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Research Article

Sterols and Lipids from *Agaricus bisporus*

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ABSTRACT
Chemical investigation of the dichloromethane extract of *Agaricus bisporus* led to the isolation of ergosterol (**1**), brassicasterol (**2**), trilinolein (**3**) and linoleic acid (**4**). The structures of **1 – 4** were identified by NMR spectroscopy.

Keywords: *Agaricus bisporus*, Agaricaceae, button mushroom, ergosterol, brassicasterol, trilinolein, linoleic acid

INTRODUCTION

There are two strains of the common button mushroom, *Agaricus bisporus* – white and brown. The white button mushroom is the more common strain sold in supermarkets, while the brown strain is marketed as "crimini" and "portabello" mushrooms[1]. There are a number of studies on the biological activities and chemical constituents of *A. bisporus.* White button mushroom lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats[2]. *A. bisporus* (brown) polysaccharide possessed strong immunostimulatory and anti-tumor bioactivity *in vivo* and *in vitro*[3]. The white button mushroom is protective against hepatic steatosis and nonalcoholic fatty liver disease in ovariectomized mice as a model for postmenopausal women[4]. It also showed antioxidant properties and reported to contain phenolics, ergothioneine and minerals[5]. The exopolysaccharides from *A. bisporus* exhibited antioxidants and anti-diabetic properties[6]. Another study reported that extracts of *A. bisporus* stipe showed antibacterial property[7]. The volatile components of *A. bisporus* were reported as 18- or 16-carbon compounds, such as octadecanoic acid, hexadecanoic acid derivatives, and other volatiles such as *dl*-limonene, *n*-nonane, benzendicarboxylic acid, and *cis*-linoleic acid esters[8]. *A. bisporus* also afforded β-glucosidase[9]. Furthermore, ergosterol was isolated in both white and brown *A, bisporus* mushrooms. The ergosterol concentration was higher in early growth stages and accumulated more in the caps after maturation[10]. The fatty acid contents of these mushrooms are mainly linoleic, palmitic, and stearic acids[10]. Recently, it was reported that the most abundant vitamin in *A. agaricus* is niacin, followed by riboflavin. Other vitamins include vitamin B_1, vitamin B_3, L-ascorbic acid and α-tocopherol[11]. This research is part of our studies on the secondary metabolites from edible mushrooms cultivated in the Philippines. We earlier reported the isolation of ergosterol, ergosterol peroxide, cerevisterol, palmitic acid, stearic acid, linoleic acid, oleic acid, and dilinoleoyl oleoyl glycerol from the oyster mushroom, *Pleurotus florida* cultivated at the Central Luzon State University[12]. Furthermore, we isolated ergosterol, triacylglycerols, and fatty acid methyl esters from the pink oyster mushroom, *P. djamor* cultivated at the Central Luzon State University[13]. Recently, we reported the isolation of ergosterol and trilinolein from the dichloromethane extract of *Lentinus edodes* obtained from the Mushroom Burger in Tagaytay City, Philippines[14]. We report herein the isolation of ergosterol (1), brassicasterol (2), trilinolein (3) and linoleic acid (4) from *Agaricus bisporus.* The structures of 1-4 are presented in Fig. 1.

MATERIALS AND METHODS

General Experimental Procedure

^{1}H (500 MHz) NMR spectra were acquired in $CDCl_3$ on a 500 MHz Agilent DD2 NMR spectrometer with referencing to solvent signals (δ 7.26). 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 by spraying with vanillin/H_2SO_4 solution followed by warming.

Sample Collection

The sample was bought from a supermarket in Metro Manila, Philippines in January 2016. It was authenticated as *Agaricus bisporus* (J.E. Lange) Emil J. Imbach at the Botany Division, Philippine National Museum.

General Isolation Procedure

A glass column 12 inches in height and 0.5 inches' 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. Ten milliliter fractions were collected. All fractions were monitored by thin layer

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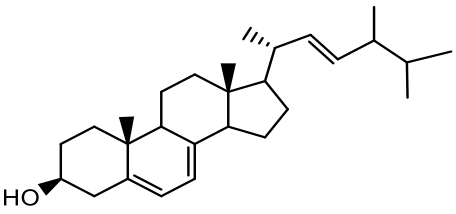

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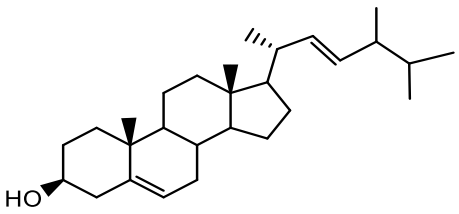

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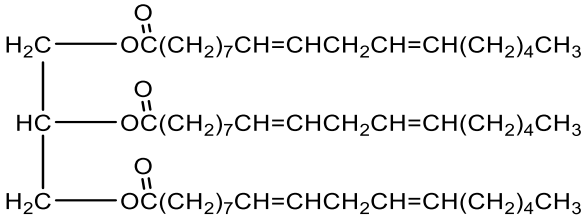

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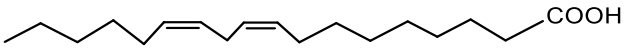

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Figure 1: Chemical structure of ergosterol (**1**), brassicasterol (**2**), trilinolein (**3**) and linoleic acid (**4**) from *Agaricus bisporus.*

chromatography. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the chemical constituents of the fruiting bodies of A. bisporus

The freeze-dried fruiting bodies of *A. bisporus* (34.93 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 (1.61 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed using 5% EtOAc in petroleum ether to yield **3** (15 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether to afford **2** (3 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether to yield **1** (8 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether to afford **4** (6 mg).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *A. bisporus* afforded ergosterol (**1**), brassicasterol (**2**), trilinolein (**3**) and linoleic acid (**4**). The NMR spectra of **1** are in accordance with data reported in the literature for ergosterol[14]; **2** for brassicasterol[15]; **3** for trilinolein[14]; and **4** for linoleic acid[16]. The fatty acid attached to the glycerol was deduced as linoleic acid based on the double allylic methylene protons at δ 2.75 (t, J = 6.6 Hz) and the terminal methyl protons at δ 0.87 (t, J = 6.6 Hz)[16]. A study reported that ergosterol (**1**) provides significant protection against the promotion of bladder tumor induced by many types of promoters in the environment[17]. Moreover, the ergosterol content of brown and white button mushrooms correlated with their antioxidant activities[10]. In another study, ergosterol was reported to have the capability to inhibit lipid peroxidation[18]. On the other hand, brassicasterol (**2**) was reported to exhibit anti-aging property[19].

Trilinolein (**3**) exhibits protective effects against cardiovascular disorders[20]. It also inhibits ischemia-induced ventricular arrhythmias and it exhibits anti-oxidant effect[21]. 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 manner[22]. 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 (**4**) 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[19] and lowers cardiovascular disease risk and inflammations[23].

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