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Chemical Constituents of *Millepora dichotoma*

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ABSTRACT

*Chemical investigation of the dichloromethane extract of the fire coral, Millepora dichotoma yielded β-sitosterol (**1**), triacylglycerols (**2**), and wax esters (**3**). The structures of **1-3** were identified by comparison of their NMR data with literature data.*

Keywords: *Millepora dichotoma,* Milleporidae, fire coral, hydrocoral, β-sitosterol, triacylglycerols, wax esters

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INTRODUCTION

Millepora spp. are important colonial hydrozoan corals that are found throughout the coral reef systems in the Atlantic and Indo-Pacific oceans, contributing on average, to about 10% of the total surface cover, although it could be much more abundant in some local areas [1. 2]. Colonies of millepores usually grow within the shallow region of the reef (< 5 m), but species have been documented to exist at depths of up to 40 meters [2, 3]. These animals, belonging to the class Hydrozoa, are not considered true stony corals (class Anthozoa). Millepores, similar to other cnidarians, have special stinging cells known as cnidocytes that contain a cocktail of toxins which is released after appropriate stimulation. They derive their common name of "fire corals" from the fact that contact with these animals result in a painful and burning sensation, usually followed by other pathological reactions [4, 5]. These corals are also referred to as hydrocorals [6].

One of the earliest work on fire coral toxins demonstrated that these bioactive molecules are proteinaceous in nature, having been purified from the nematocysts, a special organelle within the cnidocytes that contains the toxins [7-9]. These isolated toxins exhibited biological effects similar to those recorded for accidental human envenomation. More recent investigations on the crude protein extracts afforded new protein toxins milleporin-1 [10], a novel phospholipase A2 active protein, and a cytotoxic novel dermatopontin, MCTx-1 [11].In addition to these bioactivities, crude protein extracts from fire corals also exhibited vasoconstrictor, haemolytic, vasopermeable, dermonecrotic, and calcium-dependent smooth muscle excitatory effects [3, 12, 13].

Almost all of the available literature on fire coral metabolites published so far have focused on these protein toxins and their characterization. The presence of long-chain wax esters and 4-diphenylamine, and the sugar and lipid contents of *M. dichotoma* and *M.platyphylla* [14, 15]have been reported. Furthermore, Okinawan corals which included *M. murrayi* afforded monoalkyldiacylglycerol, triacylglycerols, palmitic acid, stearic acid, oleic acid, and

11 sterols, 4of which were identified as cholesterol, 24-methylenecholesterol, campesterol and β-sitosterol [6] which are of relevance to our present report.

In this study, the dichloromethane extract of *M.dichotoma* was subjected to silica gel chromatography to yield**1-3**(Fig. 1) and the structures of these compounds were determined by NMR spectroscopy. To the best of our knowledge, this is the first report of the presence of **1** and **2** from *M. dichotoma*.

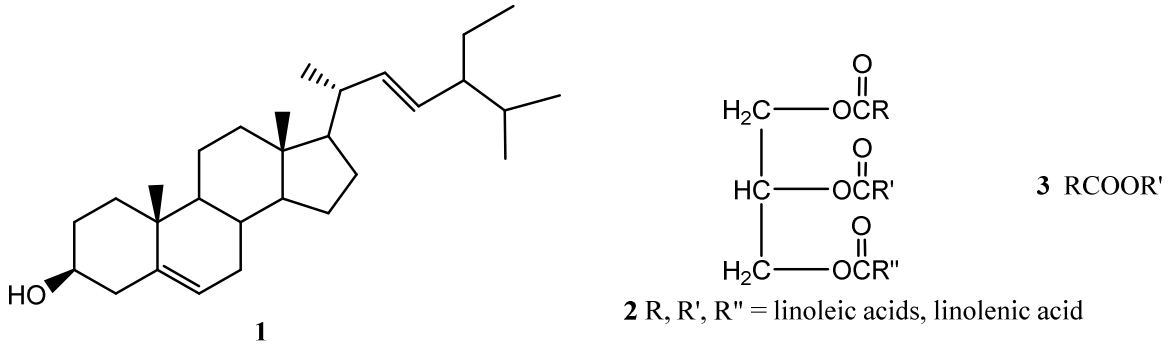

Fig. 1. Chemical structures of β-sitosterol (1), triacylglycerols (2), and wax esters (3) from *M. dichotoma*

