Four New Lanostane Triterpenoids from *Euphorbia humifusa*

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Four new lanostane triterpenoids, namely (3β)-3-hydroxy-24-methylenelanost-8-ene-7,11-dione (**1**), (3β)-3-hydroxylanosta-8,24-diene-7,11-dione (**2**), (3β,7α)-3,7-dihydroxylanosta-8,24-dien-11-one (**3**), and (3β,11β)-3,11-dihydroxylanosta-8,24-dien-7-one (**4**) were isolated from *Euphorbia humifusa*, together with 2 known compounds. The structures of these new compounds were elucidated on the basis of extensive spectroscopic analysis and comparison with the related known compounds.

Introduction. – The genus *Euphorbia*, comprising *ca*. 2000 species, is widely distributed in the world, with 80 species occurring in China [1]. Previous chemical studies on the plants of this genus have led to the isolation of diterpenoids, triterpenoids, flavanoids, and tannins [2][3]. A number of constituents have shown an array of biological activities such as skin irritating, antitumor, and tumor-promoting activities [4].

Euphorbia humifusa WILLD has been used in traditional Chinese medicine for the treatment of dysentery, enteritis, and hematochezia [5]. In continuation of our studies aimed at finding new chemical constituents from this genus, we now describe the isolation and structural elucidation of four new compounds, (3β)-3-hydroxy-24-methylenelanost-8-ene-7,11-dione (**1**), (3β)-3-hydroxylanosta-8,24-diene-7,11-dione (**2**), (3β,7α)-3,7-dihydroxylanosta-8,24-dien-11-one (**3**) and (3β,11β)-3,11-dihydroxylanosta-8,24-dien-7-one (**4**), together with two known compounds, (3β,22*E*)-cycloart-23-ene-3,25-diol and (3β,23*Z*)-cycloart-23-ene-3,25-diol (cycloartane = 9,19-cyclolanostane), from the EtOH extract of the whole plant of *E. humifusa*.

Results and Discussion. – Compound **1** was obtained as a colorless gum. Its molecular formula was determined as $C_{31}H_{48}O_3$ by HR-EI-MS (*m*/*z* 468.3613). The IR spectrum showed strong absorption bands at 3432 and 1673 cm^{-1} ascribed to OH and conjugated-carbonyl functionalities, respectively. Absorption at 269 nm in the UV spectrum confirmed the presence of a conjugated carbonyl system. From the ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), HSQC, ^{1}H,^{1}H-COSY and HMBC (*Fig. 1*), and NOESY data (*Fig. 2*), the structure of compound **1** was identified as (3β)-3-hydroxy-24-methylenelanost-8-ene-7,11-dione.

The ^{1}H- and ^{13}C-NMR spectra of **1** indicated the presence of five tertiary Me groups (δ(H) 0.80, 0.89, 1.02, 1.17, and 1.30 (5*s*, 3 H each)), three secondary Me groups (δ(H) 0.91, 1.015, 1.018 (3*d*, *J* = 6.4 Hz,

