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Chemical Constituents of *Codium edule*

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ABSTRACT

*Chemical investigation of the dichloromethane extract of Codium eduleP.C.Silva, a popular edible seaweedin the Philippines afforded monogalactosyl diacylglycerol (**1**), α-carotene (**2**), chlorophyll a (**3**), a mixture of clerosterol (**4a**) andcodisterol (**4b**) in about 3:2 ratio, and a mixture of fatty acids. The structures of **4a**and **4b** were elucidated by extensive 1D and 2D NMR spectroscopy, while those of **1**-**3** and the fatty acids were identified by comparison of their NMR data with those reported in the literature.*

Keywords: *Codium edule*, Codiaceae, monogalactosyl diacylglycerol,α-carotene, chlorophyll a, clerosterol, codisterol, fatty acids

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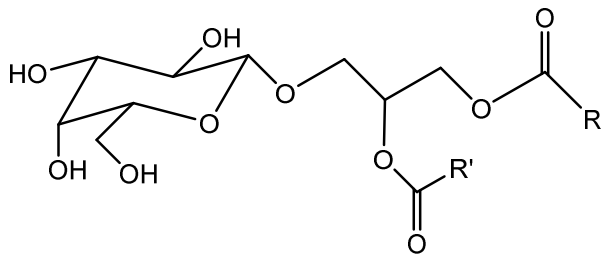
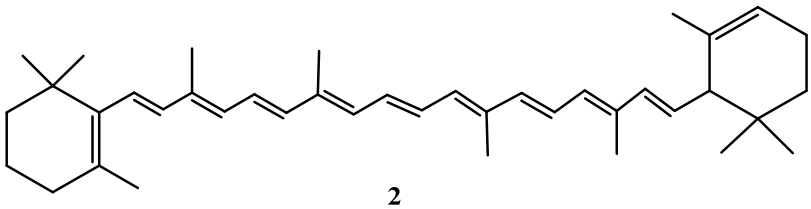
INTRODUCTION

*Codium edule*P.C.Silva, locally known as pokpoklo is a thallus intertwined green algae forming a spongy mass which is a popular edible seaweed sold in the local markets in the Northern Luzon, Philippines [1]. There are no reported secondary metabolites and biological activitiesof *C. edule.*

This study was conducted to investigate the chemical constituents of the dichloromethane extract of *C. edule* collected from Ilocos Norte, Philippines. We report herein the isolation of monogalactosyl diacylglycerol (**1**), α-carotene (**2**), chlorophyll a (**3**), a mixture of stigmasta-5,25-dien-3β-ol or clerosterol (**4a**) and codisterol (**4b**) in about3:2 ratio, and a mixture of fatty acids from *C. edule.* The structures of these **1**-**4** are presented in Fig. 1. To the best of our knowledge, this is the first report on the isolation of these compounds from *C. edule.*



1 R, R' = long chain fatty acids



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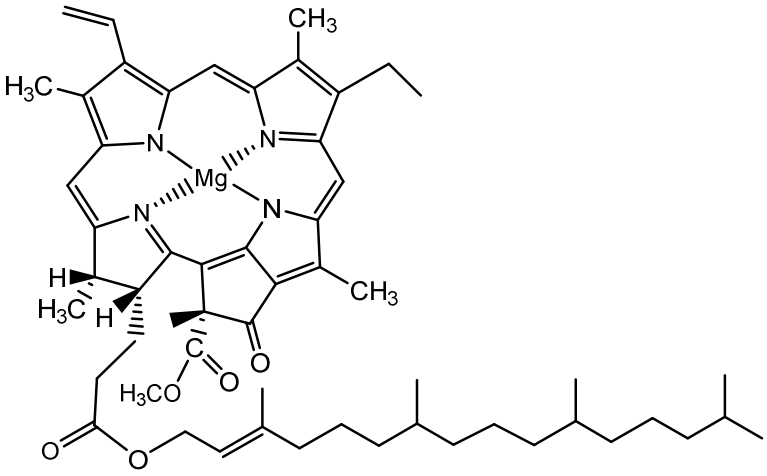

