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Chemical Constituents of *Cymodocea serrulata* R. Brown.

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ABSTRACT

Chemical investigation of the dichloromethane extract of Cymodocea serrulata has led to the isolation of bis(2-ethylhexyl) phthalate (1), chlorophyll a (2), and a mixture of β-sitosterol (3) and stigmasterol (4). The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy. The structures of 2-4 were identified by comparison of their NMR data with literature data.

Keywords: Cymodocea serrulata, Cymodoceaceae, bis(2-ethylhexyl) phthalate, chlorophyll a, β-sitosterol, stigmasterol

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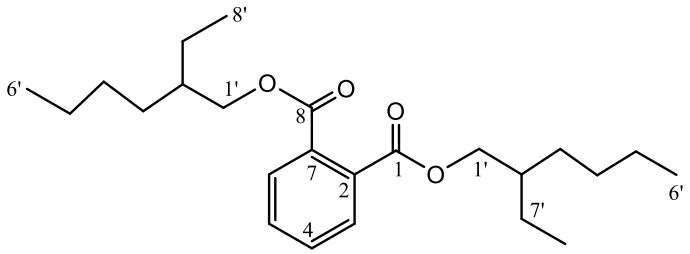
INTRODUCTION

Cymodocea serrulata is a seagrass that grows on muddy sand, fine sand or sand with coral rubble substrates in the intertidal zone [1]. A study reported that *C. serrulata* afforded a phenyl thioketone which elicited pronounced inhibitions against *Escherichia coli* with minimal inhibitory concentration values of 1-3 µg concentration using micro-dilution method [2]. Another study reported that *C. serrulata* exhibited predominant growth inhibitory activity against all the tested UTI bacteria [3]. Another study reported the isolation of the sterols: cholesterol, campesterol, stigmasterol, sitosterol and 28-isofucosterol and the major fatty acids: linolenic acid (48.6%), palmitic acid (19.2%) and linoleic acid (18.5%) [4].

We report herein the isolation of bis(2-ethylhexyl) phthalate (**1**), chlorophyll a (**2**), and a mixture of β-sitosterol (**3**) and stigmasterol (**4**) (Fig. 1) from *C. serrulata*. To the best of our knowledge this is the first report on the isolation of **1** and **2** from *C. serrulata.*



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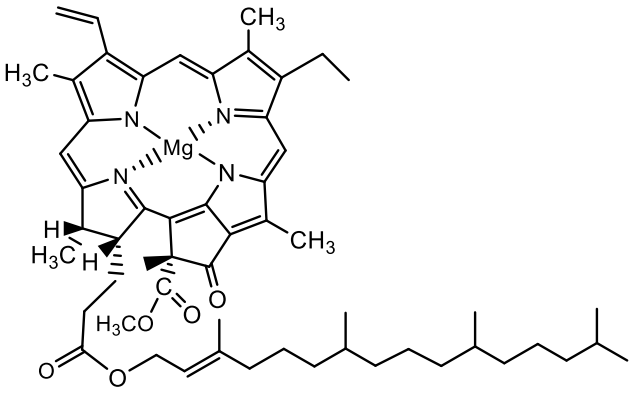

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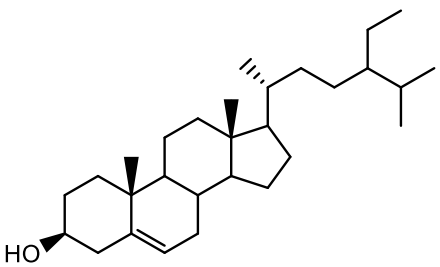

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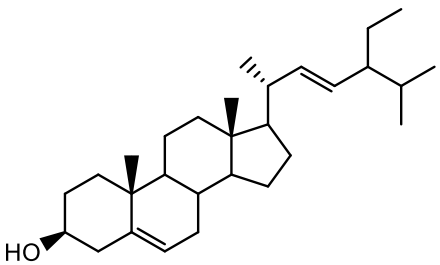

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Fig. 1. Chemical structures of bis(2-ethylhexyl) phthalate (1), chlorophyll a (2), and a mixture of β-sitosterol (3) and stigmasterol (4) from *C. serrulata*.

