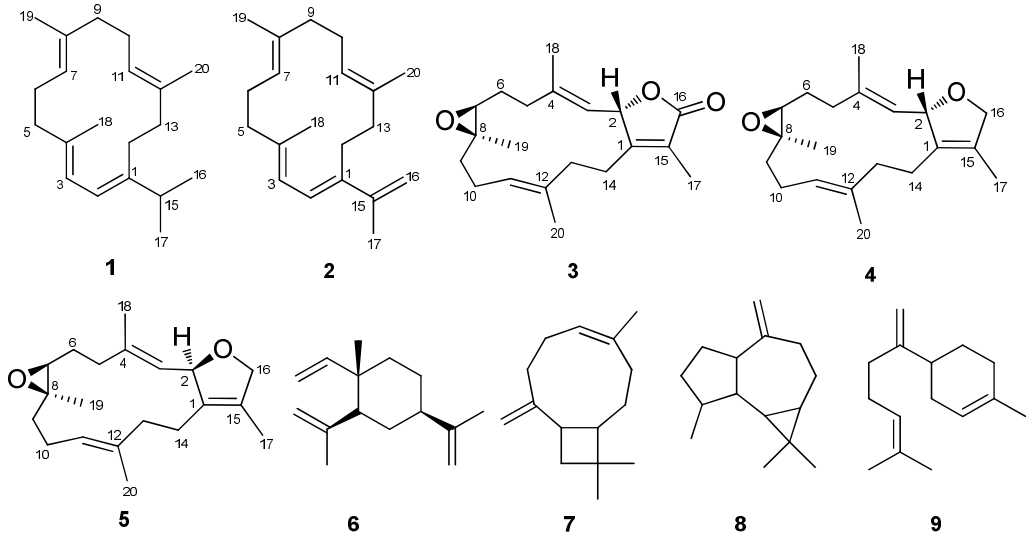

Fig. 1. Chemical structures of isolated (1-5) and detected (6-9) compounds from *Sarcophyton trocheliophorum*

Table 1. ^{13}C and ^{1}H NMR ($CDCl_3$, *J* in Hz) of (+)-sarcophine (3) along with ^{13}C NMR data of (+)-sarcophytoxide (4) and (−)-sarcophytoxide (5)

Position	3		4	5
	δ_C (125 MHz)	δ_H (300 MHz)	δ_C (125 MHz)	δ_C (125 MHz)
1	162.1	-	133.5	132.9
2	78.7	5.57 (dd, 9.9, 1.7)	83.4	85.00
3	120.5	5.04 (dd, 10.0, 1.1)	125.9	125.0
4	143.8	-	139.9	138.8
5	37.4	2.40 (ddd, 12.7, 12.7, 3.0)	38.3	37.4
6	25.2	1.70 (m), 1.90 (m)	25.9	27.2
7	61.4	2.68 (t, 8.8)	62.2	61.6
8	59.9	-	60.0	59.6
9	39.0	1.10 (td, 12.9, 2.9), 2.20 (m)	39.6	38.7
10	23.3	2.10 (m), 2.27 (m)	23.3	23.0
11	124.8	5.14 (dd, 9.6, 5.3)	123.3	123.0
12	135.4	-	136.4	135.5
13	36.4	2.10 (m), 2.24 (m)	36.4	34.8
14	27.5	2.76 (m), 2.10 (m)	25.1	23.5
15	122.8	-	127.7	127.4
16	174.5	-	78.1	78.0
17	9.0	1.85 (s)	10.2	10.0
18	16.1	1.90 (d, 1.3)	15.2	14.9
19	17.2	1.28 (s)	17.5	16.7
20	15.5	1.62 (s)	16.2	15.4

3. RESULTS AND DISCUSSION

3.1 Structural Elucidation

Structures of the well-known diterpenes, (+)-sarcophine (3) [20,21], (+)-sarcophytoxide (4) [22] and (-)-sarcophytoxide (5) were identified on the bases of NMR spectroscopy (1D&2D) (Table 1), mass spectrometry and by comparison with the literature data [20]. The sesquiterpenes, *β*-elemene (6) [23,24], caryophyllene (7) [25], alloaromadendrene (8) [26] and 1-methyl-4-(5-methyl-1-methylene-hex-4-enyl)-cyclohexene; bisabolene (9) [27,28], and dodecane were identified using GC-MS analysis (Table 2) [29]. The physico-chemical properties of the new diterpenes 1-2 are described in Table 3.

