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Chemical constituents of *Hypnea nidulans* Setchell

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ABSTRACT

*Chemical investigation of the dichloromethane extract of Hypnea nidulans Setchell led to the isolation of squalene (**1**), β-sitosterol (**2**), a mixture of ursolic acid (**3**) andoleanolic acid (**4**) in a 3:1 ratio, chrorophyll a (**5**), and hydrocarbons. The structures of these compounds were identified by comparison of their NMR data with those reported in the literature.*

Keywords: *Hypnea nidulans*, Hypneaceae, squalene, β-sitosterol, ursolic acid,oleanolic acid, chrorophyll a

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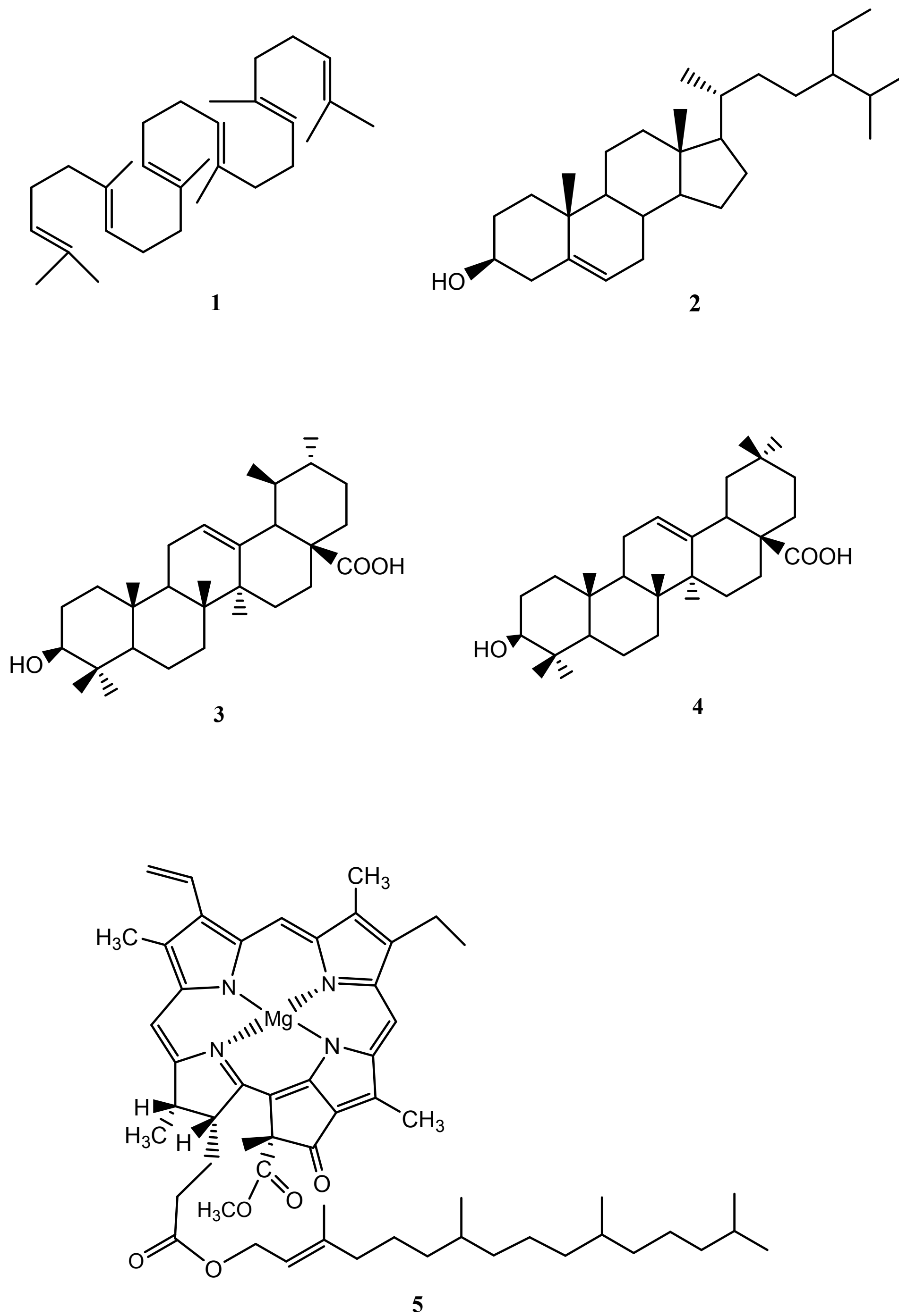
INTRODUCTION