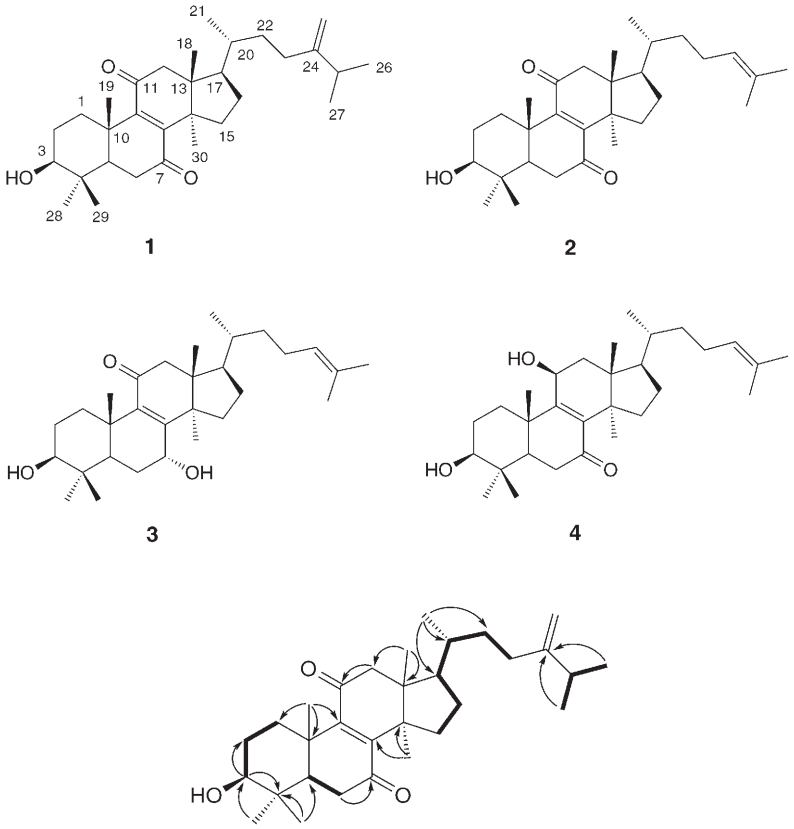

Fig. 1. *^{1}H,^{1}H-COSY* (▬) *and selected HMBC* (→) *correlations of* **1**

3 H each)), and one oxymethine (δ(H) 3.28 (*dd*, $J = 5.2$, 11.0 Hz, 1 H)). One terminal CH_2 group (δ(H) 4.66 and 4.72 (2 br. *s*, 1 H each); δ(C) 106.2 (*t*) and 156.5 (*s*) and an α,β-unsaturated diketone moiety (δ(C) 202.4, 202.2, 151.8, and 150.7 (4*s*)) were also characterized. The remaining signals were assigned to eight CH_2 and four CH groups, and four quaternary C-atoms. The molecular formula indicated the presence of eight units of unsaturation. Therefore, the compound must be tetracarbocyclic since there were only two olefinic and two ketone groups. The aforementioned data indicated that compound **1** was most likely a lanostane triterpenoid with a C_9 side-chain moiety at C(17) containing a CH_2= substituent at C(24) [6]. The gross structure of **1** was deduced from the HSQC, ^{1}H,^{1}H-COSY, and HMBC data. Inspection of the ^{1}H,^{1}H-COSY plot led to the establishment of three spin systems (*Fig. 1*). In the HMBC plot, the two geminal Me(28) and Me(29) groups at δ(H) 1.02 and 0.89 showed correlations with C(3), C(4), and C(5) at δ(C) 77.7, 38.8, and 50.2, respectively, which indicated the presence of a 3-OH group (*Fig. 1*). The HMBC cross-peaks Me(19)/C(9), Me(30)/C(8), H–C(6)/C(7), H–C(6)/C(8), H–C(12)/C(9), and H–C(12)/C(11) confirmed the presence of an 8-ene-7,11-dione moiety. The HMBC cross-peaks Me(26)/C(24), Me(27)/C(24), CH_2=C(24)/C(23), and CH_2=C(24)/C(25) revealed the CH_2=C(24) unsaturation.

The relative configuration of **1** was determined by extensive analysis of the ^{1}H-NMR (*Table 1*) and NOESY data. The large coupling constant ($J = 11.0$ Hz) between H–C(3) and H_β–C(2) clearly indicated that the 3-OH group was in equatorial β-position [7]. The NOESY correlations (*Fig. 2*) H–C(3)/H–C(5), H–C(3)/Me(28), Me(30)/H–C(17), Me(29)/Me(19), and Me(19)/Me(18) showed

Table 1. *^{1}H-NMR Data* (400 MHz, $CDCl_3$) *of Compounds* **1**–**4**. δ in ppm, J in Hz.

