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Cytotoxic Ergosterols from *Paecilomyces sp.* J300

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Seven ergosterol derivatives (**1~7**) were isolated from silkworm larvae infected with *Paecilomyces sp.* J300. On the basis of spectroscopic means, their structures have been elucidated as *3β,5α-dihydroxy-ergosta-7,22-diene* (**1**), *5α,6α-epoxy-(22E,24R)-ergosta-8(14), 22-diene-3β,7α-diol* (**2**), *5α,6α-epoxy-(22E,24R)-ergosta-8,22-diene-3β,7α-diol* (**3**), *ergosta-4,6,8(14),22-tetraene-3-one* (**4**), *ergosterol* (**5**), *ergosterol endoperoxide* (**6**), *3β,5α-dihydroxy-6β-methoxyergosta-7,22-diene* (**7**). Compounds **3~7** showed moderate cytotoxicity against five tumor cells.

Key words: *Paecilomyces sp.* J300, Silkworm larvae, Ergosterol derivatives, Cytotoxicity

INTRODUCTION

Insect larvae infected with fungus, *Cordyceps sp.* or *Paecilomyces sp.*, have been used in the Chinese traditional medicine to treat impotence, spermatorrhea and backache (Shanghai Science and Technologic Publisher and JungDam Publisher 1997; Pemberton 1999). Previous pharmacological study on fungus *Paecilomyces sp.* and *Cordyceps sp.* reported antitumor (Kinjo *et al*, 1996), immunomodulation (Shim *et al.* 2000), hypoglycemic activity (Shim *et al.*, 2000), antimicrobial (Rossi *et al.*, 1987) and antioxidative activity (Li *et al.* 2001). Leucinostatin (Rossi *et al.*, 1987), cyclopeptide (Bernardini *et al.* 1975), sphingofungin (Horn *et al.* 1992), benzanthracene (Yamashita *et al.* 1990), ergosterol (Nam *et al.* 2001) and galactomanan derivatives (Domenech *et al.* 1999) were reported from fungus *Paecilomyces sp.*. *Paecilomyces sp.* J300 was silkworm larvae inoculated by homogeneous fungi, *Paecilomyces* sp. J300 which was developed by National Institute of Agricultural Science and Technology in Korea. In the course of searching for bioactive compounds from this source, seven known ergosterols were isolated from hexane and chloroform soluble portion of methanolic extract. Their cytotoxic acitivities were evaluated by SRB bioassay against five cultured human tumor cells. Compounds **3**, **4**, **5**, **6** and **7** showed moderate cytotoxicity against five tumor cells. The present paper describes the isolation, structural characterization and cytotoxicity of these sterol derivatives.

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MATERIALS AND METHODS

General procedure

Mps: uncorr. NMR: in $CDCl_3$, Bruker AMX 500 and Varian UNITY INOVA 500. IR: Brucker Vector 22 FT-IR spectrophotometer. UV: Shimadzu UV-1601 UV-Visible Spectrophotometer. Polarimeter : JASCO P-1020. MS: JEOL JMS700 mass spectrometer (Japan). GC-MS: Hewlett-Packard 6890 GC (column : HP-5MS 30 m 0.25 mm) /Hewlett-Packard 5973 MSD system. Column Chromatography : Silica gel (Merck, 70230, 230400 mesh) and Sephadex LH-20 (Pharmacia). TLC: Merck precoated Si gel F_{254} plates and RP-18 F_{254s} plates. LPLC: Merck Lichroprep Lobar®-A Si 60 & Lobar®-A RP-18 (240× 10 mm).

Plant material

Paecilomyces sp. J300 was supplied by the National Institute of Agricultural Science and Technology, Suwon, Korea.

