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Secondary Metabolites from *Hypnea nidulans* Setchell

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ABSTRACT

*Chemical investigation of the dichloromethane extract of Hypnea nidulans Setchell has led to the isolation of monogalactosyl diacylglycerols (**1**) and zeinoxanthin (**2**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy. The structure of **2** was identified by comparison of its NMR data with those reported in the literature.*

Keywords: *Hypnea nidulans* Setchell, Hypneaceae, monogalactosyl diacylglycerols, zeinoxanthin

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INTRODUCTION

Hypnea is a red algal genus, and a well-known source of the polysaccharide carrageenan[1].*Hypnea nidulans*Setchell is a marine alga which grows in North and Central America, Africa, Asia and the Pacific Islands[2]. The species is found in crevices of branched corals up to 10 m deep[3]. There is only one reported study on the chemical constituents of *H. nidulans.* We earlier reported the isolation of squalene, β-sitosterol, ursolic acid, oleanolic acid, chrorophyll a and hydrocarbons from *H.* nidulans [4].

We report herein the isolation of monogalactosyl diacylglycerols (**1**) and zeinoxanthin (**2**) from *H. nidulans.*To the best of our knowledge this is the first reporton the isolation of these compounds from*H. nidulans.*

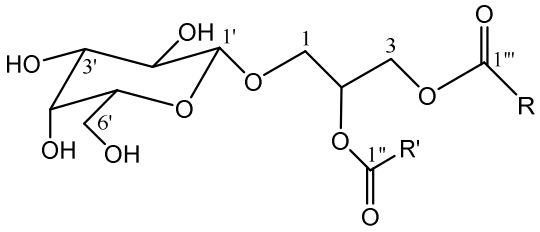
MATERIALS AND METHODS

General Experimental Procedure

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were acquired in $CDCl_3$ on a 500 MHz Agilent DD2 NMR spectrometer with referencing to solvent signals (δ 7.26 and 77.0 ppm). 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.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was used for the chromatography of the crude extracts. Twenty milliliter fractions were collected. 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. 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.



1 R, R' = long chain fatty acid alkyls

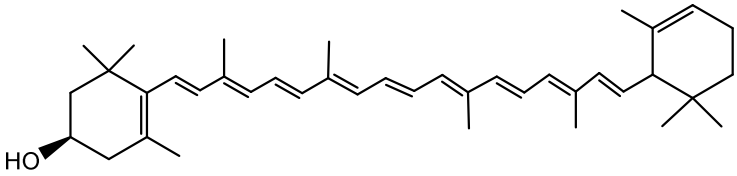


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Fig. 1. Chemical structures of monogalactosyl diacylglycerols (1) and zeinoxanthin (2) from *H. nidulans*

Plant Material

Hypnea nidulans Setchell was collected from Roxas City, Philippines in October 2014. It was authenticated at the Philippine National Museum.

Isolation of the Chemical Constituents

The freeze-dried *H. nidulans* (45.7 g) was ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.5 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc inpetroleum ether to yield **2** (2 mg) after washing with petroleum ether, followed by Et2O. The acetone fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5 by volume ratio) to afford **1** (7 mg) after trituration with petroleum ether.

***Monogalactosy diacyllglycerols* (1):** ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.90 (dd, *J* = 11.5, .5 Hz, Ha-1), 3.75 (dd, *J* = 11.5, 6.5 Hz, Hb-1), 5.32 (m, H-2), 4.39 (dd, *J* = 12, 3.5 Hz, Ha-3), 4.20 (dd, *J* = 12, 6.6 Hz, Hb-3), 4.28 (d, *J* = 7.5 Hz, H-1'), 3.64 (dd, *J* = 7.5, 9.5 Hz, H-2'), 3.59 (dd, *J* = 9.5, 3 Hz, H-3'), 4.01 (br s, H-4'), 3.55 (t, *J* = 4.5 Hz, H-5'), 3.99 (dd, *J* = 12, 6 Hz, Ha-6'), 3.87 (dd, *J* = 12, 3.5 Hz, Hb-6'),2.31, 2.32 (t, *J* = 7.5 Hz, H_2-2", H_2-2'"), 1.58–1.72 (m, H_2-3", H_2-3'"), 1.23–1.37 (m, methylenes), 2.13-1.99 (m, allylic), 5.30–5.40 (m, vinylic), 2.82 (t, *J* = 5.5 Hz, double allylic), 2.80 (t, *J* = 5.5 Hz, double allylic), 0.97, 0.88 (t, *J* = 7.5 Hz, Me); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 68.4 (C-1), 70.2 (C-2), 62.7 (C-3), 103.95 (C-1'), 71.7 (C-2'), 73.4 (C-3'), 69.5 (C-4'), 74.5 (C-5'), 63.0 (C-6'), 173.5, 173.7 (C-1"; C-1'"), 34.3, 34.1 (C-2"; C-2"), 24.8, 24.6 (C-3"; C-3"), 29.0–29.8 (CH_2"; CH_2'"), 27.2, 27.1 (CH_2"; CH_2'"), 127.1-130.7 (CH"=; CH="'), 25.5, 25.6 ($C_{(n-3)}$"; $C_{(n-3)}$'"),, 31.5, 31.9 ($C_{(n-2)}$"; $C_{(n-2)}$'"), 22.6, 22.7 ($C_{(n-1)}$"; $C_{(n-1)}$'"), 14.1, 14.2 (terminal $CH_{3(n))}$"; $CH_{3(n))}$'").

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *H. nidulans* has led to the isolation of**1** and **2**. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its NMR data with literature data [5]. The NMR spectra of **2** are in accordance with data reported in the literature for zeinoxanthin [6].

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 [7-8]. These compounds were reported to exhibit a number of biological properties, such as anti-tumor [9-10], anti-viral [11], algicidal [12] and anti-inflammatory [33-16]. Monogalactosyl diacylglycerols were also found to exhibit cytotoxic and anti-inflammatory activity in RAW 264.7 macrophage cells with IC50 values of 60.06 and 65.70 µg/mL, respectively [17]. Compound 5 was also reported to exhibit anti-inflammatory activity in human articular cartilage [18]. Itinhibited the growth of human melanoma cells in a dose-dependent manner with an IC50 value of 114 µM [19].

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