Ergostane Steroids from *Dysoxylum lukii*

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Phytochemical investigation of the 70% EtOH extract from the stem bark of *Dysoxylum lukii* led to the isolation of three new ergostane steroids, (3β)-3,20-dihydroxyergosta-5,24(28)-dien-7-one (**1**), (3β,4β,7α)-ergosta-5,24(28)-diene-3,4,7-triol (**2**), and (3β,7α)-3,20-dihydroxyergosta-5,24(28)-dien-7-yl acetate (**3**), together with the known compound (3β,4β)-ergosta-5,24(28)-diene-3,4,20-triol (**4**). Their structures were elucidated on the basis of extensive 1D- and 2D-NMR (COSY, HMQC, HMBC, and NOESY) analyses.

Introduction. – The genus *Dysoxylum* (Meliaceae) contains *ca.* 80 species that are distributed in India, Malaysia, Indonesia, Australia, and New Zealand, of which eleven species and one variety grow in the south of China [1]. The indigenous people in Asia have used many of these plant species as a traditional medicine; the bark is known to have emetic, antiperiodic, anthelmintic, and emmenagogue properties [2]. *Dysoxylum* contains a far greater range of types of compounds than all other genera of the Meliaceae family, in which the presence of triterpenoids [3–5], triterpenoid glycosides [6], diterpenoids [7][8], sesquiterpenoids [9][10], limonoids [11–13], steroids [14], and alkaloids [15] has been reported. *Dysoxylum lukii* MERR., a perennial arbor, is widely distributed throughout the south part of China. In our earlier paper [16], we reported the isolation of nine new tirucallane-type triterpenoids from the stem bark of *D. lukii*. Further work has afforded three new ergostane steroids, (3β)-3,20-dihydroxyergosta-5,24(28)-dien-7-one (**1**), (3β,4β,7α)-ergosta-5,24(28)-diene-3,4,7-triol (**2**), and (3β,7α)-3,20-dihydroxyergosta-5,24(28)-dien-7-yl acetate (**3**), as well as one known compound, (3β,4β)-ergosta-5,24(28)-diene-3,4,20-triol (**4**; *Fig. 1*). Herein, we report the isolation and structure elucidation of the new compounds by applying spectroscopic methods.

Results and Discussion. – Compound **1** was obtained as white amorphous solid. The HR-ESI-MS established the molecular formula $C_{28}H_{44}O_3$ (*m*/*z* 427.3211 ([$M-H$]$^-$; calc. 427.3212)) implying seven degrees of unsaturation. The IR spectrum indicated the

[1]) These two authors contributed equally to this work.