MATERIALS AND METHODS

General Experimental Procedures

Sample spectra were obtained on a JEOL ECA500 spectrometer with $CDCl_3$ as solvent.Normal phase, open-column chromatography was performed with silica gel 60 (70-230 mesh), while thin layer chromatography was carried out on plastic backed plates with silica gel F_{254}. Plate visualization was conducted using a vanillin/H_2SO_4 solution followed by heating.

Sample Collection

Millepora dichotoma was collected by scuba in Puerto Galera Island, Oriental Mindoro, Philippines on March 2015 by Dr. Wilfredo Licuanan of the Biology Department of De La Salle University. The samples were immediately put in ice, transported to the laboratory, and stored in a freezer prior to analysis. The sample was identified by Dr. Abdulmohsin Abdullah M. Al-Sofyani of the Marine Biology Department, King Abdulaziz University, Saudi Arabia.

General Isolation Procedure

A glass column 12 inches in height with 0.5 in internal diameter was used for the fractionation of the crude extract. Fractions of 10 mL volumes were collected and monitored by thin layer chromatography. Fractions containing spots with similar R_f values were combined and rechromatographed using the appropriate solvent. Final purification was carried out using Pasteur pipette as the column, collecting 1 mL fractions. TLC-pure isolates were combined, and after evaporation of the solvent, were subjected to NMR analysis.

Isolation of Chemical Constituents

Frozen fire coral samples, *M.dichotoma*, weighing 44 g were freeze dried overnight to yield 37.9 g of the dried sample. These were finely ground using a mortar and pestle and subsequently soaked in CH_2Cl_2 for 3 days. The extract was concentrated in a vacuum rotatory evaporator to afford 201.2 mg of the crude extract, which was then subjected to fractionation with increasing proportions of acetone in CH_2Cl_2 at 10% (v/v) increment. The 10% acetone in CH_2Cl_2 fraction was rechromatographed using 5% EtOAc in petroleum ether to afford **3**after washing with petroleum ether. The 20% acetone in CH_2Cl_2 fraction yielded**2** after rechromatography using 5% EtOAc in petroleum ether.The 40% and 50% acetone in CH_2Cl_2 fractions were combined and subjected to silica gel chromatography using 20% EtOAc in petroleum ether as eluent to afford **1**after washing with petroleum ether.

β-Sitosterol (**1**) ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.51 (m, H-3), 2.27, 2.21 (H_2-4), 5.32 (dd, *J* = 5.5, 1.5 Hz, H-6), 0.66 (s, CH_3-18), 0.99 (s, CH_3-19), 0.90 (d, J = 6.5 Hz, CH_3-21), 0.80 (d, J = 7.0 Hz, CH_3-26), 0.83 (d, J = 7.0 Hz, CH_3-27), 0.86 (t, J = 7.0 Hz, CH_3-29).

Triacylglycerols (**2**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.28 (dd, *J* = 2, 12 Hz, glyceryl CH_2O), 4.12 (dd, *J* = 6.0, 12 Hz, glyceryl CH_2O), 5.25, 5.15 (m, glyceryl CHO), 2.29 (t, *J* = 7.5 Hz, α-CH_2), 5.36 (m, olefinic H), 2.80 (m, double allylic CH_2), 1.97-2.09 (allylic, CH_2), 1.57-1.62 (β-CH_2), 1.23-1.35 (CH_2), 0.96 (t, *J* = 7.5 Hz, CH_3), 0.86 (t, *J* = 6.5 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 62.0, 62.1, 62.3, 62.8 (glyceryl CH_2), 68.9, 71.7 (glyceryl CH), 173.5, 173.3, 173.2, 172.9, 172.6, 172.4, 172.1 (COO), 31.9, 32.0, 29.70, 29.66, 29.5, 29.4, 25.6, 22.7 (CH_2), 14.3, 14.1 (CH_3).