Extraction and purification

The dried and ground *Paecilomyces sp.* J300 (2.0 kg) were extracted with MeOH five times at room temperature, and then extracted five times at 60°C. The resultant methanol extract (200 g) was suspended in water and then successively partitioned to give hexane (110 g),

chloroform (6 g) and butanol (15 g) soluble fractions. The hexane extract (110 g) was subjected to silica gel column chromatography, eluted with a gradient solvent mixture of hexane/ethylacetate to give three fractions (H1~H3). H1 fraction (70 g) was then applied to a silica gel column chromatography using methylenechloride as eluent to afford three subfractions (H11~H13). H13 fraction (4.6 g) was further subjected to silica gel column chromatography using chloroform/methanol (20 : 1) as eluent to give three subfractions (H131~H133). Each subfraction was purified with Lobar® A Si-60 column to afford **1** (8 mg), **2** (10 mg) and **3** (6 mg), respectively. The Chloroform extract (6 g) was subjected to silica gel column chromatography, eluted with a gradient solvent mixture of hexane/ethylacetate/methanol to give seven fractions (C1~C7). C1 fraction (2 g) was then applied to a silica gel column chromatography using hexane/ethylacetate (1 : 1) as eluent to afford five subfractions (C11~C15). C11 fraction (46 mg) was subjected to silica gel column chromatography using methylenechloride as eluent to give **4** (10 mg). C12 fraction (1.2 g) was washed with acetone to afford **5** (670 mg). C13 fraction (310 mg) was subjected to silica gel column chromatography using chloroform/methanol (15 : 1) and Sephadex LH-20 column chromatography using methylenechloride/methanol (1 : 1) to afford **6** (36 mg). C14 fraction (53 mg) was subjected to silica gel column chromatography using chloroform/methanol (15 : 1) to afford **7** (6 mg), respectively.

3β,5α-Dihydroxy-ergosta-7,22-diene (1): White powder, mp. 230°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 0.61 (3H, s, H-18), 0.82 (3H, d, *J* = 7.0 Hz, H-26 or H-27), 0.83 (3H, d, *J* = 7.0 Hz, H-26 or H-27), 0.91 (3H, d, *J* = 7.0 Hz, H-28), 0.96 (3H, s, H-19), 1.03 (3H, d, *J*=6.5Hz, H-21), 2.56 (1H, m, H-5), 4.04 (1H, m, H-3), 5.16 (1H, dd, *J* = 16.0, 8.0 Hz, H-22), 5.31 (1H, *J* = 16.0, 7.5 Hz, H-23), 5.65 (1H, br.d, *J* = 2.0 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 13.38 (C-18), 17.13 (C-28), 18.26 (C-19), 20.33 (C-27), 20.63 (C-26), 21.79 (C-21), 22.66 (C-11), 23.17 (C-15), 28.52 (C-16), 30.95 (C-2), 31.07 (C-6), 33.75 (C-1), 37.24 (C-12), 39.55 (C-9), 40.94 (C-20), 41.15 (C-4), 43.51 (C-24), 44.62 (C-13), 45.48 (C-10), 56.50 (C-14), 56.77 (C-17), 68.53 (C-3), 78.55 (C-5), 120.39 (C-7), 133.20 (C-23), 135.69 (C-22), 139.29 (C-8).

5α,6α-Epoxy-(22*E*, 24*R*)-ergosta-8(14),22-diene-3β,7α-diol (2): White powder, mp. 123°C; $[\alpha]_D$ -75° (c 0.1, $CHCl_3$); EI-MS m/z (rel. int.) : 428 (M^+, 4), 410 (100), 392 (33), 377 (45), 285 (20), 267 (45); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 0.82 (3H, d, *J* = 7.0 Hz H-26), 0.84 (3H, d, *J* = 7.0 Hz, H-27), 0.86 (6H, s, H-18, H-19), 0.91 (3H, d, *J* = 6.5 Hz, H-28), 1.01 (3H, d, *J* = 7.0 Hz, H-21), 3.14 (1H, m, H-6), 4.01 (1H, m, H-3), 4.42 (1H, br.s, H-7), 5.19 (1H, dd, *J* = 16.0, 7.5 Hz, H-22), 5.21 (1H, dd, *J* = 16.0, 7.5 Hz, H-23); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 17.24 (C-19), 18.32 (C-18), 18.77 (C-28), 19.70 (C-11), 20.37 (C-26), 20.68 (C-27), 21.94 (C-21), 25.66 (C-15), 27.89 (C-16), 31.81 (C-2), 32.89 (C-1), 33.79 (C-25), 36.53 (C-10), 37.29 (C-12), 39.42 (C-9), 39.97 (C-4), 40.28 (C-20), 43.54 (C-24), 43.67 (C-13), 57.51 (C-17), 62.03 (C-6), 65.77 (C-7), 68.48 (C-5), 69.40 (C-3), 125.87 (C-8), 132.93 (C-23), 135.94 (C-22), 153.30 (C-14).