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MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

Sample Collection

Samples of the seagrass, *Cymodocea serrulata* R. Brown were collected during low tide from the seagrass meadows in Caramoan, Camarines Sur, Philippines in June 2016. The whole plant including the roots were obtained using a 20 centimeter stainless cylindrical corer with a cap. The corer grabs seagrasses with their substrate from an area of about 0.031 square meter. The collected seagrasses were placed into a net bag to sieve the sediments. The *C. serrulata* samples were sorted from other seagrass species and were brought to the De La Salle University Laboratory for freeze-drying. Samples of *Cymodocea serrulata* R. Brown were authenticated at the Marine Plants Division, Philippine National Museum.

General Isolation Procedure

The crude extract was fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume as eluents. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained.

Isolation of the chemical constituents of C. serrulata

The freeze-dried *C. serrulata* (22.2 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (0.4 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The CH_2Cl_2 fraction was rechromatographed (2 ×) using 5% EtOAc in petroleum ether to afford **1** (4 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed using 7.5% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 7.5% EtOAc in petroleum ether to yield **2** after washing with petroleum ether, followed by Et_2O. The more polar fractions were combined and rechromatographed using 7.5% EtOAc in petroleum ether to afford a mixture of **3** and **4** (3 mg) after washing with petroleum ether.

Bis(2-ethylhexyl) phthalate (**1**): ^{1}H-NMR (600 MHz, CDC1$_3$): δ 7.68 (2H, dd, *J* = 3.6, 6.0 Hz, H-3, H-6), 7.51 (2H, dd, *J* = 3.6, 6.0 Hz, H-4, H-5), 4.20 (4H, m, H-1'), 1.65 (2H, m, H-2'), 1.34 (4H, m, H-3'), 1.30 (8H, m, H-4', H-5'), 0.88 (6H, t, *J* = 7.2 Hz, H-6'), 1.40 (4H, H-7'), 0.90 (6H, t, *J* = 7.2 Hz, H-8'); ^{13}C-NMR (150 MHz, CDC1$_3$): δ 167.74 (C-1, C-8), 132.45 (C-2, C-7), 130.87 (C-3, C-6), 128.78 (C-4, C-5), 68.14 (C-1'), 38.72 (C-2'), 30.34 (C-3'), 28.91 (C-4'), 22.97 (C-5'), 14.04 (C-6'), 23.73 (C-7'), 10.94 (C-8').

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *C. serrulata* yielded **1**-**4**. The NMR spectra of **1** are in accordance with data reported in the literature for bis(2-ethylhexyl) phthalate [5]; **2** for chlorophyll a [6]; **3** for β-sitosterol [7]; and **4** for stigmasterol [7].

Bis(2-ethylhexyl) phthalate (**1**) exhibited antioxidant activity with maximum activity of 77.99 % at 12 µg/ml; antitumor activity using Ehrlich cells with maximum activity of 77.29 % at 12 µg/mL; cytotoxicity against human breast adenocarcinoma cell line (MCF-7) and human alveolar basal epithelial cell line (A-549); and antiviral activity against H1N1 at different concentrations [8]. Another study reported that microbial derived plasticizers such as **1** are benign and resistant to migration, evaporation and leaching and stable to light and heat. These plasticizers also exhibit antiviral, antioxidant and antitumor activities [9-11]. In another study, **1** showed a significant decrease in viable cell count, mass gain, elevated the life span of Ehrlich ascites carcinoma

cells bearing mice and it brought back altered biochemical parameters (glucose, cholesterol, triglycerides, blood urea) to normal level [12]. Compound **1** isolated from *Calotropis gigantea* (Linn.) flower exhibited antimicrobial and cytotoxic properties [13]. This compound isolated from *Aloe vera* was found to exhibit antileukemic and antimutagenic effects [14].

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