Phytochemistry Letters 6 (2013) 383–386

Contents lists available at SciVerse ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Trochelioid A and B, new cembranoid diterpenes from the Red Sea soft coral *Sarcophyton trocheliophorum*

Mohamed-Elamir F. Hegazy [a], Tarik A. Mohamed [a], Fathy F. Abdel-Latif [b], Mansour S. Alsaid [c], Abdelaaty A. Shahat [a,c,*], Paul W. Paré [d]

[a] *Phytochemistry Department and Center of Excellence for Advanced Sciences, National Research Centre, El-Tahrir Street, Dokki, Giza 12622, Egypt*
[b] *Department of Chemistry, Faculty of Science, Minia University, El-Minia 61519, Egypt*
[c] *Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*
[d] *Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409-1061, USA*

ARTICLE INFO

Article history:
Received 31 December 2012
Received in revised form 16 April 2013
Accepted 3 May 2013
Available online 21 May 2013

Keywords:
Sarcophyton trocheliophorum
Soft coral
Diterpenes
Family Alcyoniidae

ABSTRACT

Chemical investigations of the soft coral *Sarcophyton trocheliophorum*, has led to the isolation of six cembranoids, two of which are new, Trochelioid A (1) and B (2), and one, 16-oxosarcophytonin E (3) isolated from nature for the first time. Additionally, two have been isolated from *S. trocheliophorum* for the first time (4 and 6). Structures were elucidated by employing extensive NMR and HR-FAB-MS experimentation.

1. Introduction

The Red Sea serves as an epicenter for marine biodiversity with a high endemic biota. Indeed of the 180 soft corals species identified worldwide, approximately 40% are native to the Red Sea. Cembranoids are a family of 14-membered cyclic diterpenoid natural products biosynthesized by a diverse range of marine organisms (Tursch et al., 1978) including the soft coral order Alcyonacea distinctive among coral in that they do not produce calcium carbonate skeletons (Lu et al., 2008; Anthoni et al., 1991). Soft coral of the genus *Sarcophyton* (family Alcyoniidae) are particularly abundant in cembrane terpenes (Kobayashi et al., 1983). Indeed *Sarcophyton trocheliophorum* is so rich in terpenes that the species can be toxic toward other marine life within close proximity (Rezanka and Dembitsky, 2001). From a medical prospective, cembrane-type compounds are noteworthy with regard to their bioactive activity (Bishara et al., 2007; Bensemhoun et al., 2008) with reported anti-inflammatory (Lu et al., 2008), antifouling (Anthoni et al., 1991), Ca^{2+}-antagonistic (Kobayashi et al., 1983), and Na^{+}, K^{+}-ATPase inhibition activities (Suleimenova et al., 1990). Moreover, cembrane diterpenes exhibit high potency as antitumor agents (Jia et al., 2007; Yan et al., 2007; Zhang et al., 2006).

S. trocheliophorum is found in the Red Sea and Indo-Pacific inhabiting reef flats and lagoons at depths of 2–5 m. It is a large toadstool/mushroom coral commonly referred to as elephant ear coral. From this hardy species that is rich in biologically active natural products, two new cembranoids have been isolated from an ethyl acetate extract and chemical structures are reported based on spectroscopic analysis using NMR and MS techniques.

2. Results and discussion

Two new cembranoids (**1**–**2**) have been isolated from the ethyl acetate extract of *S. trocheliophorum* (Fig. 1). The EtOAc-soluble fraction subjected to normal and reverse phase chromatography also afford four known cembranolides, 16-oxosarcophytonin E (**3**), 8-*epi*-sarcophinone (**4**), (+) sarcophine (**5**) (Chao et al., 2008; Huang et al., 2006, 2009; Cheng et al., 2008), and *ent*-sarcophine (**6**) (Huang et al., 2006, 2009). Compound **3** was isolated for the first time from nature, while **4** and **6** were isolated for the first time from this species. Preliminary ^{1}H NMR analysis established that all fractions shared a common carbon skeleton, differing either in the degree of oxidation or the configuration of one or more of the chiral centers. Precedent from soft coral literature led to the assumption of a cembranoid-skeleton backbone (Huang et al., 2006).

* Corresponding author at: Medicinal, Aromatic & Poisonous Plants Research Center, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia. Tel.: +966 537507320; fax: +966 1 4676220.
E-mail address: aashahat@hotmail.com (A.A. Shahat).

