Sterols from the Stems of *Momordica charantia*

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A new sterol, 5α,6α-epoxy-3β-hydroxy-(22*E*,24*R*)-ergosta-8,22-dien-7-one (**1**), together with eight known sterols, 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7α-diol (**2**), 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β-diol (**3**), 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7α-diol (**4**), 3β-hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one (**5**), ergosterol peroxide (**6**), clerosterol (**7**), decortinol (**8**), and decortinone (**9**), were isolated from the stems of *Momordica charantia*. Their structures were elucidated by mean of extensive spectroscopic methods, including ^{1}H, ^{13}C, 2D-NMR and HR-EI-MS, as well as comparison with the literature data. Compounds **1**, **4**, **5**, **8**, and **9** were not cytotoxic against the SK-Hep 1 cell line.

Keywords: *Momordica charantia*; Cucurbitaceae; Stem; Sterol; Ergostane.

INTRODUCTION

Momordica charantia L. (Cucurbitaceae), a slender-stemmed tendril climber, is distributed in Asian countries and widely cultivated as a vegetable crop. Tissues of this plant have extensively been used in folk medicine as a remedy for diabetes in Asian. Previous pharmacological studies have showed that the extracts or compounds of tissues of *M. charantia* possess anti-diabetic and anti-inflammatory activities.[1-3] Chemical investigations of *M. charantia* also revealed that more than seventy cucurbitane-type triterpenes have been isolated form the fruits,[3-12] seeds,[13,14] root,[15] and leaves and vines[16,17] of *M. charantia*. As part of our program aimed at the discovery of bioactive secondary metabolites from Taiwanese *M. charantia*, we had reported the isolation and structure elucidation of twenty-five cucurbitane-type triterpenoids from the MeOH extract of the stems of this plant.[18-22]

In the continuing phytochemical investigation on *M. charantia*, we further identified one new ergostane sterol, 5α,6α-epoxy-3β-hydroxy-(22*E*,24*R*)-ergosta-8,22-dien-7-one (**1**), along with eight known sterols, 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7α-diol (**2**),[23] 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β-diol (**3**),[24] 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7α-diol (**4**),[23] 3β-hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one (**5**),[25] ergosterol peroxide (**6**),[26] clerosterol (**7**),[27,28] decortinol (**8**),[27] and decortinone (**9**).[27] We describe in this paper the extraction, isolation, purification, and structure elucidation of compound **1** and the cytotoxic activity of compounds **1**, **4**, **5**, **8**, and **9**. All the known compounds except clerosterol (**7**) are isolated for the first time from the title plant.

RESULTS AND DISCUSSION

The MeOH extract of the stems of *M. charantia* was suspended in water and partitioned with EtOAc and *n*-BuOH, sequentially. The combined EtOAc soluble layer was subjected to repeated silica gel column chromatography and further purification by semi-preparative HPLC to yield compounds **1**-**9**. The eight known compounds (**2**-**9**) were identified by comparing their physical and spectral data with the reported values. The structure of the new

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Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** in $CDCl_3$ (δ, ppm)

Position	δ_C [b]	δ_H [a]	Position	δ_C [b]	δ_H [a]
1	30.4 t	1.70 m, 1.85 m	15	24.3 t	1.82 m, 1.98 m
2	30.6 t	2.02 m	16	29.5 t	1.26 m, 1.34 m
3	68.6 d	3.95 m	17	53.3 d	1.12 m
4	38.2 t	1.51 m, 2.24 m	18	11.6 q	0.56 s
5	64.5 s		19	24.1 q	1.23 s
6	62.5 d	3.27 s	20	40.4 d	2.00 m
7	196.3 s		21	21.1 q	1.00 d (6.8) [c]
8	128.8 s		22	135.3 d	5.13 m
9	158.0 s		23	132.2 d	5.20 m
10	40.5 s		24	42.8 d	1.82 m
11	25.2 t	2.24 m	25	33.0 d	1.44 m
12	35.6 t	1.44 m, 1.98 m	26	19.9 q	0.81 d (6.4)
13	42.1 s		27	19.6 q	0.79 d (6.4)
14	48.8 d	2.12 m	28	17.6 q	0.89 d (6.8)

Spectra recorded at [a] 400 MHz and [b] 100 MHz in $CDCl_3$ at 25 °C. [c] Coupling constants are presented in Hz

sterol (**1**) was elucidated as follows.

