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Research Article

Sterol and Lipid from *Pleurotus eryngii* (DC.) Quél. and *Flammulina velutipes* (Cuttis) Singer

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ABSTRACT
Chemical investigation of the dichloromethane extracts of the fruiting bodies of *Pleurotus eryngii* and *Flammulina velutipes* led to the isolation of ergosterol (**1**). *P. eryngii* also afforded trilinolein (**2**), while *F. velutipes* also yielded triacylglycerol (**3**). The structures of **1**-**3** were identified by comparison of their NMR data with literature data.

Keywords: *Pleurotus eryngii*, Pleurotaceae, *Flammulina velutipes*, Physalacriaceae, ergosterol, trilinolein, triacylglycerol.

INTRODUCTION

Pleurotus eryngii, commonly known as king oyster mushroom is sold in supermarkets throughout the Philippines. A number of studies have been conducted on the chemical constituents and biological activities of *P. eryngii.* The ethanol extract of *P. eryngii* afforded ergosterol peroxide which is an inhibitor of osteoclast differentiation. This sterol exhibited an inhibitory effect in a dose-dependent manner with an inhibition rate of up to 62% with low cytotoxicity[1]. Activity-guided fractionations led to the isolation of antitumor compound, ergosterol peroxide from the fruiting body of *P. eryngii*. The IC_{50} of ergosterol peroxide against human lung cancer cell line (A549) and human ovarian cell line (SK-OV3) were 7 μM and 14 μM, respectively. This sterol showed chromosomal DNA fragmentation and arrests G1 phase of the cell division cycle[2]. Another study reported the isolation of 5α,9α-epidioxy-8α,14α-epoxy-(22*E*)-ergosta-6,22-dien-3β-ol, 3β,5α-dihydroxyergost-7-en-6-one, 6β-acetoxy-(22E)-ergosta-7,22-diene-3β,5α-diol, 3β,5α-dihydroxy-(22*E*)-ergosta-7,22-dien-6-one, ergosterol peroxide, 2α,5α,9α–epidioxy-(22*E*)-ergosta-7,22-diene-3β,6β-diol, 5α,9α-epidioxy-3β-hydroxy-(22*E*)-ergosta-7,22-dien-6-one and 3β,5α,9α-trihydroxy-(22*E*)-ergosta-7,22-dien-6-one from *P. eryngii* (DC.: FR.)[3]. In an additional study the isolation of pleurone, ergosterol, (24*E*)-3β-hydroxycucurbita-5,24-diene-26-oic acid and nicotinic acid were reported from *P. eryngii* var. *ferulae*[4]. *Flammulina velutipes*, also known as Enoki mushroom are sold in supermarkets throughout the Philippines. Several studies were conducted on the chemical constituents of this mushroom. An earlier study reported that D-arabinitol, 9(*Z*) oleic acid, 9(*Z*),12(*Z*) linoleic acid, ergosta-5,7,22-trien-3β-ol, 5α,8α-epidioxy-ergosta-6,22-dien-3β-ol, 3β,5α,9α-trihydroxy-ergosta-7,22-dien-6-one, 5-hydroxymethyl-2-(1-methyl-ethenyl)-1-cyclohexanol, 1,3-dilinolein and hemisceramide were isolated from *F. velutipes*[5]. Furthermore, 5α,8α-epidioxy-(22E,24R)-ergost-6,22-dien-3β-ol, ergosta-4,6,8(14),22-tetraen-3-one, sterpuric acid, mannitol and ribitol were obtained from this mushroom[6]. In another study, *F. velutipes* sterols (FVS) which mainly consisted of ergosterol (54.78%), 22,23-dihydroergosterol (27.94%) and ergost-8(14)-ene-3β-ol were reported to exhibit cytotoxicity against U251 cells (IC_{50}=23.42 μg/mL)[7], This study is part of our research on the chemical constituents of Philippine edible mushrooms. We earlier reported the isolation of ergosterol, triacylglycerols and fatty acid methyl esters from *P. djamor*[8] We also reported the isolation of ergosterol, ergosterol peroxide, cerevisterol, palmitic acid, stearic acid, linoleic acid, and oleic acid, and dilinoleoyloleoylglycerol from *P. florida*[9]. Furthermore, we recently reported the isolation of ergosterol and trilinolein from *Lentinus edodes*[10]. We report herein the isolation of ergosterol (**1**) and trilinolein (**2**) from the fruiting bodies of *P. eryngii*, while the fruiting bodies of *F. velutipes* yielded **1** and triacylglycerols (**3**). The structures of **1**-**3** are presented in Fig. 1**.**

MATERIALS AND METHODS

General Experimental Procedure

^1H (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 F254 and the plates were visualized

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by spraying with vanillin/H_2SO_4 solution followed by warming.

