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Natural Products Chemistry & Research

Campos et al., Nat Prod Chem Res 2018, 6:5
DOI: 10.4172/2329-6836.1000336

Research Article Open Access

(2S*,5S*,6Z)-2,5-Epoxydocosan-6-en-21-ynoic Acid, New Fatty Acid from the Marine Sponge *Haliclona fascigera*

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Received: July 19, 2018; **Accepted:** July 30, 2018; **Published:** August 04, 2018

Abstract

Marine sponges are recognized as a rich source of new marine natural products. In this study, chemical investigation of the CH_2Cl_2-MeOH (1:1) extract from the marine sponge *Haliclona fascigera* collected in Mayotte was carried out and highlighted a new unusual acetylenic and tetrahydrofuranic fatty acid, (2S*,5S*,6Z)-2,5-epoxydocosan-6-en-21-ynoic acid (1). Its planar structure was elucidated by HRESIMS data, IR, 1D and 2D NMR spectra. The relative configuration of compound 1 was deduced from density functional theory (DFT) computational calculations fitted using the recently published DP4+ probability.

Keywords: *Haliclona fascigera*; Marine sponge; Fatty acid; Tetrahydrofuran

Introduction

Marine organisms are rich sources of biologically active metabolites [1]. Ubiquitous sponges from the genus *Haliclona* (order Haplosclerida) have been known to produce a wide diversity of bioactive compounds, alkaloids with unique structures [2-4], cyclopeptides [5] or aliphatic compounds [6,7]. In our continuing search for structurally unique metabolites from marine invertebrates, the sponge *Haliclona fascigera*, collected in Mayotte, was investigated, resulting in the isolation of the new acetylenic fatty acid, (2S*,5S*, 6Z)-2,5-epoxydocosan-6-en-21-ynoic acid (1) (Figure 1). In this study, the isolation and structure elucidation of 1 were described.

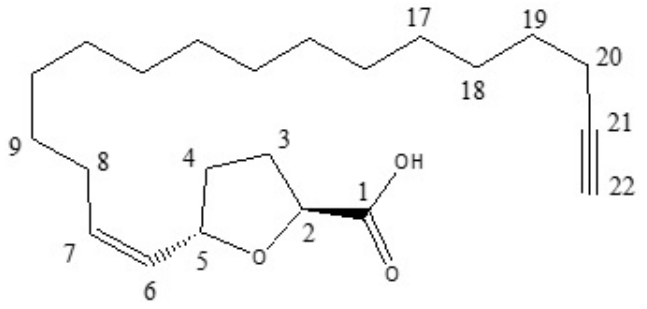

Figure 1: Structure (2S*,5S*,6Z)-2,5-Epoxydocosan-6-en-21-ynoic acid (1).

Materials and Methods

General experimental procedures

^{1}H NMR data were acquired with a Bruker Ultrashield Avance 500 MHz spectrometer. Chemical shifts were referenced using the corresponding solvent signals (δH 7.24 and δC 77.23 for $CDCl_3$). The spectra were processed using 1D and 2D NMR Notebook software. HRESIMS spectra were recorded using a Waters Acquity BEH C18, 1.7 µm, 50 × 2.1 mm column on a Waters Micromass LCT-Premier TOF mass spectrometer with a Waters Acquity UPLC system.

The sponge was lyophilized with Cosmos -80°C CRYOTEC and extracted with Dionex ASE 300. Reversed phase column chromatography separations were carried out on glass column (150 × 10 mm i.d.) packed with Acros Organics C18-RP, 23%C, silica gel (40-63 µm). Precoated TLC sheets of silica gel 60, Alugram SIL G/UV254 were used, and spots were visualized on the basis of the UV absorbance at 254 nm and by heating silica gel plates sprayed with formaldehyde-sulfuric acid or Dragendorff reagents. Analytical HPLC was carried out using a Waters Sunfire Shield RP18 (150 × 4.6 mm i.d., 5 µm) column and was performed on an Agilent 1100 series system controller equipped with a photodiode array detector (Agilent 1100 G1315B) and a mass spectrometer detector (Agilent 1100 G1956A) with Chemstation software. Preparative HPLC was carried out using a Waters Sunfire Shield RP18 prep (150 × 19 mm i.d., 5 µm) column and was performed on a Waters 600 system controller equipped with a photodiode array detector (Waters 2996 and Waters 486). All solvents were analytical or HPLC grade and were used without further purification.

Animal material

The sponge *Haliclona fascigera* (phylum Porifera, class Demospongiae, order Haplosclerida, family Chalinidae) was collected in May 2013 in Passe Bouéni, Mayotte (12°58.592' S, 44°58.005' E at 20-27 m depth). One voucher specimen (RMNH POR 8713) was deposited in the Naturalis, Netherlands Centre for Biodiversity. Sponge samples were frozen immediately and kept at -20 °C until processed.

