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Chemical Constituents of *Lentinus edodes*

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ABSTRACT

*Chemical investigation of the dichloromethane extract of Lentinus edodes led to the isolation of ergosterol (**1**) and trilinolein (**2**). The structures of **1** and **2** were identified by comparison of their NMR data with those reported in the literature.*

***Keywords*:** *Lentinus edodes*, Shiitake mushroom, Marasmiaceae, ergosterol, trilinolein

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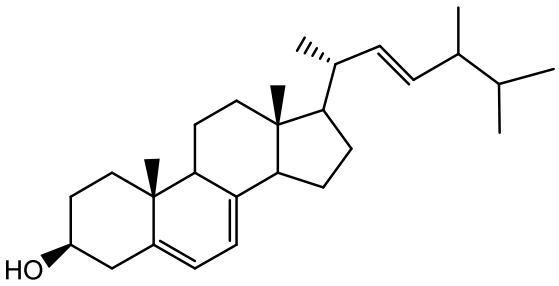
INTRODUCTION

Lentinus edodes, also known as shiitake mushroom is native to East Asia[1]. It is commercially cultivated for food and purported health benefits. *L. edodes* is used for treatment of diseases involving depressed immune function, cancer, allergies, infection, flu, colds, inflammation, heart disease, hyperlipidemia, hypertension, infectious disease, diabetes, hepatitis and regulating urinary inconsistencies. It is the source of several pharmacological preparations, containing lentinan, eritadenine, shiitake mushroom mycelium, and culture media extracts [2]. Lentinan, a polysaccharide from *L. edodes* exhibited anticancer effects in colon cancer cells [3]. It suppresses cytochrome P450 1A enzymes which metabolize pro-carcinogens to active forms [4]. Lentin, a protein from shiitake exhibits antifungal properties, inhibits proliferation of leukemic cells, and suppresses the activity of HIV-1 reverse transcriptase [5]. Studies of shiitake extracts showed antiproliferative [6], immunostimulatory [6], hepatoprotective [7] antimutagenic[8] and anticaries[9] effects in mice. Another study reported that all the polysaccharide fractions from *L. edodes* exhibited antitumor activities against Sarcoma 180 (S-180) solid tumor cells and human colorectal cancer cell lines (HT-29 and HCT-116) *in vitro* at the dose of 5mg/ml [10]. A powder formulation of *L. edodes* contained proteins, carbohydrates and unsaturated fatty acids. It also showed high antioxidant activity and high content of tocopherols and phenolic compounds (32 and 690 μg/100g) [11].Another study reported that *L. edodes* contains polysaccharides, terpenoids, sterols and lipids which are effective in treating various tumors and infections, among others [12].The ethanolic extract of *L. edodes* yielded eight known sterols: ergosterol, ergosterol peroxide, (22*E*)-ergosta-5,7,9(11),22-tetraen-3β-ol, (22*E*)-ergosta-7,9(11),22-trien-3β-ol, (22*E*)-ergosta-6,8,22-trien-3β-ol, (22*E*)-norergosta-5,7,9,22-tetraen-3β-ol, 3β,5α-dihydroxy-(22*E*)-ergosta-7,22-dien-6-one, (22*E*)-ergosta-4,6,8(14), 22-tetraen-3-one and (22*E*)-ergosta-6,22-diene-3,5α,8α-triol [13]. The lipids of the fruiting bodies of *L. edodes* contain high levels of C16:0 and C16:1 fatty acids, high monoglyceride and free fatty acid content and a low triglyceride level, while the mycelium produced in submerged culture has triglycerides and free fatty acids as its

main neutral lipids [14]. A review on the nutritional compounds and pharmacological properties of the *L. edodes* has been provided [15].

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 [16]. Recently, we isolated ergosterol, triacylglycerols, and fatty acid methyl esters from the pink oyster mushroom, *P. djamor* cultivated at the Central Luzon State University[17]. We report herein the isolation of ergosterol (**1**) and trilinolein (**2**) (Fig. 1) from the dichloromethane extract of *Lentinus edodes* obtained from the Mushroom Burger in Tagaytay City.



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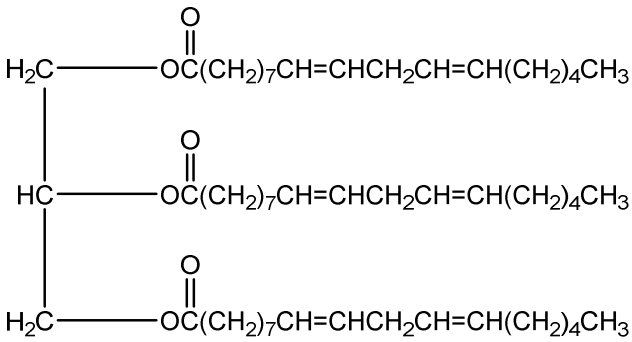

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Fig. 1 Chemical structures of ergosterol (1) and trilinolein (2) from *L. edodes.*

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

The sample was bought from Mushroom Burger in Tagaytay City, Philippines in January 2016. It was authenticated as *Lentinus edodes* at the Botany Division, Philippine National Museum.

