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Steroids and Triterpenoid from the Fruit Bodies of *Ganoderma lucidum* and Their Cytotoxic Activity

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Abstract – To determine the cytotoxic activity of natural compounds, chromatographic separation of the hexane-soluble fraction from the fruiting body of *Ganoderma lucidum* led to the isolation of four steroids and one triterpenoid. They were identified as ergosterol peroxide (**1**), stella sterol (**2**), ergosterol (**3**), 9(11)-dehydroergostrol peroxide (**4**), and ganodermanontriol (**5**) based on spectroscopic evidence and physicochemical properties. These compounds were examined for their cytotoxic activity against HL-60, MCF-7, and LLC cancer cell lines. Ganodermanontriol (**5**) showed cytotoxic activity with IC_{50} values of 24.8 and 22.9 μg/mL against HL-60 and MCF-7 cancer cell lines, respectively, whereas compounds **1 - 4** were inactive.
Keywords – *Ganoderma lucidum*, Polyporaceae, Ganoderic acid Sz, Lanostadiene triterpene, Cytotoxic activity

Introduction

Ganoderma lucidum (Fr.) Karst (Polyporaceae) is one of the famous medicinal mushrooms, which has been used as a traditional medicine for a long time in China, Japan and Korea. The potential medicinal value and wide acceptability of this edible mushroom have attracted intense interests in the search for biologically active substances in last 30 years (Wasser and Weis, 1999; Cheung *et al*., 2000). Nowadays, with the successful artificial cultivation of *G. lucidum*, this mushroom and related product are widely used as not only health food, but also clinical drugs for the prevention and treatment of hepatopathy, chronic hepatitis, nephritis, gastric ulcer, hypertension, arthritis, neurasthenia, insomnia, asthma, acute and chronic bronchitis, leucopoenia, and cancer (Wang *et al.*, 1997; Lin and Zhou, 1991). This fungus has a remarkably strong bitterness which originates from triterpenes, as a major group. During the recent decades, astonishing strides in the chemical and biological manipulation of highly oxygenated triterpenes have been observed. Several triterpenes and related constituents have been identified from the fruit bodies, cultured mycelium and spores of *G. lucidum*. Lanostane-type triterpenes are major constituents of an organic solvent-soluble fraction of *G. lucidum* (Gao *et al.*, 2003; Kubota *et al.*, 1982; Kubo *et al.*, 2003).

For a long time, polysaccharides, which were easily dissloved in water, in *G. lucidum* were known as the main constituents of anticancer (Miyazaki and Nishijima, 1981; Lin, 2001). However, *G. lucidum* contains a small amount of polysaccharides, and these are hard to determinate chemical structures. Therefore in our presence study, try to find out the materials which are major constituents of an organic solvent-soluble fraction of *G. lucidum*, and these materials also have anticancer activity. During the recent two decades, numerous studies about *G. lucidum* were advanced, and triterpenes from *G. lucidum* were divided to two groups, ganoderic alcohol and ganoderic acid. By numerous studies about *G. lucidum* and its triterpenes, ganoderic alcohol has potent cytotoxic activity than ganoderic acid (Min *et al*., 1998; Canjun, 2005). Accordingly, our studies focused on the isolation of ganoderic alcohols and their cytotoxic activit. This study is part of an ongoing investigation into cytotoxic active compounds from herbal medicines. In this study, four sterols and one triterpenoid were isolated from fruit bodies of *G. lucidum.* The structures of those isolated compounds were determined, along with their cytotoxic activity.

Experimental

General experimental procedures – Melting points

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were measured by using an Electrothermal apparatus and are not corrected. Optical rotation was determined on a JASCO DIP-100 KUY polarimeter. UV spectra were obtained with a Beckman Du-650 UV/VIS recording spectrophotometer. IR spectra were recorded on a Jasco Report-100 infrared spectrometer. Mass were carried out with a JEOL JMS-700 Mstation mass spectrometer. ^{1}H-NMR (300 and 400 MHz) and ^{13}C-NMR (75 and 100 MHz) were recorded on Bruker DRX300 and JEOL 400 spectrometers. Two-dimensional (2D) NMR spectra (^{1}H-^{1}H COSY, HMQC, and HMBC) were recorded on a Bruker Avance 500 spectrometer. For column chromatography, silica gel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, Merck) was used. Thin layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254} S (0.25 mm, Merck), and spot were detected under an UV light and by spraying with 10% H_2SO_4.

Plant material – The dried fruit bodies of *Ganoderma lucidum* (cultured at Nagano, Japan) was supplied from Prof. Masao Hattori, Toyama University, Japan, and identified by Prof. Byung-Sun Min, Catholic University of Daegu, Korea. A voucher specimen (CUD-3170) was deposited at the herbarium of the college of Pharmacy, Catholic University of Daegu, Korea.

