New Cytotoxic Triterpenoids from the Aerial Parts of *Euphorbia sieboldiana*

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Phytochemical investigation of the EtOH extract of *Euphorbia sieboldiana* led to the isolation of four new oleanane-type triterpenoids, (1*β*,2*α*,3*β*,19*β*)-1,2,3,19-tetrahydroxyolean-12-en-28-oic acid, (1*β*,3*β*,19*β*)-1,3,19-trihydroxyolean-12-en-28-oic acid, (1*β*,2*α*,3*β*,16*β*,19*β*)-1,2,3,16,19-pentahydroxyolean-12-en-28-oic acid, and (1*β*,2*α*,3*β*,19*β*,23)-1,2,3,19,23-pentahydroxyolean-12-en-28-oic acid, along with 16 known compounds. Their structures were established by extensive 1D- and 2D-NMR, as well as other spectral analyses. Biological evaluation of the four new triterpenoids revealed potent cytotoxic activities against HeLa and Hep-G2 cells.

Introduction. – The genus *Euphorbia* (Euphorbiaceae) is comprised of 2,000 species, more than 80 of which are found in China [1]. Many of them have been used as the Tibetan medicinal plants *viz E. heyneana* SPRENG., *E. hirta* L., *E. jolkinii* BOISS., *E. lathyris* L., *E. altotibetic*, *etc.* which were reported to exert antivirus, antioxidant, and anti-edema activities [2]. *Euphorbia sieboldiana* is distributed in Qinghai-Tibet Plateau of China. The roots of *E. sieboldiana* have been used as herbal medicines for treatment of tuberculosis and furunculosis [3]. Previous phytochemical studies on *Euphorbia* plants led to identification of diterpenes, triterpenoids, and flavonoids [4–6].

As part of our ongoing program to search for new biologically active components of traditional Chinese and folk medicines, three species of *Euphorbia* plants were investigated. Three new ursane-type triterpenoids were isolated from roots of *E. kansuensis*, and exhibited potent cytotoxic activity against HeLa and Hep-G2 cells [7]. Also, *E. sieboldiana* was shown to have significant biological activity [8].

In this work, the EtOH extract of *E. sieboldiana* was investigated. Herein, we describe the isolation and structural elucidation of four new oleanane-type triterpenoids, (1*β*,2*α*,3*β*,19*β*)-1,2,3,19-tetrahydroxyolean-12-en-28-oic acid (**1**), (1*β*,3*β*,19*β*)-1,3,19-trihydroxyolean-12-en-28-oic acid (**2**), (1*β*,2*α*,3*β*,16*β*,19*β*)-1,2,3,16,19-pentahydroxyolean-12-en-28-oic acid (**3**), and (1*β*,2*α*,3*β*,19*β*,23)-1,2,3,19,23-pentahydroxyolean-12-en-28-oic acid (**4**; *Fig. 1*). In addition, 16 known compounds, corosolic acid, butulin, 3*α*-hydroxy-*ent*-trachylobane, 16*β*,17-dihydroxy-*ent*-kaurane-3-one, orientin, luteolin, luteolin 5-*O*-*β*-D-glucoside, apigenin, acacetin, stigmasterol, *β*-sitosterol-3-*O*-*β*-D-glucoside, luteolin 7-*O*-*β*-D-glucoside, 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone, campesterol, 2,3-dihydroxyurs-12-en-28-oic acid, and cholesterol, were isolated. Their structures were mainly determined by spectroscopic methods. Antitumor evaluations of the four new triterpenoids against HeLa and Hep-G2 cells are also reported.