Compound **1** was obtained as amorphous white powder. The HR-EI-MS spectrum showed an $[M-H_2O]^+$ ion at *m/z* 408.2999 corresponding to a dehydrated molecular formula of $C_{28}H_{40}O_2$, which indicated seven degrees of unsaturation in **1**. A significant UV absorption maximum at 257 nm suggested the presence of an α,β-unsaturated enone. The IR spectrum showed bands attributable to hydroxyl group (3369 cm^{-1}), double-bond (3051, 1656, 970 cm^{-1}), and conjugated ketone (1656 cm^{-1}) functionalities. The ^{1}H-NMR spectrum of **1** (Table 1), obtained with the aid of a ^{1}H-^{1}H shift correlation spectroscopy (^{1}H-^{1}H COSY) spectrum, showed signals of two methyl singlets [δ_H 0.56 (3H, s, Me-18), and 1.23 (3H, s, Me-19)], four methyl doublets [δ_H 0.79 (3H, d, J = 6.4 Hz, Me-27), 0.81 (3H, d, J = 6.4 Hz, Me-26), 0.89 (3H, d, J = 6.8 Hz, Me-28), and 1.00 (3H, d, J = 6.8 Hz, Me-21)], suggesting that **1** should be an ergostane-type sterol.[19] The ^{1}H-NMR spectrum indicated a trisubstituted epoxide-bearing methine proton [δ_H 3.27 (1H, s, H-6)], a hydroxyl-bearing methine proton [δ_H 3.95 (1H, m, H-3)] and two olefinic protons [δ_H 5.13 (1H, m, H-22), δ_H 5.20 (1H, m, H-23)]. 28 carbon signals were observed in the ^{13}C-NMR spectrum of **1** (Table 1) and were assigned by DEPT experiments as six methyl, seven methylene, five methine, two quaternary, four olefinic, two tertiary and one quaternary oxygenated, and one conjugated ketone carbonyl carbons. The above data included a fully substituted double bond [δ_C 128.8 (s), δ_C 158.0 (s)], a disubstituted double bond [δ_C 132.2 (d), δ_C 135.3 (d)] and one conjugated ketone carbonyl [δ_C 196.3 (s)] carbons. Comparison of these ^{1}H- and ^{13}C-NMR spectral data of **1** with those of 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7α-diol (**2**)[19] revealed that both compounds exhibited identical structural fragments in rings A, C, and D and the side chain. The only difference was observed that the NMR signals of oxgenated methine at C-7 in **2** [δ_H 4.19 (1H, brs, H-7); δ_C 67.0 (d)] disappeared and were replaced by a conjugated ketone carbonyl [δ_C 196.3 (s)] signal in **1**. Thus, compound **1** was tentatively proposed to be an oxidized derivative of **2**. The HMBC spectrum of **1** showed long-range correlations between H-6 (δ_H 3.27)/C-7

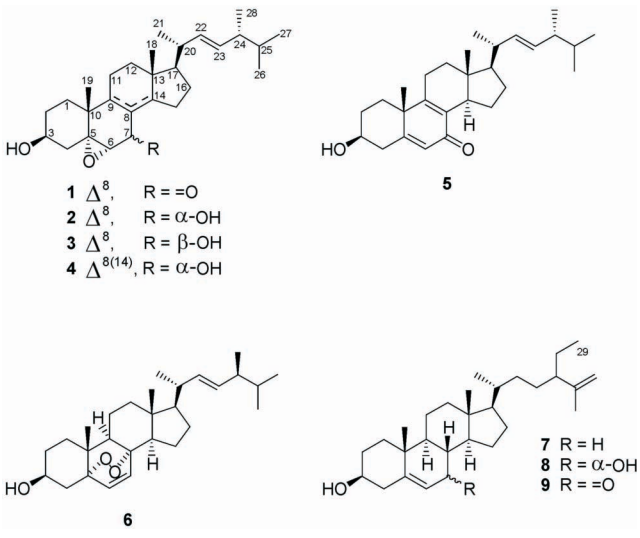

Fig. 1. Structures of compounds **1-9** from *M. charantia*.

