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Research Article

Chemical Constituents of *Coprinopsis lagopus*

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ABSTRACT
Chemical investigation of the dichloromethane extract of the fruiting bodies of *Coprinopsis lagopus* led to the isolation of 3β-linoleyloxyergosta-7,22-diene (**1**), ergosterol peroxide (**2**), linoleic acid (**3**) and triacylglycerols (**4**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy.

Keywords: *Coprinopsis lagopus*, *Coprinus lagopus*, Psathyrellaceae, 3β-linoleyloxyergosta-7,22-diene, ergosterol peroxide, linoleic acid, triacylglycerols.

INTRODUCTION

Coprinus lagopus (Fries) Fries is commonly known as harefoot mushroom due to its vague resemblance to the paw of a white rabbit[1]. *Coprinus lagopus* is the name of this mushroom until 2001 when it was named *Coprinopsis lagopus* (Fries) Redhead, Vilgalys, & Moncalvo[2]. *C. lagopus* is widely distributed throughout the world and grows on wood chips, compost heaps and vegetable refuse[2]. It is a gray to beige inedible mushroom that grows up to less than 5 cm[3]. An earlier study reported the isolation of the quinones, lagopodin A and lagopodin B from *C. lagopus*[4]. Another study reported the isolation of ergosterol and 22-dihydroergosterol from the carpophores of *C. lagopus*[5]. We report herein the isolation of 3β-linoleyloxyergosta-7,22-diene (**1**), ergosterol peroxide (**2**), linoleic acid (**3**) and triacylglycerols (**4**) from the fruiting bodies of *C. lagopus*. The structures of **1**-**4** are presented in Fig. 1. To the best of our knowledge this is the first report on the isolation of **1**-**4** from *C. lagopus.*

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

Fruiting bodies of *Coprinus lagopus* (Fries) Fries (syn. *Coprinopsis lagopus* (Fries) Redhead, Vilgalys, & Moncalvo were collected growing on rotten stumps and fallen logs located inside the campus of the College of Forestry and Natural Resources, University of the Philippines Los Baños. The collection was done between the months of May to June 2016 which marks the start of the rainy season in this climatic zone. Due to its succulent fruiting bodies, careful collection were done to ensure that the pileus will remain intact until such time it was brought to the laboratory for detailed analysis. The collected *C. lagopus* fruiting bodies were collected and identified by one of the authors (MEGD) after thorough verification with published literature about this basidiomycete.

General Isolation Procedure

The crude extract was fractionated by silica gel chromatography using increasing proportions of EtOAc in petroleum ether 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 the fruiting bodies of C. lagopus

The freeze-dried fruiting bodies of *C. lagopus* (657.3 mg) 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 (46.5 mg) which was chromatographed using increasing proportions of EtOAc in petroleum ether. The 2.5% EtOAc in petroleum ether fraction was rechromatographed (2 ×) in 1% EtOAc in

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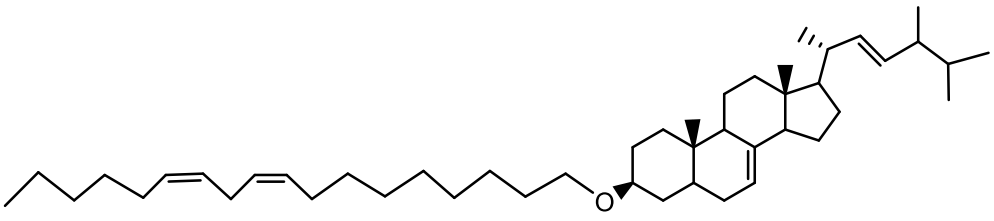

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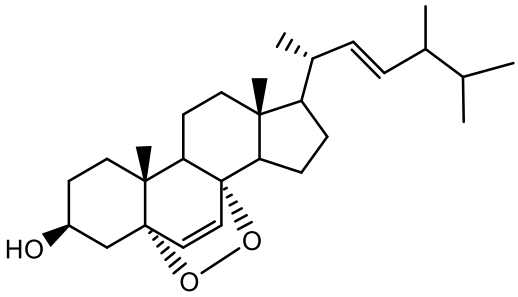

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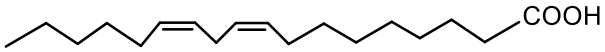

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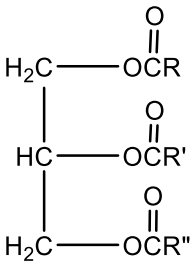

4 R, R', R" = long chain fatty acid alkyls

Figure 1: Chemical structures of 3β-linoleyloxyergosta-7,22-diene (**1**), ergosterol peroxide (**2**), linoleic acid (**3**) and triacylglycerols (**4**) from the fruiting bodies of *C. lagopus*.

petroleum ether to afford **1** (2 mg) after washing with petroleum ether. The 5% EtOAc in petroleum ether fraction was rechromatographed (3 ×) using 5% EtOAc in petroleum ether to yield **4** (2 mg). The 15% EtOAc in petroleum ether fraction was rechromatographed using 15% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to afford **3** (1 mg). The more polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to yield a mixture of **2** and **3** (1 mg) after washing with petroleum ether.

