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Four new steroids from the leaves and twigs of *Dysoxylum pallens* and their cytotoxic activities

Jia-Nan Wang[a,b,1], Zong-Yi Zhang[a,b,1], Peng Sun[a,b], Dong-Hua Cao[a,b], Yi-Dian Xiao[c,d], Xiao-Cui Shi[a,b], Chun-Fen Xiao[a], Hua-Bin Hu[a], You-Kai Xu[a,*]

[a] Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun 666303, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, PR China
[d] School of Chemical Science and Technology, Key Laboratory of Medicinal Chemistry for Nature Resource, Ministry of Education, Yunnan University, Kunming 650091, PR China

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ABSTRACT

Four previously undescribed steroids, identified as (3*S*,7*S*,8*S*,9*S*,10*R*,13*S*,14*S*,16*S*,17*R*,20*S*)-7α-methoxy-ergosta-5,24(28)-dien-3β,16β,20-triol (1), ergosta-5,24(28)-dien-3*β*,7*α*,16*β*-triol (2), ergosta-5,25-dien-3*β*,7*α*,16*β*,20-tetrol (3) and 7*α*,16*β*,24*α*-trihydroxy-varninasterol (4), as well as five known analogues (5–9), were isolated from the leaves and twigs of *Dysoxylum pallens* Hiern (Meliaceae). Their structures were elucidated based on extensive spectroscopic analysis such as HR-ESI-MS, 1D and 2D NMR, UV, and IR. The absolute configuration of compound 1 was determined by X-ray diffraction analysis. Selected compounds were evaluated for their cytotoxic activities. Compounds 1, 2, and 8 exhibited moderate cytotoxic activity against HL-60, Hela, and HepG2 tumor cell lines with IC_{50} ranged from 11.09 to 17.51 μM.

1. Introduction

The genus *Dysoxylum* (family Meliaceae), comprising about 80 species, is mainly distributed in tropical Asia, tropical and subtropical Australia, and Pacific islands. Among them, ten species and one endemic with two insufficiently known species grow in China, mainly in the tropical areas of southern areas [1]. Many species in this genus have applications in folk medicine for the treatment of fever, rigid limbs, convulsions, hemorrhage, and facial distortion in children in some areas of southeast Asia [2–4], as well as in the Dai nationality, who are mainly distributed in Yunnan Province, southwest of China [5]. Though there are abundant *Dysoxylum* plants in tropical areas of southern China, most of them remain unexploited for their constituents or potential utilities. Previous phytochemical investigations on the genus *Dysoxylum* revealed the existence of wide range of chemical constituents, such as antifeeding limonoids [6], cytotoxic tirucallane-type alkaloids [7], and antibacterial triterpenoids [8]. However, other bioactive secondary metabolites such as steroids are tended to be ignored. According to previous literature investigation, ergosterols are one of the most important steroids in the genus *Dysoxylum*, which exhibited extensive bioactivities such as anti-inflammatory [9], anti-tumor [10], antibacterial [11], and diuretic activity [12], therefore, we speculated that steroids may be one of the main effective components in the genus *Dysoxylum*.

Dysoxylum pallens Hiern, a perennial tree, is widely distributed throughout the tropical areas of southern China. To search for bioactive steroids and their analogues, 95% ethanol extract of the dried leaves and twigs of this plant were phytochemically investigated for the first time, which led to the isolation of three previously undescribed ergosterol-type steroids (1–3) and one new varninasterol-type sterols together with five known analogues (5–9) (Fig. 1). Their structures were elucidated by a combination of extensive spectroscopic (1D and 2D NMR, HR-ESI-MS, UV, and IR) data and comparison with literature date. The absolute configuration of compound 1 was determined by X-ray diffraction analysis. Herein, the isolation and structure elucidation of these compounds are described, with their cytotoxic activities against HL-60, Hela, and HepG2 cell lines also being assayed.

2. Results and discussion

Compound 1 was obtained as white lump crystals. Its HREIMS showed a molecular ion peak at *m/z* 483.3445 $[M + Na]^+$ (calcd as

* Corresponding author.
E-mail address: xyk@xtbg.ac.cn (Y.-K. Xu).
[1] These authors contributed equally to this work.