(δ_C 196.3), H-11 (δ_H 2.24)/C-9 (δ_C 158.0), and H-14 (δ_H 2.12)/C-8 (δ_C 128.8) (Fig. 2), which permitted us to confirm the 8(9)-en-7-one moiety. The HMBC correlations between H-1 (δ_H 1.70, 1.85)/C-3 (δ_C 68.6), H-2 (δ_H 2.02)/C-3, and H-4 (δ_H 1.51, 2.24)/C-3 also supported that the hydroxyl group was attached on C-3. In turn, the 5,6-epoxide was determined by the HMBC correlations between H-6/C-4 (δ_C 38.2), H-6/C-5 (δ_C 64.5), H-6/C-7 (δ_C 196.3), and H-6/C-8 (δ_C 128.8). Moreover, the structure of mono-unsaturated C_9 side chain was identified by the HMBC correlations between Me-21 (δ_H 1.00)/C-17 (δ_C 53.3), C-20 (δ_C 40.4), and C-22 (δ_C 135.3); H-22 (δ_H 5.13)/C-17, C-20, C-21 (δ_C 21.1), and C-24 (δ_C 42.8); Me-28 (δ_H 0.89)/C-23 (δ_C 132.2), C-24, and C-25 (δ_C 33.0), as well as the EI-MS fragment ion at *m/z* 301 $[M\text{-}C_9H_{17}$ (side chain)$]^+$, and 283 $[M\text{-}H_2O$-side chain$]^+$. The relative configurations of C-atoms in the tetracyclic rings were determined by significant NOE correlations between H-3 (δ_H 3.95)/H_α-1 (δ_H 1.70), and H_α-2 (δ_H 2.02); Me-18 (δ_H 0.56)/H_β-11 (δ_H 2.24), H_β-12 (δ_H 1.98), H_β-15 (δ_H 1.98), H-20 (δ_H 2.00), and Me-21 (δ_H 1.00); and between Me-19 (δ_H 1.23)/H_β-1 (δ_H 1.85), H_β-4 (δ_H 2.24), H-6 (δ_H 3.27), and H_β-11 (δ_H 2.24) in the NOESY spectrum (Fig. 3). The above NOE correlations implies that the ring A of **1** adopts boat-type conformation, as a result of incorporation of the 5α,6α-epoxide and α,β-unsaturated enone moieties. Thus, compound **1** was elucidated as 5α,6α-epoxy-3β-hydroxy-(22*E*,24*R*)-ergosta-8,22-dien-7-one.

Compounds **1**, **4**, **5**, **8**, and **9** were evaluated for their cytotoxic activity against human hepatoma SK-Hep-1 cells with 5-FU (Fluorouracil) as positive control (IC_{50} = 1.0 μM). After treatment for 48 h, compounds **1**, **4**, **5**, **8**, and **9** showed no cytotoxic activity against the SK-Hep 1 cell line with IC_{50} of values 27.7, 36.1, 31.1, 99.8, and 41.4 μM, respectively.

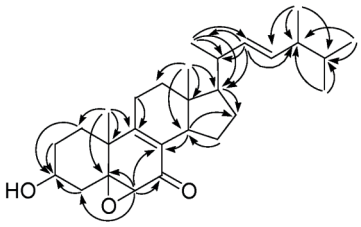

Fig. 2. Selected HMBC correlations of **1**.