Extraction and Isolation – The dried fruit bodies of *Ganoderma lucidum* (10 kg) was extracted four times with MeOH at room temperature for seven days. The MeOH solution was concentrated under reduced pressure to give a residue (392.2 g). The MeOH extract was suspended in hot-H_2O (4 L) and extracted with hexane (1 L × 2) and chloroform ($CHCl_3$, 2 L × 4), successively. The resulting fractions were concentrated in vacuo to give the hexane-soluble fraction (40.0 g) and $CHCl_3$-soluble fraction (130.0 g), respectively. The hexane soluble fraction (40.0 g) was chromatographed on a florisil column chromatography eluting with a gradient of hexane-acetone (10 : 3 → 7 : 3) to afford three fractions (Fr. H1~H3; 20.0, 6.0, 5.0 g). The fraction H1 (20.0 g) was subjected on a silica gel column chromatography eluting with gradient of hexane-acetone (20 : 3 → 10 : 3) to afford nine subfractions (Fr. H1.1~H1.9; 17.5, 0.35, 0.4, 2.0, 0.12, 0.5, 0.57, 0.1, 0.1 g). Subfraction H1.1 (17.5 g) was further chromatographed on a silica gel column chromatography eluting with a gradient of hexane-acetone (19 : 1 → 4 : 1) to afford fifteen subfractions (Fr. H1.1.1~H1.1.15; 0.6, 0.4, 0.7, 0.8, 0.3, 0.4, 0.3, 0.1, 0.3, 0.8, 1.1, 0.7, 0.5, 0.5, 0.2 g). The subfraction H1.1.11 (1.1 g) was chromatographed on a silica gel column and eluted with $CHCl_3$-acetone (55 : 1) to afford three subfractions (Fr. H1.1.11.1~H1.1.11.3; 0.03, 0.9, 0.05 g). The subfraction H1.1.11.2 (0.9 g) was further chromatographed on a silica gel column chromatography eluting with a gradient of hexane-EtOAc (10 : 1 → 2 : 1) to afford three subfractions (Fr. H1.1.11.2.1~ H1.1.11.2.3; 0.2, 0.5. 0.1 g). The H1.1.11.2.2 (0.5 g) was recrystallizde by hexane-EtOAc (10 : 1) to give compound **3** (25 mg). The subfraction H1.1.14 (0.5 g) was further purified by HPLC on a RP-18 column using a gradient of MeOH and 1% trifluoroacetic acid in H_2O as mobile phase (70 → 100%) to yield compound **1** (18 mg). The subfraction H.1.4 (2 g) was subjected to silica gel column chromatography eluting with hexane-EtOAc (4 : 1) to afford four subfractions (Fr. H1.4.1~ H1.4.4; 0.2, 0.9, 0.2, 0.1 g). The subfraction H1.4.2 (0.9 g) was subjected to silica gel column chromatography eluting with $CHCl_3$-acetone (7 : 1) to afford six subfractions (Fr. H1.4.2.1~ H1.4.2.6; 51, 350, 34, 16, 22, 28 mg). The subfraction H1.4.2.2 (350 mg) was recrystallized by $CHCl_3$-acetone (5 : 1) to give compound **2** (188 mg). The fraction H2 (6.0 g) was subjected on a silica gel column chromatography eluting with a gradient of $CHCl_3$-MeOH (30 : 1 → 10 : 1) to afford twelve subfractions (Fr. H2.1~ H2.12; 22, 18, 23, 16, 17, 35, 23, 188, 34, 167, 17, 23 mg). The subfraction H2.8 (188 mg) was further purified by HPLC on a RP-18 column using a gradient of MeOH and 1% trifluoroacetic acid in H_2O as mobile phase (90 → 100%) to yield compound **5** (5 mg). The subfraction H2.9 (34 mg) was further purified by HPLC on a RP-18 column using a gradient of MeOH and 1% trifluoroacetic acid in H_2O as mobile phase (80 → 100%) to yield compound **4** (4 mg).