5α,6α-Epoxy-(22*E*, 24*R*)-ergosta-8,22-diene-3β,7α-diol (3): White powder, mp. 170°C; $[\alpha]_D$ -34° (c 0.1, $CHCl_3$); EI-MS m/z (rel. int.) : 428 (M^+, 10), 410 (100), 392 (32), 377 (63), 285 (50), 267 (55); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 0.58 (3H, s, H-18), 0.82 (3H, d, *J* = 7.0 Hz H-26), 0.83 (3H, d, *J* = 7.0 Hz, H-27), 0.91 (3H, d, *J* = 6.5 Hz, H-28), 1.02 (3H, d, *J* = 7.0 Hz, H-21), 1.14 (3H, s, H-19), 3.31 (1H, d, *J* = 2.5 Hz, H-6), 3.98 (1H, m, H-3), 4.42 (1H, br.d, *J* = 8.0 Hz, H-7), 5.16 (1H, dd, *J* = 16.0, 7.5 Hz, H-22), 5.21 (1H, dd, *J* = 16.0, 7.0 Hz, H-23); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 11.98 (C-19), 18.36 (C-18), 20.35 (C-28), 20.67 (C-11), 21.65 (C-26), 23.53 (C-27), 24.10 (C-21), 24.55 (C-15), 29.70 (C-16), 30.89 (C-2), 31.55 (C-1), 33.78 (C-25), 36.35 (C-10) 38.68 (C-12), 39.86 (C-14), 41.12 (C-4), 42.76 (C-20), 43.53 (C-24), 50.27 (C-13), 54.33(C-17), 63.27 (C-6), 66.32 (C-7), 67.82 (C-5), 69.31 (C-3), 127.62 (C-8), 132.69 (C-23), 135.16 (C-22), 136.24 (H-9).

Ergosta-4,6,8(14),22-tetraene-3-one (4): Yellowish crystal, mp. 114°C; UV λ_{max} (MeOH) : 350 nm; EI-MS m/z (rel. int.) : 392 (33), 377 (2), 363 (1), 349 (3), 337 (4), 319 (8), 268 (100), 253 (18), 240 (7), 214 (18), 173 (11), 149 (13), 136 (14), 122 (16), 97 (18); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 0.83 (3H, d, *J* = 7.0 Hz, H-26 or H-27), 0.85 (3H, d, *J* = 7.0 Hz, H-26 or H-27), 0.93 (3H, d, *J* = 6.5 Hz, H-28), 0.96 (3H, s, H-18), 0.99 (3H, s, H-19), 1.06 (3H, d, *J* = 7.0 Hz, H-21), 5.22 (2H, dd, *J* = 15.5, 7.5 Hz, H-22), 5.27 (1H, dd, *J* = 15.5, 7.0 Hz, H-23), 6.03 (1H, d, *J* = 9.5 Hz, H-6), 6.60 (1H, d, *J* = 9.5 Hz, H-7); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 17.35 (C-19), 18.32 (C-28), 19.66 (C-2), 19.69 (C-18), 20.35 (C-26), 20.67 (C-27), 21.92 (C-21), 26.07 (C-11), 28.40 (C-16), 33.79 (C-25), 34.86 (C-1 and C-12), 36.31 (C-15), 37.47 (C-10), 39.95 (C-20), 43.58 (C-24), 44.70 (C-13), 45.06 (C-9), 56.42 (C-17), 123.71 (C-4), 125.17 (C-6 and C-8), 133.25 (C-23), 134.67 (C-7), 135.70 (C-22), 156.74 (C-14), 165.02 (C-5), 200.13 (C-3).