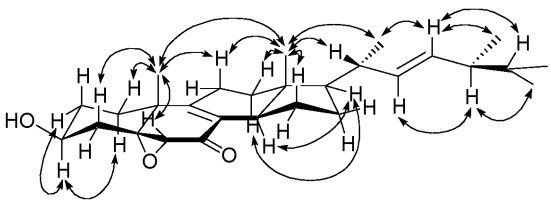

Fig. 3. Selected NOESY correlations of **1**.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were measured using a JASCO DIP-180 digital spectropolarimeter. UV spectra were measured in MeOH on a Shimadzu UV-1601PC spectrophotometer. The IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. The NMR spectra were recorded in $CDCl_3$ at room temperature on a Varian Mercury plus 400 NMR spectrometer, and the solvent resonance was used as internal shift reference (TMS as standard). The 2D NMR spectra were recorded using standard pulse sequences. EI-MS and HR-EI-MS were recorded on a Finnigan TSQ-700 and a JEOL SX-102A spectrometer, respectively. TLC was performed using silica gel 60 F_{254} plates (200 μm, Merck). HPLC was performed using a Lichrosorb Si 60 (5 μm) column (250 × 10 mm).

Plant Material

The stems of *Momordica charantia* was collected in Ping-Tung, Taiwan, in July, 2003. The plant material was identified by Prof. Sheng-Zehn Yang, a professor of the Department of Forestry, National Pingtung University of Science and Technology. A voucher specimen (no. 2013) has been deposited at the Herbarium of the Department of Forestry, National Pingtung University of Science and Technology, Pingtung, Taiwan, R.O.C.

Extraction and Isolation

Air-dried pieces of the stems of *M. charantia* (18 kg) were extracted three times with methanol (30 L) at room temperature (7 days each time). The MeOH extract was evaporated *in vacuo* to leave a black residue, which was suspended in H_2O (3 L), and then partitioned sequentially using EtOAc and *n*-BuOH. (2L × 3). The EtOAc fraction (386 g) was chromatographed on silica gel using *n*-hexane and EtOAc mixture of increasing polarity as eluent to obtain 11 fractions: fr. 1 [5000 mL, *n*-hexane], fr. 2 [4000 mL, *n*-hexane/EtOAc (49/1)], fr. 3 [4000 mL, *n*-hexane/EtOAc (19/1)], fr. 4 [4000 mL, *n*-hexane/EtOAc (9/1)], fr. 5 [4000 mL, *n*-hexane/EtOAc (17/3)], fr. 6 [4000 mL, *n*-hexane/EtOAc (8/2)], fr. 7 [4000 mL, *n*-hexane/EtOAc (7/3)], fr. 8 [3000 mL, *n*-hexane/EtOAc (5/5)], fr. 9 [3000 mL, *n*-hexane/EtOAc (4/6)], fr. 10 [(3000 mL, *n*-hexane/EtOAc (2/8)), fr. 11 (6000 mL, EtOAc). Fraction 7 was further

chromatographed on a silica gel column (5 × 45 cm, Merck 230-400 mesh), eluted with CH_2Cl_2-EtOAc (8/1 to 0/1) to resolve into seven fractions (each about 600 mL), 7A-7G. Fraction 7B was separated by HPLC on a Merck Lichrosorb Si 60 column (5 μm, 250 × 10 mn) with *n*-hexane/acetone (15/1) as eluent, 2 mL/min, to yield **1** (5 mg, t_R = 27.4 min), **6** (18 mg, t_R = 31.1 min), and **7** (13 mg, t_R = 39.4 min). Fraction 8 was further purified through a silica gel column (5 × 45 cm), eluted with CH_2Cl_2-EtOAc (7/1) to obtain six fractions (each about 500 mL), 8A-8F. Fraction 8B was separated by HPLC on a Merck Lichrosorb Si 60 column (5 μm, 250 × 10 mn) with *n*-hexane/acetone (7/3) as eluent, 2 mL/min, to yield **4** (7 mg, t_R = 26.3 min), **8** (15 mg, t_R = 30.2 min), and **9** (31 mg, t_R = 33.2 min). Fraction 9 was further chromatographed on a silica gel column (5 × 45 cm, Merck 230-400 mesh) and eluted with CH_2Cl_2/MeOH (50/1) to obtain 8 fractions, 9A-9H. HPLC of fraction 9C on a Merck Lichrosorb Si 60 column (5 μm, 250 × 10 mn) with *n*-hexane/acetone (7/3) as eluent, 2 mL/min, yield **2** (9 mg, t_R = 31.5 min), **3** (11 mg, t_R = 33.2 min), and **5** (16 mg, t_R = 39.4 min).