Compound 1 (ergosterol peroxide): White powder; mp: 182 - 184 °C; $[\alpha]_D^{22}$ –9.3° (*c* 0.1, $CHCl_3$) UV ($CHCl_3$): λ_{max} nm (log ε) 241 (3.19); EI-MS *m/z* 428 $[M]^+$ (15), 410 $[M\text{-}H_2O]^+$ (12), 396 $[M\text{-}O_2]^+$ (10), 301 $[M\text{-}C_9H_{18}]^+$ (48), 285 $[M\text{-}C_9H_{18}\text{-}H_2O]^+$ (20), 249 $[M\text{-}C_9H_{18}\text{-}H_2O\text{-}O_2]^+$ (19), 69 (80), 55(100); molecular formula: $C_{28}H_{44}O_3$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.82 (3H, d, *J* = 6.8 Hz, H-27), 0.83 (3H, s, H-18), 0.84 (3H, d, *J* = 6.8 Hz, H-26), 0.89 (3H, s, H-19), 0.91 (3H, d, *J* = 6.8 Hz, H-28), 1.00 (3H, d, *J* = 6.4 Hz, H-21), 1.21 (1H, m, H-15), 1.22 (1H, m, H-12), 1.23 (1H, m, H-17), 1.37 (1H, m, H-16), 1.37 (1H, m, H-11), 1.43 (1H, m, H-25), 1.51 (1H, m, H-15), 1.52 (1H, m, H-9), 1.54 (1H, m, H-2), 1.58 (1H, m, H-14), 1.62 (1H, m, H-11), 1.67 (1H, m, H-1), 1.74 (1H, m, H-16), 1.86 (1H, m, H-24), 1.86 (1H, m, H-2), 1.92 (1H, m, H-4), 1.95 (1H, m, H-12), 1.97 (1H, m, H-1), 2.02 (1H, m, H-20), 2.10 (1H, m, H-4), 3.97 (1H, m, H-3), 5.14 (1H, dd, *J* = 8.0, 15.2 Hz, H-22), 5.23 (1H, dd, *J* = 7.6, 15.2 Hz, H-23), 6.24 (1H, d, *J* = 8.4 Hz, H-6),

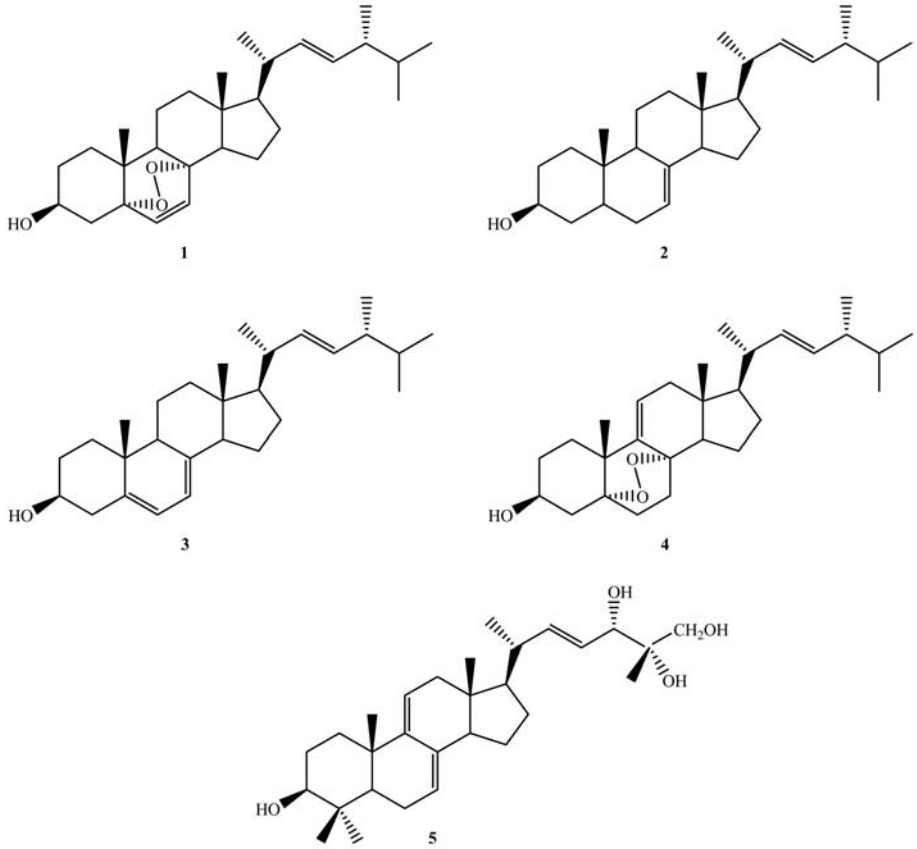

Fig. 1. Structure of isolated compounds **1 - 5**.

6.50 (1H, d, *J* = 8.4 Hz, H-7); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 34.9 (C-1), 30.3 (C-2), 66.7 (C-3), 37.1 (C-4), 79.6 (C-5), 135.6 (C-6), 131.0 (C-7), 82.4 (C-8), 51.3 (C-9), 37.2 (C-10), 20.9 (C-11), 39.5 (C-12), 44.8 (C-13), 51.9 (C-14), 23.6 (C-15), 28.9 (C-16), 56.4 (C-17), 13.1 (C-18), 18.4 (C-19), 40.0 (C-20), 21.1 (C-21), 135.4 (C-22), 132.5 (C-23), 43.0 (C-24), 33.3 (C-25), 20.2 (C-26), 19.8 (C-27), 17.8 (C-28).