3β-Linoleyloxyergosta-7,22-diene (1)

^{1}H-NMR (600 MHz, $CDC1_3$): δ 4.70 (m, H-3), 5.13 (H-7), 0.55 (s, H_3-18), 0.79 (s, H_3-19), 1.02 (d, J = 6.6 Hz, H_3-21), 5.17 (H-22), 5.18 (H-23), 0.91 (d, J = 6.6 Hz, H_3-26), 0.81 (d, J = 6.6 Hz, H_3-27), 0.83 (d, J = 6.6 Hz, H_3-28), 2.24 (t, J = 7.2 Hz, H-2'), 2.75 (t, J = 6.6 Hz, H-11'), 5.30-5.37 (4H, H-9', H-10', H-12', H-13'), 0.87 (t, J = 6.0 Hz, H-18'); ^{13}C-NMR (150 MHz, $CDC1_3$): δ 36.85 (C-1), 34.75 (C-2), 73.16 (C-3), 31.52 (C-4), 40.07 (C-5), 29.60 (C-6), 117.32 (C-7), 139.51 (C-8), 49.26 (C-9), 34.22 (C-10), 21.47 (C-11), 39.38 (C-12), 43.27 (C-13), 55.05 (C-14), 22.91 (C-15), 28.09 (C-16), 55.93 (C-17), 12.09 (C-18), 12.95 (C-19), 40.49 (C-20), 21.11 (C-21), 135.67 (C-22), 131.87 (C-23), 42.80 (C-24), 33.08 (C-25), 17.59 (C-26), 19.64 (C-27), 19.94 (C-28), 173.45 (C-1'), 33.85 (C-2'), 25.07 (C-3'), 29.10-29.34 (C-4'-7'), 27.20 (C-8'), 130.21 (C-9'), 128.03 (C-10'), 25.63 (C-11'), 127.91 (C-12'), 130.06 (C-13'), 27.20 (C-14'), 29.16 (C-15'), 31.52 (C-16'), 22.57 (C-17'), 14.07 (C-18').

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the fruiting bodies of *C. lagopus* yielded **1**-**4**. The NMR spectra of **1** are in accordance with data reported in the literature for 3β-linoleyloxyergosta-7,22-diene[6]; **2** for ergosterol peroxide[7]; **3** for linoleic acid[8]; and **4** for triacylglycerols[9]. Literature search revealed that **2**-**4** exhibited diverse biological activities. Ergosterol peroxide (**2**) isolated from *Pleurotus ostreatus* (Jacq.) P. Kumm. f. sp. Florida showed strong trypanocidal activity on the intracellular form of *T. cruzi* with an IC_{50} of 6.74 μg/mL[10].

Sterol **2** from an edible mushroom suppresses inflammatory response in RAW 264.7 macrophages and growth of HT29 colon adenocarcinoma cells[11]. Compound **2** was shown to exhibit anti-tumor activity in multiple myeloma U266 cells, Walker carcinosarcoma, human mammary adenocarcinoma, human gastric tumor (SNU-1), human hepatoma (SUN-354), human colorectal tumor (SUN-C4), and murine sarcoma-180 cell lines[12]. The IC_{50} value of **2** based on the cell viability of Hep3B was 16.7 μg/mL[13]. It exhibited an inhibitory effect on androgen-sensitive (LNCaP) and androgen-insensitive (DU-145) human prostate cancer cells at μM concentrations[14] and suppressed cell growth and STAT1 mediated inflammatory responses in HT29 cells[15]. It inhibited the growth and induced apoptosis of HL60 human leukaemia cells at a concentration of 25 μM, inhibited TPA induced inflammation and tumor promotion in mice and suppressed proliferation of mouse and human lymphocytes stimulated with mitogens[16]. It displayed potent activity against the cancer cell lines MDA-MB435, HCT-8 and SF-295[17] and induced death of miR-378 cell[18]. It exhibited significant inhibitory activities against leishmaniasis, tuberculosis, *Mycobacterium tuberculosis* H37Rv and *M.* avium[19], and inhibited the hemolytic activity of human serum against erythrocytes[20]. Sterol **2** significantly blocked MyD88 and VCAM-1 expression, and cytokine (IL-1β, IL-6 and TNF-α) production in LPS-stimulated cells and effectively inhibited NF-kB activation which indicated that it may play an important role in the immunomodulatory activity of GF[21]. It possessed marked activity against PGE2 release with an IC_{50} value of 28.7 μM. The mechanism in transcriptional level of **2** was found to down-regulate mRNA expressions of iNOS and COX-2 in dose-dependent manners[22]. Furthermore, **2** suppressed LPS-induced DNA binding activity of NF-kB and C/EBPβ and inhibited the phosphorylation of p38, JNK and ERK MAPKs. It down-regulated the expression of low-density lipoprotein receptor (LDLR) regulated by C/EBP, and HMG-CoA reductase (HMGCR) in RAW264.7 cells. Moreover, **2** induced the expression of oxidative stress-inducible genes, and the cyclin-dependent kinase inhibitor CDKN1A, and suppressed STAT1 and interferon-inducible genes[23]. Linoleic acid (**3**) belongs to the omega-6 fatty acids. It was reported to be a strong anticarcinogen in a number of animal models. It reduces the risk of colon and breast cancer[24] and lowers cardiovascular disease risk and inflammations[25]. Linolenic and linoleic acids inhibited parasites growth by 70% and 64% respectively, against *P. berghei* using the 4-day suppressive test. The two compounds, when used in combination, inhibited the parasites by 96% on day 4 of treatment[26]. Triacylglycerols (**4**) from Tuna (1000 mg/kg) have been reported to significantly inhibit the tumor growth in the spleen of mice with intrasplenically implanted Lewis lung carcinoma[27]. Triacylglycerols exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes*[28]. Another study reported that triacylglycerols showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation[29].

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