Cytotoxicity assay

The cytotoxicity of compounds **1**, **4**, **5**, **8**, and **9** were evaluated by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method based on the described procedures.[29] SK-Hep 1 cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, L-glutamine 2 mM, 1% penicillin/streptomycin (penicillin 10000 U/mL and streptomycin 10 mg/mL) in a humidified atmosphere of 5% CO_2 at 37 °C. A volume of SK-Hep 1 cells 100 μL at a density of 1×10^5 cells/mL was incubated under the same conditions for 24 h in a 96-well flat-bottomed microplate. Test samples dissolved in DMSO were added to the cultures. After a 48 h incubation, the wells were incubated with the MTT (100 μL/well concentrated at 5 mg/mL) at 37 °C for 4 h. 200 μL of DMSO was added to redissolve the formazan crystals after removing the supernatant. The absorbance of the resulting formazan was measured by an enzyme-linked immunosorbent assay plate reader at 550 nm. The results were assayed in triplicate. The ratio of cell viability (%) was calculated by using the following formula: [(experimental absorbance - background absorbance)/(control absorbance - background absorbance)] × 100. The activity is shown as IC_{50} value, which the concentration (μM) of the tested sample that results in 50% inhibition of cell growth.

5α,6α-epoxy-3β-hydroxy-(22*E*,24*R*)-ergosta-8,22-dien-7-one (1)

Amorphous white powder; $[\alpha]_D^{24}$ -10.8° (*c* 0.46, $CHCl_3$); IR (KBr) ν_{max}: 3369, 3051, 2966, 2933, 2877, 1656, 1376, 1271, 1181, 1084, 1059, 970, 796 cm^{-1}; UV (MeOH) λ_{max} (log ε) 257 (3.86) nm ^1H and ^{13}C NMR data, see Table 1; EI-MS *m/z* 426 $[M]^+$ (15), 408 (26), 392 (47), 380 (38), 283 (24), 267 (45), 251 (41), 237 (32), 212 (69), 198 (93), 173 (100), 162 (72), 137 (94), 121 (85), 115 (74), 109 (61), 91 (97), 83 (43), 69 (43), 55 (48); HR-EI-MS *m/z* $[M-H_2O]^+$ 408.2999 (calcd for $C_{28}H_{40}O_2$ 408.3029).

5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7α-diol (2)

Amorphous white powder; $[\alpha]_D^{24}$ -114.3° (*c* 0.04, $CHCl_3$); Lit: $[\alpha]_D^{25}$ -113.0° (*c* 0.9, $CHCl_3$);[30] IR (KBr) ν_{max}: 3364, 2931, 2872, 1654, 1455, 1377, 1260, 1168, 1070, 915, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.55 (3H, s, H-18), 0.78 (3H, d, *J* = 6.4 Hz, H-27), 0.80 (3H, d, *J* = 6.4 Hz, H-26), 0.88 (3H, d, *J* = 6.8 Hz, H-28), 0.98 (3H, d, *J* = 6.4 Hz, H-21), 1.10 (3H, s, H-19), 3.28 (1H, d, *J* = 3.2 Hz, H-6), 3.91 (1H, m, H-3), 4.19 (1H, brs, H-7), 5.14 (1H, m, H-22), 5.18 (1H, m, H-23); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.2 q (C-18), 17.6 q (C-28), 19.6 q (C-27), 19.9 q (C-26), 20.9 q (C-21), 22.8 q (C-19), 23.4 t (C-15), 23.8 t (C-11), 29.0 t (C-16), 30.1 t (C-1), 30.7 t (C-2), 33.0 d (C-25), 35.6 t (C-12), 37.9 s (C-10), 39.1 t (C-4), 40.4 d (C-20), 42.0 s (C-13), 42.8 d (C-24), 49.5 d (C-14), 53.6 d (C-17), 62.6 d (C-6), 65.7 s (C-5), 67.0 d (C-7), 68.4 d (C-3), 126.9 s (C-8), 131.9 d (C-23), 134.4 s (C-9), 135.5 d (C-22); EI-MS (70 eV) *m/z* 428 (4), 410 $[M-H_2O]^+$ (100), 392 (24), 377 (76), 284 (30), 239 (28), 225 (23), 213 (33), 185 (24), 91 (24), 81 (24), 69 (38), 55 (24).