Sample Collection

The samples werepurchased from a supermarket in Metro Manila, Philippines in January 2016. They were authenticated as *Pleurotus eryngii* (DC.) Quél. and *Flammulina velutipes* (Curtis) Singer at the Botany Division, Philippine National Museum.

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 by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 (10%increment) as eluents. Fifty 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. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the chemical constituents of the fruiting bodies of P. eryngii

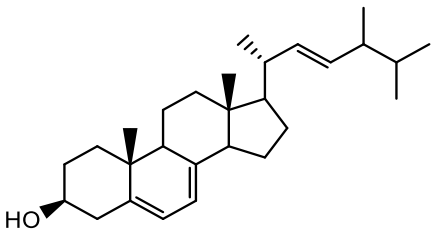
The freeze-dried fruiting bodies of *P. eryngii* (580 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (1.36 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (2 ×) using 5% EtOAc in petroleum ether to afford **2** (6 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield **1** (9 mg) after washing with petroleum ether.

Isolation of the chemical constituents of the fruiting bodies of F. velutipes

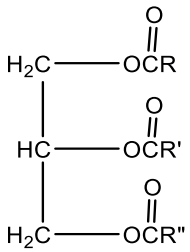
The freeze-dried fruiting bodies of *F. velutipes* (300 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (2.03 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 to 20% acetone in dichloromethane fractions were combined and rechromatographed (2 ×) using 5% EtOAc in petroleum ether to afford **3** (7 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to yield **1** (5 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the fruiting bodies of *Pleurotus eryngii* and *Flammulina velutipes* led to the isolation of **1**-**3**. The NMR spectra of **1** are in accordance with data reported in the literature for ergosterol[11]; **2** for trilinolein[11]; and **3** for triacylglycerol[12]. The fatty acids attached to the glycerol in **3** of *F. velutipes* were identified as linolenic acid, linoleic acid and oleic acid based on resonance intensities for the methyl triplet at δ 0.96 (t, *J* = 7.8 Hz), the double allylic methylenes at δ 2.78 and the olefinic protons at δ 5.34 (m) for the linolenic acid; methyl triplet at δ 0.86 (t, *J* = 6.6 Hz), the double allylic methylene at δ 2.80 and the olefinic protons at δ 5.34 (m) for the linoleic acid; and the methyl triplet at δ 0.86 (t, *J* = 6.6 Hz) and the olefinic protons at δ 5.34 (m) for the oleic acid[12]. Literature search revealed that **1**-**3** exhibited diverse biological activities. A study reported that ergosterol (**1**) provides significant protection against the promotion of bladder tumor induced by many types of promoters in the environment[13]. Moreover, the ergosterol content of brown and white button mushrooms correlated with their antioxidant activities[14]. In another study, **1** was reported to have the capability to inhibit lipid peroxidation[15]. Trilinolein (**2**) exhibits protective effects against cardiovascular disorders[17]. It also inhibits ischemia-induced ventricular arrhythmias and it exhibits anti-oxidant effect[18]. It was also reported to inhibit the growth of human non-small cell lung carcinoma A549 and induce apoptosis in a dose- and time- dependent manne[19]. Another study reported that triglycerides showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation. Linoleic acid belongs to the omega-6 fatty acids. It was reported be a strong anticarcinogen in a number of animal models. It reduces risk of colon and breast cancer[16] and lowers cardiovascular disease risk and inflammations[20]. Triacylglycerols (**3**) exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes*[25]. Another study reported that triacylglycerols showed a direct



1



2 R = R' = R" = linoleic acid

3 R, R', R" = long chain fatty acid alkyl

Figure 1: Chemical structures of ergosterol (**1**) and trilinolein (**2**) from *P. eryngii* and triacylglycerols (**3**) from *F. velutipes*.

relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation[26]. Linoleic acid 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[27] and lowers cardiovascular disease risk and inflammations[28]. 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[29].

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