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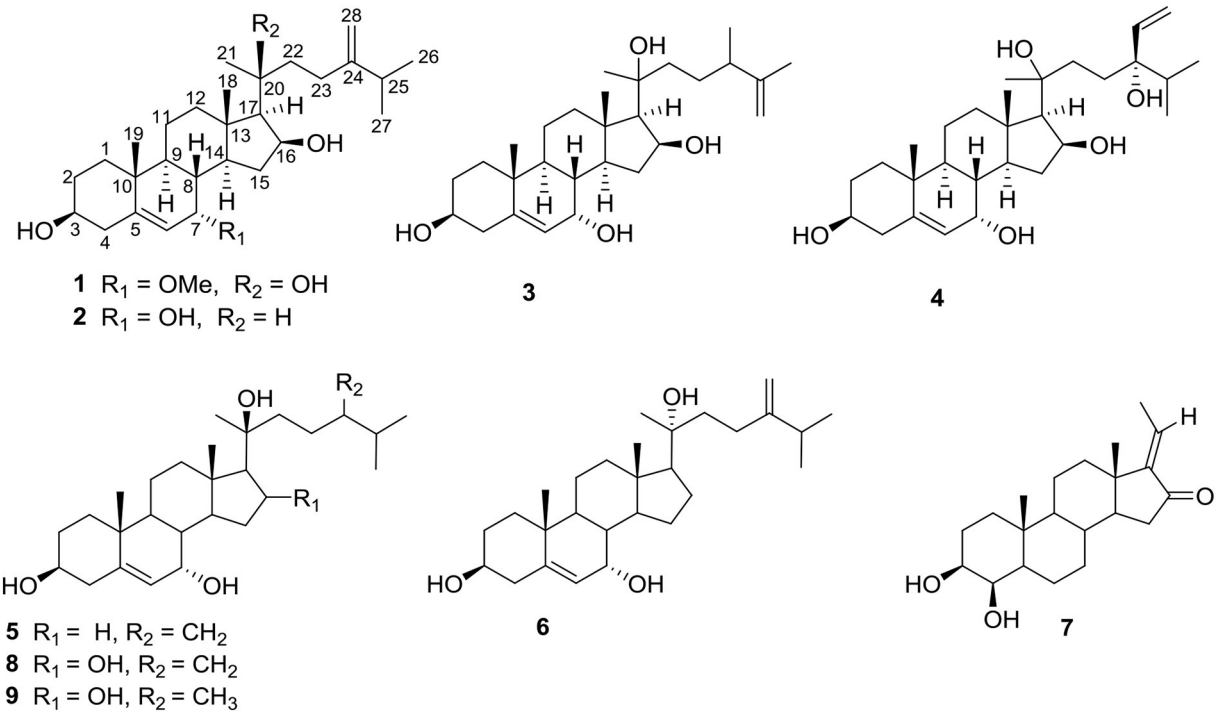

Fig. 1. The chemical structures of compounds 1–9.

Table 1
^{13}C NMR spectroscopic data of compounds 1–4.

Position	1[a]	2[a]	3[b]	4[a]
	δ_C	δ_C	δ_C	δ_C
1	38.1	38.0	38.0	38.0
2	32.1	32.1	32.1	32.1
3	72.2	72.0	72.0	72.0
4	43.1	42.9	42.9	42.9
5	148.2	146.7	146.8	146.8
6	121.3	124.9	124.8	124.8
7	74.9	65.9	65.8	65.8
8	37.6	38.6	38.1	38.1
9	44.2	43.4	43.3	43.3
10	38.7	38.5	38.5	38.5
11	21.6	21.5	21.5	21.5
12	41.1	40.9	41.1	38.3
13	44.0	43.1	43.7	43.7
14	48.6	48.5	48.5	48.6
15	38.0	37.9	37.9	37.9
16	74.5	72.8	74.6	74.5
17	60.9	62.5	60.7	61.0
18	14.8	13.3	15.0	15.0
19	18.8	18.7	18.6	18.6
20	78.0	31.4	78.1	78.1
21	26.4	18.8	26.6	26.5
22	43.7	36.0	43.1	41.2
23	30.5	32.4	31.0	34.3
24	157.6	158.1	43.2	78.6
25	35.1	35.0	151.0	37.2
26	22.4	22.4	110.2	17.0
27	22.4	22.4	19.0	18.1
28	107.0	106.6	20.5	143.2
29				113.8
7-OMe	56.7			

[a] Recorded in MeOD at 150 MHz.
[b] Recorded in MeOD at 125 MHz.

483.3450), consistent with a molecular formula of $C_{29}H_{48}O_4$, corresponding to 6 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl (3425.70 cm^{-1}) and olefinic (1637.47 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra (Table 1) of 1 exhibited the presence of twenty-nine carbons classified as six methyls (one oxygenated carbon at δ_C 56.7), one sp^2 (δ_C 107.0) and eight sp^3 methylene carbons, one sp^2 (δ_C 121.3) and eight sp^3 methines (three oxygenated carbons at δ_C 72.2, 74.5, 74.9), two sp^2 (δ_C 157.6 and 148.2) and three sp^3 (one oxygenated at δ_C 78.0) quaternary carbons. The ^{1}H NMR and ^{13}C NMR spectrum data (Tables 1 and 2) of 1 showed general features similar to 8, suggested their close structures, which was isolated from the same family Meliaceae [13]. The major differences were that 1 has one oxygenated methyl at δ_C 56.7 and one oxygenated methine at δ_C 74.9 (C-7) instead of δ_C 65.2 (C-7) in 8. The correlations (Fig. 3) between H-7/H-6 and H-7/H-8 were observed in the ^{1}H—^{1}H COSY spectrum, along with the HMBC correlations (Fig. 3) from H-6 (δ_H 5.79) to C-7 (δ_C 74.9) and from H-7 (δ_H 3.36) to OMe-7 (δ_C 56.7)/C-6 (δ_C 121.3)/C-8 (δ_C 37.6) deduced that δ_C 74.9 replaced δ_C 65.2 at C-7 in 8 and the oxygenated methyl (δ_C 56.7) at C-7.