5α,6α-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β-diol (3)

Amorphous white powder; $[\alpha]_D^{24}$ -40.9° (*c* 0.09, $CHCl_3$); Lit: $[\alpha]_D^{26}$ -37.0° (*c* 0.05, $CHCl_3$);[24] IR (KBr) ν_{max}: 3393, 2960, 2931, 2872, 1732, 1606, 1460, 1382, 1129, 1090, 1051, 856, 783, 637 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.61 (3H, s, H-18), 0.79 (3H, d, *J* = 6.4 Hz, H-27), 0.81 (3H, d, *J* = 6.4 Hz, H-26), 0.88 (3H, d, *J* = 6.8 Hz, H-28), 0.99 (3H, d, *J* = 6.4 Hz, H-21), 1.25 (3H, s, H-19), 3.12 (1H, d, *J* = 3.2 Hz, H-6), 3.90 (1H, m, H-3), 4.36 (1H, brs, H-7), 5.12 (1H, m, H-22), 5.21 (1H, m, H-23); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.5 q (C-18), 17.6 q (C-28), 19.6 q (C-27), 19.9 q (C-26), 20.9 q (C-21), 22.8 t (C-11), 23.0 t (C-15), 23.6 q (C-19), 29.2 t (C-16), 30.8 t (C-1), 30.8 t (C-2), 33.1 d (C-25), 36.1 t (C-12), 37.8 s

(C-10), 38.9 t (C-4), 40.5 d (C-20), 41.9 s (C-13), 42.8 d (C-24), 51.1 d (C-14), 54.3 d (C-17), 60.6 d (C-6), 63.2 s (C-5), 66.9 d (C-7), 68.5 d (C-3), 126.5 s (C-8), 132.1 d (C-23), 135.4 d (C-22), 137.0 s (C-9); EI-MS (70 eV) *m/z* 428 $[M]^+$ (5), 410 (100), 392 (24), 326 (30), 312 (31), 298 (11), 280 (11), 213 (33), 206 (13), 120 (31), 105 (27), 91 (48), 81 (19), 77 (30), 69 (25), 55 (61).

5α,6α-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7α-diol (4)

Amorphous white powder; $[\alpha]_D^{24}$ -108.1° (*c* 0.21, $CHCl_3$); Lit: $[\alpha]_D^{20}$ -115° (*c* 0.41, $CHCl_3$);[31] IR (KBr) ν_{max}: 3422, 2955, 2926, 2853, 1718, 1455, 1382, 1051, 749 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.79 (3H, d, *J* = 6.4 Hz, H-26), 0.81 (3H, d, *J* = 6.4 Hz, H-27), 0.84 (3H, s, H-18), 0.84 (3H, s, H-19), 0.88 (3H, d, *J* = 7.2 Hz, H-28), 0.99 (3H, d, *J* = 6.8 Hz, H-21), 3.12 (1H, d, *J* = 3.2 Hz, H-6), 3.90 (1H, m, H-3), 4.40 (1H, d, *J* = 3.2 Hz, H-7), 5.14 (1H, m, H-22), 5.20 (1H, m, H-23); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 16.5 q (C-19), 17.6 q (C-28), 18.0 q (C-18), 19.0 t (C-11), 19.7 q (C-27), 20.0 q (C-26), 21.2 q (C-21), 24.9 t (C-15), 27.2 t (C-16), 31.1 t (C-2), 32.2 t (C-1), 33.1 d (C-25), 35.8 s (C-10), 36.6 t (C-12), 38.7 d (C-9), 39.3 d (C-20), 39.5 t (C-4), 42.8 d (C-24), 42.9 s (C-13), 56.8 d (C-17), 61.3 d (C-6), 65.1 d (C-7), 67.8 s (C-5), 68.7 d (C-3), 125.1 s (C-8), 132.2 d (C-23), 135.2 d (C-22), 152.6 s (C-14); EI-MS (70 eV) *m/z* 428 $[M]^+$ (4), 410 (37), 392 (37), 374 (50), 266 (43), 249 (76), 215 (37), 179 (28), 105 (28), 91 (38), 81 (45), 69 (84), 55 (100).