Table 2. GC-MS analysis of the unpolar metabolites from fraction I

Name	R_t (min)	M. F.	M. wt.
Dodecane	9.24	$C_{12}H_{26}$	170
β-Elemene (6)	13.59	$C_{15}H_{24}$	204
Caryophyllene (7)	14.08	$C_{15}H_{24}$	204
Alloaromadendrene (8)	14.63	$C_{15}H_{24}$	204
Bisabolene (9)	15.07	$C_{15}H_{24}$	204

3.1.1 *Cis*-cembrene C

Compound 1 was isolated as colourless oil from the unpolar fraction I, applying a series of chromatographic techniques. The compound showed UV absorbance (254 nm) on TLC, and turned pink and changed later to blue on spraying with anisaldehyde/sulphuric acid and heating. The molecular weight of 1 was established as 272 Daltons, and *via* HREI MS and HRESI MS, the molecular formula was deduced as $C_{20}H_{32}$, corresponding to five double bond equivalents. Expectedly, the EI mass spectrum of 1 showed a parent molecular ion at *m/z* = 272, followed by a fragment ion at *m/z* = 270 due to the elimination of two hydrogen atoms (Table 4).

In the NMR spectra, compound 1 showed signals for 5 methyls, 6 methylenes, 5 methines and 4 quaternary carbons as matched with the molecular formula, being most likely for a diterpene system. The combination of ^{1}H, ^{13}C and HMQC NMR spectra of 1 (Table 4) revealed two 1H multiplets at *δ* 5.00 (δ_C 124.9) and 5.00 (δ_C 124.5) representing two individual 1,1,2-tri-substituted olefinic systems [-CH=C_q]. Two further 1H doublets corresponding to two conjugated 1,1,2-trisubstiuted olefinic systems of a cisoid-configuration, were visible at *δ* 5.93 (*J* = 11.2, 1.1 Hz, δ_C 121.9) and 6.01 (*J* = 11.4 Hz, δ_C 118.4). Six multiplet sp^2-bounded methylenes were present in the region of δ2.32-2.08 (δ_C 39.2~38.6, 28.1~24.6) in addition to three sp^2-attached methyl singlets (δ1.74~1.51) and two

sp^3-bounded methyl doublets (δ 1.04). The last two doublet methyls (δ_H 1.04) were linked to an sp^3 methine (δ_H 2.32, δ_C 33.8) (according to H,H COSY), representing an isopropyl group. The presence of further four quaternary carbons were established in the region δ 146.8~134.1 ppm.

Based on the H,H COSY, the six sp^2-attached methylenes mentioned above represent three ethanediyl pairs (3 × [-CH_2-CH_2-]). According to H,H COSY, two pairs of these methylenes were directly linked through two sp^2 methine carbons (124.9 &124.5) from one side, while they are connected to the quaternary carbons (134.4 & 134.6) from the other side according to HMBC connectivities (Fig. 1). Methylenes of the third ethanediyl group (H_2-14 [δ 28.1] and H_2-13 [δ 38.6]) were flanked by two sp^2 quaternary carbons, C-1 (146.8) and C-12 (134.6), respectively; such that H_2-13 (δ2.20-2.08) and H_2-14 (δ2.32) showed ^3J and ^2J correlations, respectively, at C-1 (146.8) from a side, while H_2-14 exhibited ^3J coupling at C-12 (134.6) from the other side. The doublet methyls (H_3-16,17) of the isopropyl group showed H,H COSY correlations *versus* the methine CH-15 (δ2.32), and the latter was in turn attached to C-1 (δ_C 146.8), as C-1 received ^3J and ^2J couplings from the doublet methyls (H_3-16,17) and H-15, respectively. The singlet methyl group H_3-20 (δ1.58) showed three correlations to C-13 (38.6, ^3J), CH-11 (124.5, ^3J) and C-12 (^2J), confirming the direct attachment between propene ([-CH=C(CH_3)-]) system and the methylene carbon (CH_2-13).