Compound 2 (stella sterol): White powder; $[\alpha]_D^{22}$ −32.3° (*c* 0.1, $CHCl_3$); UV ($CHCl_3$): λ_{max} nm (log ε) 274 (3.43), 284 (3.45), 296 (3.24); EI-MS *m/z* 398 $[M]^+$ (35), 355 $[M-(CH_3)_2CH]^+$ (8), 271 (100), 255 $[M-C_9H_{17}-H_2O]^+$ (56); molecular formula: $C_{28}H_{46}O$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.52 (3H, s, H-18), 0.78 (3H, s, H-19), 0.81 (3H, d, *J* = 13.2 Hz, H-26), 0.81 (3H, s, H-27), 0.89 (3H, d, *J* = 6.8 Hz, H-28), 1.00 (3H, d, *J* = 6.4 Hz, H-21), 1.08 (1H, dd, *J* = 4, 13.6 Hz, H-1), 1.20 (1H, m, H-12), 1.23 (1H, m, H-16), 1.23 (1H, m, H-2), 1.24 (1H, m, H-17), 1.29 (1H, m, H-4), 1.37 (1H, m, H-5), 1.37 (1H, m, H-6), 1.37 (2H, m, H-15), 1.45 (1H, m, H-25), 1.48 (2H, m, H-11), 1.61 (1H, m, H-9), 1.69 (1H, m, H-2), 1.69 (1H, m, H-4), 1.75 (1H, m, H-16), 1.78 (1H, m, H-14), 1.82 (1H, m, H-6), 1.83 (1H, m, H-1), 1.88 (1H, m, H-24), 2.00 (1H, m, H-12), 2.03 (1H, m, H-20), 3.57 (1H, m, H-3), 5.14 (1H, m, H-22), 5.14 (1H, m, H-7), 5.17 (1H, m, H-23); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 37.4 (C-1), 28.3 (C-2), 71.3 (C-3), 38.2 (C-4), 40.5 (C-5), 31.7 (C-6), 117.7 (C-7), 139.8 (C-8), 49.7 (C-9), 34.4 (C-10), 21.8 (C-11), 39.7 (C-12), 43.5 (C-13), 55.3 (C-14), 23.1 (C-15), 29.9 (C-16), 56.2 (C-17), 12.3 (C-18), 13.2 (C-19), 40.7 (C-20), 21.3 (C-21), 135.9 (C-22), 132.1 (C-23), 43.0 (C-24), 33.3 (C-25), 20.2 (C-26), 19.9 (C-27), 17.8 (C-28).

Compound 3 (ergosterol): White powder; mp: 164 - 165 °C; $[\alpha]_D^{22}$ −78.6° (*c* 0.1, $CHCl_3$); UV ($CHCl_3$): λ_{max} nm (log ε) 274 (3.32), 285 (3.35), 296 (3.14); EI-MS *m/z* 396 $[M]^+$ (76), 363 (100), 271 $[M-C_9H_{17}]^+$ (18), 69(62), 55(60); molecular formula: $C_{28}H_{44}O$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.61 (3H, s, H-18), 0.79 (3H, d, *J* = 7.6, H-26), 0.82(3H, d, *J* = 6.8 Hz, H-27), 0.90 (3H, d, *J* = 6.8 Hz, H-28), 0.93 (3H, s, H-19), 1.02 (3H, d, *J* = 6.8 Hz, H-21), 5.15 (1H, m, H-22), 5.19 (1H, m, H-23), 5.36 (1H, q, *J* = 2.8 Hz, H-7), 5.55 (1H, dd, *J* = 2.8, 5.4 Hz, H-6); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 38.6 (C-1), 32.2 (C-2), 70.7 (C-3), 41.0 (C-4), 140.0 (C-5), 119.8 (C-6), 116.5 (C-7),

141.6 (C-8), 46.5 (C-9), 37.2 (C-10), 21.7 (C-11), 39.3 (C-12), 43.0 (C-13), 54.8 (C-14), 23.2 (C-15), 28.5 (C-16), 55.9 (C-17), 12.3 (C-18), 16.5 (C-19), 40.6 (C-20), 21.3 (C-21), 135.8 (C-22), 132.2 (C-23), 43.0 (C-24), 33.3 (C-25), 19.9 (C-26), 20.2 (C-27), 17.8 (C-28).

