Phytochemistry Letters 26 (2018) 199–204

Contents lists available at ScienceDirect

Phytochemistry Letters

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

New isomalabaricane analogues from the sponge *Rhabdastrella providentiae* and their cytotoxic activities

Phan Van Kiem[a,b,*], Duong Thi Dung[a,b], Pham Hai Yen[a], Nguyen Xuan Nhiem[a], Tran Hong Quang[a], Bui Huu Tai[a,b], Chau Van Minh[a]

[a] Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
[b] Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

ARTICLE INFO

Keywords:
Rhabdastrella providentiae
Rhabdastrellin
Isomalabaricane analog
Marine sponge

ABSTRACT

Five new isomalabaricane analogues, named rhabdastrellins G - K (**1–5**), and three known compounds including jaspolide C (**6**), globostelletin C (**7**), and globostelletin D (**8**) were isolated from the sponge *Rhabdastrella providentiae* collected in the Eastern Sea of Vietnam. Their chemical structures were determined by HR-ESI-MS, NMR spectroscopy, experimental and calculated circular dichroism spectra. The isolated secondary metabolites were evaluated for their cytotoxicity toward HepG2, LU-1, MCF-7, HL-60, and SK-Mel2 human cancer cell lines. New isomalabaricane analogues **1**-**3** exhibited significant cytotoxicity with IC_{50} values ranging from 11.2 ± 1.4 to 84.8 ± 6.7 μM. Interestingly, the 20(22)*Z* stereoisomer (**2**) exhibited dramatic increase of cytotoxic activity in comparison with 20(22)*E* isomer (**1**).

1. Introduction

Isomalabaricanes are rare 6,6,5-tricyclic triterpenoids and differ structurally from malabaricane-type triterpenes by having *trans-syn-trans* instead of *trans-anti-trans* ring junctions. They are mainly found in, and are considered to be chemotaxonomic markers of sponges belonging to *Geodia*, *Jaspis*, *Stelletta*, and *Rhabdastrella* genera (Fouad et al., 2006; Li et al., 2010; Tanaka et al., 2011; Tasdemir et al., 2002). Chemical structure variation of isomalabaricanes results mainly from oxidation and/or degradation of the side chain (Li et al., 2010). To date, more than 30 isomalabaricanes have been reported from the sponges of *Rhabdastrella* genus. In addition to their unusual chemical structure, isomalabaricanes exhibit interesting biological activities such as selective anti-proliferative activity against vascular endothelial cells (Aoki et al., 2007), and cytotoxicity against various cancer cell lines (Bourguet-Kondracki et al., 2000; Fouad et al., 2006; Hirashima et al., 2010; Lv et al., 2008). Cytotoxic mechanisms of isomalabaricane were proposed by induction of apoptosis and regulation of ChT-L, T-L targeted proteins (Li et al., 2010), stabilized binding of DNA polymerase β to DNA (Clement et al., 2006), and inhibition of human tumor-related protein kinases (Li et al., 2012). In an effort to find cytotoxic isomalabaricane analogues from *Rhabdastrella* species, we report the isolation, structure elucidation, and cytotoxic activities of five new isomalabaricane analogues from the sponge *Rhabdastrella providentiae*.

2. Results and discussion

Fresh samples of the sponge *R. providentiae* were cut into small pieces and extracted with MeOH. The concentrated residue was partitioned in water and then separated with CH_2Cl_2 to yield corresponding soluble fractions. From the CH_2Cl_2 partition, five new and three known isomalabaricane analogues (Fig. 1) were isolated by using a combination of various chromatographic techniques.

Compound **1** was obtained as a colorless wax. The molecular formula of **1** was deduced as $C_{29}H_{38}O_4$ from the presence of a quasi-molecular ion peak at *m/z* 451.2838 $[M+H]^+$ (calcd for $C_{29}H_{39}O_4$, 451.2848) in the HR-ESI-MS spectra and in combination with ^{13}C-NMR spectra analysis, 11 degrees of unsaturation were indicated. The ^{1}H-NMR spectrum of **1** showed signals of seven tertiary methyl groups at δ_H 0.90, 1.09, 1.17, 1.53, 2.01, 2.09, and 2.35 (each 3H, s); five olefinic proton signals at δ_H 6.38 (d, J = 15.5 Hz), 6.38 (d, J = 15.5 Hz), 7.08 (d, J = 15.5 Hz), 6.63 (d, J = 11.5 Hz), 7.65 (dd, J = 11.5, 15.5 Hz). The aforementioned proton signals and 29 carbon signals in the ^{13}C-NMR spectrum (Table 1) of **1** suggested that it was a polyunsaturated nor-triterpene. Specifically, eight carbon signals in the de-shielded region of δ_C 128.6–148.0 indicated the presence of four C—C double bonds, and four carbon signals at δ_C 201.2, 203.4, 206.1, 214.9 were indicative for four ketone functional groups. The remaining 17 shielded carbon signals (δ_C 13.0–50.1) corresponded to three degrees of unsaturation. Therefore, compound **1** was characterized as a tricyclic nor-