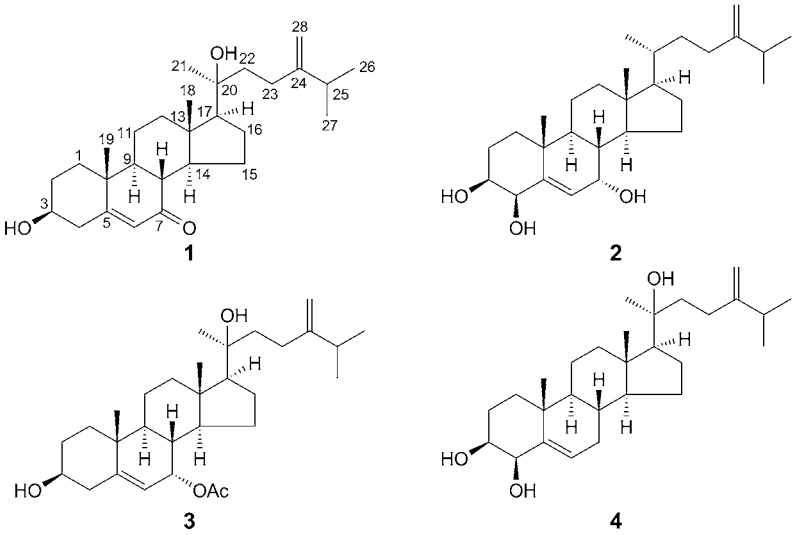

Fig. 1. *Structures of compounds* **1**–**4**

presence of OH group (3442 cm^{-1}), C=C bond (1632 cm^{-1}), and of an α,β-unsaturated C=O group (1676 cm^{-1}), which was further supported by a UV absorption band at λ_{max} 252 nm (log ε 1.89). The ^{13}C-NMR data of **1** (*Table*) revealed the presence of five Me, ten CH_2 (one olefinic), and seven CH groups (one O-bearing and one olefinic), and six quaternary C-atoms (one O-bearing, one olefinic, and one C=O group). In addition, the ^{1}H-NMR spectrum of **1** (*Table*) displayed signals of one trisubstituted C=C bond (δ(H) 5.69 (*s*)) and two geminal olefinic H-atoms (δ(H) 4.66–4.68, 4.71–4.73 (br. *s*, 1 H each)). Comparison of spectroscopic data of **1** and **4**, a known compound obtained also from this plant, revealed that **1** possesses a (8*R*,9*S*,10*S*,13*R*,14*S*,17*R*,20*R*)-24-methylcholestane skeleton with a C(5)=C(6) bond, one OH group at C(20), and an olefinic CH_2 group at C(24) [10]. The ^{13}C-NMR signals of an α,β-unsaturated ketone system (δ(C) 202.2 (*s*), 165.1 (*s*), 126.1 (*d*)) indicated the presence of the C(7)=O group which was further confirmed by correlations of H–C(9) (δ(H) 1.44–1.46) and H–C(14) (δ(H) 1.49–1.51) to C(7) ((δ(C) 202.2) in the HMBC spectrum (*Fig.* 2). The HMBCs of the signal at δ(H) 3.66–3.69 with those of C(1) (δ(C) 36.3) and C(5) (δ(C)

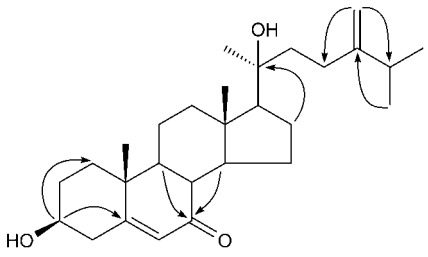

Fig. 2. *Key HMBCs* (H → C) *of* **1**

Table. *[1]H-NMR Data of* **1** *and [13]C-NMR Data of Compounds* **1**–**3**. Recorded in $CDCl_3$; δ in ppm, J in Hz.

Position	δ(H)	δ(C)		
	1	**1**	**2**	**3**
1	1.28–1.31 (overlapped, H_{ax}), 1.99–2.02 (overlapped, H_{eq})	36.3 (*t*)	37.2 (*t*)	36.9 (*t*)
2	1.59–1.62 (overlapped, H_{ax}), 1.94–1.96 (*m*, H_{eq})	31.2 (*t*)	25.5 (*t*)	31.1 (*t*)
3	3.66–3.69 (*m*, H_{ax})	70.5 (*d*)	72.8 (*d*)	71.0 (*d*)
4	2.41–2.43 (*ddd*, J = 13.8, 13.5, 4.0, H_{ax}), 2.49–2.52 (*ddd*, J = 13.5, 4.0, 3.5, H_{eq})	41.8 (*t*)	77.3 (*d*)	41.8 (*t*)
5		165.1 (*s*)	143.0 (*s*)	146.3 (*s*)
6	5.69 (*s*)	126.1 (*d*)	128.1 (*d*)	120.8 (*d*)
7	–	202.2 (*s*)	65.4 (*d*)	68.2 (*d*)
8	2.29–2.31 (*dd*, J = 13.8, 13.5)	45.4 (*d*)	37.8 (*d*)	35.6 (*d*)
9	1.44–1.46 (*ddd*, J = 13.8, 13.5, 4.0)	50.0 (*d*)	42.3 (*d*)	43.0 (*d*)
10		38.7 (*s*)	35.9 (*s*)	37.3 (*s*)
11	1.59–1.62 (overlapped)	21.2 (*t*)	21.0 (*t*)	20.6 (*t*)
12	1.28–1.31 (overlapped, H_{ax}), 2.04–2.06 (*ddd*, J = 13.5, 4.0, 3.5, H_{eq})	40.0 (*t*)	39.3 (*t*)	39.0 (*t*)
13		42.9 (*s*)	42.3 (*s*)	42.1 (*s*)
14	1.49–1.51 (*ddd*, J = 13.8, 13.5, 4.0)	49.9 (*d*)	49.7 (*d*)	49.2 (*d*)
15	1.28–1.31 (overlapped, H_{ax}), 2.38–2.40 (*m*, H_{eq})	24.6 (*t*)	24.6 (*t*)	23.6 (*t*)
16	1.34–1.36 (*m*, H_{ax}), 1.99–2.02 (overlapped, H_{eq})	22.2 (*t*)	28.4 (*t*)	22.2 (*t*)
17	1.22–1.24 (*dd*, J = 13.8, 3.5)	57.9 (*d*)	55.9 (*d*)	57.7 (*d*)
18	0.86 (*s*)	13.8 (*q*)	11.9 (*q*)	13.6 (*q*)
19	1.20 (*s*)	19.2 (*q*)	18.8 (*q*)	19.0 (*q*)
20	–	75.4 (*s*)	35.8 (*d*)	75.1 (*s*)
21	1.32 (*s*)	26.2 (*q*)	18.6 (*q*)	26.0 (*q*)
22	1.42–1.44 (*m*)	42.3 (*t*)	34.8 (*t*)	42.0 (*t*)
23	1.84–1.86, 2.04–2.06 (2*m*)	29.1 (*t*)	31.1 (*t*)	28.7 (*t*)
24		156.2 (*s*)	157.1 (*s*)	156.0 (*s*)
25	2.21–2.23 (*m*)	34.3 (*d*)	33.9 (*d*)	33.9 (*d*)
26	1.01–1.03 (*d*, J = 6.8)	22.3 (*q*)	22.2 (*q*)	21.9 (*q*)
27	1.01–1.03 (*d*, J = 6.8)	22.3 (*q*)	22.2 (*q*)	21.9 (*q*)
28	4.66–4.68, 4.71–4.73 (2 br. *s*)	106.6 (*t*)	106.2 (*t*)	106.0 (*t*)
AcO		–	–	170.5 (*s*),
		–	–	21.2 (*q*)