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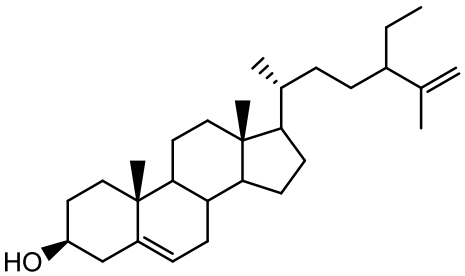
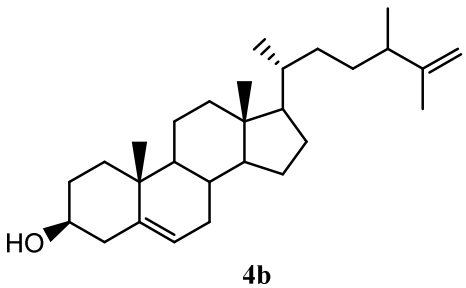


4a



4b

Fig. 1. Chemical structures of monogalactosyl diacylglycerol (1), α-carotene (2), chlorophyll a (3), clerosterol (4a) andcodisterol (4b) from *C. edule*

MATERIALS AND METHODS

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. Twenty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same $R_f$value were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Plant Material

The seaweed was collected from Ilocos Norte, Philippines in April 2016. The sample was authenticated as *Codium edule* P.C.Silva at the Philippine National Museum.

Isolation

The freeze-dried *C. edule* (208.9 g) was cut into small pieces, ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated from the filtrate under vacuum to afford a crude extract (1.3877 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increments by volume as eluents. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 5% EtOAc in petroleum ether to afford **2** (3 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield a mixture of **4a** and **4b** (5 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was rechromatographed (2×) using 15% EtOAc in petroleum ether to afford **3**(5 mg) after washing with petroleum ether, followed by Et_2O.The 60% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) usingCH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to afford a mixture of fatty acids(2 mg). The 80% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6, v/v) to afford **1** (3 mg) after trituration with petroleum ether.

Clerosterol (**4a**):^{1}H NMR (600 MHz, $CDCl_3$): δ 0.65 (3H, s H-18), 0.78 (t, *J* = 7.2 Hz, H-29), 0.88 (3H, d, *J* = 6.6 Hz, H-21), 0.98 (3H, s, H-19), 1.57 (3H, s, H-27), 3.50 (1H, m, H-3), 4.63 (dd, *J* = 1.2, 3.0 Hz, H-26a), 4.73 (dd, *J* = 1.2, 3.0 Hz, H-26b), 5.33 (t, *J* = 3.6 Hz, H-6); ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.2 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 28.2 (C-15), 29.4 (C-16), 56.0 (C-17), 11.8 (C-18), 19.4 (C-19), 35.5 (C-20), 18.7 (C-21), 33.7 (C-22), 24.3 (C-23), 49.5 (C-24), 147.6 (C-25), 111.4 (C-26), 17.8 (C-27), 26.5 (C-28), 12.1 (C-29).

Codisterol(**4b**):^{1}H NMR (600 MHz, $CDCl_3$): δ 0.65 (3H, s H-18), 0.89 (3H, d, *J* = 6.6 Hz, H-21), 0.97 (d, *J* = 6.6 Hz, H-28), 0.98 (3H, s, H-19), 1.62 (3H, s, H-27), 3.50 (1H, m, H-3), 4.62 (2H, d, *J* = 2.4 Hz, H-26), 5.33 (t, *J* = 3.6 Hz, H-6); ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.2 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 28.2 (C-15), 29.4 (C-16), 56.0 (C-17), 11.8 (C-18), 19.4 (C-19), 35.7 (C-20), 18.7 (C-21), 33.7 (C-22), 31.1 (C-23), 42.3 (C-24), 150.2 (C-25), 109.3 (C-26), 18.6 (C-27), 20.1 (C-28).

