2012 3rd International Conference on Chemistry and Chemical Engineering
IPCBEE vol.38 (2012) © (2012) IACSIT Press, Singapore

Isolation and Characterization of Fatty Acids Ester from the Thai Sea Gorgonian, *Junceella* sp.

Patchara Pedpradab[1], Wallapa Molex[1] and [2]Khanit Suwanborirux

[1] Department of Marine Science, Faculty of Sciences and Fishery Technology, Rajamangala University of Technology, Trang Campus, 92150 Thailand

[2] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand.

Abstract. This research has the main aim to search for chemical constituents from the marine organisms in the Andaman Sea. A various chromatographic methods were used to isolate the constituted fatty acids from a white gorgonian, *Juncella sp*. which was collected around Lanta Island, Krabi Province, Thailand. The isolated constituents were characterized extensively for chemical structures by nuclear magnetic resonance (nmr) and mass spectrometry (ms) methods. Three fatty acid esters namely Heptadecanoic acid pentadecyl ester (**1**), Octadecanoic acid 2-hydroxy-3-octadecanoyloxy-propyl ester (**2**), and Hexadecanoic acid 3-hexadecyloxy-2-hydroxy-propyl ester (**3**) were isolated and characterized.

Keywords: *Juncella sp*., Gorgonian, Fatty acid ester, Lanta Island

1. Introduction

Rare tropical gorgonian corals were investigated for fatty acid constituents. Based on a literature search, the gorgonian specimens collected from the Caribbean Sea were analyzed extensively. They produced polyunsaturated fatty acids, in particular of the (n-3), (n-6) series, and phospholipid (Bergé and Barnathan, 2005). For example by several genus of the family Gorgoniidae biosynthesized high amount of 6,9,12,15,18,21-tetracosahexaenoic (24:6, n-3) and 6,9,12,15,18-tetracosapentaenoic acid (24:5, n-6) such as *Plumarella* and *Paragorgia* while, the *Gorgonia* and *Pseudopterogorgia* contained phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine as major components (Carballeira *et al*., 2002; Carballeira *et al*., 1996). In addition, the genus *Eunicea* (family Plexauridae) was contained only phospholipids derivatives (Carballeira *et al*., 1997). Gorgonian samples from the Andaman Sea region are underrepresented within scientific literature as most samples originate from the Caribbean.

During our search for chemical compositions of marine organisms in the Andaman Sea, we collected 5 gorgonian specimens in the area of Lanta Island, Krai Province, Thailand. Herein, we report on the long chain saturated fatty acid ester from the white Thai gorgonian *Junceella* sp (family Ellisellidae).

2. Experimental

2.1. General Experimental Procedures

Analytical TLC (precoated aluminum sheets, DC Kieselgel 60 F_{254}, Merck, Product No. 1.05554.0001), preparative TLC (precoated glass plates, DC Kieselgel 60 F_{254}, Merck, Products No. 1.05715.0001), Darmstat, Germany, were used. Column chromatography was performed with silica gel (particle size 0.040-0.063 mm, Merck, Product No. 1.09385.1000), Darmstat, Germany. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DMX 600 spectrometer, (Fällanden, Switzerland). TMS was used as an internal standard. All measurement compounds were dissolved in $CDCl_3$. EIMS spectra obtained from MAT 95 XL Mass

Spectrometer, Thermofinigan (Egelsbach, Germany) using methane as a reagent gas. The IR spectrum of the compound in KBr discs were recorded on a Perkin Elmer FT-IR Spectrometer (Massachusetts, USA).

2.2. Isolation Procedure

The fresh gorgonian was homogenised and macerated in 10 L of methanol for 72 hrs. After filtration, the residue was repeatedly extracted and the solution was concentrated by vacuum. The aqueous methanol part was repeatedly partitioned with hexane and dichloromethane, respectively. The solvents were removed under reduced pressure to yield hexane (2 g) and CH_2Cl_2 (1.2 g) extracts. The further isolation guided by TLC analysis, the target compounds gave slightly pink spots on TLC plate after spraying with anisaldehyde reagent. The hexane part was collected and fractionationed by Si-gel vacuum liquid chromatography, using stepped gradient mixtures of C_6H_{14}, CH_2Cl_2, and MeOH to give eight fractions. The first fraction was subjected to a Si-gel column eluted with a stepwise gradient of C_6H_{14}: CH_2Cl_2 (8:2,v/v) to give **1** (10 mg). The fourth fraction was re-chromatographed on a Si-gel column eluted with C_6H_{14}-CH_2Cl_2 (8.5:1.5, 6.5:4.5, 1:1, 1.5:8.5, 2.5:7.5, 3.5:6.4, 1:1); three fractions were observed. The third fraction was further purified by preparative TLC (20×20 cm), which was triply developed using a mixture of C_6H_{14}:CH_2Cl_2 (1:1, v/v) to give **2** (7 mg) and **3** (10 mg), respectively.