The olefinic methine CH-11 (δ_H 5.00) showed H,H COSY correlations to the methylene H_2-10 (δ_H 2.20-2.08), together with two HMBC couplings at C-10 (δ_C 24.6, ^2J) and C-9 (δ_C 39.0, ^3J) and *vice versa*. On other hand, protons of the second sp^2-attached methyl group (H_3-19) displayed three HMBC correlations at C-9 (39.0, ^3J), C-8 (134.1, ^2J) and CH-7 (^3J, δ_C 124.9), confirming an attachment between the last ethanediyl group (C-9, C-10) and another propene system ([-CH=C(CH_3)-]). Likely, proton signal of the olefinic methine H-7 (δ_H 5.00) directed four HMBC correlations towards methylene carbons of the third ethanediyl group; C-6 (δ_C 25.3, ^2J), C-5 (δ_C 39.2, ^3J) along with CH_3-19 (δ_C 15.7, ^3J) and CH_2-9 (δ_C 39.0, ^3J), verifying the direct attachment between those of C-7 and C-6 of the respite ethanediyl group. Proton signals of last ethanediyl group (H_2-6 & H_2-5) showed respective ^3J and ^2J H→C correlations at the quaternary carbon C-4 (δ_C 134.4), proving the connection between CH_2-5 and C_q-4. The last olefinic quaternary carbon (C-4) received further two ^2J correlations from the vicinal H_3-18 (δ_H 1.74) and CH-3 (δ_H 5.93), in addition to an exchangeable HMBC (^3J) correlation between H_3-18 and CH-3, fixing the attachment of a third propene system [-CH=C_q(CH_3)-]. The doublet proton signal of H-3 (δ_H 5.93, J ~11.2 Hz) showed a COSY correlation to the vicinal olefinic methine H-2 (δ_H 6.01, J = 11.4 Hz), confirming their cisoid configuration, which was further established by HMBC correlations (Fig. 2). Based on these features, compound 1 was clearly deduced as cyclic diterpene of a cisoide configuration between CH-3 and CH-2. Cembrene C, transoide-configuration of compound 1, was isolated previously from a soft coral Nephthea [30], which was applied to synthetic trials, starting from farnesyl acetate [31,32] or geraniol [33]. Cembrene C; isoneocembrene A was reported by GC-MS analysis as well [34,35]. Consequently, compound 1 of cisoide-configuration, was reported here to first time, which we named as *cis*-cembrene C. It is worthy to refer herein that cembrene C (1) was reported recently [36], however, its structure was assigned on the bases comparison with non recent literatures [30,37], which have no recent spectroscopic means to find out the structure definitely, as well as its configuration.

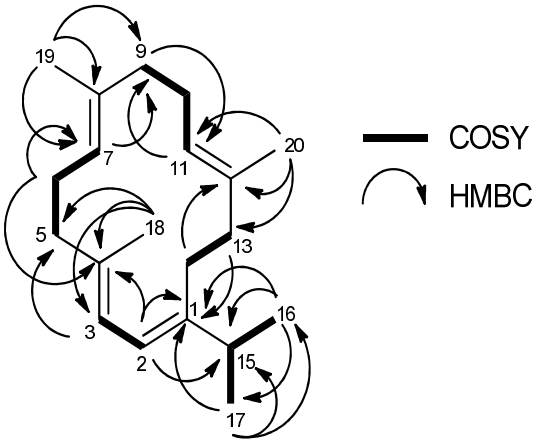

Fig. 2. Selected HMBC (→) and H,H COSY (bold lines) couplings of *cis*-cembrene C (1)