Extraction and isolation

The frozen sponge (15 g, dry weight) was chopped into small pieces and extracted by ASE first with Water (× 1) and then with MeOH/CH_2Cl_2 (1:1, v:v) (× 2). After evaporating the MeOH/CH_2Cl_2 mixture under reduced pressure, a residue (440 mg) was obtained. The extract (440 mg) was then subjected to a CC over RP silica gel in a glass column (150 × 10 mm i.d.), eluting with a combination of Water, MeOH and CH_2Cl_2 of decreasing polarity. Nine fractions were obtained (F1-F9) and F6, F7 and F8 containing each the major compound were assembled. These fractions (391 mg) were subjected to preparative HPLC (Waters Sunfire Shield RP18 prep Column, 5 µm, 150 × 19 mm i.d., 18.0 mL.min^{-1} gradient elution with 10% CAN-H_2O (+0.1% formic acid) over 5 min and 10% CAN-H_2O (+0.1% formic acid) to 100% CAN-H_2O (+0.1% formic acid) over 20 min; UV 200 nm) to give pure compound 1 ((2S*,5S*,6Z)-2,5-epoxydocosan-6-en-21-ynoic acid, 6.4 mg).

Evaluation of the biological activities

Evaluation of the antimicrobial activity: (2S*,5S*,6Z)-2,5-Epoxydocosan-6-en-21-ynoic acid (1) was tested against five marine bacterial strains commonly found on biofilms, *Roseobacter litoralis* (ATCC 495666), *Shewanella putrefaciens* (ATCC 8071), *Vibrio carchariae* (ATCC 35084), *Vibrio aestuarianus* (ATCC 35048), *Vibrio natrigens* (ATCC 14048) and *Vibrio proteolyticus* (ATCC 15338). Bacterial growth rates were determined according to the methods of Thabard et al. [8]. Bacterial suspensions (100 µ aliquots, 2 × 10^8 colony forming units/mL) were aseptically added to the compound containing microplate wells (0.01-10 µg/mL), and the plates were incubated for 48 h at 26°C. Media only was used as a blank. Bacterial growth was monitored spectroscopically at 630 nm. The minimal inhibitory concentration (MIC) for bacterial growth was defined as the lowest concentration which results in a decrease in OD.

Evaluation of the antiplasmodial activity: Activity against *Plasmodium falciparum* chloroquine-sensitive 3D7 strains was assessed following the procedure already described in Frédérich et al. [9]. The parasites were obtained from Prof. Grellier (Museum d'Histoire Naturelle, Paris, France). Each compound, fraction and extract was applied in a series of eight 2-fold dilutions (final concentrations ranging from 0.8 to 100 µg/mL for an extract and from 0.08 to 10 µg/mL for a pure substance) on two rows of a 96-well microplate and were tested in triplicate (n=3). Parasite growth was estimated by determination of lactate dehydrogenase activity as described previously [10]. Artemisinin (98%, Sigma-Aldrich) was used as positive control.

Results and Discussion

Structure elucidation

2,5-Epoxydocosan-6-en-21-ynoic acid (1) was obtained as a greenish oil. The HRESIMS spectrum exhibited a pseudo molecular ion $[M+H]^+$ at m/z 349.2751 and allowed the assignment of the molecular formula as $C_{22}H_{37}O_3$ (calcd for $C_{22}H_{37}O_3$, 349.2743) requiring five degrees of unsaturation. The IR data of 1 displayed the existence of a terminal acetylenic bond with an absorption band at 2100 cm^{-1}. The ^1H and ^{13}C NMR data of 1 displayed the resonances and correlations of one carboxylic acid group, an alkene, a terminal acetylenic bond, two oxygenated methines and fifteen methylenes (Table 1). Interpretation of the ^1H-^1H COSY correlations between H-2 (δ_H 4.55), H-3 (δ_H 2.10, 2.43), H-4 (δ_H 1.69, 2.10) and H-5 (δ_H 4.88), revealed the sequence C-2-C-3-C-4-C-5 (Figure 2). The chemical shifts of the methines C-2 (δ_C 76.4) and C-5 (δ_C 77.6) and the possibilities left by the molecular formula suggested that C-2 and C-5 were linked to the same oxygen. The tetrahydrofuran ring sequence O-C-2-C-3-C-4-C-5- was therefore deduced. The methine proton H-2 and the methylene protons H-3 showed both HMBC correlations to the carbon C-1 of the carboxylic acid (δ_C 174.1). 1H-1H COSY correlations (Figure 2) between H-6 (δ_H 5.40) and H-7 (δH 5.55) revealed a 6,7-double bond. The cis geometry of the double bond was indicated by the coupling constant (J=10.6 Hz). The correlation between H-5 and H-6 indicated the link between the double bond and the tetrahydrofuran ring. Other COSY correlations between H-7, H-8 (δ_H 2.10), H-9 (δ_H 1.35) and H-10 (δ_H 1.24) revealed the chain of 3 methylenes C-8-C-9-C-10 linked to C-7. In addition, correlations between H-17 (δ_H 1.24), H-18 (δ_H 1.35), H-19 (δ_H 1.50) and H-20 (δ_H 2.15) indicated the second part of the chain of 4 methylenes C-17-C-18-C-19–C-20. The ^1H-^{13}C HBMC correlations (Figure 2) also indicated that this butyl spin system was linked to terminal acetylenic carbons. The methylene H-19 showed correlations to the acetylenic carbons C-21 (δ_C 85.6), H-20 showed correlations to the two acetylenic carbons C-21 and C-22 (δ_C 68.3) and the acetylenic proton H-22 showed correlations to C-21 (δ_C 85.6). At last, the length of the linear fatty chain between C-8 and C-20 (13 methylenes) was deduced thanks to the molecular formula obtained by HRESIMS.