Compound 4 (9(11)-dehydroergosterol peroxide): Colorless amorphous powder; mp: 164 - 165 °C; $[\alpha]_D^{22}$ −84.3° (*c* 0.1, MeOH); UV (MeOH): λ_{max} nm (log ε) 205 (3.36); EI-MS *m/z* 426 $[M]^+$ (15), 394 $[M-O_2]^+$ (28), 376 $[M-O_2-H_2O]^+$ (25), 251 $[M-C_9H_{18}-O_2-H_2O]^+$ (100), 75 (95); molecular formula: $C_{28}H_{44}O_3$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.57 (3H, s, H-18), 0.83 (3H, d, *J* = 6.4 Hz, H-27), 0.84 (3H, d, *J* = 6.8 Hz, H-26), 0.92 (3H, d, *J* = 6.8 Hz, H-28), 0.98 (3H, s, H-19), 1.03 (3H, d, *J* = 6.4 Hz, H-21), 1.21 (1H, m, H-15), 1.22 (1H, m, H-12), 1.23 (1H, m, H-17), 1.34 (1H, m, H-7), 1.37 (1H, m, H-16), 1.37 (1H, m, H-11), 1.43 (1H, m, H-25), 1.47 (1H, m H-7), 1.51 (1H, m, H-15), 1.52 (2H, m, H-6), 1.54 (1H, m, H-2), 1.58 (1H, m, H-14), 1.67 (1H, m, H-1), 1.74 (1H, m, H-16), 1.86 (1H, m, H-24), 1.86 (1H, m, H-2), 1.92 (1H, m, H-4), 1.95 (1H, m, H-12), 1.97 (1H, m, H-1), 2.02 (1H, m, H-20), 2.10 (1H, m, H-4), 4.01 (1H, m, H-3), 5.03 (1H, brs, H-11), 5.16 (1H, dd, *J* = 7.6, 15.2 Hz, H-23), 5.23 (1H, dd, *J* = 7.2, 15.2 Hz, H-22); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 34.9 (C-1), 30.3 (C-2), 67.6 (C-3), 37.1 (C-4), 70.5 (C-5), 41.3 (C-6), 20.9 (C-7), 76.2 (C-8), 142.2 (C-9), 37.2 (C-10), 119.7 (C-11), 39.5 (C-12), 44.8 (C-13), 54.9 (C-14), 23.6 (C-15), 28.9 (C-16), 56.4 (C-17), 12.4 (C-18), 18.0 (C-19), 40.0 (C-20), 21.3 (C-21), 135.6 (C-22), 132.3 (C-23), 43.0 (C-24), 33.3 (C-25), 19.9 (C-26), 20.1 (C-27), 17.8 (C-28).

Compound 5 (ganodermanontriol): White powder; mp: 161 - 162 °C; $[\alpha]_D^{22}$ +16.6° (*c* 0.1, DMSO); UV ($CHCl_3$): λ_{max} nm (log ε) 236 (3.26), 243 (3.28), 251 (3.14); EI-MS *m/z* 472 $[M]^+$ (11), 454 $[M-H_2O]^+$ (12), 439 $[M-H_2O-Me]^+$ (75) 396 $[M-C_9H_{18}]^+$ (13), 309 (25), 281 (48), 258 (70), 207 (100), 69 (31), 55 (43); molecular formula: $C_{30}H_{48}O_4$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.58 (3H, s, H-18), 0.86 (3H, s, H-28), 0.92 (3H, d, *J* = 6.1 Hz, H-21), 1.07 (3H, s, H-19), 1.10 (3H, s, H-29), 1.11 (3H, s, H-26), 1.18 (3H, s, H-27), 1.23 (3H, s, H-30), 2.35 (1H, m, H-1β), 2.76 (1H, dt, *J* = 5.6, 14.8 Hz, H-1α), 3.45 (1H, m, H-24), 3.45 (1H, m, H-26), 3.82 (1H, d, *J* = 10.4 Hz, H-26), 5.37 (1H, d, *J* = 5.2 Hz, H-7), 5.49 (1H, d, *J* = 6.8 Hz, H-11); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 36.5 (C-1), 34.8 (C-2), 217.1 (C-3), 47.4 (C-4), 50.2 (C-5), 23.6 (C-6), 120.1 (C-7), 143.0 (C-8), 144.7 (C-9), 37.7 (C-10), 117.5 (C-11), 37.1 (C-12), 43.6 (C-13), 50.6 (C-14), 27.8 (C-15), 28.8 (C-16), 50.9 (C-17), 15.7 (C-18), 22.4 (C-19), 36.5 (C-20), 18.6 (C-21), 31.4 (C-22), 33.5 (C-23), 79.6 (C-24), 76.1 (C-25), 68.2 (C-26), 22.0 (C-27), 25.4 (C-28), 25.4 (C-29), 20.8 (C-30), 20.0 ($OCOCH_3$).

Cytotoxic activity assay method – The cancer cell lines (MCF-7, HL-60, and LLC) were maintained in RPMI 1640 that included L-glutamine (GIBCO) with 10% FBS (GIBCO) and 2% penicillin-streptomycin (GIBCO). Cells were cultured at 37 °C in a 5% CO_2 incubator. Cytotoxicity was measured using a modified MTT assay (Mosmann, 1983). Viable cells were seeded in the growth medium (180 μL) into 96-well microtiter plates (1×10^4 cells per well) and incubated at 37 °C in a 5% CO_2 incubator. The test sample was dissolved in DMSO and adjusted to final sample concentrations ranging from 5.0 to 100 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 wells. Forty eight hours after the test sample was added, 20 μL of MTT was also added to the each well (final concentration, 5 μg/mL). Two hours later, the plate was centrifuged for 5 min at 1500 rpm, the medium was removed, and the resulting formazan crystals were dissolved with DMSO 150 μL. 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 which reduced absorbance by 50% relative to the vehicle-treated control (Mosmann, 1983).

