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Cytotoxicity of Ergosterol Derivatives from the Fruiting Bodies of *Hygrophorus russula*†

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Abstract – Bioassay-guided fractionation of the $CHCl_3$-soluble fraction of a MeOH extract of the fruiting bodies of *Hygrophorus russula* led to the isolation of five ergosterol derivatives (**1** - **5**). The structures of these compounds were identified as ergosterol peroxide (**1**), ergosta-4,6,8(14),22-tetraen-3-one (**2**), ergosta-7,22-diene-3β,5α,6α-triol (**3**), ergosta-7,22-diene-3β,5α,6β,9α-tetraol (**4**), and 5α,6α-epoxy-ergosta-8(14),22-diene-3β,7α-diol (**5**) by comparing their physicochemical and spectral data with those in the literature. These compounds were evaluated for *in vitro* cytotoxicity against A549 and XF498 cancer cell lines. Most of the tested compounds, except for compound **3**, exhibited moderate cytotoxicity against both A549 and XF498 cell lines with IC_{50} values ranging from 10.2 to 18.3 μg/mL and from 11.4 to 24.6 μg/mL, respectively.
Keywords – *Hygrophorus russula*, Hygrophoraceae, Ergosterol derivatives, Cytotoxicity

Introduction

Higher Basidiomycetes have been used in folk medicine throughout the world since ancient times. The spectrum of detected pharmacological activities from Basidiomycetes is very broad (Abraham, 2001; Brizuela *et al.*, 1998; Lindequist *et al.*, 2005; Wasser and Weis, 1999). Among their biological effects, antifungal, anti-inflammatory, antitumor, antiviral, antibacterial, antiparasitic, immunomodulating, and hepatoprotective activities are notable; equally promising is their role in the regulation of blood pressure, as well as in the cure of cardiovascular disorders, hypercholesterolemia and diabetes. This is due to the large number of biologically active metabolites they contain (Lorenzen and Anke, 1998; Wasser, 2002; Yassin *et al.*, 2003; Zjawiony, 2004). A number of metabolites that were isolated from fungi are part of medical applications such as natural products, starting materials for pharmaceuticals or as lead structures for the development of pharmaceutical products.

The genus *Hygrophorus* (wax caps) is comprised of over 60 species assigned to several subsections. They are obligate symbionts (mycorrhiza) with coniferous or deciduous trees. Previous phytochemical investigations of some species demonstrate the presence of several bioactive constituents, including hygrophorones (Lübken *et al.*, 2004) and unusual 4-oxo-2-alkenoic fatty acids with a g-oxocrotonate partial structure (Teichert *et al.*, 2005). However much less is known about the constituents and the biological activities of *Hygrophorus russula*.

H. russula (Hygrophoraceae) is an edible fungus with a reddish cap and close pale gills and dry stalk, that is found under hardwoods. In searching for novel antitumor agents from natural resources, we found that the $CHCl_3$-soluble fraction from the MeOH extract of the fruiting bodies of *H. russula* had cytotoxicity (> 60% inhibition at 30 μg/mL) against the human lung cancer A549 cell line. Further phytochemical investigation on this fraction resulted in the isolation of five ergosterol derivatives (**1** - **5**) (Fig. 1). This report describes the isolation and structural elucidation of these compounds, as well as the characterization of their *in vitro* cytotoxicities against the A549 and XF498 cancer cell lines.

Material and Methods

General Experimental Procedures – Melting points were measured on an Electrothemal apparatus. Optical

†Dedicated to professor KiHwan Bae for his leading works on bioactive natural products.
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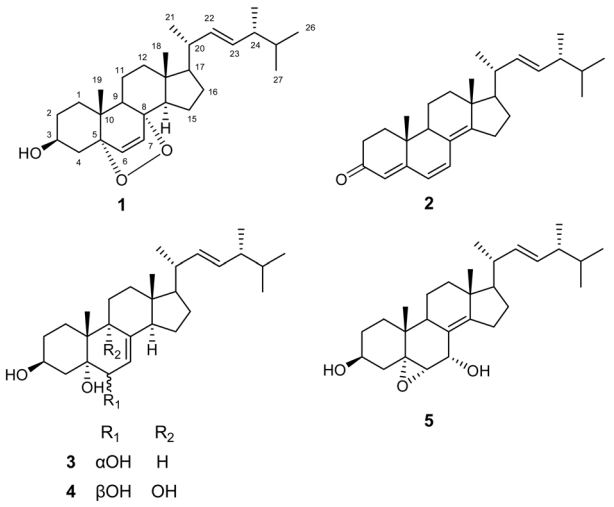