The NOESY correlations (Fig. 4) that H-8 with H-19/H-18/H-7/H-15β deduced that these protons are on the same side, leaving H-3 with H-2α/H-4α and H-17 with H-16/H-15α at the opposite orientation in the spatial configuration of 1. At this point, their relative configuration was confirmed. The absolute configuration of 1 was further confirmed by X-ray crystallographic diffraction analysis (Fig. 2) with Cu Kα radiation, indicated that the secondary hydroxy groups at C-3, C-7, C-16 were of the *S, R, S*-configurations respectively, and the tertiary hydroxy group at C-20 was of the *S*-configuration, respectively. Thus, the structure of 1 was elucidated as depicted and name was (3*S*,7*S*,8*S*,9*S*,10*R*,13*S*,14*S*,16*S*,17*R*,20*S*)-7α-methoxy-ergosta-5,24(28)-dien-3β,16β,20-triol.

Compound 2 was obtained as white amorphous powder. Its HREIMS showed a molecular ion peak at *m/z* 453.3337 [M + Na]$^+$ (calcd as 453.3345), consistent with a molecular formula of $C_{28}H_{46}O_3$, corresponding to 6 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl (3497.77 cm^{-1}) and olefinic (1633.42 cm^{-1}). Detailed analysis of the 1D and 2D NMR spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectra of 2 (Tables 1 and 2) with 8 ($C_{28}H_{46}O_4$) [13], indicated that both compounds were similar, except the absence of a quaternary hydroxy group in 2. The significant

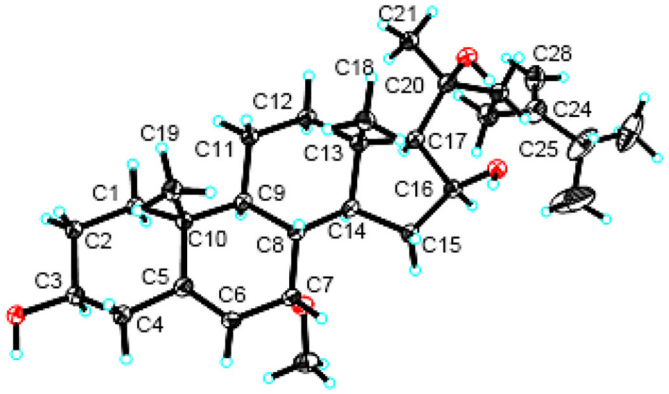

Fig. 2. The X-ray crystal data of compound 1.

downfield shift for C-23 ($\Delta\delta_C$ 3.0) and upfield shift for C-20, C-21 and C-22 ($\Delta\delta_C$ −45.3, 7.9, and 6.6, respectively) in 2 indicated this absence of a hydroxy group was at C-20. This difference was confirmed by analyzing the ^{1}H−^{1}H COSY and HMBC correlations (Fig. 3). Based on biogenetic consideration and by comparison of the ^{1}H and ^{13}C NMR spectra of 2 with 1 (Tables 1 and 2), it was found that chemical shift of C-3 was very close between 1 (C-3 δ_C 72.2, δ_H 3.46, m) and 2 (C-3 δ_C 72.0, δ_H 3.48, m), suggesting that two compounds have same configuration at H-3 as α-oriented. The NOESY correlations (Fig. 4) that H-8 with H-19/H-7 deduced that these protons are on the same side, leaving H-3 with H-2α/H-4; H-16 with H-17/H-15α at the opposite orientation. It was known that H-3 was α oriented, other configurations can be assumed as β and α at H-7 and H-16, respectively. At this point, their relative configuration was confirmed. Thus, the structure of 2 was elucidated as depicted, and its name was determined to be ergosta-5,24(28)-dien-3β,7α,16β-triol.

Compound 3 was obtained as white needle-like crystals. Its HREIMS showed a molecular ion peak at m/z 469.3288 $[M + Na]^+$ (calcd as 469.3294), consistent with a molecular formula of $C_{28}H_{46}O_4$, corresponding to 6 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl (3405.35 cm^{-1}) and terminal olefinic (2928.80 and 1637.47 cm^{-1}) groups. Detailed analysis of the 1D and 2D NMR spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectra of 3 (Tables 1 and 2) with 8 [13], indicated that both compounds shared the same skeleton, and the major differences were the presence of a C-25—C-26 double bond in 3 instead of a C-24—C-28 one in 8. The conclusion was supported by the significant downfield shift for C-24 and C-26 ($\Delta\delta_C$ 9.3 and 3.0, respectively) and upfield shift for C-25 ($\Delta\delta_C$ -5.4). The COSY correlation (Fig. 3) of δ_H 4.69 to H-27 and δ_H 2.11 to H-28/H-23 indicated that double bond is not at C-24. The HMBC

Table 2
^{1}H NMR spectroscopic data of compounds 1–4.