3. Results and Discussion

Compound **1** was isolated as a white amorphous material. Its ^{1}H NMR spectral data (Table S1) showed five signals resonating at δ_H 4.10 (t, *J* = 7.0 Hz, H-1′), 2.30 (t, *J* = 7.0 Hz, H_2-2), 1.60 (q, *J* = 7.0, H_2-3 and H_2-2′), 1.30 (H_2-3′, H_2-4, H_2-4′, H_2-5), and 0.85 (t, *J* = 7.0, H_3-6 and H_3-5′). The integration measurement revealed that the signals at δ_H 1.60 and 0.85 contained four and six protons, respectively, while the overlapping methylene signal at δ_H 1.30 was measured for 52 protons. The CH-direct correlation analysed through HSQC spectral data found the cross peaks at δ_H 1.60/δ_C 26.0 (C-3), 25.5 (C-2′) and δ_H 1.30/δ_C 23.0 (C-5), 32.0 (C-4′), 30.0 (C-3′), 28.5 (C-4). This information implied that **1** contained two similar parts and a long methylenes chain. ^{1}H-^{1}H COSY continuously crosses peaks between δ_H 4.10 and 2.30/δ_H 1.60/δ_H 1.30/δ_H 0.85, while HMBC spectral data showed ^3J-correlation between δ_H 4.10 and 2.30 with δ_H 1.30, confirming the appearance of two sets of -$CH_2CH_2CH_2CH_2CH_3$ spin systems. Hence, the number of protons at δ_H 1.30 was finally calculated (52-4) as 48 atoms, which were equally divided due to the identical spin systems. Both were linked to ester carbonyl by HMBC cross peaks between H_2-1′/C-1 and H_2-3/C-1. Compound **1** exhibited a pseudomolecular (HRESIMS) ion peak $[M+H]^+$ at *m/z* 481.4981, which was compatible with the molecular formula $C_{32}H_{65}O_2$. It was identified as Heptadecanoic acid pentadecyl ester.

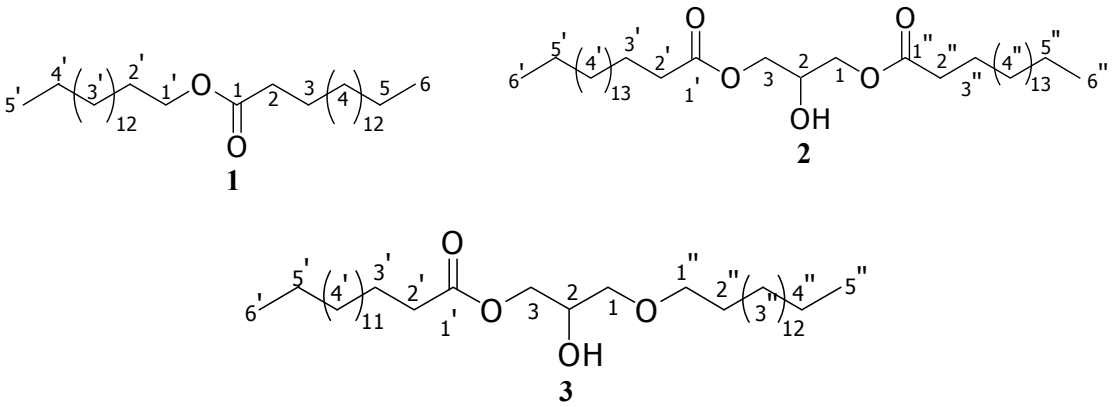

Fig. 1: Chemical structures of the isolated fatty acids.

Compound **2** was also isolated as a white amorphous material. Both ^{1}H NMR (δ_H 4.09-4.19) and ^{13}C NMR (δ_C 65.0-71.0) exhibited the characteristics of a glycerol backbone, which was further confirmed by ^{1}H-^{1}H COSY correlation cross peaks at δ_H 4.09 (p, *J* = 4.4 Hz, H-2)/δ_H 4.19 (dd, *J* = 4.4, 11.4 Hz, H_a-1,3) and δ_H 4.10(dd, *J* = 4.4, 11.4 Hz, H_b-1,3), and by a HMBC cross peak between $H_{a,b}$-1/δ_C 65.0 (C-3). The long