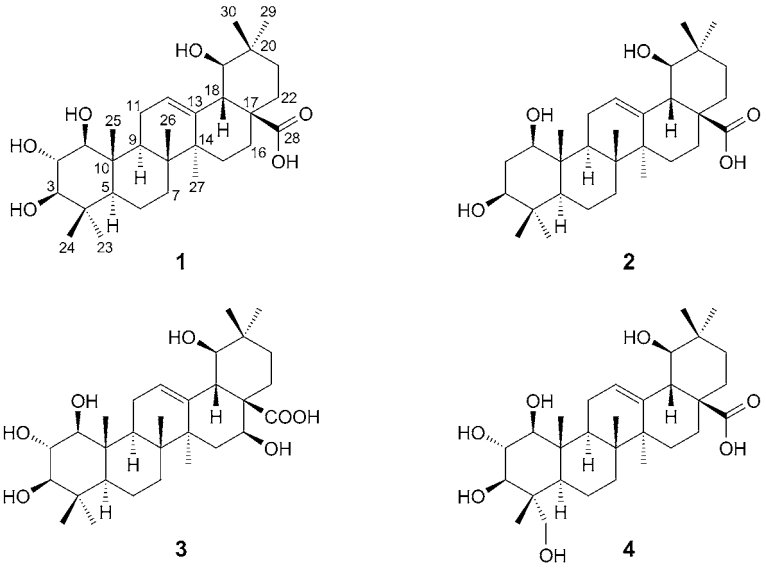

Fig. 1. *Structures of compounds* **1**–**4**, *isolated from* Euphorbia sieboldiana

Results and Discussion. – The petroleum ether-soluble fraction, AcOEt-soluble fraction, and BuOH-soluble fraction of the EtOH/H_2O 95 : 5 extract of the aerial parts of *E. sieboldiana* were submitted to multiple chromatographic steps to afford four new oleanane-type triterpenoids, **1**–**4**, together with 16 known compounds.

Compound **1** was obtained as colorless crystals with $[\alpha]_D^{20} = +4.1$ ($c = 0.01$, MeOH). The negative-ion-mode HR-ESI-MS of compound **1** showed a molecular-ion peak at m/z 503.3372 ($[M-H]^-$). In conjunction with its ^{13}C-NMR data, it indicated the molecular formula $C_{30}H_{48}O_6$. The assignment of ^{1}H- and ^{13}C-NMR spectroscopic data (*Tables 1* and *2*) of **1** was based on HSQC, HMBC, and ROESY spectra.

The ^{13}C-NMR spectrum ($CDCl_3$) of **1** exhibited seven Me signals at δ(C) 18.8, 19.1, 19.8, 23.1, 28.3, 30.9, and 31.9, a COOH C-atom signals at δ(C) 180.8, and a pair of olefinic C-atom signals at δ(C) 121.7 and 140.8, typical for a C(12)=C(13) bond in oleanane-type triterpenes [9]. In addition, seven Me signals at δ(H) 0.68, 0.81, 0.83, 0.85, 0.92, 0.93, and 1.01, and an olefinic H-atom signal at δ(H) 5.36 (*s*, 1 H) could be detected in the ^{1}H-NMR of **1**.

The ^{1}H-NMR spectrum ($CDCl_3$) indicated four O-bearing CH H-atoms (δ(H) 3.29 (d, $J = 3.6$, 1 H), 3.49 (*s*, 1 H), 3.52 (d, $J = 6.8$, 1 H), and 3.95 (d, $J = 6.8$, 1 H)). The four O-bearing CH C-atoms were also indicated in the ^{13}C-NMR spectrum ($CDCl_3$) of **1** (δ(C) 71.8, 76.7, 77.3, and 80.2). Thus, the structure of **1** was elucidated as a polyhydroxyolean-12-ene triterpenoid derivative.

In the HMBC spectrum of **1** (*Fig. 2*), the correlations of H–C(3) (δ(H) 3.49), and H–C(5) (δ(H) 1.33–1.35) with C(1) at (δ(C) 76.7), of H–C(2) (δ(H) 3.52) with C(4) (δ(C) 40.3), of H–C(1) (δ(H) 3.95) with C(3) (δ(C) 77.3), of H–C(1) (δ(H) 3.95) with C(5) (δ(C) 50.2), and of H–C(5) (δ(H) 1.33–1.35) with C(3) (δ(C) 77.3), revealed the

Table 1. *^{1}H-NMR Data of* **1**–**4** (at 500 MHz, in $CDCl_3$ at 27°; δ in ppm)