Position	1[a]	2[a]	3[b]	4[a]
	δ_H m (*J* in Hz)	δ_H m (*J* in Hz)	δ_H m (*J* in Hz)	δ_H m (*J* in Hz)
1α	1.10, overlapped	1.16, overlapped	1.15, overlapped	1.16, overlapped
1β	1.87, overlapped	1.87, dt (12.48, 3.06)	1.86, dt (13.5, 3.35)	1.86, dt (13.32, 3.00)
2α	1.80, overlapped	1.79, overlapped	1.81, overlapped	1.78, overlapped
2β	1.50, overlapped	1.49, overlapped	1.49, overlapped	1.51, d (13.02)
3	3.46, m	3.48, m	3.48, m	3.46, m
4α	2.32, m (2H)	2.26, overlapped	2.30, m (2H)	2.27, m (2H)
4β		2.30, overlapped		
5				
6	5.79, d (4.8)	5.55, d (5.16)	5.55, d (5.13)	5.54, d (4.38)
7	3.36, dd (4.8, 4.14)	3.77, dd (5.16, 3.84)	3.76, dd (5.13, 4.30)	3.75, dd (4.38, 3.78)
8	1.64, td (11.28, 4.14)	1.51, overlapped	1.55, overlapped	1.56, overlapped
9	1.33, overlapped	1.31, s	1.33, overlapped	1.31, overlapped
10				
11α	1.59, overlapped	1.55, overlapped (2H)	1.60, overlapped (2H)	1.59, overlapped
11β	1.55, overlapped			1.57, overlapped
12α	1.18, overlapped	1.13, overlapped	1.18, overlapped	1.81, overlapped
12β	2.13, dt (12.84, 4.32)	2.03, dt (12.48, 3.06)	2.14, overlapped	1.69, overlapped
13				
14	1.36, overlapped	1.32, overlapped	1.31, overlapped	1.29, overlapped
15α	2.25, overlapped	2.40, m	2.36, m	2.37, m
15β	1.34, overlapped	1.18, overlapped	1.29, overlapped	1.28, overlapped
16	4.62, m	4.24, td (7.56, 4.8)	4.58, m	4.62, m
17	1.31, overlapped	1.08, dd (11.1, 7.56)	1.28, overlapped	1.27, overlapped
18	1.14, s	0.91, s	1.14, s	1.13, s
19	1.03, s	1.01, s	1.03, s	1.02, s
20		1.92, m		
21	1.29, s	1.02, d (6.0)	1.26, s	1.25, s
22α	1.77, dd (12.9, 4.56)	1.22, m	1.73, m	1.18, overlapped
22β	1.91, overlapped	1.82, overlapped	1.52, overlapped	2.13, m
23α	2.10, overlapped	2.15, ddd (14.34, 12.18, 4.32)	1.39, overlapped (2H)	1.54, overlapped
23β	2.05, overlapped	1.98, overlapped		1.62, overlapped
24			2.11, d (6.90)	
25	2.27, overlapped	2.28, overlapped		1.75, m
26	1.05, d (6.84)	1.03, d (2.7)	4.69, s	0.91, d (6.9)
27	1.06, d (6.84)	1.04, d (2.64)	1.67, s	0.89, d (6.9)
28	4.72, s	4.71, s	1.05, d (6.85)	5.82, dd (17.40,10.98)
	4.75, s		1.15, overlapped	
29				5.21, dd (17.40,1.68)
				5.14, dd (11.04,1.68)
7-OMe	3.34, s			

[a] Recorded in MeOD at 600 MHz.
[b] Recorded in MeOD at 500 MHz.

