Tetrahedron 74 (2018) 1933–1941

Contents lists available at ScienceDirect

Tetrahedron

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

Structural, stereochemical, and bioactive studies of cembranoids from Chinese soft coral *Sarcophyton trocheliophorum*

Lin-Fu Liang [a, b], Tibor Kurtán [d, *], Attila Mándi [d], Li-Gong Yao [b], Jia Li [b], Le-Fu Lan [b], Yue-Wei Guo [b, c, **]

[a] College of Materials Science and Engineering, Central South University of Forestry and Technology, 498 South Shaoshan Road, Changsha, 410004, PR China
[b] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhang Jiang Hi-Tech Park, Shanghai, 201203, PR China
[c] Open Studio for Drugability Research of Marine Natural Products, Qingdao National Laboratory for Marine Science and Technology, 1 Wenhai Road, Aoshanwei, Jimo, Qingdao, 266237, PR China
[d] Department of Organic Chemistry, University of Debrecen, POB 400, 4002, Debrecen, Hungary

ARTICLE INFO

Article history:
Received 15 January 2018
Received in revised form
14 February 2018
Accepted 23 February 2018
Available online 26 February 2018

Keywords:
Sarcophyton trocheliophorum
Cembranoid
Structure elucidation
Absolute configuration
Biological activities

ABSTRACT

A series of highly oxidative new cembranoids with diverse structural features such as a dienoate moiety (sarcophytonolides S – U, **1**–**3**) or an α,β-unsaturated ε-lactone (sartrolides H – J, **4**–**6**) were obtained from Hainan soft coral *Sarcophyton trocheliophorum*, along with known related analogues **7**–**13**. It is an extremely challenging work to determine the absolute configurations of these metabolites. For compounds **1**, **3** and **4**, solution TDDFT calculation of ECD and specific rotation were applied in combination with conformational analysis and NMR data to determine their relative and absolute configurations, leading to the revision of relative configuration of **14**. The absolute configurations of compounds **8**–**10** were determined by the solid-state TDDFT-ECD approach, and that of **8** was further confirmed by single-crystal X-ray diffraction experiment with Cu Kα radiation. In the bioassays, compound **8** exhibited not only moderate protein tyrosine phosphatase 1B (PTP1B) inhibitory activity ($IC_{50} = 15.4\ \mu M$) but also moderate antibacterial activity against *Staphylococcus aureus* Newman strain ($MIC_{50} = 250\ \mu M$).

1. Introduction

The soft corals of genus *Sarcophyton* (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonaceae, family Alcyoniidae) are abounded in the South China Sea, which have been reported to contain a variety of diterpenes, among which cembranes represent the most commonly encountered structural type. The cembranes possess a 14-membered carbocyclic skeleton containing one isopropyl and three methyl groups, in which methyl group was often oxidized to hydroxymethyl or carboxylic acid, further leading to the formation of lactone rings. This type of secondary metabolites not only display a library of diverse intriguing structural features, but also exhibit a wide spectrum of biological activities, including cytoprotective, cytotoxic, anti-inflammatory, and other bioactive properties,[1,2] making them attractive targets for chemical synthesis.[3,4]

The soft coral *Sarcophyton trocheliophorum* is a widespread member of the coral reef in the South China Sea. Recently, as part of our ongoing research project aimed at discovering bioactive substances from Chinese marine invertebrates,[5–9] we collected the title soft coral, off Yalong Bay, Hainan Province, China. Previous chemical studies on this organism by our group resulted in the identification of a novel class of cyclobutane-containing diterpenoids possessing PTP1B inhibitory activity,[5] two rare sarsolenane and three capnosane diterpenes with PTP1B inhibitory activity.[10] Inspired by the previous work, a subsequent detailed chemical investigation of another collection of the title animal was carried out, which led to the isolation of six new cembranolides, sarcophytonolides S – U (**1**–**3**) and sartrolides H – J (**4**–**6**), along with seven known related analogues **7**–**13**. In this paper we discuss the isolation and the structural elucidation of all the new compounds **1**–**6** and the stereochemical study for compounds **1**, **3**, **4** and **8**–**10**. For compounds **1**, **3** and **4**, solution TDDFT calculation of ECD and

* Corresponding author.
** Corresponding author. State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhang Jiang Hi-Tech Park, Shanghai, 201203, PR China.
E-mail addresses: kurtan.tibor@science.unideb.hu (T. Kurtán), ywguo@simm.ac.cn (Y.-W. Guo).

https://doi.org/10.1016/j.tet.2018.02.059
0040-4020/

specific rotation were applied in combination with conformational analysis and NMR data to determine their relative and absolute configurations, leading to the revision of relative configuration of **14**. The absolute configurations of compounds **8**–**10** were determined by the solid-state TDDFT-ECD approach, and that of **8** was further confirmed by single-crystal X-ray diffraction experiment with Cu Kα radiation. This is first time to determine the absolute configurations of cembranoids with an α,β-unsaturated ε-lactone using TDDFT-ECD approach. In addition, the evaluations of their PTP1B inhibitory and antibacterial activities were reported herein.