Position	**1**	**2**	**3**	**4**
1	3.95 (*d*, *J* = 6.8)	3.95 (*d*, *J* = 6.8)	3.94 (*d*, *J* = 6.8)	4.52 (*d*, *J* = 6.8)
2	3.52 (*d*, *J* = 6.8)	1.49–1.51 (*m*, H_a), 1.28–1.31 (*m*, H_b)	3.52 (*d*, *J* = 6.8)	4.48 (*d*, *J* = 6.8)
3	3.49 (*s*)	3.54 (*s*)	3.49 (*s*)	4.31 (*s*)
4	–	–	–	–
5	1.33–1.35 (*m*)	1.32–1.35 (*m*)	1.33–1.35 (*m*)	1.87–1.90 (*m*)
6	1.54 (*d*, *J* = 4.0, H_a), 1.25 (*d*, *J* = 4.0, H_b)	1.54 (*d*, *J* = 4.0, H_a), 1.26 (*d*, *J* = 4.0, H_b)	1.54 (*d*, *J* = 4.0, H_a), 1.26 (*d*, *J* = 4.0, H_b)	1.69 (*d*, *J* = 4.0, H_a), 1.57 (*d*, *J* = 4.0, H_b)
7	1.51 (*d*, *J* = 4.5, H_a), 1.31 (*d*, *J* = 4.5, H_b)	1.50 (*d*, *J* = 4.5, H_a), 1.30 (*d*, *J* = 4.5, H_b)	1.50 (*d*, *J* = 4.5, H_a), 1.30 (*d*, *J* = 4.5, H_b)	1.90 (*d*, *J* = 4.5, H_a), 1.87 (*d*, *J* = 4.5, H_b)
8	–	–	–	–
9	1.38–1.42 (*m*)	1.39–1.42 (*m*)	1.39–1.43 (*m*)	1.91–1.93 (*m*)
10	–	–	–	–
11	1.89–1.91 (*m*, H_a), 1.83–1.86 (*m*, H_b)	1.89–1.92 (*m*, H_a), 1.84–1.87 (*m*, H_b)	1.89–1.91 (*m*, H_a), 1.84–1.87 (*m*, H_b)	1.88–1.92 (*m*, H_a), 1.85–1.88 (*m*, H_b)
12	5.36 (*s*)	5.38 (*s*)	5.36 (*s*)	5.18 (*s*)
13	–	–	–	–
14	–	–	–	–
15	1.49–1.52 (*m*, H_a), 1.24–1.27 (*m*, H_b)	1.49–1.52 (*m*, H_a), 1.24–1.27 (*m*, H_b)	1.49–1.52 (*m*, H_a), 1.25–1.27 (*m*, H_b)	1.91–1.94 (*m*, H_a), 1.58–1.60 (*m*, H_b)
16	1.48–1.51 (*m*, H_a), 1.25–1.26 (*m*, H_b)	1.48–1.51 (*m*, H_a), 1.25–1.26 (*m*, H_b)	2.91–2.94 (*m*)	2.00–2.07 (*m*, H_a), 1.84–1.87 (*m*, H_b)
17	–	–	–	–
18	2.28 (*d*, *J* = 3.6)	2.29 (*d*, *J* = 3.6)	2.28 (*d*, *J* = 3.6)	2.49 (*d*, *J* = 9.5)
19	3.29 (*d*, *J* = 3.6)	3.14 (*d*, *J* = 3.6)	3.13 (*d*, *J* = 3.6)	3.87 (*d*, *J* = 7.0)
20	–	–	–	–
21	1.60–1.64 (*m*, H_a), 1.46–1.49 (*m*, H_b)	1.59–1.63 (*m*, H_a), 1.45–1.48 (*m*, H_b)	1.60–1.64 (*m*, H_a), 1.46–1.48 (*m*, H_b)	1.89–1.93 (*m*, H_a), 1.55–1.59 (*m*, H_b)
22	1.68–1.71 (*m*, H_a), 1.53–1.56 (*m*, H_b)	1.69–1.72 (*m*, H_a), 1.53–1.57 (*m*, H_b)	1.68–1.72 (*m*, H_a), 1.51–1.55 (*m*, H_b)	1.67–1.70 (*m*, H_a), 1.60–1.64 (*m*, H_b)
23	1.01 (*s*, 3 H)	1.01 (*s*, 3 H)	1.01 (*s*, 3 H)	4.00 (*d*, *J* = 9.5, H_a), 3.75 (*d*, *J* = 9.5, H_b)
24	0.93 (*s*, 3 H)	0.93 (*s*, 3 H)	0.96 (*s*, 3 H)	1.13 (*s*, 3 H)
25	0.85 (*s*, 3 H)	0.85 (*s*, 3 H)	0.86 (*s*, 3 H)	0.95 (*s*, 3 H)
26	0.83 (*s*, 3 H)	0.83 (*s*, 3 H)	0.84 (*s*, 3 H)	0.93 (*s*, 3 H)
27	0.92 (*s*, 3 H)	0.92 (*s*, 3 H)	0.92 (*s*, 3 H)	1.10 (*s*, 3 H)
28	–	–	–	–
29	0.81 (*s*, 3 H)	0.81 (*s*, 3 H)	0.81 (*s*, 3 H)	0.92 (*s*, 3 H)
30	0.68 (*s*, 3 H)	0.68 (*s*, 3 H)	0.68 (*s*, 3 H)	0.85 (*s*, 3 H)