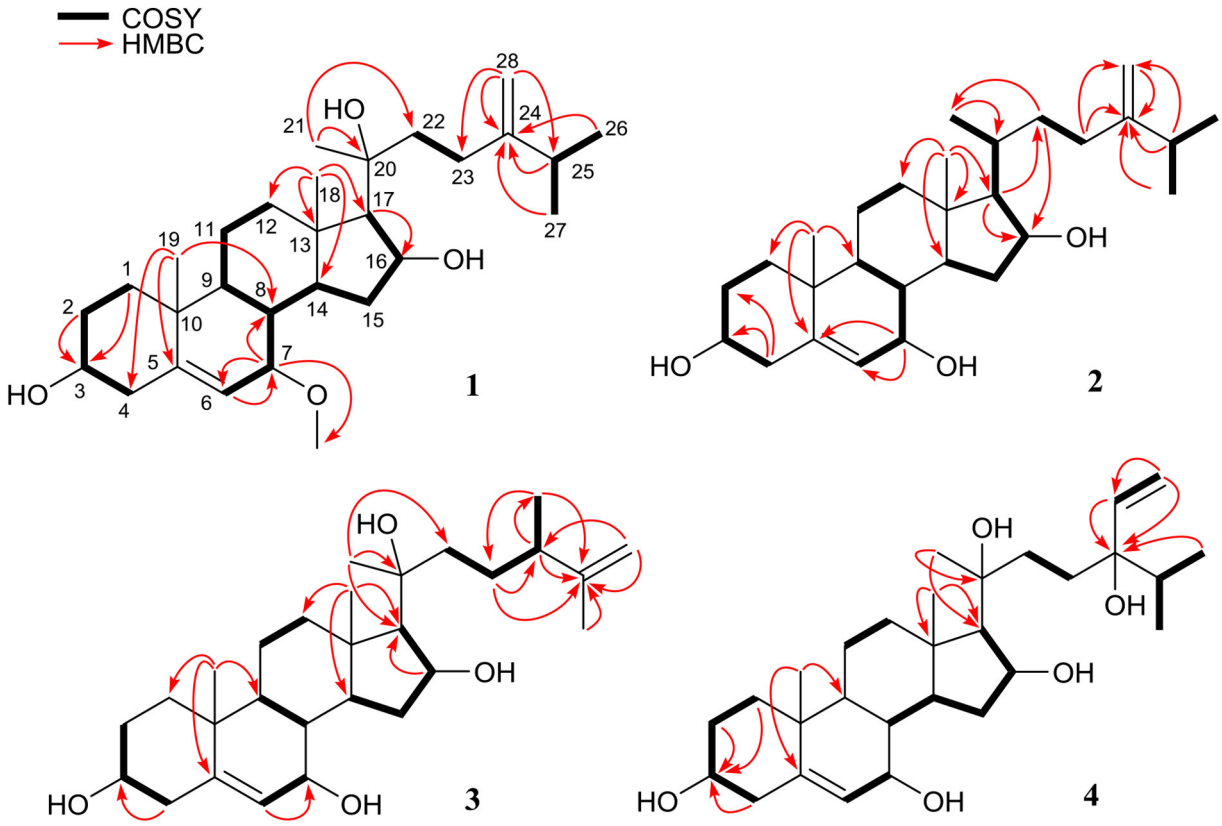

Fig. 3. The COSY and HMBC correlations of compound 1–4.