Table 3. Physico-chemical properties of *cis*-cembrene C (1) and *cis*-cembrenene C (2)

	1	2
Appearance	**Colourless oil**	**Colourless oil**
R_f	0.31 (n-pentane)	0.51 (n-pentane)
Colouration with anisaldehyde/ sulphuric acid	blue turned later to dark green	pink turned later to blue
Solubility	Soluble in hexane, DMSO, MeOH, EtOH, EtOAc and CH_2Cl_2. Insoluble in H_2O.	Soluble in hexane, DMSO, MeOH, EtOH, EtOAc and CH_2Cl_2. Insoluble in H_2O.
Molecular formula	$C_{20}H_{32}$	$C_{20}H_{30}$
EI-MS: *m/z* (%)	272 ($[M]^{+\bullet}$, 100), 270 ($[M\text{-}2H]^+$, 14), 229 (8), 189 (8), 161 (20), 136 (82), 121 (88), 119 (40), 107 (30), 91 (30), 81 (27), 68 (36), 55 (24), 41 (38)	
HREI-MS: *m/z*		
Found	272.2496 $[M]^{+\bullet}$	
Calcd.	272.2498 for $C_{20}H_{32}$	
(-)-HRESI-MS		
Found	271.242439 $[M-H]^-$	269.22658 $[M-H]^-$
Calcd.	271.24244 for $C_{20}H_{31}$	269.22659 for $C_{20}H_{29}$

Table 4. ^{13}C of and ^1H NMR ($CDCl_3$, *J* in Hz) of *cis*-cembrene C (1) and *cis*-cembrenene C (2)

Position	1		2		Yalongenes A & B [36]	
	δ_C (125MHz)	δ_H (300 MHz)	δ_C (125MHz)	δ_H (300 MHz)	$\delta_{C\text{-}trans}$ (Yalongene A)	$\delta_{C\text{-}cis}$ (Yalongene B)
1	146.8	-	143.5	-	143.5 ($^{\delta}\Delta$ =0)	143.6 ($^{\delta}\Delta$ = 0.1)
2	118.4	6.01 (d, 11.4)	124.7	6.38 (d, 11.3)	124.8 ($^{\delta}\Delta$=0.1)	124.8 ($^{\delta}\Delta$ =0.1)
3	121.9	5.93 (dd,1.2,1.1)	123.8	6.04 (d, 11.6)	122.6 ($^{\delta}\Delta$=-0.2)	123.8 ($^{\delta}\Delta$ = 0)
4	134.4	-	134.3	-	138.3 ($^{\delta}\Delta$=4.0)	137.9 ($^{\delta}\Delta$ =3.6)
5	39.2	2.20-2.08 (m)	38.9	2.18-2.10 (m)	39.5 ($^{\delta}\Delta$=0.6)	31.2 ($^{\delta}\Delta$ =−7.7)
6	25.3	2.20-2.08 (m)	25.4	2.18-2.10 (m)	25.3 ($^{\delta}\Delta$= −0.1)	26.3 ($^{\delta}\Delta$ =−0.9)
7	124.9	5.00 (m)	124.3	5.04-4.87 (m)	124.3 ($^{\delta}\Delta$=0)	123.3 ($^{\delta}\Delta$ =−1.0)
8	134.1	-	134.3	-	134.4 ($^{\delta}\Delta$=0.1)	134.5 ($^{\delta}\Delta$ =0.2)
9	39.0	2.20-2.08 (m)	39.0	2.18-2.10 (m)	38.8 ($^{\delta}\Delta$=−0.2)	39.6 ($^{\delta}\Delta$ =0.6)
10	24.6	2.20-2.08 (m)	26.7	2.18-2.10 (m)	24.6 (Δ=−2.1)	25.3 ($^{\delta}\Delta$ =−1.4)
11	124.5	5.00 (m)	125.0	5.04-4.87 (m)	125.1 ($^{\delta}\Delta$=0.1)	127.1 ($^{\delta}\Delta$ =2.0)
12	134.6	-	132.0	-	135.3 ($^{\delta}\Delta$=3.3)	132.1 ($^{\delta}\Delta$ =0.1)
13	38.6	2.20-2.18(m)	38.5	2.18-2.10 (m)	39.0 ($^{\delta}\Delta$=0.5)	38.5 ($^{\delta}\Delta$ =0)
14	28.1	2.32 (m)	24.8	2.18-2.10 (m)	26.5 ($^{\delta}\Delta$=1.7)	24.2 ($^{\delta}\Delta$ =−0.6)
15	33.8	2.32 (m)	136.1	-	139.1 ($^{\delta}\Delta$=3.0)	136.1 ($^{\delta}\Delta$ =0)
16	22.4	1.04 (d, 6.8)	111.1	4.99 (s), 4.91(s)	111.8 (Δ=0.7)	111.2 ($^{\delta}\Delta$ =0.1)
17	22.4	1.04 (d, 6.8)	21.6	1.91 (d, 1.2)	21.3 ($^{\delta}\Delta$=−0.3)	21.5 ($^{\delta}\Delta$ =−0.1)
18	17.0	1.74 (s)	16.0	1.51 (s)	17.2 ($^{\delta}\Delta$=1.2)	23.8 ($^{\delta}\Delta$ =7.8)
19	15.7	1.51 (s)	15.8	1.51 (s)	15.9 ($^{\delta}\Delta$=0.1)	15.7 ($^{\delta}\Delta$ =−0.1)
20	17.2	1.58 (s)	16.2	1.54 (d, 1.6)	17.2 ($^{\delta}\Delta$=1.0)	16.1 ($^{\delta}\Delta$ =−0.1)