presence of three OH groups located at C(1), C(2), and C(3). Correlations of H–C(19) (δ(H) 3.29) with C(17), C(18), C(20), C(29), and C(30) (δ(C) 46.1, 43.4, 39.8, 31.9 and 30.9, resp.) demonstrated that one OH group was attached to C(19).

Correlations of H–C(18) (δ(H) 2.28) with C(12) (δ(C) 121.7) and of H–C(19) (δ(H) 3.29) with C(13) (δ(C) 140.8), revealed the presence of a C(12)=C(13) bond. Correlations of H–C(18) (δ(H) 2.28) with C(28) (δ(C) 180.8), revealed a C=O group at C(17).

Table 2. *^{13}C-NMR Data of* **1**–**4** (at 125 MHz, in $CDCl_3$ at 27°; δ in ppm)

Position	**1**	**2**	**3**	**4**
1	76.7	76.7	76.7	76.6
2	71.8	37.3	71.8	72.6
3	77.3	77.3	77.3	77.4
4	40.3	40.3	40.3	39.6
5	50.2	50.2	50.2	41.8
6	21.1	21.1	21.1	18.3
7	33.9	33.9	33.9	33.3
8	42.4	42.4	42.4	38.3
9	39.8	39.8	39.8	47.7
10	37.3	37.3	37.3	37.2
11	23.1	23.1	23.1	23.5
12	121.7	121.7	121.7	124.3
13	140.8	140.8	140.8	145.2
14	45.8	45.8	45.8	39.8
15	29.2	29.2	29.2	28.4
16	26.2	26.2	71.7	26.9
17	46.1	46.1	46.1	47.2
18	43.4	43.4	43.4	46.8
19	80.2	80.2	80.2	81.0
20	39.8	39.8	39.8	34.7
21	36.5	36.5	36.5	28.4
22	31.7	31.7	31.7	32.6
23	23.1	23.1	23.1	71.7
24	19.1	19.1	19.1	17.5
25	19.8	19.8	19.8	16.7
26	18.8	18.8	18.8	17.5
27	28.3	28.3	28.3	26.2
28	180.8	180.8	180.8	171.0
29	31.9	31.9	31.9	28.0
30	30.9	30.9	30.9	25.9

The relative configuration of **1** was assigned on the basis of ROESY (*Fig. 3*) experiment. ROESY Correlations H–C(5)/Me(23), H–C(9)/Me(27), and H–C(18)/Me(30) indicated the usual triterpenoid ring junctions (*A*/*B trans*, *B*/*C trans*, *C*/*D trans*, *D*/*E cis*). The key ROE correlations H–C(1)/H–C(3), H–C(2)/Me(25), H–C(3)/Me(23), and H–C(19)/Me(29) indicated that H–C(1), H–C(3), and H–C(19) were α-oriented, and H–C(2) had the β-orientation. Further, ROESY correlations H–C(1)/H–C(5), H–C(2)/Me(24), H–C(3)/H–C(5), and H–C(5)/H–C(9) completed the relative configuration of **1** [10]. Thus, compound **1** was determined to be (1β,2α,3β,19β)-1,2,3,19-tetrahydroxyolean-12-en-28-oic acid.

Compound **2** was obtained as colorless crystals with $[\alpha]_D^{20} = +3.7$ ($c = 0.01$, MeOH). The molecular formula of **2** was established as $C_{30}H_{48}O_5$ by HR-ESI-MS (*m*/*z* 487.3422 ($[M-H]^-$)).