165.1) indicated that a second OH group was positioned at C(3). The NOESY correlations H–C(3)/H_α–C(1) established that H–C(3) was β-oriented. Based on the above evidences, the structure of compound **1** was determined as (3β)-3,20-dihydroxyergosta-5,24(28)-dien-7-one.

Compound **2** exhibited a quasi-molecular-ion peak (*m*/*z* 429.3365 ([$M-H$]$^-$; calc. 429.3369) in its HR-ESI-MS, accounting for the molecular formula $C_{28}H_{46}O_3$, which suggested six degrees of unsaturation. The IR spectrum indicated the presence of OH and C=C moieties. The NMR data of **2** evidenced a (8*R*,9*S*,10*S*,13*R*,14*S*,17*R*,20*R*)-24-methylcholestane skeleton similar to that of **1** with the exception of the side-chain resonances. The upfield shift of the C(20) signal from δ(C) 75.4 in **1** to 35.8 in **2** indicated that the O-bearing quarternary C-atom was replaced by a CH group. Three signals appearing at δ(C) 65.4, 72.8, and 77.3 were due to the presence of three OH groups. The HMBCs of the H-atom signal at δ(H) 3.50–3.52 with those of C(1) and C(5) indicated the location of one OH group at C(3). In the ^{1}H,^{1}H-COSY spectrum, the correlations between a signal at δ(H) 4.11–4.12 with that of H–C(3) suggested that another OH group was at C(4), which was supported by the HMBCs between the signal of H–C(4) and those of C(2) (δ(C) 25.5) and C(6) (δ(C) 128.1). The HMBCs of the signal at δ(H) 3.82–3.84 with those of C(5), C(9), and C(14) indicated that the third OH group was at C(7). The NOESY correlations H–C(3)/H_α–C(1), H–C(3)/H–C(4), and H–C(7)/H–C(8) established that H–C(3), H–C(4), and H–C(7) were β-, β-, and α-oriented, respectively. Accordingly, compound **2** was elucidated as (3β,4β,7α)-ergosta-5,24(28)-diene-3,4,7-triol.

Compound **3** was obtained as a white amorphous solid. Its positive-ion-mode HR-ESI-MS spectrum exhibited a quasi-molecular-ion peak (*m*/*z* 471.3475 ([$M-H$]$^-$; calc. 471.3474), consistent with the molecular formula $C_{30}H_{48}O_4$, accounting for seven degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectra of **3** showed resonances characteristic of an Ac group and a (8*R*,9*S*,10*S*,13*R*,14*S*,17*R*,20*R*)-24-methylcholestane skeleton identical with that of **1** except for the chemical shifts of a HO–CH(7) (δ(C) 68.2 and δ(H) 3.78–3.80) in **3** replacing the α,β-unsaturated ketone in **1**. The AcO group was at C(7) based on the HMBC of the signal of H–C(7) (δ(H) 3.78–3.80) with that of the AcO group (δ(C) 170.5). The NOESY correlations H–C(3)/H_α–C(1) and H–C(7)/H–C(8) established that H–C(3) and H–C(7) were β- and α-oriented, respectively. Thus, the structure of **3** was determined as (3β,7α)-3,20-dihydroxyergosta-5,24(28)-dien-7-yl acetate.