	1	**2**	**3**	**4**
H_α–C(1)	1.20–1.25 (*m*)	1.20–1.26 (*m*)	1.10–1.18 (*m*)	1.52–1.60 (*m*)
H_β–C(1)	2.89 (*dt*, *J* = 13.8, 3.6)	2.89 (*dt*, *J* = 13.8, 3.6)	3.01 (*dt*, *J* = 13.8, 4.0)	2.40–2.46 (*m*)
CH_2(2)	1.68–1.78 (*m*)	1.66–1.78 (*m*)	1.65–1.74 (*m*)	1.70–1.79 (*m*)
H–C(3)	3.28 (*dd*, *J* = 5.2, 11.0)	3.27 (*dd*, *J* = 5.2, 11.0)	3.31 (*dd*, *J* = 5.6, 11.2)	3.31 (*dd*, *J* = 5.6, 10.4)
H–C(5)	1.54 (*dd*, *J* = 5.2, 12.2)	1.51 (*dd*, *J* = 5.0, 12.2)	1.28 (*dd*, *J* = 2.0, 12.0)	1.60–1.68 (*m*)
CH_2(6)	2.46–2.52 (*m*)	2.46–2.52 (*m*)	1.72–1.79 (*m*)	2.40–2.48 (*m*)
H–C(7)	–	–	4.38 (*d*, *J* = 4.2)	–
H–C(11)	–	–	–	4.69 (*t*, *J* = 7.2)
H_α–C(12)	2.76 (*d*, *J* = 16.0)	2.74 (*d*, *J* = 16.0)	2.68 (*d*, *J* = 17.2)	1.74–1.81 (*m*)
H_β–C(12)	2.61 (*d*, *J* = 16.0)	2.60 (*d*, *J* = 16.0)	2.46 (*d*, *J* = 17.2)	2.35–2.43 (*m*)
H_α–C(15)	2.25–2.28 (*m*)	2.10–2.17 (*m*)	1.84–1.90 (*m*)[a])	2.12–2.19 (*m*)
H_β–C(15)	1.74–1.81 (*m*)	1.70–1.79 (*m*)	1.65–1.71 (*m*)[a])	1.37–1.42 (*m*)
H_α–C(16)	1.98–2.04 (*m*)	1.93–2.02 (*m*)	2.00–2.04 (*m*)	1.88–1.94 (*m*)
H_β–C(16)	1.38–1.42 (*m*)	1.30–1.38 (*m*)	1.38–1.44 (*m*)	1.28–1.32 (*m*)
H–C(17)	1.64–1.72 (*m*)	1.62–1.70 (*m*)	1.70–1.75 (*m*)[a])	1.55–1.60 (*m*)
Me(18)	0.80 (*s*)	0.80 (*s*)	0.78 (*s*)	0.70 (*s*)
Me(19)	1.30 (*s*)	1.30 (*s*)	1.05 (*s*)	1.25 (*s*)
H–C(20)	1.40–1.48 (*m*)	1.36–1.50 (*m*)[a])	1.38–1.46 (*m*)[a])	1.40–1.46 (*m*)[a])
Me(21)	0.91 (*d*, *J* = 6.4)	0.90 (*d*, *J* = 6.4)	0.88 (*d*, *J* = 6.4)	0.86 (*d*, *J* = 6.4)
CH_2(22)	1.10–1.18 (*m*)	1.06–1.10 (*m*)	1.02–1.08 (*m*)	1.08–1.13 (*m*)
	1.65–1.69 (*m*)	1.43–1.48 (*m*)[a])	1.38–1.46 (*m*)[a])	1.56–1.62 (*m*)[a])
CH_2(23)	1.82–1.91 (*m*)	1.82–1.90 (*m*)	1.84–1.88 (*m*)	1.84–1.90 (*m*)
	2.07–2.15 (*m*)	2.00–2.08 (*m*)	2.00–2.07 (*m*)	1.98–2.02 (*m*)
H–C(24)	–	5.08 (*t*, *J* = 7.0)	5.08 (*t*, *J* = 7.0)	5.08 (*t*, *J* = 7.2)
H–C(25)	2.20–2.27 (*m*)	–	–	–
Me(26)	1.015 (*d*, *J* = 6.4)[b])	1.68 (*s*)	1.68 (*s*)	1.68 (*s*)
Me(27)	1.018 (*d*, *J* = 6.4)[b])	1.60 (*s*)	1.60 (*s*)	1.61 (*s*)
Me(28)	1.02 (*s*)	1.02 (*s*)	1.04 (*s*)	0.99 (*s*)
Me(29)	0.89 (*s*)	0.88 (*s*)	0.83 (*s*)	0.90 (*s*)
Me(30)	1.17 (*s*)	1.17 (*s*)	1.23 (*s*)	1.13 (*s*)
CH_2–C(24)	4.66, 4.72 (2 br. *s*)	–	–	–