Ergosterol (5): White powder, mp. 150°C, EI-MS m/z (rel. int.) : δ 396 (M^+, 58), 378 (5), 363 (100), 337 (22), 314 (5), 271 (30), 253 (64), 239 (10), 211 (41); ^{1}H-NMR ($CDCl_3$, 500 MHz): 0.63 (3H, s, H-18), 0.82 (3H, d, *J* = 6.5 Hz, H-26 or H-27), 0.84 (3H, d, *J* = 6.5 Hz, H-26 or H-27), 0.92 (3H, d, *J* = 7.0 Hz, H-28), 0.95 (3H, s, H-19), 1.04 (3H, d,

J = 7.0 Hz, H-21), 3.65 (1H, m, H-3), 5.20 (2H, m, J = 15.5, 7.5 Hz, H-22), 5.22 (1H, dd, J = 15.5, 7.0 Hz, H-23), 5.38 (1H, m, H-7), 5.57 (1H, m, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 12.75 (C-18), 16.98 (C-19), 18.31 (C-28), 20.35 (C-21), 20.66 (C-26), 21.81 (C-11, 15), 23.70 (C-27), 28.99 (C-12), 32.69 (C-2), 33.79 (C-25), 37.73 (C-10), 39.07 (C-1), 39.78 (C-16), 41.13 (C-20), 41.49 (C-4), 43.52 (C-24, C-13), 46.94 (C-9), 55.26, 56.42 (C-17, C-14), 71.16 (C-3), 116.98 (C-7), 120.28 (C-6), 132.67 (C-23), 136.26 (C-22), 140.78 (C-8), 142.07 (C-5).

Ergosterol endoperoxide (6): White powder, mp. 182°C, EI-MS (m/z) : δ 428 (M^+, 2), 410 (4), 396 (30), 337 (12), 285 (4), 251 (14), 211 (8), 157 (16), 119 (20), 81 (52), 69 (100); ^{1}H-NMR ($CDCl_3$, 500 MHz) : 0.81-0.84 (9H, m, H-18, H-27, H-28), 0.89 (3H, s, H-19), 0.91 (3H, d, J = 7.0 Hz, H-28), 1.00 (3H, d, J = 6.5 Hz, H-21), 3.97 (1H, m, H-3), 5.13 (1H, dd, J = 15.0, 8.5 Hz, H-22), 5.22 (1H, dd, J = 15.0, 8.0 Hz, H-23), 6.24 (1H, d, J = 8.5 Hz, H-7), 6.50 (1H, d, J = 8.5 Hz, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 12.98 (C-18), 17.55 (C-28), 18.16 (C-19), 19.63 (C-26), 19.93 (C-27), 20.63 (C-15), 20.88 (C-21), 23.41 (C-11), 28.62 (C-16), 30.16 (C-2), 33.07 (C-25), 34.72 (C-1), 36.97 (C-4, C-10), 39.37 (C-12), 39.69 (C-20), 42.78 (C-24), 44.57 (C-13), 51.14 (C-9), 51.71 (C-14), 56.25 (C-17), 66.47 (C-3), 79.41 (C-8), 82.14 (C-5), 130.76 (C-7), 132.34 (C-23), 135.20 (C-22), 135.41 (C-6).

3β, 5α-Dihydroxy-6β-methoxyergosta-7, 22-diene (7): White powder, mp. 175°C; $[\alpha]_D$ -54° (c 0.08, $CHCl_3$); EI-MS m/z (rel. int.) : 444 (M^+, 2), 426 (100), 411 (28), 393 (64), 377 (39), 337 (9), 269 (14), 251 (30), 225 (8), 209 (7), 197 (10); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 0.52 (3H, s, H-18), 0.75 (3H, d, J = 7.0 Hz, H-26 or H-27), 0.76 (3H, d, J = 7.0 Hz, H-26 or H-27), 0.84 (3H, d, J = 6.5 Hz, H-28), 0.93 (3H, s, H-19), 0.95 (3H, d, J = 6.5 Hz, H-21), 3.09 (1H, br.d, J = 4.5 Hz, H-6), 3.30 (3H, s, OMe), 3.98 (1H, m, H-3), 5.10 (2H, m, J = 16.0, 8.0 Hz, H-22), 5.15 (1H, dd, J = 16.0, 7.5 Hz, H-23), 5.33 (1H, m, H-7); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 13.01 (C-18), 18.30 (C-28), 19.05 (C-19), 20.35 (C-21), 20.66 (C-26), 21.81 (C-27), 22.86 (C-11), 23.58 (C-15), 28.64 (C-16), 31.58 (C-2), 33.45 (C-1), 33.78 (C-2), 37.95 (C-10), 40.05 (C-4), 40.28 (C-12), 41.00 (C-20), 43.52 (C-24), 44.55 (C-9 or 13), 44.57 (C-13 or 9), 55.65 (C-14), 56.68 (C-17), 59.01 (OCH_3), 68.53 (C-3), 77.03 (C-5), 83.14 (C-6), 115.67 (C-7), 132.80 (C-23), 136.14 (C-22), 144.35 (C-8).