Similarities of H- and C-atom resonances of **2** with those of **1**, substantiated by the 1D- and 2D-NMR results, confirmed that **2** was a polyhydroxyolean-12-en-28-oic acid.

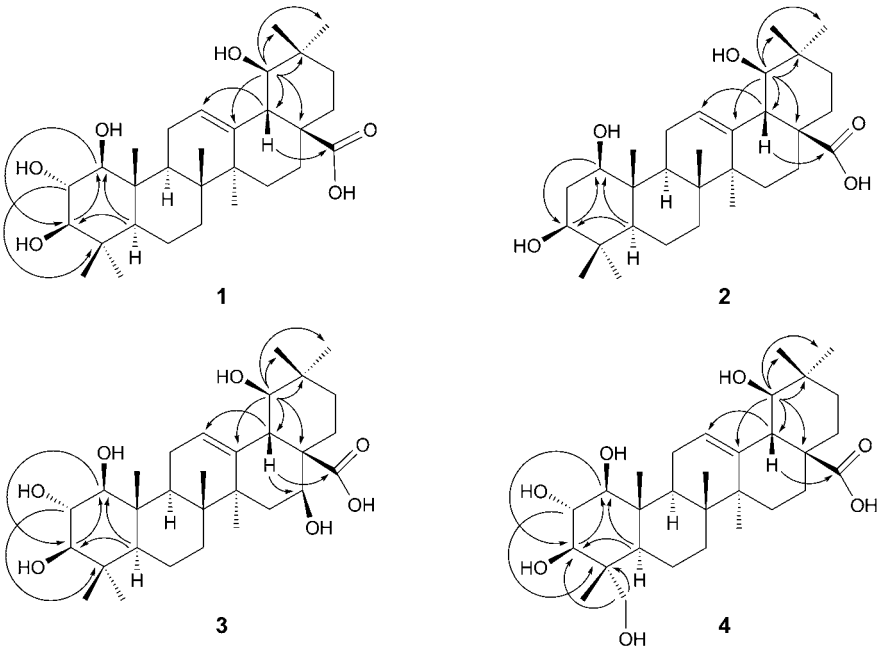

Fig. 2. *Key HMBCs* (H → C) *of compounds* **1**–**4**

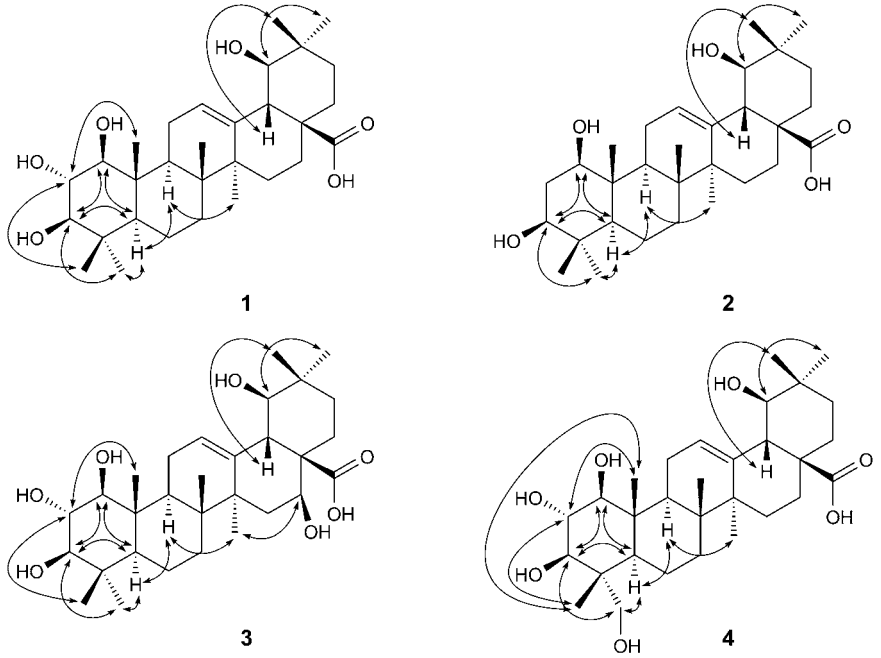

Fig. 3. *Key ROESY correlations* (H ↔ H) *of compounds* **1**–**4**

The OH group at C(2) in **1** disappeared in **2**. This was confirmed by the HMBC features of H–C(3) (δ(H) 3.54) and H–C(5) (δ(H) 1.32 – 1.35) with C(1) (δ(C) 76.7), of H_a–C(2) (δ(H) 1.49 – 1.51) and H_b–C(2) (δ(H) 1.28 – 1.31) with C(4) (δ(C) 40.3), and of H–C(1) (δ(H) 3.95) with C(3) (δ(C) 77.3). ROESY Correlations H–C(5)/Me(23), H–C(9)/Me(27), and H–C(18)/Me(30) indicated the usual triterpenoid ring junctions (*A*/*B trans*, *B*/*C trans*, *C*/*D trans*, *D*/*E cis*). ROE Correlations H–C(1)/H–C(3), H–C(3)/Me(23), and H–C(19)/Me(29) indicated that the H-atoms at C(1), C(3), and C(19) are α-oriented. Further, ROESY correlations H–C(1)/H–C(5), H–C(3)/H–C(5), and H–C(5)/H–C(9) completed the relative configuration of **2**. Therefore, compound **2** was identified as (1β,3β,19β)-1,3,19-trihydroxyolean-12-en-28-oic acid.

Compound **3** was obtained as colorless crystals with $[\alpha]_D^{20} = +3.9$ ($c = 0.01$, MeOH). The molecular formula of **3** was established as $C_{30}H_{48}O_7$ by HR-ESI-MS (*m*/*z* 519.3321 ($[M-H]^-$)).

Similarities of H- and C-atom resonances of **3** with those of **1**, together with the 1D- and 2D-NMR results, confirmed that **3** was anadditional polyhydroxyolean-12-en-28-oic acid.