3β-hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one (5)

Amorphous white powder; $[\alpha]_D^{24}$ -24.1° (*c* 0.12, $CHCl_3$); Lit: $[\alpha]_D^{24}$ -28.3° (*c* 0.1, $CHCl_3$);[25] IR (KBr) ν_{max}: 3364, 2960, 2872, 1664, 1616, 1455, 1372, 1211, 1065, 754 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.62 (3H, s, H-18), 0.80 (3H, d, *J* = 6.4 Hz, H-27), 0.82 (3H, d, *J* = 6.4 Hz, H-26), 0.90 (3H, d, *J* = 6.8 Hz, H-28), 1.03 (3H, d, *J* = 6.8 Hz, H-21), 1.34 (3H, s, H-19), 3.67 (1H, m, H-3), 5.18 (1H, dd, *J* = 6.8, 15.2 Hz, H-22), 5.23 (1H, dd, *J* = 6.8, 15.2 Hz, H-23), 6.02 (d, *J* = 1.2 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.1 q (C-18), 17.8 q (C-28), 19.8 q (C-27), 20.2 q (C-26), 21.3 q (C-21), 23.9 q (C-19), 24.8 t (C-11), 24.9 t (C-15), 29.6 t (C-16), 30.8 t (C-2), 33.3 d (C-25), 34.8 t (C-1), 35.7 t (C-12), 40.5 d (C-20), 42.0 t (C-4), 42.1 s (C-10), 42.5 s (C-13), 43.0 d (C-24), 48.6 d (C-14), 53.5 d (C-17), 72.1 d (C-3), 126.9 d (C-6), 132.3 d (C-23), 134.2 s (C-8), 135.6 d (C-22), 161.4 s (C-9), 161.9 s (C-5), 186.5 d (C-7); EI-MS (70 eV) *m/z* (rel. int.) 410 $[M]^+$ (100), 395 (85), 377 (18), 285 (33), 267 (85), 253 (45), 229 (24), 173 (31), 213 (29), 191 (32), 173 (31), 125 (52), 69 (53), 55 (24).

ergosterol peroxide (6)

Amorphous white powder; $[\alpha]_D^{24}$ -46.3° (*c* 0.26, $CHCl_3$); IR (KBr) ν_{max}: 3349, 2931, 2843, 1659, 1592, 1518, 1460, 1382, 1275, 1163, 1026, 866, 822 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.81 (3H, s, H-18), 0.81 (3H, d, *J* = 7.0 Hz, H-27), 0.83 (3H, d, *J* = 7.0 Hz, H-26), 0.88 (3H, s, H-19), 0.90 (3H, d, *J* = 6.5 Hz, H-28), 1.00 (3H, d, *J* = 7.0 Hz, H-21), 3.96 (1H, m, H-3), 5.14 (1H, dd, *J* = 8.5, 15.5 Hz, H-22), 5.22 (1H, dd, *J* = 8.5, 15.5 Hz, H-23), 6.24 (1H, d, *J* = 8.5 Hz, H-6), 6.50 (1H, d, *J* = 8.5 Hz, H-7); EIMS *m/z* 428 $[M]^+$ (6), 410 (16), 396 (38), 377 (5), 363 (18), 251 (7), 69 (100).