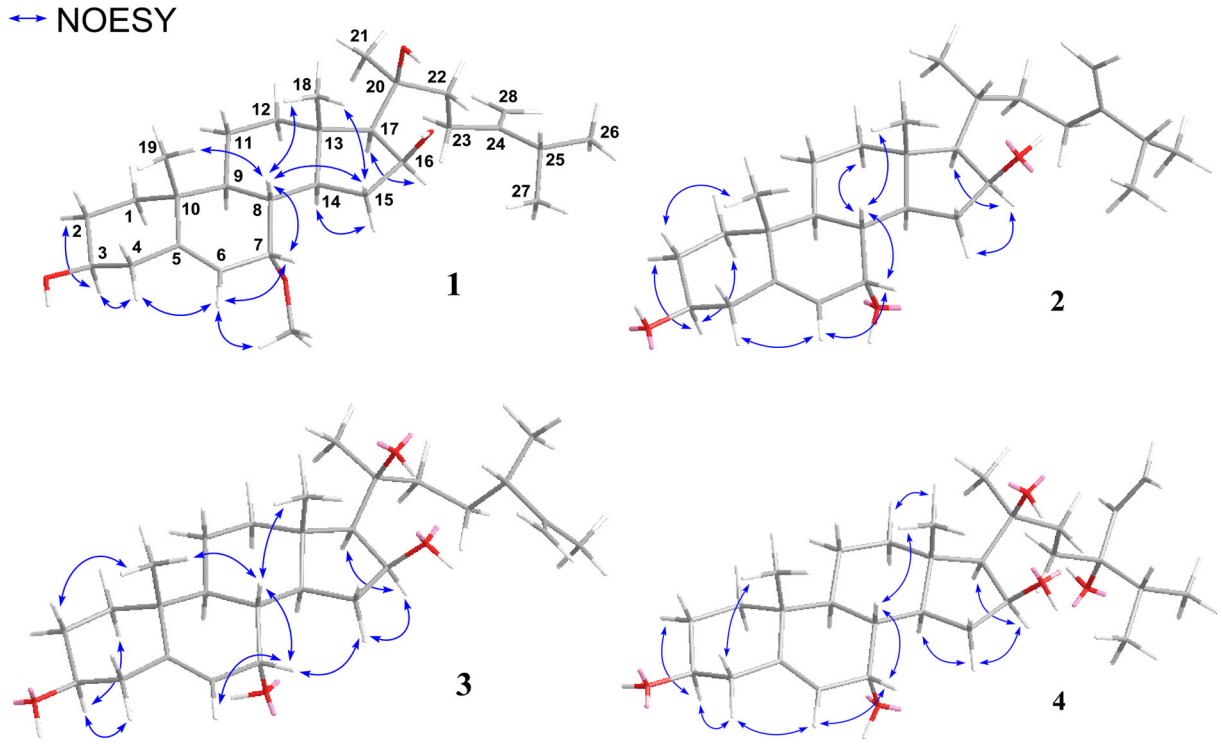

Fig. 4. The NOESY correlations of compound 1–4.

correlations (Fig. 3) from H-27/H-23/H-28/H-26/H-24 to δ_C 151.0 and from H-24/H-23 to C-28 indicated double bond is at C-25—C-26. Similar to compound 2, the relative configuration of 3 was determined by comparison of the ^{1}H and ^{13}C NMR spectra between compound 1 and 3, as well as the NOESY correlations (Fig. 4) of 3. At this point, their relative configuration was confirmed. Thus, the structure of 3 was elucidated as depicted, and its name was determined to be ergosta-5,25-dien-3*β*,7*α*,16*β*,20-tetrol.

Compound 4 was obtained as white needle-like crystals from a mixture of MeCN-CH_2Cl (3,1, *v*/v). Its HREIMS showed a molecular ion peak at *m/z* 499.3392 $[M + Na]^+$ (calcd as 499.3399), consistent with a molecular formula of $C_{29}H_{48}O_5$, corresponding to 6 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl (3425.32 cm^{-1}) and olefinic (1633.33 cm^{-1}) groups. A comparison of the ^1H and ^{13}C NMR spectra of 4 (Tables 1 and 2) with the known steroid varninasterol [14], indicated they had very similar structures, except for the presence of two oxy methine signal and one oxy quaternary carbon instead of two methylenes (H-7, H-16) and one methine (H-24) in 4. Detailed 1D and 2D NMR (COSY, HSQC) analysis of 4 led to the assignments of two oxymethines each located at C-7[H-7: δ_H 3.75(1H, t, J = 3.78 Hz)] and C-16[H-16: δ_H: 4.62(1H, m)]. The HMBC correlations from H-28/H-29/H-26 to δ_C 78.6 deduced that the oxygenated quaternary carbon δ_C 78.6 was at C-24. Similar to compound 3, the relative configuration of 4 was determined by comparison of the ^1H and ^{13}C NMR spectra between compound 1 and 4, as well as the NOESY correlations (Fig. 4) of 4, which assumed that the secondary hydroxy groups at C-3, C-7, C-16 were of the *β*-, *α*-, *β*-configurations, respectively. Thus, the structure of 4 was elucidated as depicted, and its name was determined to be 7*α*,16*β*,24*α*-trihydroxy-varninasterol.

Additionally, five known compounds were elucidated as 3*β*,7*α*,20*β*-trihydroxyergosta-5,24(24′-diene) (5) [15], 3*α*,7*α*,20*α*-trihydroxyergosta-5,24(24′-diene) (6) [15], Lansisterone E (7) [16], (20*S*)-5,24(28)-ergostadiene-3*β*,7*α*,16*β*,20-tetrol (8) [13], (20*S*)-5-ergostene-3*β*,7*α*,16*β*,20-tetrol (9) [13] by comparison of their spectroscopic data with those in the literature. All the compounds were isolated from the plant for the first time.

Compounds 1–4, 8, and 9 were obtained in sufficient amounts to be evaluated for their cytotoxic activity against human myeloid leukemia (HL-60), human cervical cancer cell (Hela) and human hepatoma carcinoma cell lines (HepG2). Compound 3 exhibited potent cytotoxicity against the Hela cell lines with IC_{50} values of 11.09 ± 0.06, and compounds 2, 3, and 8 just show moderate cytotoxicity against HL-60 and HepG2 cell lines, with IC_{50} values in the range 14.19 to 17.51 μM (Table 3).

3. Experimental procedures

3.1. General experimental procedures

ESI-MS and HR-ESIMS were recorded on an Auto Spec Premier P776 instrument. UV spectra were measured with a Shimadzu UV-2401A instrument. IR spectra (KBr) were determined on a Bruker Tensor-27 infrared spectrometer. Optical rotations were obtained with a JASCO P-1020 polarimeter. 1D and 2D NMR spectra were recorded on Bruker DRX-500 and Bruker Avance III 600 spectrometers with TMS as an internal standard. Semi-preparative HPLC was performed on a Waters 600 pump system with a 2996 photodiode array detector by using a YMC-Pack ODS-A column (300 × 10 mm, S-5 μm). MCI gel (CHP20/P120, 75–150 μm, high-porous polymer, Mitsubishi Chemical Corporation, Tokyo, Japan), Silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 gel (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden) and C18-reversed phase silica gel (ODS-A-HG, AAG12S50, YMC Co. Ltd., Japan) were used for column chromatography (CC). Pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd., China) were used for analytical TLC. All solvents used for CC were of analytical grade (Shanghai Chemical Reagents Co. Ltd., China), and all solvents used for HPLC were of spectral grade.