General Isolation Procedure

A glass column 12 inches in height and 0.5 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. Ten 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. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the chemical constituents of the fruiting bodies of L. edodes

The freeze-dried fruiting bodies of*L. edodes* (99.85 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 (0.65 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed using 2.5% EtOAc in petroleum ether to afford **2**(6 mg). The 20% and 30% acetone in CH_2Cl_2 fractions were combined and washed with petroleum ether to yield **1**(8 mg).

Ergosterol (**1**): ^{1}H-NMR (600 MHz, $CDC1_3$): δ 5.55 (m, H-6), 5.36 (m, H-7), 5.20 (dd, *J* = 7.8, 15.0 Hz, H-23), 5.15 (dd, *J* = 7.8, 15.0 Hz, H-22), 3.62 (m, H-3), 1.03 (d, *J* = 6.6 Hz, H-21), 0.93 (s, H-19), 0.90 (d, *J* = 6.6 Hz, H-28), 0.83 (d, *J* = 6.6 Hz, H-26), 0.81 (d, *J* = 7.2 Hz, H-27), 0.61 (s, H-18). ^{13}C-NMR (150 MHz, $CDC1_3$): δ 38.36 (C-1), 31.99 (C-2), 70.46 (C-3), 40.79 (C-4), 139.77 (C-5), 119.58 (C-6), 116.27 (C-7), 141.37 (C-8), 46.23 (C-9), 37.02 (C-10), 21.10(C-11), 39.07 (C-12), 42.82, 42.81 (C-13, C-24), 54.55 (C-14), 22.99 (C-15), 28.29 (C-16), 55.71 (C-17), 12.04 (C-18), 17.60 (C-19), 40.43 (C-20), 21.10(C-21), 135.56 (C-22), 131.96 (C-23), 33.08 (C-25), 19.64 (C-26), 19.95 (C-27), 16.28 (C-28).

Trilinolein (**2**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.27 (dd, *J* =4.2, 12 Hz, glyceryl CH_2O), 4.12 (dd, *J* = 6, 12 Hz, glyceryl CH_2O), 5.24 (m, glyceryl CHO), 2.29 (t, *J* = 7.2 Hz, α-CH_2), 5.30-5.37 (m, olefinic H), 2.75 (t, *J* = 6.6 Hz, double allylic CH_2), 2.01-2.04 (allylic, CH_2), 1.57-1.60 (β-CH_2), 1.23-1.35 (CH_2), 0.87 (t,*J* = 6.6 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 62.09 (glyceryl CH_2), 68.85 (glyceryl CH), 173.25 (C=O α), 172.84 (C=O β), 34.01, 34.04, 34.17 (C-2), 22.56, 22.70, 24.82, 24.86, 25.61, 27.18, 29.03, 29.07, 29.11, 29.12, 29.17, 29.19, 29.26, 29.33, 29.60, 29.61, 29.65, 29.69, 29.75, 31.51, 31.91 (CH_2), 130.21, 130.00, 129.97, 128.06, 128.04, 127.88, 127.866(CH=CH), 14.07, 14.11 (terminal CH_3).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the fruiting bodies of *L. edodes* yielded **1** and**2**. The structures of **1** and **2** were identified by comparison of their NMR data with those reported in the literature for ergosterol [16] and trilinolein [18], respectively. 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 δ0.87 (t, *J* = 6.6 Hz) [19].

A study reported that ergosterol (**1**)provides significant protection against the promotion of bladder tumor induced by many types of promoters in the environment [20]. Moreover, the ergosterol content of brown and white button mushrooms correlated with their antioxidant activities [21].In another study, ergosterol was reported to have the capability to inhibit lipid peroxidation [22].

Trilinolein (**2**)exhibits protective effects against cardiovascular disorders [23]. It also inhibits ischemia-induced ventricular arrhythmias and it exhibits anti-oxidant effect [24]. 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 [25]. Another study reported that triglycerides showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation. Trilinolenin (18:3; µ-3) was toxic only after prolonged incubation [26]. 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 [27] and lowers cardiovascular disease risk and inflammations [28].