Result and Discussion

Compound **1** was obtained as a white powder. The UV spectrum exhibited absorption at 241 nm. The molecular formula of $C_{28}H_{44}O_3$ was estimated from a molecular ion peak $[M]^+$ at *m/z* 428 in the EI-MS. The ^{13}C NMR spectrum including DEPT experiment of compound **1** showed signals for four olefinic carbons at δ 135.6 (C-6), 135.4 (C-22), 132.5 (C-23) and 131.0 (C-7), two oxygenated quaternary carbons at δ 82.4 (C-8) and 79.6 (C-5), one oxygenated methine peak at δ 66.7 (C-3), and six methyl carbons at δ 13.1 (C-18), 18.4 (C-19), 21.1 (C-21), 20.2 (C-26), 19.8 (C-27) and 17.8 (C-28) indicating the presence of a sterol skeleton, compared with that of stigmasterol. The ^{1}H NMR spectrum of compound **1** showed signals for two olefinic methines at δ 6.50 (1H, d, *J* = 8.4 Hz, H-7) and 6.24 (1H, d, *J* = 8.4 Hz, H-6), two other olefinic methines at δ_H 5.23 (1H, dd, *J* = 7.6, 15.2 Hz, H-23), and 5.14 (1H, dd, *J* = 8.0, 15.2 Hz, H-22), and one oxygenated methine at δ_H 3.97 (1H, m, H-3). In

addition, it exhibited signals for four secondary methyls at δ_H 0.82 (3H, d, *J* = 6.8 Hz, H-27), 0.84 (3H, d, *J* = 6.8 Hz, H-26), 0.91 (3H, d, *J* = 6.8 Hz, H-28) and 1.00 (3H, d, *J* = 6.4 Hz, H-21), and two tertiary methyls at δ_H 0.83 (3H, s, H-18) and 0.89 (3H, s, H-19). Compound **1** was finally identified as 5α,8α-epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-ol (ergosterol peroxide) by comparison of the physical and spectral data with those in the references (Joachim and Wilfried, 2000; Arisawa *et al.*, 1986).

Compound **2** was obtained as a white powder, $[\alpha]_D^{22}$ −32.3° (*c* 0.1, $CHCl_3$). The UV spectrum exhibited absorption at 274, 284, and 296 nm. The molecular formula of $C_{28}H_{46}O$ was estimated from a molecular ion peak $[M]^+$ at *m/z* 398 in the EI-MS. The ^{13}C NMR spectrum including DEPT experiment of compound **2** showed signals for six methyl carbons at δ 12.3 (C-18), 13.2 (C-19), 21.3 (C-21), 20.2 (C-26), 19.9 (C-27) and 17.8 (C-28), eight methylene carbons at δ 37.4 (C-1), 28.3 (C-2), 38.2 (C-4), 31.7 (C-6), 21.8 (C-11), 39.7 (C-12), 23.1 (C-15) and 29.9 (C-16), eleven methins at δ 71.3 (C-3), 40.5 (C-5), 117.7 (C-7), 49.7 (C-9), 55.3 (C-14), 56.2 (C-17), 40.7 (C-20), 135.9 (C-22), 132.1 (C-23), 43.0 (C-24) and 33.3 (C-25), and three quaternary carbons at *ä* 139.8 (C-8), 34.4 (C-10) and 43.5 (C-13). Furthermore, compound **2** exhibited characteristic signals due to four olefinic carbons at δ 117.7 (C-7), 139.8 (C-8), 135.9 (C-22) and 132.1 (C-23), and one oxygenated carbon at δ 71.3 (C-3) in the ^{13}C NMR spectrum, indicated the presence of the steroid skeleton in compound **2** compared with that of compound **1** (Jeffrey and Wright, 1981). In addition, the ^1H NMR spectrum showed signals due to three olefinic protons at δ 5.14 (1H, m, H-22), 5.15 (1H, m, H-7) and 5.17 (1H, m, H-23), and six methyl protons at δ 0.52 (3H, s, H-18), 0.78 (3H, s, H-19), 0.81 (3H, d, *J* = 13.2 Hz, H-26), 0.81 (3H, s, H-27), 0.89 (3H, d, *J* = 6.8 Hz, H-28), and 1.00 (3H, d, *J* = 6.4 Hz, H-21). The structure of this steroid was determined as (22*E*, 24*R*)-24-methyl-5α-cholesta-7,22-diene-3β-ol by comparison with the reported ^1H- and ^{13}C NMR spectra data (Jeffrey and Wright, 1981).