Experimental Part

General. All solvents were distilled before use. TLC: Silica gel *GF$_{254}$* (SiO_2, 10–40 μm; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). Column chromatography (CC): silica gel (SiO_2, 200–300 mesh, 10–40 μm; *Qingdao Marine Chemical Factory*) and *Sephadex LH-20* (*Amersham Pharmacia Biotech*, Sweden). Optical rotations: *JASCO-20C* digital polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *577* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-400* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *VG AutoSpec-3000* mass spectrometer; in *m*/*z*. HR-ESI-MS: *API QSTAR Pulsar-1* mass spectrometer; in *m*/*z*.

Plant Material. The stem bark (3.9 kg) of *D. lukii* was collected in Zhongdian, Yunnan Province, P. R. China, in May 2010. A specimen (DL20090501), identified by one of the authors (*J.-G. C.*), was

deposited with the Herbarium of the College of Biological Resources and Environment Science, Qujing Normal University, Qujing, Yunnan Province, P. R. China.

Extraction and Isolation. The air-dried stem bark (3.9 kg) of *D. lukii* was ground into powder and extracted thrice with 70% EtOH. After evaporation of the EtOH, the crude extract (328 g) was partitioned between H_2O and AcOEt. The AcOEt-soluble portion (118 g) was purified by CC (SiO_2; $CHCl_3$/MeOH 100:1 → 1:1) to afford ten fractions, *Frs. 1–10*. *Fr. 4* (7.6 g) was subjected to CC (*MCI*; MeOH/H_2O 70 → 95%) to yield six subfractions, *Frs. 4.A – Fr. 4.F*. *Fr. 4.B* (1.7 g) was separated by repeated CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1; and SiO_2), to afford **3** (78 mg). *Fr. 4.C* (1.9 g) was purified by CC (*Sephadex LH-20*; MeOH; and *ODS*; MeOH) to yield compound **1** (69 mg) and **2** (81 mg).

(3β)-3,20-Dihydroxyergosta-5,24(28)-dien-7-one (=*(3S,8S,9S,10R,13S,14S,17S)-1,2,3,4,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-3-hydroxy-17-[(2S)-2-hydroxy-6-methyl-5-methylideneheptan-2-yl]-10,13-dimethyl-7H-cyclopenta[a]phenanthren-7-one*; **1**). White amorphous solid. M.p. 197–199°. $[\alpha]_D^{23.3} = -83.3$ ($c = 0.98$, MeOH). UV (MeOH): 252 (1.89). IR (KBr): 3442, 2976, 1676, 1632, 1110, 1078. ^{1}H- (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): see the *Table*. FAB-MS (neg.): 427 ($[M-H]^-$). HR-EI-MS: 427.3211 ($[M-H]^-$, $C_{28}H_{43}O_3^-$; calc. 427.3212).