Fig. 1. Structures of compounds **1 - 5** isolated from the fruiting bodies of *H. russula*.

rotation was measured in $CHCl_3$ on a JASCO DIP-370 digital polarimeter. UV and IR spectra were recorded on JASCO V-550 UV/VIS and JASCO 100 IR spectrometers, respectively. ESI-MS data was recorded on a HP 1100 series LC/MSD spectrometer. ^1H (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Bruker DRX-300 spectrometer with tetramethylsilane (TMS) as the internal standard. Two-dimensional (2D) NMR experiments (HMQC, HMBC, and NOESY) were run on a Bruker Avance 500 spectrometer. Column chromatography was carried out using silica gel (Kieselgel 70 - 230 and 230 - 400 mesh, Merck), and thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F_{254} plates (0.25 mm, Merck).

Plant Material – The dried fruiting bodies of *H. russula* were collected at Mt. Seolak, Kangwondo, Korea in August 2006, and identified by Prof. KiHwan Bae. A voucher specimen (CNU-987) has been deposited in the herbarium at the College of Pharmacy, Chungnam National University, Daejeon, Korea.

Extraction and Isolation – The dried fruiting bodies of *H. russula* (2.0 kg) were extracted three times with MeOH at room temperature for 7 days, filtered and concentrated to give a MeOH extract (70 g). The MeOH extract was suspended in H_2O (2 L) and then partitioned successively with $CHCl_3$ (2 L × 3), EtOAc (2 L × 3), and BuOH (2 L × 3) to afford $CHCl_3$- (8 g), EtOAc- (3 g), and BuOH-soluble fractions (12 g), respectively. The $CHCl_3$-soluble fraction (8 g) with cytotoxicity against the A549 cancer cell line was chromatographed on a silica gel column (30 × 10.0 cm) eluting with a gradient of hexane-EtOAc (100 : 1 → 1 : 1) to afford four fractions (Fr. A-D). Fraction B was rechromatographed on a silica gel column (50 × 5.0 cm) with a gradient of hexane-EtOAc (20 : 1 → 10 : 1) to give compound **1** (10 mg). Fraction C was applied to a silica gel column and eluted using a hexane-EtOAc gradient (20 : 1 → 5 : 1), yielding four subfractions (C1-C4). Subfractions C2 and C3 were then separately chromatographed on a YMC RP-18 column (50 × 3.5 cm). Elution of C2 with a gradient of MeOH-H_2O (8 :1 → 10 : 1) yielded **2** (4 mg). Elution of C4 with a gradient of MeOH-H_2O (6 : 1 → 10 : 1) yielded **3** (5 mg). Chromatography of fraction D on a silica gel column (60 × 6.5 cm) using a gradient solvent system of $CHCl_3$-MeOH (50 : 1 → 10 : 1) yielded four subfractions (D1-D4). Subfraction D3 was further purified by a YMC RP-18 column (50 × 3.5 cm) using a gradient of MeOH-H_2O (4 : 1 → 8 : 1), which yielded **4** (3 mg) and **5** (2 mg), respectively.

Ergosterol peroxide (1): White powder; $[\alpha]_D^{25}$ –26.0 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3440, 2960, 1635, 1458, 1380; ESI-MS *m/z*: 451 $[M+Na]^+$; ^1H NMR ($CDCl_3$, 300 MHz): δ 6.50 (1H, d, *J* = 8.5 Hz, H-7), 6.24 (1H, d, *J* = 8.5 Hz, H-6), 5.24 (1H, dd, *J* = 15.4, 7.2 Hz, H-23), 5.15 (1H, dd, *J* = 15.4, 7.8 Hz, H-22), 3.96 (1H, m, H-3), 1.00 (3H, d, *J* = 6.6 Hz, H-21), 0.92 (3H, d, *J* = 6.9 Hz, H-28), 0.88 (3H, s, H-19), 0.84 (3H, d, *J* = 6.9 Hz, H-26), 0.82 (3H, d, *J* = 6.9 Hz, H-27), 0.81 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz): see Table 1.

