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A Sterol from *Auriculariaauricula-judae*

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ABSTRACT

*Silica gel chromatography of the dichloromethane extract of the fruiting bodies of Auriculariaauricula-judaeled to the isolation of ergosterol peroxide (**1**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its ^{1}H and $^{13}CNMR$ data with literature data.*

Keywords: *Auriculariaauricula-judae*, Auriculariaceae, ergosterol peroxide

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INTRODUCTION

Auriculariaauricula-judae Schrot., also known as Judas's ear and locally known as taingan-daga is an edible saprophytic fungus, growing in tree stumps in damp moist forests throughout the Philippines [1]. A number of studies have been conducted on the medicinal properties of *A.auricula-judae*. The dichloromethane extract of *A.auricula-judae* inhibited lipopolysaccharide (LPS) -induced nitric oxide (NO) production significantly in a dose-dependent manner in the concentration ≥10 μg/ml ($p < 0.05$) [2]. The extract also markedly reduced the expressions of inflammatory cytokines (IL-6, TNF-α and IL-1β) mRNA in LPS-treated murine RAW 264.7 macrophages [2]. The polysaccharide extract of *A.auricula-judae* exhibited hypoglycemic [3], hypocholostemic [4], anticomplement [5], antioxidant [6-8], antitumor [9-11], hypolipidemic [12],anti-inflammatory [12], antithrombotic [13], and wound healing [14] properties. A recent study reported that the dichloromethane extract of *A.auricula-judae* yielded thirty-six volatile compounds, ergosterin andergost-5,8-dien-3β-ol, while the diethyl ether extract yielded twenty-six volatile compounds, ergosterin and linoleic acid [15].

We report herein the isolation of ergosterol peroxide (**1**)from the fruiting bodies of *Auriculariaauricula-judae*. The chemical structure of **1** is shown in Fig. 1.

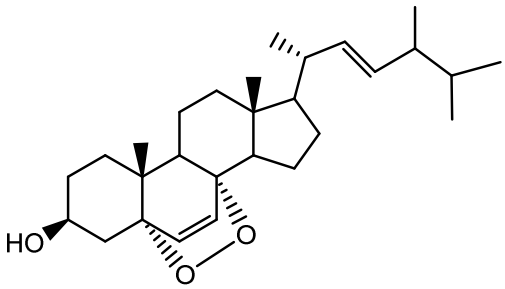

Fig. 1. Chemical structure of ergosterol peroxide (1) from *A. auricula-judae*

MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in CDCl3 at 600 MHz for ^{1}H NMR and 150 MHzfor^{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 withsilica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

Sample Collection

Matured fruiting bodies of *A. auricular-judae* were collected from various sources including rotten stumps, fallen logs and small cut branches of several forest tree species located inside the campus of the College of Forestry and Natural Resources, University of the Philippines Los Baños. The collected specimen ranged from light brown to dark brown in color, soft and with gelatin-like texture. It was noticeable that most of the fruiting bodies collected were also consumed by ants causing some degree of difficulty in the collection process. The collection was done between the months of May to June 2016 which marks the start of the rainy season in this climatic zone. The matured *A. auricular-judae* fruiting bodies were collected and identified by one of the authors (MEGDC).

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 *Rf* values were combined and rechromatographed in appropriate solvent systems until TLCpure 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 milliliterfractions were collected.

Isolation of the chemical constituents of the fruiting bodies of A. auricula-judae

The freeze-dried fruiting bodies of *A. auricula-judae* (11.53 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 (0.1036 g) which was chromatographed using increasing proportions of EtOAc in petroleum ether at 5% increment. The 15% EtOAc in petroleum ether fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to yield**1**(7 mg) after trituration with petroleum ether.

Ergosterol peroxide (**1**): ^{1}H-NMR (500 MHz, $CDC1_3$): δ 6.48 (d, *J*=8.4, H-6), 6.22 (d, J= 8.4, H-7),5.12 (dd, *J* = 8.4,15.6 Hz, H-22), 5.20 (dd, *J* = 7.8, 15 Hz, H-23), 3.95 (m, H-3), 0.80 (s, Me-18), 0.86 (s, Me-19), 0.98 (d, *J*=6.6 Hz,Me-21*)*, 0.81 (3H, d,*J* = 6.6 Hz, H-26), 0.82 (3H, d, *J* = 7.2 Hz, H-27), 0.89 (3H, d, *J* = 7.2 Hz, H-28);^{13}C-NMR (125MHz, $CDC1_3$): δ34.68 (C-1), 30.12 (C-2), 66.47 (C-3), 36.92, 36.95 (C-4, C-10), 82.14 (C-5), 135.39 (C-6), 130.74 (C-7),79.49 (C-8), 51.67 (C-9), 20.87 (C-11), 39.33 (C-12), 44.55 (C-13), 51.07 (C-14), 23.39 (C-1 5), 28.64 (C-16), 56.19 (C-17),12.86 (C-18), 18.17 (C-19), 39.72 (C-20), 19.63 (C-21), 135.19 (C-22), 132.30 (C-23), 42.76 (C-24), 33.06 (C-25), 19.94 (C-26), 20.62 (C-27), 17.55 (C-28).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *Auricularia auricula-judae* led to the isolation of ergosterol peroxide (**1**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its ^{1}H NMR data with literature data [16].

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 strongtrypanocidal activity on the intracellular form of *T. cruzi* with an $IC_{50}$of 6.74 μg/mL [17]. Sterol **1**from an ediblemushroom suppresses inflammatory response in RAW 264.7 macrophages and growth of HT29 colonadenocarcinoma cells [18]. Compound **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 **2** based on thecell viability of Hep3B was 16.7 μg/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 growthand 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]. Itdisplayed 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, *Mycobacteriumtuberculosis* H37Rv and *M.* avium [26], and inhibited the hemolytic activity of human serum against erythrocytes [27]. Sterol **1**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 [28]. It possessed marked activity against PGE2release with an IC_{50} value of 28.7 μM. The mechanism in transcriptional level of **1**was found to down-regulate mRNA expressions of iNOS and COX-2 in dose-dependent manners [29].Furthermore, **1**suppressed LPS-induced DNA binding activity of NF-kB and C/EBPβ and inhibited the phosphorylation of p38, JNK and ERK MAPKs. Itdown-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 [30].

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