1874-3900/$ – see front matter © 2013 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.phytol.2013.05.005

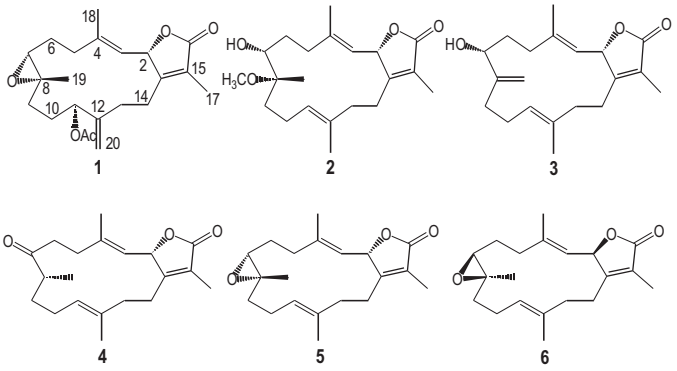

Fig. 1. Structures of metabolites **1**–**6**.

Compound **1** was obtained as colorless oil, $[\alpha]_D^{25}$ +10.0 (*c* 0.005, MeOH). The HR-FAB-MS exhibited a *m*/*z* of 397.2093 $[M+Na]^+$, indicating a molecular formula of $C_{22}H_{30}O_5Na$, appropriate for seven degrees of unsaturation. The IR spectrum indicated the presence of an α,β-unsaturated-γ-lactone (1753 and 1686 cm^{-1}), an epoxide (1254 cm^{-1}) and a keto group (1706 cm^{-1}). These structural features were supported by NMR data. The ^{13}C NMR and DEPT spectrum (Table 1) exhibited 22 carbon signals establishing: four methyls (including one acetate methyl), seven methylenes, four methines, and seven quaternary carbons (including two ester carbonyls). The spectrum also revealed the presence of an exomethylene functionality at δ_C 111.5/144.3, three oxymethine carbons at δ_C 61.2, 73.9 and 79.0, one oxygenated quaternary carbon at δ_C 61.4, and two olefinic carbons at δ_C 119.9 and 145.8. ^{13}C NMR analysis indicated that two oxygens contributed to an α,β-unsaturated-γ-lactone with appropriate signals at δ_C 174.5 and 79.0 for the carbonyl and oxymethine carbons, respectively. The olefinic methyl group at δ_H 1.83 (CH_3-17) exhibited an HMBC correlation with a low-filed ^{13}C NMR resonance for a keto group in association with the α,β-unsaturated-γ-lactone ring at δ_C 174.5 (C-16); CH_3-17 also showed HMBC correlations with carbons at δ_C 124.5 (C-15) and δ_C 161.1 (C-1). Carbon signals at δ_C 124.5 (C-15) and 161.1 (C-1) were consistent with α and β olefinic carbons of an α,β-unsaturated-γ-lactone system. The carbon signal at δ_C 79.0 (C-2) was consistent with an oxymethine carbon while the oxymethine proton at δ_H 5.48 (d, J = 9.6 Hz; H-2) exhibited a strong correlation with a one-proton doublet at δ_H 5.09 (J = 9.6 Hz; H-3) in the ^1H–^1H COSY spectrum (Fig. 2). The olefinic methyl group proton signal at δ_H 1.93 (H_3-18) also shows an HMBC correlation with an olefinic methine carbon signals at δ_C 119.9 (C-3) as well as correlations with δ_C 145.8 (C-4) and δ_C 36.1 (C-5). The location of the epoxide ring at C-7/C-8 was determined from HMBC correlations observed between C-7 (δ_C 61.2) and CH_3-19 and H-9 (1.87 m); and between C-8 (δ_C 61.4) and H-9 and CH_3-19 (Fig. 2). The downfield signal at δ_H 5.24 (J = 5.0 Hz; H-11) integrating for one proton was consistent with an attached acetate unit. The proton on the oxymethine carbon at C-11 (δ_C 73.9) exhibited correlations with methylene signals at H-9 (δ_H 1.17, m) and exomethylene signals at H_2-20 (δ_H 4.97, s and 5.03, s) in the ^1H–^1H COSY spectrum (Fig. 2). The position of the acetate was confirmed by HMBC correlations between H-11 and C-9 (32.0, t), C-12 (144.3, s), and C-20 (111.5, t). Exomethylene protons at δ_H 4.97 (s) and 5.03 (s) (H_2-20) showed strong correlations with carbon signals at δ_C 111.5 (C-20) and δ_C 144.3 (C-12) in HMQC and HMBC analyses, respectively. Furthermore, the acetoxy group positioned at C-11 was confirmed by HMBC correlations between methyl protons of the acetate (δ_H 2.07) and the ester carbonyl carbon at δ_C 170.1 (C=O) as well as the oxymethine carbon at 73.9 (C-11). The relative configuration of **1** was determined on the basis of coupling constants and NOESY experiments. The vicinal coupling constant of 9.6 Hz between H-2 and H-3 suggested a *cis* configuration between the γ-lactone proton (H-2) and the olefinic proton (H-3) which was confirmed by observed NOESY correlations (Fig. 3). The NOE correlation observed between H-11 and H-10β (δ_H 1.50), H-9β (δ_H 1.87), and H-7β (δ_H 2.46) reflects an α-orientation of the acetate substituent at C-11. Furthermore, a NOESY experiment exhibited an NOE interaction between CH_3-17 and H-2, revealing an *E* geometry for the C-3/C-4 double bond (Hegazy et al., 2011). From the above evidence and the other NOE correlations (Fig. 2), the structure of **1** was established unambiguously to be 11β-acetoxy-sarcoph-12(20)-ene (**1**).