Ergosta-4,6,8(14),22-tetraen-3-one (2): Yellowish crystal; $[\alpha]_D^{25}$ +52.0 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} nm: 345; IR (KBr) ν_{max} cm^{-1}: 2850, 1665, 1585, 1460; ESI-MS *m/z*: 415 $[M+Na]^+$; ^1H NMR ($CDCl_3$, 300 MHz): δ 6.60 (1H, d, *J* = 9.6 Hz, H-7), 6.02 (1H, d, *J* = 9.6 Hz, H-6), 5.27 (1H, dd, *J* = 15.0, 7.2 Hz, H-23), 5.22 (1H, dd, *J* = 15.0, 7.2 Hz, H-22), 1.06 (3H, d, *J* = 6.9 Hz, H-21), 1.00 (3H, *s*, H-19), 0.97 (3H, s, H-18), 0.93 (3H, d, *J* = 6.9 Hz, H-28), 0.85 (3H, d, *J* = 6.9 Hz, H-26), 0.83 (3H, d, *J* = 6.9 Hz, H-27); ^{13}C NMR ($CDCl_3$, 75 MHz): see Table 1.

Ergosta-7,22-diene-3β,5α,6α-triol (3): White powder; $[\alpha]_D^{25}$ –2.0 (*c* 0.1, $CDCl_3$); IR (KBr) ν_{max} cm^{-1}: 3400, 2960, 1650, 1460, 1385; ESI-MS *m/z*: 437 $[M+Na]^+$; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.25 (1H, dd, *J* = 16.0, 8.0 Hz, H-23), 5.15 (1H, dd, *J* = 16.0, 7.5 Hz, H-22), 5.03 (1H, d, *J* = 1.5 Hz, H-7), 4.03 (1H, m, H-3), 3.97 (1H, br s, H-6), 1.03 (3H, d, *J* = 6.6 Hz, H-21), 0.98 (3H, s, H-19), 0.93 (3H, d, *J* = 6.9 Hz, H-28), 0.85 (3H, d, *J* = 6.9 Hz, H-26), 0.83 (3H, d, *J* = 6.9 Hz, H-27), 0.57 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz): see Table 1.

Ergosta-7,22-diene-3β,5α,6β,9α-tetraol (4): White powder; $[\alpha]_D^{25}$ –8.0 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}:

3440, 2955, 1660, 1450, 1380; ESI-MS *m/z*: 453 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$+MeOD, 300 MHz): δ 5.32 (1H, dd, *J* = 5.4, 2.4 Hz, H-7), 5.20 (1H, dd, *J* = 15.4, 6.6 Hz, H-23), 5.15 (1H, dd, *J* = 15.4, 6.9 Hz, H-22), 4.00 (1H, m, H-3), 3.63 (1H, m, H-6), 1.08 (3H, s, H-19), 1.00 (3H, d, *J* = 6.6 Hz, H-21), 0.90 (3H, d, *J* = 6.6 Hz, H-28), 0.82 (3H, d, *J* = 6.6 Hz, H-26), 0.80 (3H, d, *J* = 6.6 Hz, H-28), 0.61 (3H, s, H-18); ^{13}C-NMR ($CDCl_3$+MeOD, 75 MHz): see Table 1.

5α,6β-Epoxy-ergosta-8(14),22-diene-3β,7α-diol (5): white powder; $[\alpha]_D^{25}$ –7.5 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3436, 2960, 1650, 1460, 1377; ESI-MS *m/z*: 451 $[M+Na]^+$; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.22 (1H, dd, *J* = 16.0, 7.5 Hz, H-23), 5.18 (1H, dd, *J* = 16.0, 7.2 Hz, H-22), 4.42 (1H, br s, H-7), 3.94 (1H, m, H-3), 3.14 (1H, m, H-6), 1.02 (3H, d, *J* = 6.6 Hz, H-21), 0.92 (3H, d, *J* = 6.6 Hz, H-28), 0.87 (3H, s, H-19), 0.84 (3H, d, *J* = 6.6 Hz, H-26), 0.82 (3H, d, *J* = 6.6 Hz, H-28), 0.82 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz): see Table 1.