* Corresponding author at: Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Caugiay, Hanoi, Vietnam.
E-mail address: phankiem@yahoo.com (P.V. Kiem).

https://doi.org/10.1016/j.phytol.2018.06.003
Received 8 February 2018; Received in revised form 9 May 2018; Accepted 11 June 2018

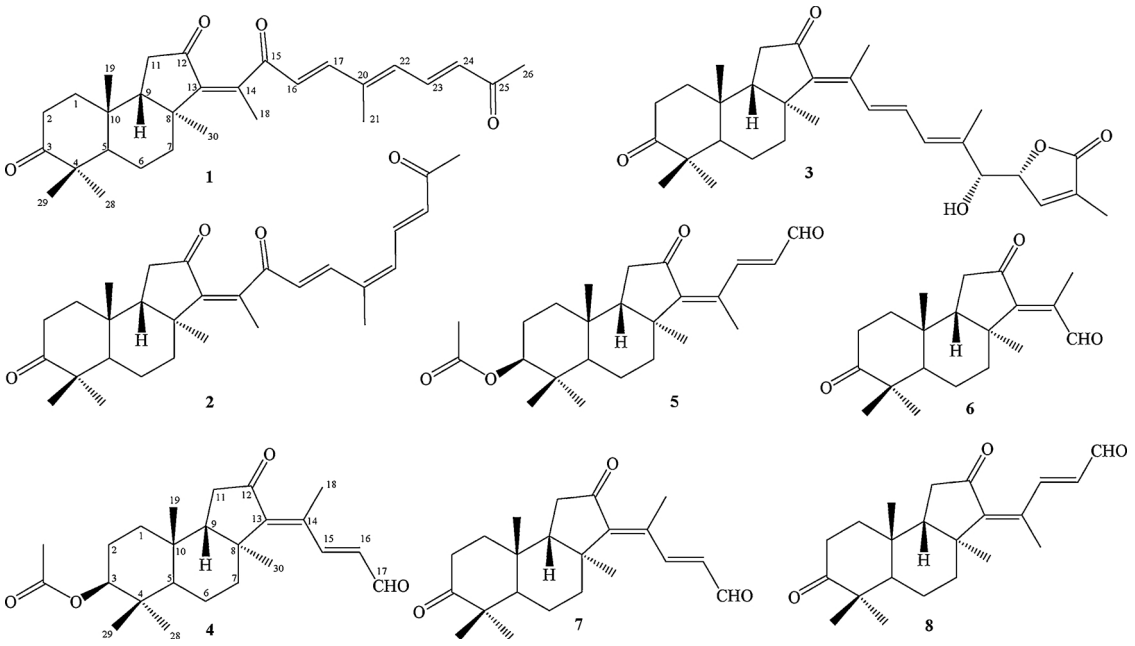

Fig. 1. Chemical structures of isomalabaricane analogues **1**-**8** isolated from *R. providentiae*.

triterpene, a rare class of secondary metabolites occurring in nature. The structure of the side chain was established by HMBC correlations of H-26 (δ_H 2.35)/ C-25 (δ_C 201.2), C-24 (δ_C 134.2); COSY cross peaks of H-24 (δ_H 6.38)/ H-23 (δ_H 7.65)/ H-22 (δ_H 6.63); HMBC correlations of H-21 (δ_H 2.09)/ C-22 (δ_C 137.4), C-20 (δ_C 144.2), C-17 (δ_C 147.7); HMBC correlations of H-17 (δ_H 7.08)/ C-16 (δ_C 128.6), C-15 (δ_C 203.4); and HMBC correlations of H-18 (δ_H 2.01)/ C-15 (δ_C 203.4), C-14 (δ_C 144.4), C-13 (δ_C 148.0). Furthermore, HMBC correlation between H-18 and quaternary olefinic carbon C-13 indicated that the side chain was attached to the tricyclic system through a double bond C-13/C-14 (Fig. 2). HMBC correlations of methyl protons H-28 (δ_H 1.17), H-29 (δ_H 1.09) with C-3 (δ_C 214.9) confirmed a ketone functional group at C-3. The ketone functional group at C-12 was deduced from COSY correlations of H-9 (δ_H 2.06)/H-11 (δ_H 2.20 and 2.29) and from HMBC correlations of H-11/C-12 (δ_C 206.1). Finally, relative stereochemistry of **1** was determined by NOESY spectral analysis (Fig. 3). NOESY correlation between H-9 (δ_H 2.06) and H-19 (δ_H 0.90) indicated the *β*-orientation of H-9, C-19. Meanwhile, a NOESY correlation between H-5 (δ_H 2.56) and H-30 (δ_H 1.53) confirmed the *α*-orientation of H-5, C-30. The above-mentioned results were in agreement with the structure of the *trans-syn-trans* 6,6,5-tricyclic system in the isomalabaricane (8,9-diepimer of malabricane) triterpene. The double bond configurations were determined to be 13*Z*, 16*E*, 20(22)*E*, and 23*E* by NOESY correlations of H-30 (δ_H 1.53)/H-18 (δ_H 2.01), H-16 (δ_H 6.38)/ H-21 (δ_H 2.09), H-21 / H-23 (δ_H 7.65), and H-22 (δ_H 6.63)/ H-24 (δ_H 6.38), respectively. The large *J* coupling constants of H-16/H-17 and H-23/H-24 (each 15.5 Hz) were also supported by 16*E* and 23*E* configurations of those double bonds. Consequently, compound **1** was determined to be a new isomalabaricane analog and was named rhabdastrellin G.