[a]) δ(H) are overlapped. [b]) δ(H) are interchangeable.

that H–C(3), H–C(5), H–C(17), Me(28), and Me(30) were all in α-orientation, whereas Me(18), Me(19), and Me(29) were in β-orientation. The NOESY correlations H–C(20)/Me(18), and Me(21)/H–C(17) indicated that Me(21) was α-orientated [8].

Compound **2** was obtained as a colorless gum. The molecular formula $C_{30}H_{46}O_3$ was established by HR-EI-MS (*m*/*z* 454.3455), indicating the presence of 8 degrees of unsaturation. The comparison of NMR data (*Tables 1* and *2*) showed that **1** and **2** were very closely related to each other, including configuration, except for the monounsaturated side-chain moiety. The differences between these two compounds were explained by the absence of the terminal methylene group and the presence of a trisubstituted C=C bond (δ(H) 5.08 (*t*, *J* = 7.0 Hz, 1 H); δ(C) 124.8 (*d*) and 131.3 (*s*)) at C(24) in **2**. Thus compound **2** was determined to be (3β)-3-hydroxylanosta-8,24-diene-7,11-dione.

Table 2. *^{13}C-NMR Data* (100 MHz, $CDCl_3$) *of Compounds* **1**–**4**. δ in ppm.

	1	**2**	**3**	**4**
C(1)	34.1	34.1	34.1	33.6
C(2)	27.7	27.7	27.9	27.8
C(3)	77.7	77.7	78.5	78.2
C(4)	38.8	38.9	38.5	39.0
C(5)	50.2	50.2	45.9	49.2
C(6)	36.4	36.5	28.1	35.8
C(7)	202.2	202.2	68.2	200.1
C(8)	150.7	150.7	160.3	140.4
C(9)	151.8	151.8	141.1	161.2
C(10)	39.8	39.8	38.9	39.6
C(11)	202.4	202.5	200.9	68.1
C(12)	51.7	51.7	51.9	42.8
C(13)	48.9[a])	48.9[a])	50.8[a])	48.0[a])
C(14)	47.4[a])	47.4[a])	47.4[a])	46.2[a])
C(15)	32.2	32.2	30.1	31.8
C(16)	27.4	27.4	27.2	27.3
C(17)	49.1	49.1	50.1	48.6
C(18)	16.8	16.8	16.8	16.2
C(19)	17.6	17.58	17.2	19.6
C(20)	36.2	35.9	36.0	35.6
C(21)	18.6	18.5	18.3	18.7
C(22)	34.8	36.1	36.03	35.5
C(23)	31.2	24.8	24.8	24.8
C(24)	156.5	124.8	124.8	124.9
C(25)	33.8	131.3	131.2	131.1
C(26)	22.0[a])	25.7	25.7	25.68
C(27)	21.9[a])	17.65	17.6	17.7
C(28)	27.9	27.9	28.1	27.6
C(29)	15.5	15.5	15.9	15.2
C(30)	25.9	25.9	27.4	25.73
CH_2–C(24)	106.2	–	–	–

[a]) δ(C) are interchangeable.