Wax esters (**3**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, J = 7.0 Hz), 1.20-1.30 (m), 1.55-1.62 (m), 1.97-2.09 (m), 2.27 (t, *J* =7.5 Hz), 2.79 (t, *J* = 6 Hz), 2.82 (t, J = 6 Hz), 4.03 (t, *J* = 7 Hz, CH_2O), 5.26-5.40(m, CH=); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.2 (COO), 132.2, 129.4, 128.8, 128.5, 128.4, 128.3, 128.1, 127.2 (CH=CH), 64.8, 64.6 (CH_2O), 32.1, 29.92, 29.88, 29.7, 29.6, 25.8, 22.9 (CH_2), 14.5, 14.3 (CH_3).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the fire coral, *M. dichotoma* yielded β-sitosterol **(1)**[16], triacylglycerols (**2**) [17], and wax esters (**3**) [18]. The structures of **1-3** were identified by comparison of their NMR data with those reported in the literature. The extract yielded two fractions containing mixtures of sterols with β-sitosterol **(1)** as the major sterol.

The major fatty acids attached to the glycerol in the triacylglycerols (**3**)were identified as linoleic acid and linolenic acid as deduced from the integrations and intensities of the ^{1}H NMR resonances at δ 2.80 for the double allylic olefinicprotons and at δ 5.36 for the olefinic protons of linoleic and linolenic acids, and the methyl protons at δ 0.86 and 0.96 for the terminal methyls of linoleic acid and linolenic acid, respectively [19].Based on integrals of the methyl protons at δ 0.86 and 0.96, the ratio of the linoleic acid to linolenic acid is 2:1. The ^{13}C NMR spectrum of **3** indicated resonances for carboxylates at δ 172.1-173.5, olefinic carbons at δ 127.0-132.0, oxymethine carbons at δ 68.9 and 71.7, oxymethylene carbons at δ 62.0-62.8, methylene carbons at δ 22.7-31.9, and methyl carbons at δ 14.1 and 14.3. The presence of four resonances for the oxymethylene carbons at δ 62.0-62.8 suggested that **3** is a mixture of triacylglycerols.

The wax esters (**3**)are mixtures of compounds containing linoleic acid, linolenic acid and saturated fatty acids. These were deduced from the integrations and intensities of the resonances for linolenic and linoleic acids [18]and the methyl triplet at δ 0.86 for the saturated fatty acids and linoleic acid. The oxymethylene protons at δ 4.03 confirmed the presences of CH_2OCO in **3**. Furthermore, the ^{13}C NMR spectrum of **3** gave resonances for an ester carbonyl at δ 174.2, olefinic carbons at δ 127.2-132.2, oxymethylene carbons at δ 64.8 and 64.6, methylene carbons at δ 22.9-32.2 and methyl carbons at δ 14.5 and 14.3 [18]. The presence of oxymethylene carbons at δ 64.8 and 64.6 suggested that **3** is a mixture of wax esters. Wax esters have been previously identified as constituents of *M. dichotoma* [15].

CONCLUSION

The dichloromethane extract of *M. dichotoma* yielded β-sitosterol **(1)**, triacylglycerols (**2**), and wax esters (**3**). The major fatty acids attached to the glycerols of **2**and the wax esters (**3**)are linolenic acid and linoleic acid which belong to the omega-3 and omega-6 fatty acids, respectively. It is interesting to note that **1** [20-26] and **2** [27, 28]which are reported for the first time from this fire coral,as well as the omega-3 [29-31] and omega-6 [32, 33] fatty acids have been reported to exhibit anticancer properties.

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