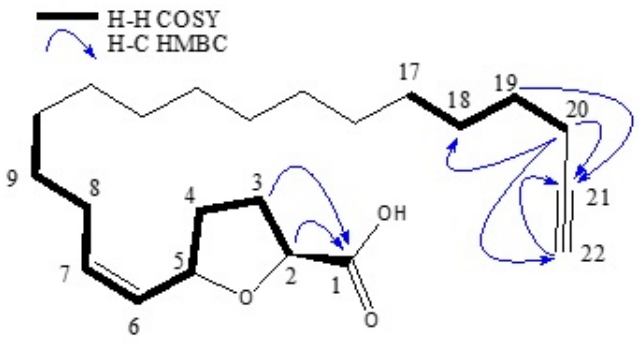

Figure 2: Key ^1H-^1H COSY and ^1H-^{13}C HMBC correlations for 1.

Position	δ_C, type	δ_H (J in Hz)	COSY (^1H-^1H)	HMBC (^1H-^{13}C)
1	174.1, C	-	-	-

2	76.4, CH	4.55, t (7.4)	3	1, 3
3	30.4, CH_2	2.10, m; 2.43, m	2, 4	1, 2, 4
4	33.0, CH_2	1.69, m; 2.10, m	3, 5	3, 5, 6, 7
5	77.6, CH	4.88, dt (8.4, 5.2)	4, 6	4, 6
6	128.8, CH	5.40, t (10.6)	5, 7	7, 8
7	134.5, CH	5.55, dt (10.6, 7.3)	6, 8	5, 6, 9
8	28.1, CH_2	2.10, m	7, 9	6, 7
9	28.7, CH_2	1.35, m	8, 10	7, 10
Oct-17	29.3-29.9, CH_2	1.24, m	-	-
18	29.0, CH_2	1.35, m	17, 19	17
19	28.0, CH_2	1.50, m	18, 20	18, 20, 21
20	18.8, CH_2	2.15, m	19, 22	18, 21, 22
21	85.6, C	-	-	-
22	68.3, CH	1.90, t (2.6)	20	21

Table 1: ^1H and ^{13}C NMR data for 2,5-epoxydocosan-6-en-21-ynoic acid (1) (^1H 500 MHz, ^{13}C 125 MHz, $CDCl_3$).

Relative configuration

Even in the golden age of NMR, incorrectly assigned natural products are not uncommon. Hundreds of structural revisions have been published in the last decades, ranging from profound connectivity to stereochemical errors [11]. Modern computational chemistry, especially the successful application of NMR calculations in the assignment or reassignment of complex molecular structures, has significantly contributed to prevent these misinterpretations [12,13].

In order to determine relative configuration of C-2 and C-5 atoms of 1, Density Functional Theory (DFT) calculations were done on 2*R*, 5*S* and 2*S*,5*S* diastereoisomers. (6*Z*)-2,5-epoxynon-6-enoic acid (2) was studied instead of 1 to reduce the number of conformers to be optimized and so computational cost (Figure 3).

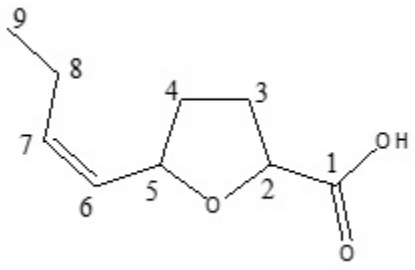

Figure 3: Structure of (6Z)-2,5-Epoxynon-6-enoic acid (2) used for DFT calculations.