Compound **2** was obtained as colorless oil, $[\alpha]_D^{25}$ +86.7 (*c* 0.02, MeOH). The HR-FAB-MS exhibited a *m*/*z* of 371.2209 $[M+Na]^+$ suggesting a molecular formula of $C_{21}H_{32}O_4Na$ with seven degrees of unsaturation. The IR spectrum revealed the presence of an α,β-unsaturated-γ-lactone (1750 and 1686 cm^{-1}), a broad absorption band for OH stretching (3000–3350 cm^{-1}) and a keto group (1707 cm^{-1}). All these predicted functional groups were supported by NMR data. ^{13}C NMR (Table 1) and DEPT spectral data indicted 21 carbons present in the configuration of: five tertiary methyls (including one methoxy methyl), six methylenes, four methines, and six quaternary carbons. Chemical shift data suggested a lactone carbonyl at δ_C 175.0, a methoxyl at δ_C 49.2, two oxymethine carbons at δ_C 79.5 and 72.8, one oxygenated quaternary carbon at δ_C 79.1, and four olefinic carbons at δ_C 121.0, 144.2, 125.5 and 134.4. ^1H and ^{13}C NMR data for **2** were almost identical to 7β-hydroxyl-8α-methoxydeepoxysarcophine previously isolated from the soft coral *S. trocheliophorum* (Grote et al., 2006). However two differences between the ^1H NMR chemical shifts for **2** and the literature reported compound were observed at CH_3-19 (δ_H 1.11) and H-7 (δ_H 3.41) with both proton signals exhibiting a relative down field shift for **2** compared with 7β-hydroxyl-8α-methoxydeepoxysarcophine. These down-field shifts can be explained by an alternative stereochemistry at C-7. The relative configuration of **2** was determined on the basis of coupling constants and a NOESY experiment. The vicinal coupling constant of 10.3 Hz between H-2 and H-3 established a *cis* configuration between γ-lactone (H-2) and the olefinic proton (H-3). The NOE correlation observed between H-7 and the methyl group at CH_3-19 indicated that both are on the same side of the molecule in a β-orientation and that correlations between H-3, H-5 and H-7 reflect an α-orientation of the hydroxyl group substituent at C-7. These results, together with other detailed NOE correlations of **2** (Fig. 3) established the structure of 7α-hydroxyl-8α-methoxydeepoxysarcophine (**2**).

Compound (**3**) was obtained as colorless oil, $[\alpha]_D^{25}$ +22.5 (*c* 0.005, MeOH). The HR-FAB-MS exhibited an *m*/*z* of 339.43 $[M+Na]^+$ establishing a molecular formula of $C_{20}H_{28}O_3Na$. Seven degrees of unsaturation were predicted and the IR spectrum revealed the presence of carbonyl (1749 cm^{-1}) and hydroxyl (3446 cm^{-1}) groups supported by NMR data. Carbon signals were observed by ^{13}C NMR (Table 1) and classified by DEPT analysis. Twenty carbon signals were observed as follows: three methyls, seven methylenes, four methines, and six quaternary carbons. The ^{13}C NMR spectrum revealed the presence of an exomethylene functionality at δ_C 110.1/154.5, two oxymethine carbons at δ_C 70.6 and 78.8, and four olefinic carbons at δ_C 121.5, 143.7, 125.9 and 135.1. NMR analysis also indicated the presence of an α,β-unsaturated-γ-lactone with appropriate signals at δ_C 174.9 and 78.8 for the carbonyl and oxymethine carbons, respectively. Carbon signals at δ_C 123.0 (C-15) and 162.7 (C-1) were consistent with α and β olefinic carbons of an α,β-unsaturated-γ-lactone system. The olefinic methyl groups at δ_H 1.83 (H_3-17), 1.87 (H_3-18) and 1.61 (H_3-20) also showed HMBC correlations with carbon signals at δ_C 174.9 (C-16), 121.5 (C-3) and 125.9 (C-11), respectively. The carbon signal at δ_C 78.8 (C-2) is consistent with an oxymethine carbon while the oxymethine proton at δ_H 5.60 (d,