Hypnea is a red algal genus, and a well-known source of the polysaccharide carrageenan [1].*Hypnea nidulans* Setchell is a marine algae which grows in North and Central America, Africa, Asia and the Pacific Islands [2]. They are found in crevices of branched corals up to 10 m deep [3]. There are no reported chemical and biological activity studies on *H. nidulans.*However, congeners of this red algae were studied for their chemical constituents and biological activities. A previous study reported the isolation of β-sitosterol and fatty acids from *H. musciformis*[4].The pentane, ethyl acetate, and n-butanol extracts of *H. musciformis*gave DPPH radical scavenging activity (EC_{50} values) of >10.0, 7.1 ± 0.15 and 5.2 ± 0.01 µg/mL [4]. Another study reported the isolation of 22-dehydrocholesterol, cholesterol, cholesterol oleate, (22*E*)-cholesta-5,22-dien-3*β*-ol-7-one and oleic acid from *H. flagelliformis* [5].The methanol extract of *H. flagelliformis* exhibited antimicrobial activity against *S. Aureus, S. epidemidis,* and *B. subtilis* [6].Another *Hypnea* species, *H. charoides* Lamx. yielded ergost-5,22-dien-3-ol, ergost-5-en-3-ol, stigmasta-5,22-dien-3-ol, cholesta-5,22-dien-3-ol, cholesta-5-en-3-ol,stigmasta-5-en-3-ol, and a ceramide [7].Furthermore, *H. pannosa* J. Ag. afforded 10-bromo-7, 12-dihydroxy-$\Delta^{3,4}$-laurene, filiformin and filiforminol [8]. Another study reported the presence of fatty acids, sesquiterpenes and sterols in*H. cornuta, H. musciformis, H. Pannosa,* and *H. valentiae* [9].

We report herein the isolation of squalene (**1**), β-sitosterol (**2**), a mixture of ursolic acid (**3**) andoleanolic acid (**4**) in a 3:1 ratio, chrorophyll a (**5**), and hydrocarbons from *H. nidulans.* To the best of our knowledge this is the first report on the isolation of these compounds from *H. nidulans.*



Chemical structures of squalene (**1**), β-sitosterol (**2**), ursolic acid (**3**), oleanolic acid (**4**), and chrorophyll a (**5**) from*Hypnea 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 visualizedby 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.

Plant material
Hypnea nidulans Setchellwas 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 CH_2Cl_2 fraction was rechromatographed using petroleum ether. The less polar fractions were rechromatographed using petroleum ether to afford the hydrocarbons (8 mg). The more polar fractions were rechromatographed using 1% EtOAc in petroleum ether to yield **1** (3 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 20% EtOAc in petroleum ether to afford **2** (5 mg) and **5** (6 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2fraction was rechromatographed (3 ×) using Et_2O:CH_3CN:CH_2Cl_2 (1:1:8, v/v) ether to yield a mixture of**3** and **4**(3 mg) after washing with petroleum ether.

Squalene (**1**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.08-5.13 (6H, =CH), 1.58 (18H, allylic CH_3, cis), 1.66(6H, allylic CH_3, trans), 1.94-2.07 (20H, allylic CH_2).

β-Sitosterol (**2**): ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.50 (m, H-3), 2.26, 2.21 (H_2-4), 5.33 (dd, J = 5.0, 2.0 Hz, H6),0.66 (s, CH_3-18), 0.99 (s, CH_3-19), 0.90 (d, J = 7.0 Hz, CH_3-21), 0.79 (d, J = 7.0 Hz, CH_3-26), 0.82 (d, J = 7.0 Hz, CH_3-27), 0.85 (t, J = 7.0 Hz, CH_3-29).

Ursolic acid (**3**): ^{1}H NMR ($CDCl_3$, 600 MHz): δ 3.20 (dd, J = 4.2, 11.4 Hz, H-3α), 5.28 (t, J = 3.6 Hz, H-12), 2.18 (d, J =11.4 Hz, H-18), 1.23 (s,CH_3-23), 0.97 (s, CH_3-24), 0.77 (s, CH_3-25), 1.06 (s, CH_3-26), 1.12 (s, CH_3-27), 0.93 (d, J = 6.6 Hz, CH_3-29), 0.91 (d, J = 5.9 Hz, CH_3-30).

Oleanolic acid (**4**):^{1}H NMR (500 MHz, $CDCl_3$): δ 3.20 (dd, J = 4.2, 11.4 Hz, H-3α), 5.26 (t, J = 3.6 Hz, H-12), 2.81 (dd, J = 4.2, 13.8 Hz, H-18), 0.96 (s, H_3-23), 0.73 (s, H_3-24), 0.89 (s, H_3-25), 0.75 (s, H_3-26), 1.11 (s, H_3-27), 0.91 (s, H_3-29), 0.88 (s, H_3-30).