Table 3
Cytotoxic activity (IC_{50} μM) of selected compounds.

Compound	IC_{50}/μM		
	HL-60	Hela	HepG2
2	14.69 ± 0.42	NA	16.12 ± 0.33
3	14.19 ± 0.51	11.09 ± 0.06	16.06 ± 0.46
8	14.72 ± 0.74	NA	17.51 ± 0.22
Cisplatin[a]	6.56 ± 1.30	7.61 ± 0.43	7.11 ± 0.36

[a] Positive control.

3.2. Plant material

Leaves and twigs of *D. pallens* were collected from Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Science (CAS), Menglun Town, Mengla Country, Yunnan Province, People's Republic of China in June 2014, and identified by professor Jianwu Li. A voucher specimen (HITBC-162435) was deposited in the herbarium at XTBG.

3.3. Extraction and isolation

The air-dried and powered plant materials (5.6 kg) were extracted three times (each for 3 days) with 95% aqueous EtOH (25 L) at room temperature. Removal of the solvent from the combined extracts in vacuo afforded a crude residue (280 g). The EtOH extracts was then suspended in water and partitioned sequentially with petroleum ether, EtOAc and *n*-BuOH. The EtOAc-soluble fraction (86 g) was subjected to macroporous adsorbent resin (D-101) column and eluted with EtOH-H_2O (30:70, 60:40, 85:15, 100:0, *v*/v, each 8 L) to give four fractions. The second fraction (23 g) was chromatographed on a silica gel column (6 cm × 40 cm, 200–300 mesh) with gradient mixtures of CH_2Cl_2-MeOH(100:0, 50:1, 40:1, 25:1, 10:1, 5:1, 0:100, v/v, each 4 L)elution to yield seven fractions, Frs. A-G (5.4, 7.2, 3.6, 2.1, 2.0, 6.1 g and 3.2 g, respectively). Fr. B (7.2 g) was subjected to Sephadex LH-20 (2 cm × 100 cm) eluted with MeOH: H_2O (90% to 100%, *v*/v) to give four sub-fractions (B1 ~ B4). Sub—Fr. B2 (13 mg) and B4 (22 mg) was purified by semipreparative HPLC (10 mm × 300 mm, MeCN / H_2O, 75:25, *v*/v, 3 mL/min) to yield respectively 7 (1 mg), 5 (2 mg) and 6 (2 mg). Fr. C (3.6 g) was subjected to Sephadex LH-20 (2 cm × 100 cm) eluted with MeOH: H_2O (85% to 100%, *v*/v) to give three sub-fractions (C1-C3). Sub-fraction C1 was fractionated by a silica gel column (3 cm × 25 cm, 200–300 mesh) eluted with CH_2Cl_2/MeOH (100:1, 50:1, 25:1, 20:1, v/v, each 200 mL) to yield four sub-fractions C-1a to C-1d. Sub-fraction C-1b was further purified by semipreparative HPLC (10 mm × 300 mm, MeCN/H_2O, 80:20, v/v, 3 mL/min) to yield 1 (4 mg), and sub-fraction C-1d was also purified by semipreparative HPLC (10 mm × 300 mm, MeCN/H_2O, 60:40, v/v, 3 mL/min) to yield 2 (3 mg). Fr. D (2.1 g) was purified initially by a silica gel column (3 cm × 25 cm, 200–300 mesh) and then by semipreparative HPLC (10 mm × 300 mm, MeCN/H_2O, 60:40, v/v, 3 mL/min) to yield respectively 3 (6 mg), 8 (6 mg) and 9 (8 mg). Fr. E (2.0 g) was purified by a silica gel column (3 cm × 25 cm, 200–300 mesh) and then by Sephadex LH-20 (2 cm × 100 cm) eluted with 75% MeOH-H_2O to yield 4 (9 mg).

3.3.1. (3S,7S,8S,9S,10R,13S,14S,16S,17R,20S)-7α-methoxy-ergosta-5,24(28)-dien-3β,16β,20-triol (1)

White lump crystal; $C_{29}H_{48}O_4$; $[\alpha]_D^{26.4}$ − 170.0 (*c* 0.03, MeOH); HR-EIMS *m/z* 483.3445 $[M + Na]^+$ (calcd as 483.3450); IR (KBr) ν_{max}: 3426 (br), 2928, 2854, 1637, 1464, 1384; ^1H NMR (600 MHz, MeOH) and ^{13}C NMR (150 MHz, MeOH) data, see Tables 1 and 2.