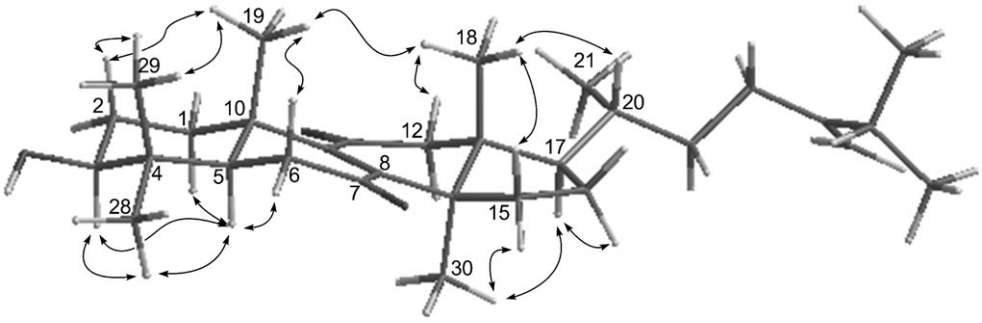

Fig. 2. *Key NOESY correlations of* **1**

Compound **3** was obtained as a white amorphous powder. The molecular formula $C_{30}H_{48}O_3$ was established by HR-EI-MS (*m/z* 456.3604), indicating the presence of 7 degrees of unsaturation. IR-Absorption bands at 3423 and 1641 cm^{-1} indicated the presence of OH and conjugated-carbonyl groups. The UV absorption at 255 nm was characteristic of an α,β-unsaturated ketone [9]. The comparison of NMR data (*Tables 1* and *2*) showed that **2** and **3** were similar in structure and configuration, except that the ketone function at C(7) of **2** was replaced by a secondary-alcohol function in **3**, which was confirmed by HMBC cross-peaks (H–C(7) (δ(H) 4.38)/C(5), C(8), and C(9)). The relative configuration of H–C(7) was deduced from the multiplicity of its signal (*d*, $J = 4.2$ Hz) [10], which was further supported by the significant ROESY correlation H–C(7)/Me(18). Compound **3** was accordingly elucidated as (3β,7α)-3,7-dihydroxylanosta-8,24-dien-11-one.

Compound **4** was obtained as a white amorphous powder. The molecular formula $C_{30}H_{48}O_3$ was established by HR-EI-MS (*m/z* 456.3609), indicating the presence of 7 degrees of unsaturation. The comparison of the NMR data (*Tables 1* and *2*) showed that **2** and **4** were similar in structure and configuration, except that the ketone function at C(11) of **2** was replaced by a secondary-alcohol function in **4**, which was confirmed by HMBC cross-peaks (H–C(11) (δ(H) 4.69)/C(8), C(9), and C(12)). The relative configuration of H–C(11) was deduced from the multiplicity of its signal (*t*, $J = 7.2$ Hz) [11], which was further supported by the ROESY correlations H–C(11)/H_α–C(1) and H_α–C(12). Thus, compound **4** was determined as (3β,11β)-3,11-dihydroxylanosta-8,24-dien-7-one.

The two known compounds (3β,23*E*)-cycloart-23-ene-3,25-diol [12], and (3β,23*Z*)-cycloart-23-ene-3,25-diol [13] were identified by comparison of their ^{1}H- and ^{13}C-NMR as well as MS data with those reported in the literature.

Experimental Part

General. All solvents used were of anal. grade *(Shanghai Chemical Plant*, Shanghai, P. R. China). Column chromatography (CC): silica gel (200–300 mesh). Optical rotation: *Perkin-Elmer 341* polarimeter. IR Spectra: *Perkin-Elmer 577* spectrometer; in cm^{-1}. NMR Spectra: *Bruker AM-400* spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. EI-MS and HR-EI: *Finnigan MAT-95* mass spectrometer; in *m/z* (rel. %). HPLC: *Agilent 1100* apparatus with an *Agilent DAD* spectrophotometer set.

Plant Material. The whole plant of *Euphorbia humifusa* was collected in September 2006 in Anhui Province, P. R. China. The plant material was authenticated by Prof. *Hu-Biao Chen*. The voucher specimen (SC0092006) is available for inspection at the Institute of Materia Medica, SIBS, CAS.