RESULTS AND DISCUSSSION

Silica gel chromatography of the dichloromethane extracts of *C. edule* yielded **1**–**4**and a mixture of fatty acids. The NMR spectra of **1** are in accordance with data reported in the literature formonogalactosyl diacylglycerol (**1**) [2]; **2** for α-carotene [3];**3** for chlorophyll a [4];**4a** for clerosterol [5];**4b** forcodisterol [6]; and fatty acids [7] from *C. edule*.

The mixture of **4a** and **4b** was deduced from the integrations and intensities of the methylene olefinic protons at δ 4.63 (dd, *J* = 1.2, 3.0 Hz) and 4.73 (dd, *J* = 1.2, 3.0 Hz) for **4a** and δ 4.62 (2H, d, *J* = 2.4 Hz) for **4b**. Furthermore, two almost overlapping methyl doublets were found at δ 0.88 (*J* = 6.6 Hz) for **4a** and δ 0.89 (*J* = 6.6 Hz) for **4b**; and a methyl triplet atδ 0.78 (*J* = 7.2 Hz) for **4a** and a methyl doublet at δ 0.97(*J* = 6.6 Hz) for **4b**. Based on the integrations and intensities of these proton resonances, the ratio of **4a** and **4b** is about 3:2.

Although no biological activity tests were conducted on the isolated compounds, a literature search of **1**-**4** revealed that these have diverse bioactivities.

Monogalactosyl diacylglycerols (**1**)and dinogalactosyl diacylglycerols are the most widespread non-phosphorous polar lipids in nature, constituting about 80% of membrane lipids in plants and more than half of all lipids in algae [8, 9]. These compounds were reported to exhibit a number of biological properties, such as anti-tumor [10, 11], anti-viral [12], algicidal [13] and anti-inflammatory [14-17]. Monogalactosyl diacylglycerols were also found to exhibit cytotoxic and anti-inflammatory activity in RAW 264.7 macrophage cells with IC_{50} values of 60.06 and 65.70 µg/mL, respectively [18]. Compound **1** was also reported to exhibit anti-inflammatory activity in human articular cartilage [19]. Itinhibited the growth of human melanoma cells in a dose-dependent manner with an IC_{50} value of 114 µM [2].

A study reported that α-carotene (**2**) suppressed spontaneous liver carcinogenesis and promoting stage of lung and skin carcinogenesis in mice [20]. Another study reported that **2** inhibited colonic aberrant crypt foci formation in rats [21]. Compound **2** also inhibited the proliferation of the human neuroblastoma cell line GOTO in a dose- and time-dependent manner [22].

Chlorophyll a (**3**) and its various derivatives are used in traditional medicine and for therapeutic purposes [23]. Natural chlorophyll and its derivatives have been studied for wound healing [24], anti-inflammatory properties [25], control of calcium oxalate crystals [26], utilization as effective agents in photodynamic cancer therapy [27-29], and chemopreventive effects in humans [30, 31]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided [32].

Clerosterol (**4a**)inhibited the growth of human melanoma cells (A2058) with an IC_{50} of 150 µM by inducing apoptotic cell death [33].Furthermore, **4a** was reported to be cytotoxic to A549 lung cancer cells [34]. Sterol **4a** from the leaves and roots of *C. infortunatum* is used as an antitumor agent [35]. It was also shown to exhibit trypanocidal activity against Trypanosoma brucei with an ED_{50} of 134.34 µM [2]. Another study reported that **4a** exhibited weak antitrypanosomal activity and nontoxic to mammalian cells [36].

CONCLUSION

Codium edule, a popular edible seaweed in the Northern Philippines with no reported chemical constituents and medicinal properties yielded **1**-**4** which were reported to exhibit anticancer properties.

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