Test for cytotoxicity *in vitro* SRB bioassay was used as a cytotoxicity screening method. Activities of each compound were monitored at several concentration levels against five cultured human tumor cells; A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon) *in vitro* (Skehan *et al.* 1990).

RESULTS AND DISCUSSION

Compound **1** was obtained as amorphous powder. The ^{1}H-NMR spectrum showed six methyl signals at δ 0.61 (3H, s), 0.82 (3H, d, J = 7.0 Hz), 0.83 (3H, d, J = 7.0 Hz), 0.91 (3H, d, J = 7.0 Hz), 0.96 (3H, s) and 1.03 (3H, d, J = 6.5 Hz), an oxygenated proton signal at δ 4.04 (1H, m) and olefinic proton signals at δ 5.16 (1H, dd, J = 16.0, 8.0 Hz), 5.31 (1H, dd, J = 16.0, 7.5 Hz) and 5.65 (1H, br.d). The ^{13}C-NMR spectrum showed total 28 carbon signals contained four olefinic carbons at δ 120.39, 133.20, 135.69 and 139.29 and two oxygenated carbon signals at δ 68.53 and 78.55. The spectral data of **1** were very similar to those of (22*E*)-5α-ergosta-7,22-dien-3β-ol (Goad and Akihisa

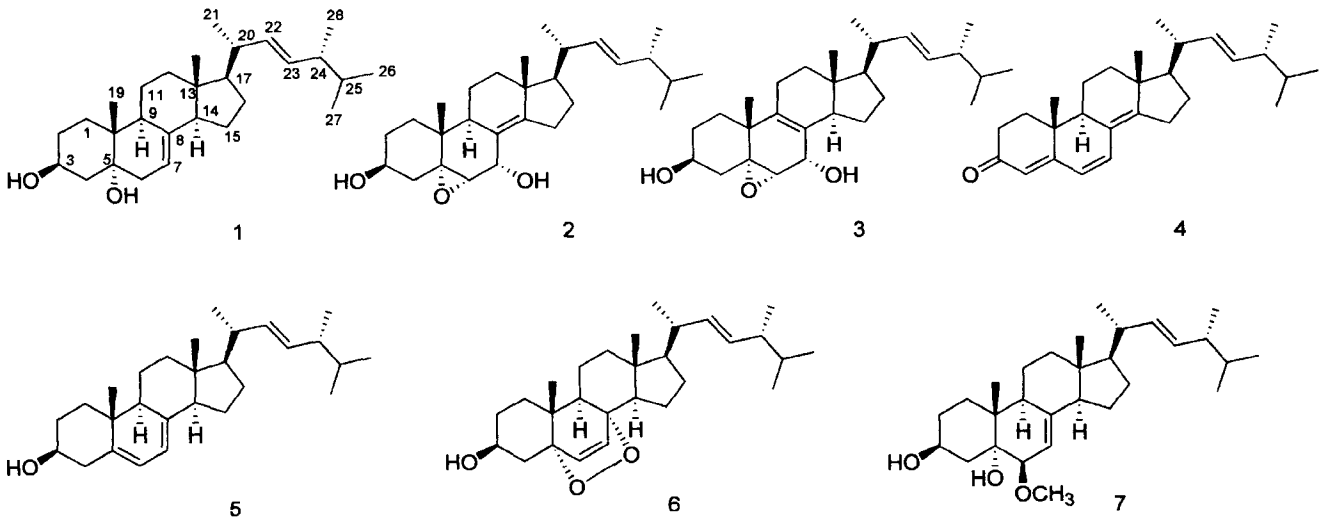

Fig. 1. Structures of compounds **1~7** isolated from *Paecilomyces sp.* J300.