Extraction and Purification. The powdered whole plant of *E. humifusa* (4 kg) was percolated with 95% EtOH. After evaporation of the solvent, the crude extract (450 g) was dispersed in H_2O and then extracted with AcOEt to afford a dark viscous residue (110 g), which was separated by CC (SiO_2, petroleum ether/acetone 100:1 → 1:1): *Fractions A–F. Fr. B* (28 g) was separated by CC (SiO_2, petroleum ether/acetone 30:1 → 10:1): *Fr. B1–B4. Fr. B2* (1.9 g) was subjected to CC (SiO_2, petroleum ether/AcOEt 15:1): (3β,23*E*)-cycloart-23-ene-3,25-diol (30 mg) and (3β,23*Z*)-cycloart-23-ene-3,25-diol (17 mg). *Fr. C* (16 g) was separated by CC (SiO_2, petroleum ether/acetone 20:1 → 5:1): *Fr. C1–C4. Fr. C1* (1.1 g) was separated by CC (SiO_2, petroleum ether/AcOEt 10:1) followed by semi-prep. reversed-phase HPLC (*ODS-HG-5* (5 μ; 250 × 10 mm), MeOH/H_2O 4:1, 2.0 ml/min): **1** (3 mg) and **2** (5 mg). *Fr. C3* (0.9 g) was separated by CC (SiO_2, petroleum ether/AcOEt 10:1) followed by semi-prep. reversed-phase HPLC (*ODS-HG-5* (5 μ; 250 × 10 mm), MeOH/H_2O 3:1, 2.0 ml/min): **3** (6 mg) and **4** (7 mg).

(3β)-3-Hydroxy-24-methylenelanost-8-ene-7,11-dione (**1**): Colorless gum. $[\alpha]_D^{25} = +35$ ($c = 0.084$, MeOH). UV (MeOH): 269 (4.52). IR (KBr): 3432, 3030, 2962, 1673, 1641, 1461, 1378, 1234, 887. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 468 (100, M^+), 450 (10), 384 (50), 343 (16), 316 (37), 289 (16), 187 (24), 109 (46), 69 (32), 55 (24). HR-EI-MS: 468.3613 (M^+, $C_{31}H_{48}O_3^+$; calc. 468.3603).

(3β)-3-Hydroxylanosta-8,24-diene-7,11-dione (**2**): Colorless gum. $[\alpha]_D^{25} = +54$ ($c = 0.087$, MeOH). UV (MeOH): 269 (4.30). IR (KBr): 3421, 2933, 1672, 1627, 1450, 1236, 1037. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 454 (100, M^+), 436 (10), 343 (18), 276 (20), 255 (18), 69 (42). HR-EI-MS: 454.3455 (M^+, $C_{30}H_{46}O_3^+$; calc. 454.3447).

(3β,7α)-3,7-Dihydroxylanosta-8,24-dien-11-one (**3**): White amorphous powder. $[\alpha]_D^{25} = +143$ ($c = 0.12$, MeOH). UV (MeOH): 255 (3.77). IR (KBr): 3423, 2966, 1641, 1459, 1376, 1026. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 456 (100, M^+), 438 (25), 317 (18), 277 (16), 251 (36), 69 (32). HR-EI-MS: 456.3604 (M^+, $C_{30}H_{48}O_3^+$; calc. 456.3603).

(3β,11β)-3,11-Dihydroxylanosta-8,24-dien-7-one (**4**): White amorphous powder. $[\alpha]_D^{25} = +6$ ($c = 0.1$, MeOH). UV (MeOH): 254 (3.80). IR (KBr): 3426, 2966, 1650, 1456, 1377, 1035. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 456 (100, M^+), 438 (10), 423 (40), 343 (16), 302 (40), 121 (24), 69 (52). HR-EI-MS: 456.3609 (M^+, $C_{30}H_{48}O_3^+$; calc. 456.3603).

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Received August 20, 2007