All DFT calculations were performed using the Gaussian 09 program [14] using tight convergence criteria and an ultrafine grid. ωB97XD/6-31+G(d,p)/SMD(chloroform) DFT level [15-17] was used to compute geometries. All stationary points were confirmed as true minima by vibrational frequency calculations. For (2*R*,5*S*,6*Z*)-2,5-epoxynon-6-enoic acid (2a), 16 conformers were optimized with relative free energies lower than 3.0 kcal/mol. For (2*S*,5*S*,6*Z*)-2,5-epoxynon-6-enoic acid (2b), only 10 conformers were found in the same energy range. In the most stable conformers of 2a and 2b (Figure 4), interatomic distance values between H-2 and H-5 were close (respectively 3.4 and 3.8 Å). Hence, experimental NOESY correlations might not be confidently used to carry out relative stereochemistry of C-2 and C-5. Therefore DP4+ probability [13] was used to assign relative stereochemistry of 1. The DP4 probability [12] is one of the most sophisticated and popular approaches for the stereochemical assignment of organic molecules using Gauge-Independent Atomic Orbital (GIAO) NMR chemical shift calculations when only one set of experimental data is available. DP4+ probability is an evolution of DP4. ^1H and ^{13}C chemical shifts were computed at the GIAO/mPW1PW91/6-31+G(d,p)/PCM(chloroform) level on previously optimized geometries of 2a and 2b [18]. Then calculated chemical shifts were averaged according to the Boltzmann populations of the conformers at 298 K. The obtained sets of ^1H and ^{13}C chemical shifts for 2a and 2b were compared to the experimental data of 1 via DP4+ probability. A 100% probability DP4+ value in favor of 2b was concordant with the anti (2S*,5S*) relative stereochemistry of the new compound (2S*,5S*,6Z)-2,5-epoxydocosan-6-en-21-ynoic acid (1).

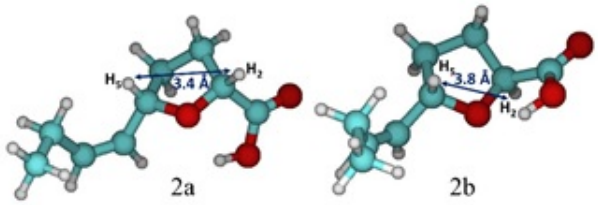

Figure 4: Interatomic distance values between H-2 and H-5 for conformers (2*R*,5*S*,6*Z*)-2,5-Epoxynon-6-enoic acid (2a) and (2*S*,5*S*,6*Z*)-2,5-Epoxynon-6-enoic acid (2b).

Although furan fatty acids (F-acids) are well known in plants, fish lipids [19], and even from a sponge [20], our compound is unusual because it presents the novelty of an unoxidized tetrahydrofuran with an acetylene at the end of the chain. The role of such tetrahyfuran compound in nature is interesting not only from the chemistry and biochemistry point of view, but also for biosynthetic questions.

Characteristics of compound 1

(2S*,5S*,6Z)-2,5-Epoxydocosan-6-en-21-ynoic acid (1). Greenish oil; $[\alpha]^{25}$:-10,5 (*c* 0,5 mg/100 mL, DCM); IR (ν_{max} cm^{-1}):2957, 2924, 2854, 2100, 1128, 1270, 1072, 1039. ^1H and ^{13}C NMR data, (Table 1); HRESIMS m/z 349.2751 $[M+H]^+$ (calcd for $C_{22}H_{37}O_3$, 349.2743).

Biological activity

(2S*,5S*,6Z)-2,5-Epoxydocosan-6-en-21-ynoic acid (1) was tested against five marine bacterial strains commonly found on biofilms, *Roseobacter litoralis* (ATCC 495666), *Shewanella putrefaciens* (ATCC 8071), *Vibrio carchariae* (ATCC 35084), *Vibrio aestuarianus* (ATCC 35048), *Vibrio natrigens* (ATCC 14048) and *Vibrio proteolyticus* (ATCC 15338) and also against the protozoan parasite *Plasmodium*

falciparum (3D7 strain). The compound 1 did not show antimicrobial or antimalarial activities at the concentration tested.

Acknowledgements

This project was supported by the Regional Council of Reunion Island and the ANR 2011-EBIM-006-01 (ERA-NET Netbiome project POMARE). The authors also express their gratitude to Prof. M. E. Remanevy for assistance in sponge collection and to the "Centre de Calcul de l'Université de La Réunion (CCUR)" for computer time.

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