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Sterols from *Trametes versicolor.*

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ABSTRACT

Chemical investigation of the dichloromethane extract of the fruiting bodies of *Trametes versicolor* yielded ergosterol peroxide (**1**) and a mixture of stellasterol (**2**) and ergosterol (**3**) in a 3.6:1 ratio. The structures of **1**-**3** were identified by comparison of their NMR data with literature data.

Keywords: *Trametes versicolor,* Polyporaceae, ergosterol peroxide, stellasterol, ergosterol

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INTRODUCTION

*Trametes versicolor*also known as Turkey tail mushroom grows on decaying hardwood or at the base of trees [1].Itis known to possess diverse biological activities including immune-enhancing [2], antitumor [3], and antiviral [4] activities. A protein-bound polysaccharide, polysaccharide-K(PSK or Krestin),extracted from *T. versicolor*,is used in cancer treatment [5, 6]. The anticancer effects and mechanisms of PSK for cancer immunotherapy has been reported [7].The ethyl acetate and the ethanol extracts of *T. versicolor* exhibited anti-leishmanial activity with IC_{50}values of 101.8 ± 4.2 µg/mL and 97.4 ± 2.0 µg/mL, respectively [8]. The hexane extract of the fruiting bodies of *T. versicolor* yielded ergosterol peroxide, stellasterol, and trametenolic acid [9]. Another study reported the isolation of 4-isobutoxyphenyl palmitate, cerebroside, 3β-linoleyloxyergosta-7,22-diene, 3β-linoleyloxyergosta-7-ene, betulinic acid, ergosterol, ergosterol peroxide, trilinolein, ergosta-7, 22-dien-3β-ol, and betulin [10].

This study is part of our research on the chemical constituents of mushrooms found and cultivated in the Philippines. We earlier reported the isolation of ergosterol peroxide from *Auricularia auricula-judae* [11]; ergosterol, brassicasterol, trilinolein and linoleic acid from *Agaricus bisporus* [12]; ergosterol and trilinolein from *Lentinus edodes* [13]; ergosterol, triacyl glycerols and fatty acid methyl esters from *Pleurotus djamor* [14]*;* ergosterol and triacylglycerols from *Phellinus gilvus* [15]; and ergosterol, ergosterol peroxide, cerevisterol, palmitic acid, stearic scid, linoleic acid, oleic acid and dilinolenoyloleoylglycerol from *Pleurotus florida* [16].

We report herein the isolation of ergosterol peroxide (**1**) and a mixture of stellasterol (**2**) and ergosterol (**3**) from *T. versicolor.* The structures of **1**-**3** are presented in Fig. 1