3.1.2 *Cis*-cembrenene C

Closely related to *cis*-cembrene C (1), compound 2 was obtained as colourless oil from the second fast fraction II, with less polarity than 1, showing stronger UV absorbance (254 nm) on the TLC than 1 in addition to similar reaction with anisaldehyde/sulfuric acid, indicating its terpenoidal nature (Table 3). The molecular formula was established as $C_{20}H_{30}$ based on HRESIMS (Table 3), with less 2H than 1 corresponding to six double bond equivalents. The ^1H and ^{13}C NMR spectra (Table 4) exhibited the same pattern as in 1, except the replacement

of the doublet methyls of the isopropyl group attached to C-1 in 1 by an isopropenyl group, at where two singlet proton signals for a terminal-methylene (H_2-16), were visible at 4.99 and 4.91 (δ_C 111.1) along with a further sp^2-attached doublet methyl signal (H_3-17, δ_H 1.91, J~1.2 Hz) and the in-between quaternary carbon (C-15) was proved at δ_C 136.1. The ^{1}H NMR, ^{13}C NMR and HMQC spectra of 2 (Table 4) showed in accordance, 20 carbon signals comprising five quaternary carbons, four sp^2-attached methyls, six sp^2-attached methylenes, four sp^2-methines and one exo-sp^2-methylene. According to further studies using H,H COSY and HMBC correlations (Fig. 3), structure of 2 was proved as newly ene-derived diterpene of *cis*-cembren C (1), which we named as *cis*-cembrenene C (2).

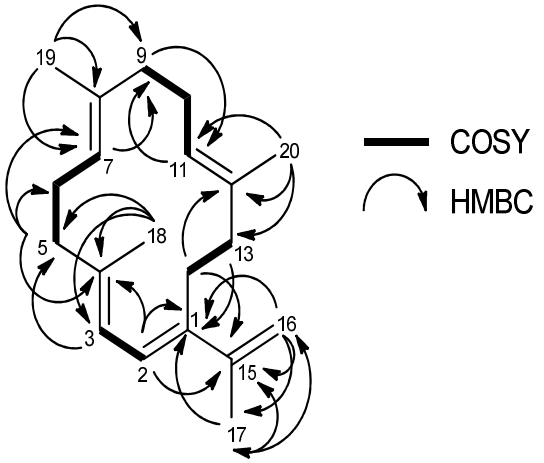

Fig. 3. Selected HMBC (→) and H,H COSY (bold lines) couplings of *cis*-cembrenene C (2)