Chlorophyll a (**5**):^{1}H NMR (500 MHz, $CDCl_3$): δ 3.40 (3H, s, H-1a), 7.98 (1H, dd, J = 18, 12 Hz, H-2a), 6.18 (2H, dd, J = 11.4, 1.2 Hz, H_2b), 6.26 (1H, dd, J = 12, 18 Hz, H-$_2$b), 3.23 (3H, s, H-3a), 3.68 (2H, m, H-4a), 1.69 (3H, t, J = 7.2, H-4b), 3.69 (3H, s, H-5a), 4.42 (1H, m, H-7), 2.15, 2.48 (2H, m, H-7a), 2.33, 2.63 (2H, m, H_2-7b), 4.22 (1H, m, H-8), 1.80 (3H, d, J = 7.2 Hz, H-8a), 6.27 (1H, s, H10), 3.87 (3H, s, H-10b), 9.44 (1H, s, H-α or H-β), 9.58 (1H, s, Hα or H-β), 8.62 (s, H-δ), 4.46 (2H, m, H-1'), 5.11 (1H, t, J = 7.2 Hz, H-2'), 1.56 (3H, br s, H-17'), 0.82 (6H, d, J = 6.6 Hz, H-18' and H-19'), 0.76 (3H, d, J = 6.6 Hz, H-16'), 0.74 (3H, d, J = 6.6 Hz, H-20').

Hydrocarbons: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, *J* = 6.5 Hz, CH_3), 1.23 [br s, $(CH_2)_n$].

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the freeze-dried *H. nidulans*led to the isolation of squalene (**1**) [10], β-sitosterol (**2**) [10], a mixture of ursolic acid (**3**) [11] andoleanolic acid (**4**) [12] in a 3:1 ratio, chrorophyll a (**5**) [13], and hydrocarbons [14]. The structures of these compounds were identified by comparison of their NMR data with those reported in the literature.

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

Squalene (**1**) was reported to significantly suppress colonic ACF formation and crypt multiplicity which strengthened the hypothesis that it possesses chemopreventive activity against colon carcinogenesis [15]. It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [16]. A recent study reported that tocotrienols, carotenoids, squalene and coenzyme Q10 have anti-proliferative effects on breast cancer cells [17]. The preventive and therapeutic potential of **1** on tumor promotion and regression have been reported [18]. A recent review on the bioactivities of **1** has been provided [19].

β-Sitosterol (**2**) was reported to exhibit growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [20]. It was shown to be effective for the treatment of benign prostatic hyperplasia [21]. It attenuated β-catenin and PCNA expression, as well as quenched radical in-vitro, making it a potential anticancer drug for colon carcinogenesis [22]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [23]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [24].

Ursolic acid (**3**) was found to induce apoptosis in tumor cells by activation of caspases and modulation of other pathways involved in cell proliferation and migration. It decreases proliferation of cells and induces apoptosis, thereby inhibiting growth of tumor cells both in vitro and in vivo [25]. An earlier study reported that **3** exhibited anti-tumor activity against human colon carcinoma cell line HCT15 [26]. Moreover, **3** inhibits the growth of colon cancer-initiating cells by targeting STAT3 [27]. Furthermore, **3** has potential therapeutic use in prostate cancer through its antiproliferative and apoptotic effects [28]. A recent study reported that **3** inhibited cell growth and proliferation of Jurkat leukemic T-cells, as well as suppressed PMA/PHA induced IL-2 and TNF-α production in a concentration and time dependent manner [29]. Another study reported that ursolic acid-activated autophagy induced cytotoxicity and reduced tumor growth of cervical cancer cells TC-1 in a concentration-dependent manner [30].

Oleanolic acid (**4**) exhibited anti-inflammatory effects by inhibiting hyperpermeability, the expression of CAMs, and the adhesion and migration of leukocytes [31]. It showed anti-inflammatory activities through the inhibition of the HMGB1 signaling pathway [32]. It exhibited anti-inflammatory, hepatoprotective, gastroprotective, immunoregulatory and anti-ulcer activities [33], and gastroprotective effect on experimentally induced gastric lesions in rats and mice [34]. It was also reported to inhibit mouse skin tumor [35], protect against hepatotoxicants and treat hepatitis [36], and showed significant antitumor activity on human colon carcinoma cell line HCT 15 [37].

Chlorophyll a (**5**)and its various derivatives are used in traditional medicine and for therapeutic purposes [38]. Natural chlorophyll and its derivatives have been studied for wound healing [39], anti-inflammatory properties [40], control of calcium oxalate crystals [41], utilization as effective agents in photodynamic cancer therapy [42-44], and chemopreventive effects in humans [45, 46]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided [47].

CONCLUSION

The dichloromethane extract of *H. nidulans*yielded squalene (**1**), β-sitosterol (**2**), ursolic acid (**3**),oleanolic acid (**4**), chrorophyll a (**5**) [13], and hydrocarbons which were isolated for the first time from this red macroalgae. Compounds **1**-**5** were reported to exhibit anticancer properties. These compounds were also reported to exhibit diverse biological activities.

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