2. Results and discussion

Freshly collected animals of *S. trocheliophorum* were immediately put at −20 °C, and kept frozen prior to extraction. The usual workup[5] of the Et_2O-soluble fraction of the acetone extract of the *S. trocheliophorum* yielded thirteen cembrane derivatives (**1**–**13**) (Fig. 1). Among them, seven known compounds were readily identified as deacetylemblide (**7**),[11] 4*Z*,12*Z*,14*E*-sarcophytolide (**8**),[12] sarcrassin D (**9**),[13] emblide (**10**),[11] sarcophytonolide A (**11**),[14] (*E*,*E*,*E*)-7,8-epoxy-l-isopropyl-4,8,12-trimethylcyclotetradeca-l,3,11-triene (**12**),[15] and (4*Z*,8*S*,9*R*,12*E*,14*E*)-9-hydroxy-1-isopropyl-8,12-dimethyloxabicyclo[9.3.2]-hexadeca-4,12,14-trien-18-one (**13**),[12] respectively, by comparison of their spectral data with those reported in the literature.

A preliminary MS and ^{1}H NMR analysis revealed that six new compounds **1**–**6** should share a cembrane framework. The following careful analysis of ^{1}H and ^{13}C NMR data revealed that sarcophytonolides S − U (**1**–**3**) possessed a dienoate moiety (C-1 − C-4 and C-18), whereas sartrolides H − J (**4**–**6**) had a lactone ring, namely an α,β-unsaturated ε-lactone (C-8 − C-12 and C-20). The planar structures and relative configuration of compounds **1**–**6** have been elucidated by means of a detailed 1D and 2D NMR analysis aided by comparison with data of related derivatives, and their absolute configurations were determined by TDDFT-ECD approach. Herein we will describe firstly the structural analyses of epoxidic cembranes **1**–**3** followed by those of the lactonic cembranes **4**–**6**.

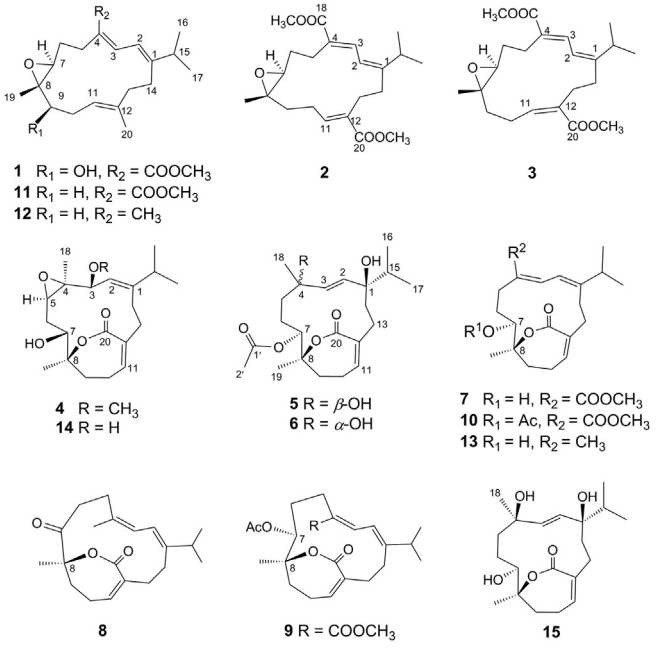

Fig. 1. The structures of compounds **1**–**15**.