Compared with **1**, an additional OH group was found to be located at C(16) in **3**. This was confirmed by the HMBC features of H–C(16) (δ(H) 2.31 – 2.94) with (C(18) and C(28) at δ(C) 43.4 and 180.8, resp.).

The relative configuration of **3** was assigned on the basis of a ROESY experiment. ROESY Correlations H–C(5)/Me(23), H–C(9)/Me(27), and H–C(18)/Me(30) indicated the usual triterpenoid ring junctions (*A*/*B trans*, *B*/*C trans*, *C*/*D trans*, *D*/*E cis*). ROE Correlations H–C(1)/H–C(3), H–C(2)/Me(25), H–C(3)/Me(23), H–C(16)/Me(27), and H–C(19)/Me(29) indicated that the H-atoms at C(1), C(3), C(16), and C(19) were α-oriented, and H–C(2) had β-orientation. Further, ROESY correlations H–C(1)/H–C(5), H–C(2)/Me(24), H–C(3)/H–C(5), and H–C(5)/H–C(9) completed the relative configuration of **3**. Therefore, compound **3** was identified as (1β,2α,3β,16β,19β)-1,2,3,16,19-pentahydroxyolean-12-en-28-oic acid.

Compound **4** was obtained as colorless crystals with $[\alpha]_D^{20} = +3.3$ ($c = 0.01$, MeOH). The molecular formula of **3** was established as $C_{30}H_{48}O_7$ by HR-ESI-MS (*m*/*z* 519.3322 ($[M-H]^-$)).

Similarities of H- and C-atom resonances of **4** with those of **1**, together with the result of 1D- and 2D-NMR, confirmed that **4** was also a polyhydroxyolean-12-en-28-oic acid derivative.

Compared to compound **1**, **4** had a OH group at C(23) in this was confirmed by the HMBC features of H_a–C(23) (δ(H) 4.00) and H_b–C(23) (δ(H) 3.75) with C(3) and C(5) (δ(C) 77.4 and 41.8, resp.).