1997). The major difference was the chemical shift of C-5 signal in the ^{13}C-NMR spectrum [**1**: δ 78.55, (22*E*)-5α-ergosta-7,22-dien-3β-ol; δ 40.08], indicated hydroxyl group bonded at C-3 and C-5 in **1**. The structure of **1**, therefore, was determined as (22*E*)-3β, 5α-dihydroxy-ergosta-7, 22-diene. The ^{1}H-NMR data and physicochemical properties were same with those in previous literature (Goldstein 1996).

Compound **2** was obtained as colorless amorphous powder. The ^{1}H-NMR spectrum showed two methyl singlets at δ 0.86 (6H, s), four methyl doublets at δ 0.82 (3H, d, *J* = 7.0 Hz), 0.84 (3H, d, *J* = 7.0 Hz), 0.91 (3H, d, *J* = 6.5 Hz) and 1.01 (3H, d, *J* = 7.0 Hz), four oxygenated proton signals at δ 3.14 (1H, m), 4.01 (1H, m) and 4.42 (1H, br.s) and two olefinic proton signals at δ 5.19 (1H, dd, *J* = 16.0, 7.5 Hz) and 5.21 (1H, dd, *J* = 16.0, 7.5 Hz). The ^{13}C-NMR spectrum showed total 28 carbon signals contained four olefinic carbon signals at δ 125.87, 132.93, 135.94 and 153.30 and four oxygenated carbon signals at δ 62.03, 65.77, 68.48 and 69.40. The spectral data of **2** were similar with those of **1**, **7** and **9**, indicated **2** was ergostane type sterol. Signals at δ 62.03 and 68.48 in the ^{13}C-NMR spectrum suggested the presence of an epoxy ring. On the basis of above evidences, the structure of **2** was determined as 5α,6α-epoxy-(22*E*, 24*R*)-ergosta-8(14),22-diene-3β,7α-diol (Greca *et al.* 1993).

The spectral data of compound **3** were very similar to those of **2.** The major difference was olefinic carbon signals at δ 127.62 and 136.24 in the ^{13}C-NMR spectrum, indicated the position of double bond at C-8 and C-9. The structure of **2**, therefore, was determined as 5α,6α-epoxy-(22*E*, 24*R*)-ergosta-8,22-diene-3β,7α-diol (Yue *et al.*, 2001).

Compound **4** was yellowish crystal with λ_{max} 350 nm in UV spectrum. The ^{1}H-NMR spectrum showed two methyl singlets at δ 0.96 and 0.99, four methyl doublets at δ 0.83 (3H, d, *J* = 7.0 Hz), 0.85 (3H, d, *J* = 7.0 Hz), 0.93 (3H, d, *J* = 6.5 Hz) and 1.06 (3H, d, *J* = 7.0 Hz), and four olefinic proton signals at δ 5.22 (2H, m, *J* = 15.5, 7.5 Hz), 5.27 (1H, dd, *J* = 15.5, 7.0 Hz), 6.03 (1H, d, *J* = 9.5 Hz) and 6.60 (1H, d, *J* = 9.5 Hz). The ^{13}C-NMR spectrum showed 28 carbon signals contained eight olefinic carbon signals δ 123.71, 125.17 (×2), 133.25, 134.67, 135.70, 156.74 and 165.02 and a ketone signal at δ 200.13. On the basis of aboved evidences, the structure of **4** was determined as ergosta-4,6,8(14),22-tetraene-3-one (Schulte *et al.* 1968; Jinming *et al.* 2001).