Cytotoxicity Assay – The cancer cell lines (A549 and XF498) were maintained in RPMI 1640 which included L-glutamine (JBI) with 10% FBS (JBI) and 2% penicillin-streptomycin (GIBCO). Cells were cultured at 37 °C in a 5% CO_2 incubator. Cytotoxicity was measured by a modified Microculture Tetrazolium (MTT) assay (Mosmann, 1983). Viable cells were seeded in the growth medium (180 μL) into 96 well microtiter plates (1×10^4 cells per each well) and incubated at 37 °C in 5% CO_2 incubator. The test sample was dissolved in DMSO and adjusted to final sample concentrations ranging from 1 to 30 μg/mL by diluting with the growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to < 0.1%. After standing for 2 h, 20 μL of the test sample was added to each well. The same volume of DMSO was added to the control group well. Forty-eight hours after the test sample was added, 20 μL MTT was also added to the each well (final concentration, 5 μg/mL). Two hours later, the plate was centrifuged for 5 minutes at 1500 rpm, the medium was then removed and the resulting formazan crystals were dissolved with 150 μL DMSO. The optical density (O.D.) was measured at 570 nm using a Titertek microplate reader (Multiskan MCC/340, Flow). The IC_{50} value was defined as the concentration of sample to reduce absorbance by 50% relative to the vehicle-treated control.

Results and Discussion

The MeOH extract of the fruiting bodies of *H. russula* was suspended in H_2O and successively partitioned with $CHCl_3$, EtOAc and BuOH. The $CHCl_3$-soluble fraction with cytotoxicity against the A549 cancer cell line was subjected to a series of chromatographic techniques and led to the isolation of 5 ergosterol derivatives (**1** - **5**).

Table 1. ^{13}C (75 MHz) NMR data of compounds **1** - **5**

No	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[a]
1	34.8	34.1	38.8	26.9	32.4
2	30.3	34.1	30.9	30.3	31.4
3	66.6	199.5	67.7	67.0	68.9
4	37.2	122.9	31.9	39.4	39.8
5	82.3	164.4	76.3	77.4	68.0
6	135.6	124.5	70.6	72.6	61.6
7	130.9	134.0	119.8	119.0	65.3
8	79.6	124.4	142.3	143.0	125.4
9	51.2	44.3	43.6	74.7	39.4
10	37.1	36.7	39.0	40.2	36.0
11	23.6	18.9	22.9	27.9	19.2
12	39.9	35.6	39.5	35.1	36.8
13	44.7	44.0	44.0	43.6	43.2
14	51.8	156.1	55.0	50.6	152.8
15	20.8	25.4	21.6	22.9	25.2
16	28.8	27.7	28.3	28.2	27.4
17	56.4	55.7	56.2	55.2	57.1
18	13.0	18.9	12.4	11.6	18.3
19	18.3	16.6	18.0	20.9	16.7
20	40.6	39.3	40.6	40.4	40.5
21	21.1	21.2	21.3	21.4	21.4
22	135.4	135.0	135.6	135.5	135.7
23	132.5	132.5	132.4	132.0	132.5
24	42.9	33.1	43.0	47.9	43.0
25	33.2	42.9	33.3	33.0	33.3
26	19.8	19.6	20.2	19.5	20.1
27	20.1	12.0	19.9	19.8	21.1
28	17.7	17.6	17.8	17.4	18.3

[a] in $CDCl_3$, [b] in $CDCl_3$+MeOD

Compound **1** was obtained as a white powder with $[\alpha]_D^{25}$ –26.0 (*c* 0.2, $CHCl_3$). It gave a positive Liebermann-Burchard test. The ESI-MS revealed a molecular ion peak at *m/z* 451 $[M+Na]^+$ and its ^{13}C NMR spectrum (Table 1) showed 28 carbon signals, suggesting the molecular formula $C_{28}H_{44}O_3$. The ^{1}H NMR spectrum showed two tertiary methyl signals at δ_H 0.81 (3H, s, H-18) and 0.88 (3H, s, H-19), four secondary methyl signals at δ_H 0.82 (3H, d, *J* = 6.9 Hz, H-27), 0.84 (3H, d, *J* = 6.9 Hz, H-26), 0.92 (3H, d, *J* = 6.9 Hz, H-28), and 1.00 (3H, d, *J* = 6.6 Hz, H-21), a carbinol proton signal at δ_H 3.96 (1H, m, H-3), and four olefinic proton signals at δ_H 5.15 (1H, dd, *J* = 15.4, 7.8 Hz, H-22), 5.24 (1H, dd, *J* = 15.4, 7.2 Hz, H-