Sarcophytonolide S (**1**) was isolated as colorless oil. Its molecular formula $C_{21}H_{32}O_4$ was deduced from its HR-ESI-MS ($[M + Na]^+$ at *m/z* 371.2172). Thus, six degrees of unsaturation were determined for **1**. Compound **1** exhibited IR absorptions indicative of the presence of hydroxyl and ester carbonyl moieties (ν_{max} 3498, 1713 cm^{-1}).[14] An intense UV absorption at λ_{max} 283 nm (log ε 3.33) indicated the presence of a dienoate moiety,[14] which was confirmed by ^{1}H and ^{13}C NMR data of **1**: δ_C 158.1 (*s*, C-1), 119.5 (*d*, C-2), 136.6 (*d*, C-3), 126.2 (*s*, C-4), 168.0 (*s*, C-18), 51.4 (*q*, C-1′) and δ_H 6.88 (*d*, *J* = 11.5 Hz, H-2), 6.67 (*d*, *J* = 11.5 Hz, H-3), 3.77 (*s*, Me-1′). Its NMR spectra also indicated the presence of an isolated trisubstituted double bond [δ_C 118.8 (*d*, C-11), 137.6 (*s*, C-12) and δ_H 5.03 (*td*, *J* = 7.2, 1.2 Hz, H-11)], a trisubstituted epoxidic ring [δ_C 62.7 (*d*, C-7), 63.5 (*s*, C-8), δ_H 2.86 (*dd*, *J* = 6.6, 5.2 Hz, H-7)] and an oxymethine [δ_C 78.0 (*d*, C-9) and δ_H 3.16 (*dd*, *J* = 8.8, 6.1 Hz, H-9)] (Table 1). Comparison of these data with those of co-isolated cembranoid sarcophytonolide A (**11**), previously reported by our group,[14] suggested that **1** and **11** actually share the same carbon framework and they only differ in the presence of a hydroxyl group at C-9 of **1**, in agreement with the 16 mass units difference. This was confirmed further by the HMBC correlations of Me-19/C-7, C-8, and C-9, and H-7/C-9 (Fig. 2). The geometry of the Δ^1, Δ^3, and Δ^{11} double bonds, were suggested to be the same as in **11** on the basis of the almost identical chemical shift values for C-1 − C-4, C-11, C-12, and C-20 in these two compounds. The relative configuration of the three stereogenic centers C-7, C-8 and C-9 was tentatively established as (7*R**,8*R**,9*S**) by the ROESY correlations of H-7/H-9, H-7/H_a-6 and Me-19/H_b-6 in its ROESY spectrum (Fig. 2). Unfortunately, the attempt to determine the absolute configuration of **1** through the Mosher's method failed indeed, due to the insufficient amount to apply the method.

Instead, the solution TDDFT-ECD calculation method was applied, the efficiency of which has been demonstrated earlier in the stereochemical studies of conformationally flexible macrolides.[16,17] Since elucidation of the relative configuration of **1** was tentative, we investigated *in silico* two diastereomers, the (7*S*,8*S*,9*R*)-**1** and the (7*S*,8*S*,9*S*)-**1** in order to confirm the assignment of the relative configuration. The initial MMFF (Merck Molecular Force Field) conformational analysis of (7*S*,8*S*,9*R*)-**1** resulted in 300 conformers (Fig. S41), while that of (7*S*,8*S*,9*S*)-**1** gave 339 conformers (Fig. S42). These geometries were reoptimized by various DFT methods [B3LYP/6-31G(d) *in vacuo*, B3LYP/TZVP with PCM for MeCN, B97D/TZVP[16,18] with PCM for MeCN and CAM-B3LYP/TZVP[19,20] with PCM for MeCN] and ECD spectra were computed at the B3LYP/TZVP, BH&HLYP/TZVP, CAM-B3LYP/TZVP and PBE0/TZVP levels. Interestingly, for both diastereomers we got opposite results by using different sets of DFT conformers. While gas-phase calculations for both diastereomers suggested (*S*) absolute configuration for the chirality centers C-7 and C-8, the B97D functional suggested opposite absolute configuration. In the case of (7*S*,8*S*,9*R*)-**1**, the solvent model B3LYP and CAM-B3LYP reoptimizations yielded similar ECD spectra to the gas-phase calculations (Fig. 3), while for (7*S*,8*S*,9*S*)-**1** the experimental high-wavelength intense positive Cotton effect (CE) was computed negative but the low-wavelength one was found positive at these levels (Fig. 4).

These contradicting results derived from the rather different Boltzmann-distribution of the conformers estimated by the different methods and ECD spectra were found highly sensitive to the conformation of the macrocycle.[16] For the configurational assignment of highly flexible derivatives, it is advisable and helpful to apply the combination of more than one chiroptical method.[21,22] Thus conformers were also reoptimized with PCM for $CHCl_3$ and optical rotation (OR) values were computed for the gas-phase and the PCM/$CHCl_3$ optimized conformers.[23] For most low-energy