The relative configuration of **4** was assigned on the basis of a ROESY experiment. ROESY Correlations H–C(5)/Me(23), H–C(9)/Me(27), and H–C(18)/Me(30) indicated the usual triterpenoid ring junctions (*A*/*B trans*, *B*/*C trans*, *C*/*D trans*, *D*/*E cis*). ROE Correlation Me(24)/Me(25) suggested that the $HOCH_2$(23) group was oriented. Strong ROE correlations H–C(1)/H–C(3), H–C(2)/Me(25), H–C(3)/Me(23), and H–C(19)/Me(29) indicated that H-atoms at C(1), C(3), and C(19) were α-oriented, and H–C(2) had the β-orientation. Further, ROESY correlations H–C(1)/H–C(5), H–C(2)/Me(24), H–C(3)/H–C(5), and H–C(5)/H–C(9) completed the relative

configuration of **4**. Therefore, compound **4** was identified as (1*β*,2*α*,3*β*,19*β*,23)-1,2,3,19,23-pentahydroxyolean-12-en-28-oic acid.

The other 16 known compounds were identified by comparison of their spectroscopic data with those in the literature.

In summary, we have described the isolation of four new oleanane-type triterpenoids, along with 16 known compounds from the EtOH extract of *E. sieboldiana*.

Also, cytotoxic activities of the four new oleanane-type triterpenoids, **1**–**4**, against HeLa and Hep-G2 cells were evaluated. Matrine was used as a positive control. In the test, IC_{50} values of higher than 10 μM were defined as inactive. The four new triterpenoids revealed significant antitumor activities *in vitro* (*Table 3*).

Table 3. *Minimal Inhibitory Concentrations* (*MIC*s [μM]) *of Compounds* **1**–**4**

Compound	HeLa Cells[a])	Hep-G2 Cells[a])
1	2.57	3.37
2	2.86	4.09
3	3.27	4.42
4	3.46	5.0
Matrine[b])	1.17	0.93

[a]) Clinical strain. [b]) Positive control.

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Experimental Part

General. Anal. TLC: silica-gel plates (SiO_2; *Yantai Institute of Chemical Technology*); visualization under UV light and by spraying with 10% aq. H_2SO_4, followed by heating. Column chromatography (CC): SiO_2 (200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*) and *Sephdex LH-20* (*Mitsubishi Chemical Industries Ltd.*). M.p.: *X-4* digital display micro-melting point apparatus. Optical rotation (ORD): *JASCO P-1020* spectropolarimeter. IR Spectra: *Avatar 360-ESP* spectrophotometer (*Thermo Nicolet*), as KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *DRX-500* spectrometer, in $CDCl_3$; δ in ppm, *J* in Hz. HR-ESI-MS: *Bruker APEX 7.0 TESLA* FT-MS apparatus; in *m*/*z*.

Plant Material. The dried aerial parts of *E. sieboldiana* were collected in August, 2010 in the Hainan Tibetan Autonomous Prefecture of Qinghai Province, China. The plant was identified by Prof. *Hong-Fa Sun* of Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining, China. A voucher specimen (No. 10-01-02) was deposited with the laboratory of *Z.-X. L.*, Southeast University, Nanjing, China.

Extraction and Isolation. The dried and powdered plant (5.5 kg) of *E. sieboldiana* was percolated four times with 95% EtOH at r.t. The filtrates were combined and evaporated to dryness *in vacuo*. The residue (193.0 g) was suspended in H_2O (1.5 l), and extracted with petroleum ether (3 × 500 ml), AcOEt (3 × 500 ml), and BuOH (3 × 500 ml), successively.

The petroleum ether extract (54.0 g) was subjected to CC (SiO_2; petroleum ether/AcOEt 40:1 to 0:100) to give three fractions, *Frs. 1–3*. *Fr. 2* (petroleum ether/AcOEt 18:1) was further separated by CC (*Sephadex LH-20*; EtOH/H_2O 7:3) to yield **1** (18.0 mg) and **2** (15.0 mg). *Fr. 3* (petroleum ether/AcOEt 8:1) was further submitted to CC (*Sephadex LH-20*; EtOH/H_2O 4:1) to yield stigmasterol (27.0 mg) and cholesterol (21.0 mg).