Compound **3** was obtained as a white powder with an optical rotation $[\alpha]_D^{22}$ −78.6° (*c* 0.1, $CHCl_3$). The UV spectrum exhibited absorption at 274, 285, and 296 nm. The molecular formula of $C_{28}H_{44}O$ was estimated from a molecular ion peak $[M]^+$ at *m/z* 396 in the EI-MS. The ^{13}C NMR spectrum including DEPT experiment of compound **3** showed signals for six methyl signals at δ 12.3 (C-18), 16.5 (C-19), 21.3 (C-21), 19.9 (C-26), 20.2 (C-27) and 17.8 (C-28), seven methylenes at δ 38.6 (C-1), 32.2 (C-2), 41.0 (C-4), 21.7 (C-11), 39.3 (C-12), 23.2 (C-15) and 28.5 (C-16), eleven methines at δ 70.7 (C-3), 119.8 (C-6), 116.5 (C-7), 46.5 (C-9), 54.8 (C-14), 55.9 (C-17), 40.6 (C-20), 135.8 (C-22), 132.2 (C-23), 43.0 (C-24) and 33.3 (C-25), and four quaternary carbons at δ 140.0 (C-5), 141.6 (C-8), 37.2 (C-10) and 43.0 (C-13). Furthermore, compound **3** exhibited characteristic signals due to six olefinic carbons at δ 140.0 (C-5), 119.8 (C-6), 116.5 (C-7), 141.6 (C-8), 135.8 (C-22) and 132.2 (C-23), and one oxygenated carbon at δ 70.7 (C-3), which were assignable to ergosterol skeleton, compared with that of ergosterol (Rubinstein and Goad, 1976). This result was further confirmed by the ^1H NMR spectrum signals for four olefinic protons at δ 5.15 (1H, m, H-22), 5.19 (1H, m, H-23), 5.36 (1H, q, *J* = 2.8 Hz, H-7) and 5.55 (1H, dd, *J* = 2.8, 5.4 Hz, H-6), and six methyl protons at δ 0.61 (3H, s, H-18), 0.79 (3H, d, *J* = 7.6 Hz, H-26), 0.82 (3H, d, *J* = 6.8 Hz, H-27), 0.90 (3H, d, *J* = 6.8 Hz, H-28), 0.93 (3H, s, H-19) and 1.02 (3H, d, *J* = 6.8 Hz, H-21). Base on the above evidence and comparison with the literature data, compound **3** was identified as (22*E*,24*R*)-24-methyl-5α-cholesta-5,7,22-triene-3β-ol (Rubinstein and Goad, 1976).

Compound **4** was isolated as a colorless amorphorus powder. The UV spectrum exhibited absorption at 205 nm. The molecular formula of $C_{28}H_{44}O_3$ was estimated from a molecular ion peak $[M]^+$ at *m/z* 426 in the EI-MS. The ^{13}C including DEPT and ^1H NMR spectra of compound **4** showed closely to **1,** such as four olefinic carbons at δ 142.2 (C-9), 135.6 (C-22), 132.3 (C-23) and 119.7 (C-11), two oxygenated quaternary carbons at δ 76.2 (C-8) and 70.5 (C-5), one oxygenated methine peak at δ 67.6 (C-3), and six methyl carbons at δ 12.4 (C-18), 18.0 (C-19), 21.3 (C-21), 19.9 (C-26), 20.1 (C-27) and 17.8 (C-28), except the location of double bond at 9(11) instead of 6(7) which were indicated by 142.2 (C-9), 119.7 (C-11) in **4** and 135.6 (C-6), 131.0 (C-7) of **1**. The above NMR data indicates the presence of a sterol skeleton, compared with that of ergosterol peroxide. The ^1H NMR spectrum of compound **4** showed signals for two olefinic methines at δ_H 5.16 (1H, dd, *J* = 7.6, 15.2 Hz, H-23), 5.23 (1H, dd, *J* = 7.2, 15.2 Hz, H-22), one other olefinic methines at δ_H 5.03 (1H, brs, H-11), and one oxygenated methine at δ_H 4.01 (1H, m, H-3), In addition, it exhibited signals for four secondary methyls at δ_H 0.83 (3H, d, *J* = 6.4 Hz, H-27), 0.84 (3H, d, *J* = 6.8 Hz, H-26), 0.92 (3H, d, *J* = 6.8 Hz, H-28) and 1.03 (3H, d, *J* = 6.4 Hz, H-21), and two tertiary methyls at δ_H 0.57 (3H, s, H-18) and 0.98 (3H, s, H-19). Compound **4** was finally identified as be 5α ,8α -epidioxy-22*E*-ergosta-6,9 (11),22-trien-3β-ol by comparison of the physical and spectral

data with those in the literature (Kobori *et al*., 2006).