23), 6.24 (1H, d, J = 8.5 Hz, H-6), and 6.50 (1H, d, J = 8.5 Hz, H-7), suggesting an ergostane skeleton including a nine carbon side chain (Adler *et al.*, 1977). The ^{13}C NMR chemical shifts of two oxygenated quaternary carbons at δ_C 79.6 and 82.3 as well as the unsaturation degree indicated the presence of an epidioxy group. The chemical shift value and the multiplicity of the hydroxymethine proton at C-3 [$_H$ 3.96 (1H, m, $W_{1/2}$ = 22 Hz) was characteristic of 3-hydroxy-5-oxygenated A/B *trans* sterols (Ishizuka *et al.*, 1998). In addition, the HMBC analysis indicated the position of an epidioxy group between C-5 and C-8. By comparing this data with the literature (Kwon *et al.*, 2002), **1** was recognized as ergosterol peroxide.

Compound **2** was obtained as yellowish crystal with $[\alpha]_D^{25}$ +52.0 (*c* 0.1, $CHCl_3$). The ESI-MS analysis yielded a molecular ion peak at *m/z* 415 $[M+Na]^+$, in accordance with the molecular formula $C_{28}H_{40}O$. The compound exhibited a UV absorption peak at 345 nm and an infrared band at 1665 cm^{-1}, suggesting the presence of a long conjugated ketone system. The ^{1}H NMR spectrum showed two tertiary methyl signals at 0.97 (3H, s, H-18) and 1.00 (3H, s, H-19), four secondary methyl signals at δ_H 0.83 (3H, d, J = 6.9 Hz, H-27), 0.85 (3H, d, J = 6.9 Hz, H-26), 0.93 (3H, d, J = 6.9 Hz, H-28), and 1.06 (3H, d, J = 6.9 Hz, H-21), and four olefinic proton signals at δ_H 5.22 (1H, dd, J = 15.0, 7.2 Hz, H-22), 5.27 (1H, dd, J = 15.0, 7.2 Hz, H-23), 6.02 (1H, d, J = 9.6 Hz, H-6), and 6.60 (1H, d, J = 9.6 Hz, H-7), indicating the presence of an ergostane skeleton. The ^{13}C NMR (Table 1), combined with DEPT, revealed that **2** contained 28 carbon signals including a carbonyl carbon at δ_C 199.5 and six olefinic carbons at δ_C 122.9, 124.4, 124.5, 134.0, 156.1 and 164.4 which were assigned to C-4, C-8, C-6, C-7, C-14, and C-5, respectively, by analysis of HMQC and HMBC spectra. On the basis of the above data, the structure of **2** was established as ergosta-4,6,8(14),22-tetraen-3-one (Lee *et al.*, 2005).

Compound **3** was obtained as a white powder with $[\alpha]_D^{25}$ –2.0 (*c* 0.1, $CHCl_3$). The ESI-MS of **3** exhibited a molecular ion peak at *m/z* 437 $[M+Na]^+$ corresponding to the molecular formula $C_{28}H_{46}O_3$. The signals for six methyl groups at δ_H 0.57 (3H, s, H-18), 0.83 (3H, d, J = 6.9 Hz, H-27), 0.85 (3H, d, J = 6.9 Hz, H-26), 0.93 (3H, d, J = 6.9 Hz, H-28), 0.98 (3H, s, H-19), and 1.03 (3H, d, J = 6.6 Hz, H-21) in the ^{1}H NMR spectrum indicated an ergostane skeleton. The ^{13}C NMR spectrum (Table 1) showed 28 carbon signals including four olefinic carbon signals at δ_C 119.8, 132.4, 135.6, and 142.3 and three oxygenated carbon signals at δ_C 67.7, 70.6, and 76.3 which were assigned to C-3, C-6, and C-5, respectively. The -configuration of the hydroxyl group at C-6 was deduced from the NOE cross peak between δ_H 3.97 (H-6) and 0.98 (Me-19) in the NOESY spectrum. Thus, the structure of **3** was identified as ergosta-7,22-diene-3,5,6-triol (Yaoita *et al.*, 1998).