The AcOEt fraction (55.0 g) was fractionated by CC (SiO_2; petroleum ether followed by increasing concentration of AcOEt) to yield three fractions, *Frs. 1–3*. *Fr. 1* was first subjected to CC (petroleum ether/AcOEt 30:1 to 0:100); and the fraction from petroleum ether/AcOEt 12:1 was further separated by CC (*Sephadex LH-20*; EtOH/H_2O 4:1) to yield **3** (17.0 mg), **4** (11.0 mg), and 2,3-dihydroxyurs-12-en-28-oic acid (22.0 mg). *Fr. 2* was subjected to CC (SiO_2; petroleum ether/AcOEt 20:1 to 8:1) to yield corosolic acid (52.0 mg), butulin (33.0 mg), and 3α-hydroxy-*ent*-trachylobane (25.0 mg). *Fr. 3* was further purified by CC (SiO_2; ether/AcOEt 15:1) to 1:1) to give 16β,17-dihydroxy-*ent*-kauran-3-one (32.0 mg), luteolin (29.0 mg), apigenin (12.0 mg), acacetin (24.0 mg), and β-sitosterol 3-*O*-β-D-glucoside (27.0 mg).

Also the BuOH extract (32.0 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 40:1 to 1:1) to yield two fractions, *Frs. 1* and *2*. *Fr. 1* ($CHCl_3$/MeOH 35:1) was further separated by CC (*Sephadex LH-20*; EtOH/H_2O 7:3) to yield orientin (19.0 mg), 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone (18.0 mg), and campesterol (15.0 mg). *Fr. 2* ($CHCl_3$/MeOH 25:1) was further separated by CC (*Sephadex LH-20*; EtOH/H_2O 4:1) to yield luteolin 5-*O*-β-D-glucoside (24.0 mg) and luteolin 7-*O*-β-D-glucoside (12.0 mg).

(1β,2α,3β,19β)-1,2,3,19-Tetrahydroxyolean-12-en-28-oic Acid (**1**). Colorless crystals. M.p. 259–260°. $[\alpha]_D^{20} = +4.1$ ($c = 0.01$, MeOH). IR (KBr): 3436, 2930, 2863, 1642, 1464, 1381, 1216. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 503.3372 ($[M-H]^-$, $C_{30}H_{47}O_6^-$; calc. 503.3373).

(1β,3β,19β)-1,3,19-Trihydroxyolean-12-en-28-oic Acid (**2**). Colorless crystals. M.p. 261–262°. $[\alpha]_D^{20} = +3.7$ ($c = 0.01$, MeOH). IR (KBr): 3432, 2930, 2865, 1642, 1464, 1380, 1210. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 487.3422 ($[M-H]^-$, $C_{30}H_{47}O_5^-$; calc. 487.3424).

(1β,2α,3β,16β,19β)-1,2,3,16,19-*Pentahydroxyolean-12-en-28-oic Acid* (**3**). Colorless crystals. M.p. 260–261°. $[\alpha]_D^{20} = +3.9$ ($c = 0.01$, MeOH). IR (KBr): 3430, 2935, 2862, 1640, 1464, 1382, 1210. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 519.3321 ($[M-H]^-$, $C_{30}H_{47}O_7^-$; calc. 519.3322).

(1β,2α,3β,19β,23)-1,2,3,19,23-Pentahydroxyolean-12-en-28-oic Acid (**4**). Colorless crystals. M.p. 262–263°. $[\alpha]_D^{20} = +3.3$ ($c = 0.01$, MeOH). IR (KBr): 3435, 2930, 2865, 1642, 1460, 1380, 1210. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 519.3322 ($[M-H]^-$, $C_{30}H_{47}O_7^-$; calc. 519.3322).

Cytotoxic Activity Experiments. Antitumor activities of compounds **1**–**4** against HeLa and Hep-G2 cells were evaluated. HeLa and Hep-G2 cells were cultured in DMEM (*Dulbecco*'s Modified Eagle Medium) at 37° in 5% CO_2. The media were supplemented with 10% (*v*/*v*) heat-inactivated fetal bovine serum. The cells were routinely cultured in 96-well tissue culture microplates, and the plates were subsequently inoculated with HeLa and Hep-G2 cells with 100 µl of cultures per well. Samples were diluted in the culture medium at different concentrations. Samples were cultured at 37° in 5% CO_2 for 3 d. The cells were evaluated using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay, and following cultivation every well was injected with 15 µl of MTT (5 mg/ml) and then maintained for 4 h at 37°. The clear supernatant was removed, and 150 µl of DMSO was injected into each well and lightly shaken [11]. Matrine was used as positive control.

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