3.3.2. Ergosta-5,24(28)-dien-3β,7α,16β-triol (2)

White amorphous powder; $C_{28}H_{46}O_3$; $[\alpha]_D^{19.9}$ − 312.81 (*c* 0.069, MeOH); HR-EIMS *m/z* 453.3337 $[M + Na]^+$ (calcd as 453.3345); IR (KBr) ν_{max}: 3427 (br), 2929, 2855, 1633, 1464, 1383; ^1H NMR (600 MHz, MeOH) and ^{13}C NMR (150 MHz, MeOH) data, see Tables 1 and 2.

3.3.3. Ergosta-5,25-dien-3β,7α,16β,20-tetrol (3)

White needle-like crystal; $C_{28}H_{46}O_4$; $[\alpha]_D^{22.5}$ − 65.91 (*c* 0.044, MeOH); HR-EIMS m/z 469.3288 $[M + Na]^+$ (calcd as 469.3294); IR (KBr) ν_{max}: 3405 (br), 2929, 2854, 1643, 1462, 1384; ^1H NMR (500 MHz, MeOH) and ^{13}C NMR (125 MHz, MeOH) data, see Tables 1 and 2.

3.3.4. 7α,16β,24α-trihydroxy-varninasterol (4)

White needle-like crystal; $C_{29}H_{48}O_5$; $[\alpha]_D^{25.3}$ − 214.59 (*c* 0.017, MeOH); HR-EIMS m/z 499.3392 $[M + Na]^+$ (calcd as 499.3399); IR (KBr) ν_{max}: 3425 (br), 2922, 2852, 1633, 1466, 1384; ^1H NMR (600 MHz, MeOH) and ^{13}C NMR (150 MHz, MeOH) data, see Tables 1 and 2.

3.4. X-ray crystallographic data of compound 1

Colorless crystals of 1 were obtained by recrystallization from a mixture of MeOH/CH_3COCH_3 (*v*/v, 5:2). The X-ray crystallographic data were obtained on a Bruker APEX DUO CCD diffractometer equipped with graphite monochromatic Cu-Kα radiation (λ = 1.54178 Å) at 100 (2) K. The structure was solved by direct method with SHELXS-97 (Sheldrick 2008) and refined with full-matrix least-squares calculations on F2 by using SHELXS-97 (Sheldrick 2008). All non-hydrogen atoms were refined anisotropically. The hydrogen atom position was geometrically idealized and allowed to ride on their parent atoms. Crystal data of 1, $C_{29}H_{48}O_4$, M = 460.67, a = 13.5028(4) Å, b = 14.8477(5) Å, c = 28.2346(10) Å, α = 90°, β = 90°, γ = 90°, V = 5660.6(3) Å^3, T = 100.(2) K, space group $P212121$, Z = 8, μ (Cu Kα) = 0.545 mm^{-1}, 205,964 reflections measured, 11,232 independent reflections (R_{int} = 0.1339). The final R_1 values were 0.0684 ($I > 2\sigma(I)$). The final wR (F^2) values were 0.1908 ($I > 2\sigma(I)$). The final R_1 values were 0.0780 (all data). The final wR (F^2) values were 0.2005 (all data). The goodness of fit on F^2 was 1.035. Flack parameter = 0.11 (7). CCDC number 2002700 for compound 1 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html(or from the CCDC, 12 Union Road, Cambridge CB21EZ, UK; fax: +441, 223,336, 033; e-mail: deposit@ccdc.cam.ac.uk).

4. Cytotoxicity assay

The MTS method was used for assessing the cytotoxicity of the compounds against three tumor cell lines (HL-60 human myeloid leukemia, Hela human cervical cancer, and HepG2 human hepatoma carcinoma). All cells were cultured in RPMI 1640 or DMEM medium containing 10% fetal bovine serum. Then, 100 μL of adherent cells was seeded into each well (0.3–1.5 × 10^4 cells/well) of 96-well cell culture plates and allowed to adhere for 12 h at 37 °C before test drug additions. Each tumor cell line was exposed to a test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40 μM in DMSO in triplicate for 48 h, with cisplatin as the positive control. After 48 h incubation, 20 μL of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium] solution and 100 μL cell-culture medium was added to each well, which were incubated for another 4 h to give a formazan product. The OD value of each well was measured at 492 nm using a MULTISKAN FC instrument. The IC_{50} value of each compound was calculated by the Reed and Muench method [17].

Declaration of Competing Interest

All authors involved have no commercial association or other arrangement that might pose or imply a conflict of interest in connection with the submitted article.

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Appendix A. Supplementary data

UV, IR, HR-ESI-MS, 1D and 2D NMR spectra of compounds 1–4. Supplementary data associated with this article can be found online at XXXX. Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.fitote.2020.104696.

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