Compound **5** was obtained as amorphous powder. Molecular ion peak at m/z 396 in EI-MS and ^{13}C-NMR spectrum (28 C) suggested the molecular formular to be $C_{28}H_{44}O$. The ^{1}H-NMR spectrum showed six methyl signals at δ 0.63 (3H, s), 0.82 (3H, d, *J* = 6.5 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.92 (3H, d, *J* = 7.0 Hz), 0.95 (3H, s) and 1.04 (3H, d, *J* = 7.0 Hz), a carbinol proton signal at δ 3.65 (1H, m) and four olefinic proton signals at δ 5.20 (1H, dd, *J* = 15.5, 7.5 Hz), 5.22 (1H, dd, *J* = 15.5, 7.0 Hz), 5.38 (1H, m) and 5.57 (1H, m). The ^{13}C-NMR spectrum showed 28 carbon signals contained six olefinic carbon signal at δ 116.98, 120.28, 132.67, 136.26, 140.78 and 142.07 and oxygenated carbon signal at δ 71.16. The spectral and physicochemical data of **5** led to the structure of ergosterol (Goad and Akihisa 1997).

Compound **6** was obtained as amorphous powder and was positive in peroxide reagent (Lee 1991). From the EI-MS and ^{13}C-NMR (28C) spectra, the molecular formula was deduced to be $C_{28}H_{44}O_3$. The ^{1}H-NMR spectrum showed three methyl signals at δ 0.81~0.83, a methyl singlet at 0.89 (3H, s), two methyl doublets at δ 0.91 (3H, d, *J* = 7.0 Hz) and δ 1.00 (3H, d, *J* = 6.5 Hz), a carbonyl proton signal at δ 3.97 (1H, m) and four olefinic proton signals at δ 5.13 (1H, dd, *J* = 15.0, 8.5 Hz), 5.22 (1H, dd, *J* = 15.0, 8.0 Hz), 6.24 (1H, d, *J* = 8.5 Hz) and 6.50 (1H, d, *J* = 8.5 Hz). The ^{13}C-NMR spectrum showed 28 carbon signals contained four carbon signals at δ 135.20, 132.34, 135.41 and 130.76, a carbinol carbon signal at δ 66.50 and two adjacent carbon to peroxy group at δ 82.14 and 79.41. On the basis of above evidences, the structure of **6** was determined as ergosterol endoperoxide (Kwon *et al.* 1999).

Compound **7** was obtained as amorphous powder and EI-MS spectrum showed a molecular ion peak at m/z 444. From the EI-MS and ^{13}C-NMR (29C) spectra, the molecular formula was deduced to be $C_{29}H_{48}O$. The ^{1}H-NMR spectrum showed six methyl signals at δ 0.52 (3H, s), 0.75 (3H, d, *J* = 7.0 Hz), 0.76 (3H, d, *J* = 7.0 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.93 (3H, s) and 0.95 (3H, d, *J* = 6.5 Hz), two oxygenated proton signals at 3.09 (1H, br.d, *J* = 4.5 Hz), and 3.98 (1H, m), a methoxy group at δ 3.30 (3H, s) and three olefinic proton signals at δ 5.10 (1H, dd, *J* = 16.0, 8.0 Hz), 5.15 (1H, dd, *J* = 16.0, 7.5 Hz) and 5.33 (1H, m). The ^{13}C-NMR spectrum showed 29 carbon

Table I. Cytotoxicity of Compounds **1~7**

	EC_{50} values* (μM)				
Compounds	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	>30.0	>30.0	>30.0	>30.0	>30.0
2	>30.0	>30.0	>30.0	>30.0	14.26
3	23.17	20.86	20.41	18.04	>30.0
4	27.66	23.09	23.43	34.71	23.07
5	23.11	12.03	12.36	18.90	16.04
6	15.81	11.93	9.76	13.61	12.24
7	12.26	12.30	12.46	13.41	12.33

*EC_{50} value of compound against each cancer cell line, which was defined as a concentration (μM) that caused 50% inhibition of cell growth *in vitro*

signals contained four olefinic carbon signals at δ 115.67, 132.80, 136.14 and 144.35, three oxygenated carbon signals at δ 68.53, 77.03 and 83.14 and a methoxy group at δ 59.01. On the basis of the above data, the structure of **7** was determined as 3β, 5α-dihydroxy-6β-methoxyergosta-7, 22-diene (Kawagishi *et al.* 1988).

Compounds (**1~7**) were tested for their cytotoxicity against human tumor cell lines (Table I). Compounds **3~7** showed non-specific moderate cytotoxicities against tested human cancer cells Compounds **1** and **2** were of little activity against the tested five human cancer cell lines.

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