(3β,4β,7α)-Ergosta-5,24(28)-diene-3,4,7-triol (=*(3S,4R,7S,8S,9S,10R,13R,14S,17R)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-10,13-dimethyl-17-[(2R)-6-methyl-5-methylideneheptan-2-yl]-1H-cyclopenta[a]phenanthrene-3,4,7-triol*; **2**). White amorphous solid. M.p. 211–212°. $[\alpha]_D^{23.3} = -88.7$ ($c = 0.33$, MeOH). IR (KBr): 3445, 3030, 1640, 1622, 1107, 830. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.70 (*s*, Me(18)); 0.94 (*s*, Me(21)); 0.98 (*s*, Me(19)); 0.99 (*d*, $J = 7.0$, Me(26,27)); 1.09–1.10 (overlapped, H_{ax}–C(1), H_{ax}–C(15)); 1.16–1.17 (*m*, H_{ax}–C(12)); 1.18–1.19 (overlapped, H–C(17), H_a–C(22)); 1.19–1.20 (*m*, H–C(9)); 1.25–1.26 (*m*, H_{ax}–C(16)); 1.39–1.41 (overlapped, H–C(14), H–C(20)); 1.47–1.49 (*m*, H–C(8)); 1.49–1.50 (*m*, H_{ax}–C(2)); 1.50–1.53 (overlapped, CH_2(11), H_b–C(22)); 1.68–1.69 (*m*, H_{eq}–C(15)); 1.79–1.80 (*m*, H_{eq}–C(2)); 1.83–1.85 (*m*, H_{eq}–C(16)); 1.85–1.86 (*ddd*, $J = 13.6$, 4.5, 4.2, H_{eq}–C(1)); 1.87–1.89 (*m*, H_a–C(23)); 1.98–1.99 (*m*, H_{eq}–C(12)); 2.04–2.05 (*m*, H_b–C(23)); 2.18–2.19 (*m*, H–C(25)); 3.50–3.52 (*m*, H–C(3)); 3.82–3.84 (*dd*, $J = 7.0$, 4.0, H–C(7)); 4.11–4.12 (*d*, $J = 4.0$, H–C(4)); 4.64–4.65 (br. *s*, H_a–C(28))); 4.72–4.74 (br. *s*, H_b–C(28)); 5.76–5.78 (*d*, $J = 7.0$, H–C(6)). ^{13}C-NMR (125 MHz, $CDCl_3$): see the *Table*. FAB-MS (neg.): 429 ($[M-H]^-$). HR-EI-MS: 429.3365 ($[M-H]^-$, $C_{28}H_{45}O_3^-$; calc. 429.3369).

(3β,7α)-3,20-Dihydroxyergosta-5,24(28)-dien-7-yl Acetate (=*(3S,7S,8S,9S,10R,13S,14S,17S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-3-hydroxy-17-[(2S)-2-hydroxy-6-methyl-5-methylideneheptan-2-yl]-10,13-dimethyl-1H-cyclopenta[a]phenanthren-7-yl Acetate*; **3**). White amorphous solid. M.p. 204–205°. $[\alpha]_D^{23.3} = -91.3$ ($c = 0.20$, MeOH). IR (KBr): 3440, 3025, 1735, 1640, 1620, 825. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.88 (*s*, Me(18)); 1.05–1.06 (*d*, $J = 7.0$, Me(26,27)); 1.20 (*s*, Me(19)); 1.20–1.21 (*m*, H–C(9)); 1.29–1.30 (overlapped, H_{ax}–C(1), H_{ax}–C(12)); 1.24–1.25 (*dd*, $J = 13.8$, 4.0, H–C(17)); 1.30–1.31 (*m*, H_{ax}–C(15)); 1.33 (*s*, Me(21)); 1.35–1.37 (*m*, H_{ax}–C(16)); 1.44–1.46 (*m*, CH_2(22)); 1.49–1.51 (*m*, H–C(14)); 1.85–1.87 (*m*, H_a–C(23)); 1.92–1.93 (*m*, H–C(8)); 1.60–1.62 (overlapped, H_{ax}–C(2), CH_2(11)); 2.40–2.42 (*m*, H_{eq}–C(15)); 1.95–1.97 (*m*, H_{eq}–C(2)); 1.98–2.01 (overlapped, H_{eq}–C(1), H_{eq}–C(16)); 2.01 (*s*, MeCO)); 2.05–2.06 (overlapped, H_{eq}–C(12), H_b–C(23)); 2.19–2.21 (*m*, H–C(25)); 2.34–2.36 (*ddd*, $J = 14.0$, 13.8, 3.6, H_{ax}–C(4)); 2.52–2.53 (*ddd*, $J = 13.8$, 4.0, 3.6, H_{eq}–C(4)); 3.50–3.52 (*ddd*, $J = 13.8$, 13.5, 4.0, H–C(3)); 4.68–4.69 (br. *s*, H_a–C(28)); 4.72–4.74 (br. *s*, H_b–C(28)); 4.93–4.95 (*dd*, $J = 7.0$, 4.0, H–C(7)); 5.57–5.59 (*d*, $J = 7.0$, H–C(6)). ^{13}C-NMR (125 MHz, $CDCl_3$): see the *Table*. FAB-MS (neg.): 471 ($[M-H]^-$). HR-EI-MS: 471.3475 ($[M-H]^-$, $C_{30}H_{47}O_4^-$; calc. 471.3474).

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