clerosterol (7)

Amorphous white powder; $[\alpha]_D^{24}$ -48.1° (*c* 0.23, $CHCl_3$); Lit: $[\alpha]_D^{25}$ -45.2° (*c* 0.01, $CHCl_3$);[32] IR (KBr) ν_{max}: 3354, 2936, 2858, 1654, 1601, 1514, 1455, 1382, 1275, 1163, 1041, 915, 817, 749 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.77 (3H, t, *J* = 7.6 Hz, H-29), 0.88 (3H, d, *J* = 6.4 Hz, H-21), 0.98 (3H, s, H-19), 1.54 (3H, s, H-26), 3.55 (1H, m, H-3), 4.62 (1H, brs, H-27), 4.70 (1H, brs, H-27), 5.33 (1H, d, *J* = 5.2 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.8 q (C-18), 12.1 q (C-29), 17.7 q (C-26), 18.6 q (C-21), 19.4 q (C-19), 21.1 t (C-11), 24.3 t (C-15), 26.5 t (C-28), 28.2 t (C-16), 29.7 t (C-23), 31.6 t (C-2), 31.9 d (C-7), 31.9 d (C-8), 33.7 d (C-22), 35.5 d (C-20), 36.5 s (C-10), 37.2 t (C-1), 39.7 t (C-12), 42.3 t (C-4), 42.3 s (C-13), 49.5 d (C-24), 50.7 d (C-9), 56.0 d (C-17), 56.7 d (C-14), 71.8 d (C-3), 111.4 t (C-27), 121.7 d (C-6), 140.7 s (C-5), 147.6 s (C-25); EI-MS (70 eV) *m/z* 412 $[M]^+$ (40), 394 (14), 314 (16), 299 (20), 271 (34), 255 (22), 213 (28), 187 (22), 173 (25), 159 (40), 145 (45), 133 (37), 121 (39), 105 (55), 95 (68), 81 (80), 69 (71), 55 (100).

decortinol (8)

Amorphous white powder; $[\alpha]_D^{24}$ -55.4° (*c* 0.36, $CHCl_3$); IR (KBr) ν_{max}: 3417, 2945, 2872, 1718, 1455, 1377, 1216, 1070, 886, 759 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.65 (3H, s, H-18), 0.78 (3H, t, *J* = 8.5 Hz, H-29), 0.89 (3H, d, *J* = 6.4 Hz, H-21), 0.97 (3H, s, H-19), 1.54 (3H, s, H-26), 3.55 (1H, m, H-3), 3.83 (1H, brs, H-7), 4.62 (1H, brs, H-27), 4.70 (1H, brs, H-27), 5.58 (1H, dd, *J* = 2.0, 6.2 Hz, H-6); EI-MS (70 eV) *m/z* (rel. int.) 428 $[M]^+$ (4), 410, (96), 392 (100), 377 (15), 351 (5), 273 (10), 253 (14), 143 (12), 119 (8), 95 (7), 81 (9), 55 (16).

decortinone (9)

Amorphous white powder; $[\alpha]_D^{24}$ -37.9° (*c* 0.90,

$CHCl_3$); IR (KBr) ν_{max}: 3393, 2955, 2963, 2877, 1718, 1445, 1372, 1207, 1036, 968, 890, 759 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.65 (3H, s, H-18), 0.78 (3H, t, J = 7.2 Hz, H-29), 0.89 (3H, d, J = 6.4 Hz, H-21), 1.17 (3H, s, H-19), 1.54 (3H, s, H-26), 3.65 (1H, m, H-3), 4.62 (1H, brs, H-27), 4.70 (1H, brs, H-27), 5.66 (1H, dd, J = 1.2, 2.0 Hz, H-6); EI-MS (70 eV) *m/z* (rel. int.) 426 $[M]^+$ (20), 408, (72), 393 (15), 343 (11), 310 (16), 285 (20), 269 (45), 187 (73), 174 (100), 161 (48), 81 (25), 55 (29).

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