Compound **2** was isolated as a colorless wax. In the HR-ESI-MS spectra of **2**, a quasi-molecular ion peak at *m/z* 451.2846 $[M+H]^+$ indicated that compounds **2** and **1** had the same molecular formula, $C_{29}H_{38}O_4$ (calcd for $C_{29}H_{39}O_4$, 451.2848). ^{1}H- and ^{13}C-NMR spectral analyses of **2** revealed that resonant signals of **2** were almost identical with those of **1**, except for slight differences at signals of the side chain. Additionally, a similar pattern of HMBC and COSY correlations between **1** and **2** suggested that compound **2** was a geometric isomer of **1**. The down-field shifted δ value of C-21 (δ_C 20.4) in comparison with that of **1** (C-21, δ_C 13.0) suggested that the configuration of the double bond at C-20/C-22 had *Z* geometry (Fig. 1). The 20(22)*Z* configuration was also supported by a NOESY correlation between H-21 (δ_H 2.07) and H-22 (δ_H 6.53). Similar with compound **1**, the configurations of remaining double bonds (13*Z*, 16*E*, 23*E*) were deduced from NOSEY correlations of H-30 (δ_H 1.58)/H-18 (δ_H 2.04), H-16 (δ_H 6.35)/ H-21 (δ_H 2.07), H-22 (δ_H 6.53)/ H-24 (δ_H 6.36) and in combination with *J* coupling constants of H-16/H-17 (15.5 Hz), H-23/H-24 (15.5 Hz). Therefore, compound **2** was determined and was named rhabdastrellin H.

A quasi-molecular ion peak at *m/z* 481.2948 $[M+H]^+$ in the HR-ESI-MS of **3** suggested its molecular formula to be $C_{30}H_{40}O_5$ (calcd for $C_{30}H_{41}O_5$, 481.2954). Detailed ^{1}H- and ^{13}C-NMR spectral analyses of **3** and comparison with those of **1** and **2** (Table 1) indicated that all three compounds shared the same tricyclic nucleus. The structure of the side chain was established by 2D-NMR spectra. HMBC correlations of H-18 (δ_H 2.34)/ C-13 (δ_C 147.3), C-14 (δ_C 143.7), C-15 (δ_C 133.9) and COSY cross peaks of H-15 (δ_H 6.74)/ H-16 (δ_H 7.08)/ H-17 (δ_H 6.40) indicated location of double bonds at C-13/C-14, C-15/C-16, and C-17/C-20. HMBC correlations of H-21 (δ_H 1.97)/ C-17 (δ_C 128.7), C-20 (δ_C 141.9), C-22 (δ_C 77.3) and the chemical shift value of C-22 indicated the presence of a hydroxy group at C-22. Moreover, COSY cross peaks of H-22 (δ_H 4.25)/ H-23 (δ_H 5.08)/ H-24 (δ_H 7.28) and HMBC correlations of H-26 (δ_H 1.92)/ C-24 (δ_C 148.7), C-25 (δ_C 131.8), C-27 (δ_C 176.4) demonstrated another C−C double bond at C-24/C-25 and a carbonyl functional group at C-27. A γ-lactone bridge between C-23 and the carbonyl group C-27 was suggested by deshielded signals of C-27 (δ_C 176.4), C-23 (δ_C 83.9), and H-23 (δ_H 5.08). Finally, the stereogenic centers of **3** were further determined by NOESY and CD spectral analyses. NOESY correlations of H-30 (δ_H 1.47)/ H-15 (δ_H 6.74), H-15 / H-17 (δ_H 6.40), and H-16 (δ_H 7.08)/ H-21 (δ_H 1.97) confirmed the geometric configurations of the corresponding double bonds to be 13*E*, 15*E*, and 17*E*, respectively. The chemical shift values of C-22 and C-23 (δ_C 77.3 and 83.9) suggested a *threo*-relationship of their two substitutions in comparison with those reported of similar chemical structures (xestospongienes I and J, *erythro*, δ_C 72.8 and 82.3; xestospongienes K and L, *threo*, δ_C 75.1 and 82.7) (Jiang et al., 2011). Additionally, the CD analysis of **3** revealed a negative Cotton effect at wavelength of 207 nm (−0.29) and a positive Cotton effect at a wavelength of 233 nm (+0.56), in agreement with the calculated CD spectrum of the (22R,23R)-isomer of **3** (Fig. 4). Therefore, the chemical structure of compound **3** was determined and was name rhabdastrellin I.