Yalongenes A and B are closely related stereoisomers to *cis*-cembrenene C (**2**), which were isolated from the South China Sea soft coral *Sarcophyton trocheliophorum* Marenzeller, and assigned as transoide (A) and cisoide (B) isomers, respectively [36]. On the bases of comparison of the ^{13}CNMR data of compound 2 with both isomers, it is shown a difference in some carbon values between either of these two isomers *versus* 2. Particularly, the *cis*-configured Yalongene B showed a $^{\delta}\Delta$ =3.6 ppm higher in C-4 than those of 2, while C-5 showed $^{\delta}\Delta$ =-7.7 ppm lower than 2. In contrast, C-11 and C-18 of yalongene B showed respective higher values ($^{\delta}\Delta$ =2.0 and 7.8) than those of 2. Alternatively, the coupling constant of the cisoide isomer of yalongene B (J = 10.8 Hz), while its reported transoide one (yalongene A, J = 11.4) is very close to 2 (J_2 = 11.3, J_3 = 11.6 Hz). In accordance, there is a difference in the assignment of the carbon signals for 2 in comparison with both isomers of yalongene. However, it is worthy to know that $^3J_{trans} > {}^3J_{cis}$, and typical values are ~17 and 10 Hz, respectively [38].

Alternatively, the determination of the geometric configuration for compound 2 whereas *cis* or *trans* by X-ray analysis is not possible, since all attempts to obtain suitable crystals using various crystallization conditions were unsuccessful. Pure unpolar cembrenes and their congeners are known for their poor crystallization or the formation of small crystals, which are not suitable for X-ray analysis. Based on the NMR spectroscopy, proton signals of the NOE (1D) spectrum for the desired assignments (H_3-17, H-2, H-3, H_3-18 and H_2-5) showed high overlapping with other signals of the molecule, and it was not possible to fix the configuration (*cis*/*trans*) herein for 2. Furthermore, compound 2 showed no attached hydroxyl or amino groups, and hence the attempts to determine the configuration on the bases of chiral reagents (e.g. Mosher or other chiral reagents) were not possible as well. Therefore, the coupling constant of the olefinic protons at H-2 and H-3 in 2, was the sole technique which was able herein to fix the configuration of 2 as *cis*, is a good decision in this case, which was supported by comparison with the related structures from literatures.

3.2 Biological Activities

The crude extract of *Sarcophyton trocheliophorum* and the isolated compounds were antibiotically inactive against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Candida albicans*, *Mucor miehi*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, *Rhizoctonia solani*, and *Pythium ultimum* at 40 *μ*g/disk. The resulted diterpenes 1-5 and the crude extract were further examined for cytotoxicity against brine shrimp at a concentration 10 *μ*g/mL (24 hr). *Cis*-cembrene C (1) showed a weak cytotoxicity with a mortality of 22.5%, while the remaining compounds 2-5 exhibited no cytotoxcity (0%). In addition, the crude extract of *Sarcophyton trocheliophorum* showed no cytotoxicity with a mortality rate of 1.7% [17].

4. CONCLUSION

During our continual bioassay guided fractionation of the soft coral *Sarcophyton trocheliophorum* collected from Red Sea [17], two new unpolar cembrane diterpenes namely *cis*-cembrene C (1) and *cis*-cembrenene C (2) were isolated along with (+)-sarcophine (3), (+)-sarcophytoxide (4) and (-)-sarcophytoxide (5). Four unpolar sesquiterpenes; β-elemene (6), caryophyllene (7), alloaromadendrene (8) and 1-methyl-4-(5-methyl-1-methylene-hex-4-enyl)-cyclohexene; bisabolene (9) were further identifed by GC-MS analysis. Structure of the new diterpenes 1-2 were identified by spectroscopic data (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, HMQC, HMBC, HREI-MS and HRESI-MS) interpretation and by comparison with related structures. The antimicrobial and cytotoxic activities of compounds 1-5 were further investigated.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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