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REFERENCES

[1] B. R. Cassileth. Shiitake mushroom (*Lentinula edodes*) | Cancer Network. Downloaded from www.cancernetwork.com/integrative.../shiitake-mushroom-lentinula-edo... on March 12, **2016**.
[2] P. S. Bisen, R. K. Baghel, B. S. Sanodiya, G. S. Thakur, G. B. Prasad,*Curr. Med. Chem.*, **2010**, 17(22), 2419-30.
[3] M. L. Ng, A. T. Yap,*J. Altern. Complement. Med*., **2002**, 8, 81-9.
[4] T. Okamoto, R. Kodoi, Y. Nonaka,*Biofactors*, **2004**, 21, 407-9.
[5] P. H. Ngai, T. B. Ng,*Life Sci*.,**2003**, 73, 3363-74.
[6]. C. Israilides, D. Kletsas, D. Arapoglou, *Phytomed*.,**2008**, 15, 512-9.
[7]. S. Akamatsu, A. Watanabe, M. Tamesada, *Biol. Pharm. Bull*., **2004**, 27, 1957-60.
[8]. P. L. de Lima, R. D. Delmanto, M. M. Sugui, *Mutat. Res*., **2001**, 496, 23-32.
[9]. N. Shouji, K. Takada, K. Fukushima, M. Hirasawa,*Caries Res.*,**2000**, 34, 94-8.
[10] Y. Zheng, M. Hao, H. Nan, I. Jeff, Y. Zhou, Y. Gao,*Pak. J. Pharm. Sci*., **2015**, 28(3 Suppl.), 1069-74.
[11] A. A. Carneiro, I. C. Ferreira, M. Dueñas, L. Barros, R. da Silva, E. Gomes, C. Santos-Buelga,*Food Chem*., **2013**, 138(4), 2168-73.
[12] X. Wang, L. Zhang, *Carbohydr. Res*.,**2009**, 344, 2209-2216.
[13] A. Rivera, O. L. Benavides, J. Rios-Motta,*Nat. Prod. Res*.,**2009**, 23(3), 293-300.
[14] V. E. Nikitina, O. M. Tsivileva, A. N. Pankratov, N. A. Bychkov,*Food Technol. Biotechnol*.**2007**,45(3), 230–237.
[15] T. C. Finimundy, A. J. P. Dillon, J. A. P. Henriques, M. R. Ely,*Food Nutr. Sci*., **2014**, 5, 1095-1105.
[16] C. Y. Ragasa, V. D. Ebajo Jr., R. G. Reyes, R. Brkljača, S. Urban, *Der Pharma Chemica*.**2015**,7(10), 331-336.
[17] C. Y. Ragasa, M. C. S. Tan, J. Ting, R. G. Reyes, R. Brkljača, S. Urban, *Der Pharma Chemica*. **2016**, 8(2), 343-346.
[18] R. S. Del Fierro, Q. M. A. Maquilang, R. A. S. Sanjorjo, M. D. Tradio, C.-C. Shen, C. Y. Ragasa,*J. Med. Plants Res*., **2012**, *6*(11), 2146-2149.
[19] Human Metabolome Database. Linoleic acid. Downloaded from http://www.hmdb.ca/spectra/nmr_one_d/1471 on Nov. 27, 2013.
[20] Y. Yazawa, M. Yokota, K. Sugiyama,*Biol. Pharm. Bull*.,**2000**, 23(11), 1298–302.
[21] S.-Q. Shao, M. Hernandez, J. K. G. Kramer, D. L. Rinker, R. Tsao, *J. Agric. Food Chem.*, **2010**, 58(22), 11616-11625.
[22] D. P. Dissanayake, D. T. U. Abeytunga, N. S. Vasudewa, W. D. Ratnasooriya, *Phcog. Mag*., **2009**, 5(19), 266-271.
[23] P. Chan, G. N. Thomas, B. Tomlinson, *Acta Pharmacol. Sin*., **2002**, 23(12), 1157-1162.
[24] P. Chan, P. F. Kao, B. Tomilson, *Acta Cardio Sin*., **2005**, 21, 71-76.
[25] P. Y. Chou, G. J. Huang, C. H. Pan, Y. C. Chien, Y. Y. Chen, C. H. Wu, M. J. Sheu, H. C. Cheng, *Amer. J. Chin. Med*., **2011**, 39(4), 803-15.
[26] S. J. Hardwick, K. H. Carpenter, N. S. Law, C. Van Der Veen, C. E. Marchant, R. Hird, M. J. Mitchinson, *Free Radic. Res*., **1997**, 26 (4), 351-362.
[27] P. Chan, G. N. Thomas, B. Tomlinson, *Acta Pharmacol. Sin*., **2002**, 23(12), 1157-1162.
[28] J. Whelan, *Prostaglandins, Leukot. Essent. Fatty Acids*, **2008**, 79(3-5), 165-167.