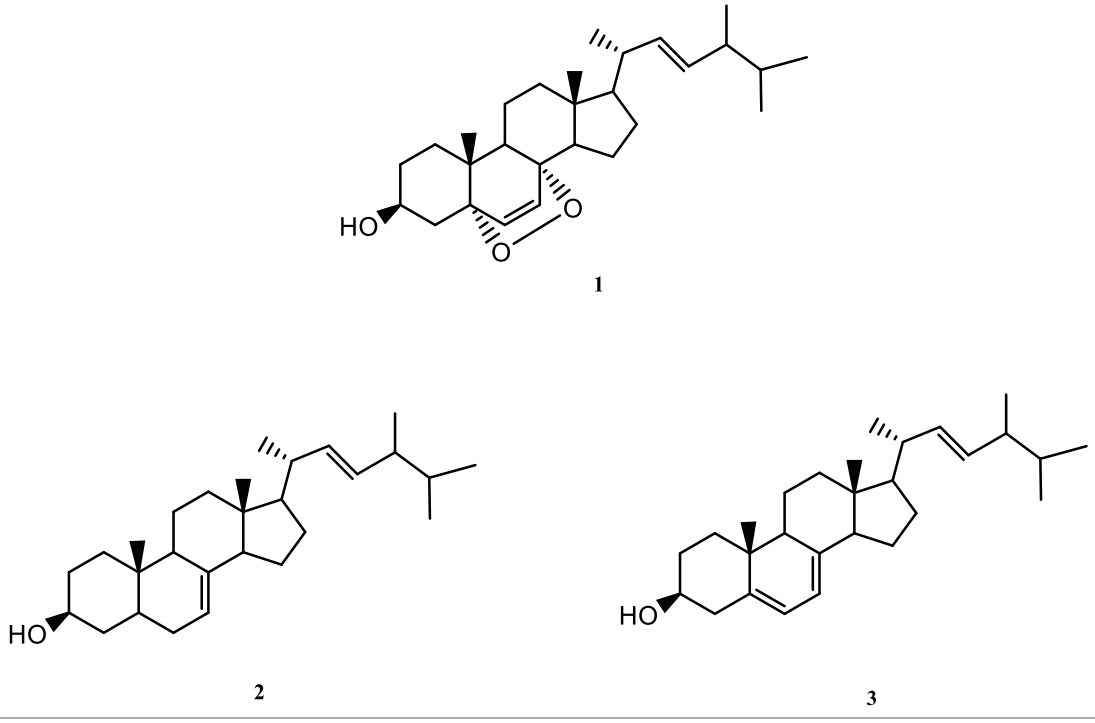

Fig 1: Ergosterol peroxide (1) and a mixture of stellasterol (2) and ergosterol (3) from *T. versicolor.*

MATERIALS AND METHODS

General Experimental Procedure

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Ascend 400 in $CDCl_3$ at 400 MHz. Column chromatography was performed with silica gel 60 (70-230 mesh, Merck). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} (Merck) and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming. All solvents used were ofanalytical grade.

Sample Collection

The sample was collected from Zamboanga, Philippines in January, 2016. It was authenticated as *Trametes versicolor*by one of the authors (MEDC) based on the available literature.

General Isolation Procedure

A glass column 18 inches in height and 1 inch internal diameter was used for the fractionation of the crude extracts. Eleven 20 mL fractions were collected. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography of fractions from the crude extracts. 2 mL fractions were collected. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. Rechromatography and final purifications were conducted using Pasteur pipettes as columns. 1 mL fractions were collected.

Isolation of Chemical Constituents

The air-dried *A. scholaris* leaves(25.3 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 (0.5 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 in 10% increments by volume. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford a mixture of **2** and **3**(3 mg) afterwashing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether. Fractions from this column were rechromtographed using 20% EtOAc in petroleum ether to yield **1** (2 mg) after washing with petroleum ether.

Ergosterol peroxide (**1**): ^{1}H-NMR (400 MHz, CDC13): δ 6.49 (d, *J*=8.4, H-6), 6.22 (d, J= 8.4, H-7), 5.12 (dd, *J* = 8, 15.2 Hz, H-20), 5.22 (dd, *J* = 7.2, 15.2 Hz, H-23), 3.95 (m, H-3), 0.80 (s, Me-18), 0.86(s, Me-19), 0.97 (d, *J*=8 Hz,Me-21*),* 0.79 (3H, d,*J* = 6.8 Hz, H-26), 0.81 (3H, d, *J* = 6.4 Hz, H-27), 0.88 (3H, d, *J* = 6.8 Hz, H-28).

Stellasterol (**2**): ^{1}H-NMR (400 MHz, $CDCl_3$): (δ, ppm) 0.52 (s, H_3-18), 0.80 (d, *J* = 6.4 Hz, H_3-26), 0.82 (d, *J* = 6.4 Hz, H_3-27), 0.78 (s, H_3-19), 0.90 (d, *J* = 6.8 Hz, H_3-28), 0.99 (d, *J* = 6.4 Hz, H_3-21), 3.6 (m, H-3), 5.20 (t, *J* = 6.8 Hz, H-7), 5.15 (m, 2H, H-22, H-23);^{13}C-NMR (100 MHz, $CDCl_3$): (δ, ppm): 37.98 (C-1), 31.47 (C-2), 71.06 (C-3), 37.13 (C-4), 49.44 (C-5), 29.69 (C-6), 117.45 (C-7), 139.56 (C-8), 40.47 (C-9), 34.21 (C-10), 21.53 (C-11), 39.44 (C-12), 43.28 (C-13), 55.10 (C-14), 22.92 (C-15), 28.09 (C-16), 55.95 (C-17), 12.08 (C-18), 13.03 (C-19), 40.25 (C-20), 21.10 (C-21), 131.87 (C-22), 135.66 (C-23), 42.80 (C-24), 33.08 (C-25), 19.63 (C-26), 19.94 (C-27), 17.58 (C-28).