Compound **5**, obtained as a white powder. The UV spectrum exhibited absorptions at 236, 243 and 251 nm. The molecular formula of $C_{30}H_{48}O_4$ was estimated from a molecular ion peak $[M]^+$ at *m/z* 472 in the EI-MS. In the ^1H and ^{13}C NMR spectra including DEPT of compound **5** showed seven methyl signals, including six tertiary methyls at δ 15.7 (C-18), 22.4 (C-19), 18.6 (C-21), 22.0 (C-27), 25.4 (C-28), 25.4 (C-29) and 20.8 (C-30), nine methylenes (including one oxygenal methylene) at δ 36.5 (C-1), 34.8 (C-2), 23.6 (C-6), 37.1 (C-12), 50.6 (C-15), 28.8 (C-16), 31.4 (C-22), 33.5 (C-23) and 68.2 (C-26), three methines (including one oxygenated methan) at δ 50.2 (C-5), 36.5 (C-20) and 79.6 (C-24), one ketone carbonyl at δ 217.1 (C-3), four olefinic carbons at δ 120.1 (C-7), 143.0 (C-8), 144.7 (C-9) and 117.5 (C-11), and five quaternary carbons (among of them, one is oxygenated carbon) at δ 47.4 (C-4), 37.7 (C-10), 43.6 (C-13), 50.6 (C-14) and 76.1 (C-25) indicating the presence of a sterol skeleton, compared with that of compound **1**. The ^1H NMR spectrum of compound **5** showed signals for six tertiary methyls at δ 0.58 (H-18), 0.86 (H-28), 1.07 (H-19), 1.10 (H-29), 1.18 (H-27), and 1.23 (H-30), one secondary methyl proton at δ 0.92 (H-21), and two olefinic protons at δ 5.37 (1H, d, J = 5.2 Hz, H-7) and 5.49 (1H, d, J = 6.8 Hz, H-11). Thus, the structure of compound **5** was determined to be 24(*S*),25,26-trihydroxy-5α-lanosta-7,9(11)-dien-3-one by comparison with reported data (Fujita *et al*., 1986).

Five compounds isolated from fruit bodies of *Ganoderma lucidum* were tested for their cytotoxic activity against HL-60, MCF-7 and LLC cancer cell lines, and showed their results in Table 1. Compounds **1** - **4** were inactive against HL-60, MCF-7 and LLC cancer cell lines. Compound **5** showed moderate activity against HL-60 and MCF-7 with IC_{50} values as 24.8 and 22.9 μg/mL, respectively. It is well documented that the extracts including polysaccharide or triterpene-enriched extract from *G. lucidum* inhibit cancer proliferation, induce cell cycle arrest, or apoptosis of human and mouse carcinoma cell lines (Hu *et al*., 2002; Lu *et al*., 2003). The extracts of *G. lucidum* inhibited primary solid-tumor growth in the spleen, liver metastasis, and secondary metastatic tumor growth in the liver in intrasplenic Lewis lung carcinoma (LLC)-implanted mice, inhibited Martigel-induced angiogenesis (Kimura *et al*., 2002), induced actin polymerization in bladder cancer cells in vitro, inhibited tumor cell adhesion, inhibited induced oxidative stress-invasiveness of cancer cells through suppression of interleukin (IL)-8 secretion, and suppressed motility and invasiveness of highly invasive breast and prostate cancer cells (Lu *et al*., 2004; Wu *et al*., 2006; Thyagarajan *et al*., 2006; Sliva *et al*., 2002; Wang *et al*., 2007). Recently, the effect of treterpenoids such as lucidenic acids on induction of cell apoptosis and the apoptotic pathway in HL-60 cells were investigated. The results demonstrated that lucidenic acids decreased cell population growth of HL-60 cells, assessed with the MTT assay. The cell cycle assay indicated that treatment of HL-60 cells with lucidenic acid caused cell cycle arrest in the G1 phase (Chen *et al*., 2008). Of the our experiment results, compound **5** with 24(*S*),25,26-trihydroxy-5α-lanosta group in the chemical structure, showed the most potent inhibitory activity. In the accordance with previous results, this compound and other active components might play important role in the anti-cancer activity of *G. lucidum*.

Table 1. Cytotoxic activity of compounds **1** - **5**

Compound	IC_{50} (μg/mL)		
	HL-60	MCF-7	LLC
1	> 100	> 100	> 100
2	> 100	> 100	> 100
3	> 100	> 100	> 100
4	> 100	> 100	> 100
5	28.8 ± 1.5	22.9 ± 2.1	> 100
Adriamycin[a]	3.0 ± 0.4	2.4 ± 0.2	2.8 ± 0.3

[a] Positive control

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