Compound **4** was obtained as a white powder with $[\alpha]_D^{25}$ –8.0 (*c* 0.1, $CHCl_3$). It gave a molecular ion peak at *m/z* 453 $[M+Na]^+$ in the ESI-MS, consistent with the molecular formula $C_{28}H_{46}O_4$. The ^{13}C NMR spectrum (Table 1) showed 28 carbon signals including four olefinic carbon signals at δ_C 119.0, 132.0, 135.5 and 143.0, as well as four oxygenated carbon signals at δ_C 67.0, 72.6, 74.7, and 77.4 which were assigned to C-3, C-6, C-9 and C-5, respectively. Comparison of the ^{13}C NMR data of **4** with that of **3** revealed that they were very similar, except for the presence of an oxygenated quaternary carbon signal at C-9 (δ_C 74.7) in **4** instead of a methine group at C-9 (δ_C 43.6) in **3**. Furthermore, the NOE cross peak observed between δ_H 3.63 (H-6) and 4.00 (H-3) in the NOESY spectrum indicated a -configuration of the hydroxy group at C-6 rather than -configuration found in **3**. Based on the above data and comparison with the literature (Ishizuka *et al.*, 1998), the structure of **4** was deduced to be ergosta-7,22-diene-3β,5α,6β,9α-tetraol.

Compound **5** was obtained as a white powder with $[\alpha]_D^{25}$ –7.5 (*c* 0.1, $CHCl_3$). The ESI-MS showed a molecular ion at *m/z* 451 $[M+Na]^+$ corresponding to the molecular formula $C_{28}H_{44}O_3$. The ^{1}H NMR spectrum at δ_H 3.14 (1H, m, H-6) and ^{13}C NMR spectrum (Table 1) at δ_C 61.6 and 68.0 inferred the presence of a trisubstituted 5α,6β-epoxy ring (Kobayashi and Kanda, 1991). The proton at δ_H 4.42 (1H, br s) for H-7β and the resonance at δ_C 65.3 for C-7 suggested the presence of a 7α-hydroxyl group. The two quaternary carbons at δ_C 125.4 and 152.8 for a fully substituted double bond suggested possible assignments at $\Delta^{8,9}$ or $\Delta^{8(14)}$. The assignment of a $\Delta^{8(14)}$ skeleton seemed to be favorable from the comparison of its ^{1}H and ^{13}C NMR spectra with those of melithasterol B (Kobayashi and Kanda, 1991). Thus, the structure of **3** was identified as 5α,6α-epoxy-ergosta-8(14),22-diene-3β,7α-diol (Greca *et al.*, 1993).

All the isolates (**1** - **5**) were evaluated for *in vitro* cytotoxicities against A549 (human lung) and XF498 (human skin) cancer cell lines using the MTT assay, and the results are presented in Table 2. Most of the tested compounds, except for ergosta-7,22-diene-3β,5α,6α-triol (**3**), exhibited moderate cytotoxicity against A549 and XF498 cell lines with IC_{50} values ranging from 10.2 to

Table 2. Cytotoxicity of compounds **1 - 5** from *H. russula*

Compound	IC_{50} (μg/mL)	
	A549	XF498
1	10.2	11.4
2	18.8	24.6
3	> 30	> 30
4	17.1	16.5
5	15.3	15.1
Adriamycin[a]	1.2	1.1

[a] positive control

18.8 μg/mL and from 11.4 to 24.6 μg/mL, respectively, compared to the positive control, adriamycin (1.2 and 1.1 μg/mL, respectively). Of the tested compounds, ergosterol peroxide (**1**) having a 5α,8α-epidioxy group exhibited the strongest cytotoxicity against A549 and XF498 cell lines with IC_{50} values of 10.2 and 11.4 μg/mL, respectively. Furthermore, 5α,6α-epoxy-ergosta-8(14),22-diene-3β,7α-diol (**5**) bearing a 5α,6α-epoxy group also exhibited significant cytotoxicity against both A549 and XF498 cell lines. This result is in accordance with previous studies (Kwon *et al.*, 2002; Sheu *et al.*, 2000), suggesting that an epidioxy or epoxy functional group on the ring structure of ergosterol derivatives increases their cytotoxic properties.

In summary, bioassay-guided fractionation of the $CHCl_3$-soluble fraction of a MeOH extract of the fruiting bodies of *H. russula* led to the isolation of five ergosterol derivatives (**1 - 5**). The structures of these compounds were elucidated on the basis of physicochemical and spectroscopic analyses. To the best of our knowledge, this is the first report of the isolation of ergosterol derivatives from *H. russula*. These compounds were evaluated for *in vitro* cytotoxicity against A549 and XF498 cell lines. Most of the tested compounds, except for compound **3**, exhibited moderate cytotoxicity against both A549 and XF498 cell lines. This result indicated that ergosterol derivatives (**1 - 5**) isolated in this study might be the cytotoxic principles of *H. russula*.

Acknowledgments

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