Ergosterol (**3**): ^{1}H-NMR (400 MHz, $CDC1_3$): δ 5.57 (dd, *J* = 2.8, 5.6 Hz, H-6), 5.38 (dd, *J* = 2.8, 5.6 Hz, H-8),5.22(m, H-23), 5.15 (m, H-22), 3.63 (m, H-3), 1.01 (d, *J* = 8.4 Hz, H-21), 0.93 (s, H-19),0.91 (d, *J* = 6.0 Hz, H-28), 0.85 (d,*J* = 6 Hz, H-26), 0.82 (d, *J* = 6 Hz, H-27), 0.61 (s, H-18).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *Trametes versicolor* yielded ergosterol peroxide (**1**) and a mixture of stellasterol (**2**) and ergosterol (**3**). TheNMR spectra of **1**are in accordance with data reported in the literature for ergosterol peroxide [16]; **2**for stellasterol [9]; and **3** for ergosterol [16]. The 3.6:1 ratio of stellasterol (**2**) and ergosterol (**3**) was deduced from integrations and intensities of the resonances for methyl protons at δ 0.52 (s, H_3-18) for **2** and δ 0.61(s, H_3-18) for **3**.

Although bioassays were not conducted on the isolated compounds, there were previous studies that reported ontheir biological activities.

A number of studies have been conducted on the biological activities of ergosterol peroxide (**1**). Compound **1** isolated from *Pleurotus ostreatus* (Jacq.) P. Kumm. f. sp. florida showed strongly panocidal activity on the intracellular form of *T. cruzi* with an IC_{50} of 6.74 µg/mL [17]. Sterol **1**from an edible mushroom suppresses inflammatory response in RAW 264.7 macrophages and growth of HT29 colonadenocarcinoma cells [18]. Inaddition, **1**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 [19].The IC_{50} value of **1**based on the

cell viability of Hep3B was 16.7µg/mL [20]. It exhibited an inhibitory effect on androgen-sensitive (LNCaP) and androgen-insensitive (DU- 145)human prostate cancer cells at µM concentrations [21]and suppressed cell growth and STAT1 mediated inflammatory responses in HT29 cells [22].It inhibited the growth and induced apoptosis ofHL60 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 [23]. It displayed potent activity against the cancer cell lines MDA-MB435, HCT-8 and SF-295 [24] and induced death ofMIR-378 cell [25]. It exhibited significant inhibitory activities against leishmaniasis, tuberculosis, *Mycobacterium tuberculosis* H37Rv and *M.avium* [26], and inhibited the hemolytic activity of human serum againsterythrocytes [27]. Sterol **1**significantlyblocked 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 *Grifola frondosa* [28]. It possessed marked activity against PGE_2 release with an IC_{50} value of28.7 µM. The mechanism in transcriptional level of **1**was found to down-regulate mRNA expressions of iNOS andCOX-2 in dose-dependent manners [29].Furthermore, **1**suppressed LPS-induced DNA binding activity of NF-kBand C/EBPβ and inhibited the phosphorylation of p38, JNK and ERK MAPKs. It down-regulated the expression oflow-density lipoprotein receptor (LDLR) regulated by C/EBP, and HMG-CoA reductase (HMGCR) in RAW264.7cells. Moreover, **2** induced the expression of oxidative stress-inducible genes, and the cyclin-dependent kinase inhibitor CDKN1A, and suppressed STAT1 and interferon-inducible genes [30].

Stellasterol (**2**) showed antibiotic activity against gram positive bacteria [31].Another study reported that cell cycle arrest against the human cancer cell lines, MCF-7 and SH-SY5Y was exhibited by stellaterol [32]. Furthermore, **2** exhibited anti-inflammatory activity against iNOS, CHOP and IKB-α expression [33].

A study reported that ergosterol (**3**)provides significant protection against the promotion of bladder tumor induced by many types of promoters in the environment [34]. Oral administration of **3** (400 and 800 mg/kg) for 20 days to sarcoma 180 bearing mice significantly reduced tumor growth [35]. Furthermore, **3** showed antifibrotic effect *invivo* and *invitro* [36].

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