Chemical Constituents of Codium edule

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ABSTRACT

Chemical investigation of the dichloromethane extract of Codium edule P.C.Silva, a popular edible seaweed in the Philippines afforded monogalactosyl diacylglycerol (1), α-carotene (2), chlorophyll a (3), a mixture of clerosterol (4a) and codisterol (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

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 activities of 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 about 3:2 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.
Fig. 1. Chemical structures of monogalactosyl diacylglycerol (1), α-carotene (2), chlorophyll a (3), clerosterol (4a) and codisterol (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 with 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 Rf 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 CH2Cl2 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 CH2Cl2 at 10% increments by volume as eluents. The 20% acetone in CH2Cl2 fraction was rechromatographed (2 ×) using 5% EtOAc in petroleum ether to afford 2 (3 mg) after washing with petroleum ether. The 30% acetone in CH2Cl2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield a mixture of 3a and 3b (5 mg) after washing with petroleum ether. The 60% acetone in CH2Cl2 fraction was rechromatographed (2 ×) using CH3CN:EtO:CH2Cl2 (0.5:0.5:9, v/v) to afford a mixture of fatty acids (2 mg). The 80% acetone in CH2Cl2 fraction was rechromatographed (3 ×) using CH3CN:EtO:CH2Cl2 (2:2:6, v/v) to afford 1 (3 mg) after trituration with petroleum ether.

Clerosterol (4a):1H NMR (600 MHz, CDCl3): δ 0.65 (3H, s H-18), 0.78 (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); 13C NMR (150 MHz, CDCl3): 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):1H NMR (600 MHz, CDCl3): 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); 13C NMR (150 MHz, CDCl3): 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 DISCUSSION
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 from monogalactosyl diacylglycerol (1) [2]; 2 for α-carotene [3]; 3 for chlorophyll a [4]; 4a for clerosterol [5]; 4b for codisterol [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 at δ 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 digalactosyl 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\textsubscript{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]. It inhibited the growth of human melanoma cells in a dose-dependent manner with an IC\textsubscript{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\textsubscript{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 was used as a multitumor agent [35]. It was also shown to exhibit trypanocidal activity against Trypanosoma brucei with an ED\textsubscript{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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REFERENCES

Der Pharma Chemica, 2015, 7(3), 94-100.
[8] S. V. Khotimchenko, 
[9] P. Dormann, C. Benning, 