chain acyl part was constructed by using ^{1}H NMR, ^{1}H-^{1}H COSY, HSQC, and HMBC spectral data. The integration of metylene signals at δ_H 1.25 was determined for 56 atoms; each of δ_H 2.35 and 1.62 was 4, while δ_H 0.86 was measured for 6 protons. This indicated that **2** contained two sets of the same fragments and a long chain methylene at δ_H 1.25, but the analysis of HSQC spectral data revealed that this proton signal showed C-H direct correlation to δ_C 23.0 (C-4′, 4″), 30.0 (C-5′), 31.0 (C-5″). This implies that it contains three methylene groups, one of which is the proton of two long chain methylene fragments. Therefore, the signal at δ_H 1.25 was finally calculated to be (56-4) 52 atoms. Those of fragments were connected by analysis of ^{1}H-^{1}H COSY correlation cross peaks at δ_H 2.35 (t, J = 7.6 Hz, H_2-2′)/δ_H 1.62 (p, J = 7.6 Hz, H_2-3′); and δ_H 1.25 (H_2-5′)/δ_H 0.83 (t, J= 6.3, H_3-6) indicated the presence of -CH_2CH_2- and -CH_2CH_3 spin systems. HMBC spectra analysis found that δ_H 1.62 and 2.35 showed ^{3}J- correlation to δ_C 31.0.(C-5′), 23.0 (C-4′), respectively, and hence the –CH_2(C-2′)CH_2(C-3′)CH_2(C-4′)CH_2(C-5′)CH_3(C-6′) spin system was established. Due to the symmetrical molecule, which was determined by integrating the ^{1}H-NMR spectrum, the number of methylene protons at C-4′ was equally divided for 26 atoms or $(CH_2)_{13}$, and full assignment for two sets of –$CH_2CH_2(CH_2)_{13}CH_2CH_3$ fragments was completed. Both fragments were connected to the glycerol unit by HMBC cross peaks at δ_H 4.19, 4.10 and 1.62/δ_C 173.0. The HRESIMS spectral data displayed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 625.5777 that is compatible with $C_{39}H_{79}O_5$. Therefore, **2** was identified as octadecanoic acid 2-hydroxy-3-octadecanoyloxy-propyl ester.

Compound **3** was isolated as a white amorphous material. ^{1}H and ^{13}C NMR signals revealed a typical glycerol backbone and long acyl chains, as found in **2**. The molecular feature of **3** contained ester and ether functions linked to *sn-1* and *sn-3* of glycerol backbone analysed by the appearance of up field signals at δ_H 3.49 (dd, J = 4.2, 9.5, H_a-3), 3.41 (dd, J = 4.2, 9.5, H_b-3), methylenes at δ_H 3.45 (dd, J = 3.4, 13.2, H_2-1″) and 2.30 (m, H_2-2′), which were confirmed by HMBC cross peak at δ_H 4.15 (H_a-1), 4.12 (H_b-1)/δ_C 174.0 (C-1′) and δ_H 3.49, 3.41/δ_C 174.0 (C-1″), respectively. Similar to **2**, the integration measurement of methylene at δ_H 1.65 (p, H_2-2″,3′), 1.30 (H_2-3″, 4′), 1.51(m, H_2-4″,5′), and methyl at δ_C 0.85 (t, J = 7.0, 10.5, H_3-5″, 6′) were determined for 4, 46, 4 and 6 atoms, respectively. This information indicated the presence of two similar fragments of the long acyl chain (-$CH_2CH_2CH_2CH_3$), which was constructed by ^{1}H-^{1}H COSY cross peaks analysis. The completed structure proposed was accordance to a pseudomolecular ion peak at *m/z* 555.5344 (calculated for $C_{35}H_{70}O_4$). Hence, **3** was identified as hexadecanoic acid 3-hexadecyloxy-2-hydroxy-propyl ester.

4. Acknowledgements

This research was financially supported by the Thailand Research Fund (grant number MRG5080328), RMUTSV (2553), BNPME is supported by the Grant for Center of Excellence from the Commission on Higher Education, Thailand. We are deeply thanks Dr. Victor Wray, Department of Structural Biology, Helmholtz Centre for Infection Research, Germany, for his suggestion, nmr and hrms determination

5. References

[1] J. P. Bergé, and G. Barnathan. Fatty Acids from Lipids of Marine Organisms: Molecular Biodiversity, Role as Marker, Biologically Active Compounds, and Economical Aspects. *Adv. Biochem. Eng./Biotechnol.* 2005, **96**: 49-125.

[2] N.M. Carballeira, C. Miranda, and A.D. Rodríguez. Phospholipid Fatty Acid Composition of *Gorgonia mariae* and *Gorgonia ventalina*. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.,* 2002, **131**: 83-87.

[3] N.M. Carballeira, A. Sostre, and A.D. Rodríguez. Phospholipid fatty acid composition of gorgonians of the genus *Eunicea*: further identification of tetracosapolyenoic acids. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol*, 1997, **118**: 257-260.

[4] N.M. Carballeira, A. Sostre, and A.D. Rodríguez. Phospholipid fatty acid composition of gorgonians of the genus *Pseudopterogorgia*: Identification of tetracosapolyenoic acids. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol* 1996, **118**:781-783.