Two Novel Olean Triterpenoids from *Celastrus hypoleucus*

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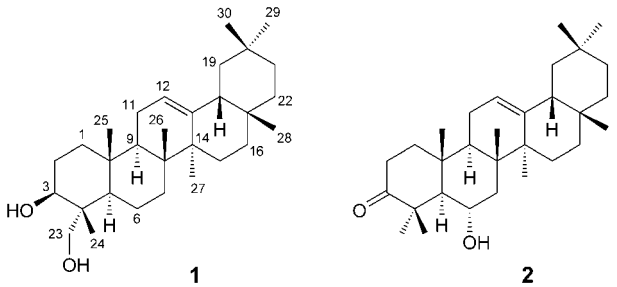
Two new triterpenoids, (3β)-olean-12-ene-3,23-diol (**1**) and (6α)-6-hydroxyolean-12-en-3-one (**2**) were isolated from the MeOH extract of the stalks of *Celastrus hypoleucus* (OLIV.) WARB., together with the seven known compounds (3β,22α)-3,22-dihydroxyolean-12-en-29-oic acid, β-amyrin and β-amyrin palmitate, wilforlide A, wilforilide B, palmitic acid, and β-sitosterol. Their structures were elucidated on the basis of spectroscopic data and, in the case of **2**, by X-ray crystallography. Compound **1** showed moderate cytotoxicity against human cervical squamous carcinoma (Hela) cells.

Introduction. – Celastraceae plants have been the subject of continued and growing interest due to the range of biological activities shown by many members of this family [1–4]. Pharmaceutical studies and clinical practice have demonstrated that Celastraceae sesqui- and triterpenes possess notable antibacterial, antitumor, insect antifeedant, and cytoxic activities [5].

In this paper, we report the isolation and identification of the two novel triterpenes (3β)-olean-12-ene-3,23-diol (**1**) and (6α)-6-hydroxyolean-12-en-3-one (**2**) isolated from the MeOH extract of the stalks of *Celastrus hypoleucus* (OLIV.) WARB., a perennial creeping plant. Also isolated were the following known compounds: (3β,22α)-3,22-dihydroxyolean-12-en-29-oic acid [6], β-amyrin [7], wilforlide A [8], wilforilide B [8], β-amyrin palmitate [9], palmitic acid, and β-sitosterol.



1 **2**

Results and Discussions. – Compound **1**, isolated as white powder (m.p. 206–208°), showed a positive *Liebermann–Burchard* reaction, indicating a triterpene. The molecular formula was established as $C_{30}H_{50}O_2$ by FT-ICR-MS (m/z at 443.3888 ($[M+H]^+$, calc. 443.3884)) and NMR experiments. The IR spectrum showed OH and olefinic resonances at 3458 and 1629 cm^{-1}, respectively. From the six degrees of

unsaturation, it was, thus, concluded that **1** contained five rings. The assignments of all the ^{1}H- and ^{13}C-NMR signals were successfully carried out with ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Table 1*).

The ^{13}C-NMR and DEPT spectra (125 MHz, $CDCl_3$) of compound **1** allowed the assignment of 30 ^{13}C signals to seven Me, eleven CH_2, and five CH groups, as well as seven quaternary C-atoms. The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) showed seven Me *singlets* at δ(H) 0.83 (Me(28)), 0.87 (Me(29) and Me(30)), 0.91 (Me(24)), 0.97 (Me(26)), 0.99 (Me(25)), and 1.13 (Me(27)). A *triplet* at δ(H) 5.18 ($J = 4.8$, H–C(12)) corresponded to the olefinic H-atom, which was correlated with the signal at δ(C) 121.9 (C(12)). The quaternary olefinic C(13)-atom appeared at δ(C) 145.4. These data and the molecular formula suggested that **1** was an olean-12-ene triterpene with two OH groups [10].

The resonance at δ(H) 3.44, 3.76 (2*d*, $J = 10.0$ each), which was coupled to δ(C) 72.5 (*t*, C(23)), was assigned to the CH_2(23) moiety bearing an OH function. From the ^{1}H-NMR multiplicity, this OH group could only be located at one of the C-atoms in positions 23–30. The ^{13}C-NMR signals ascribable to C(3) to C(6), C(23), and C(24) were different from those in β-amyrin [7], and, in particular, the Me signal at δ(C) 11.6 (C(24)) attached to C(4) showed an upfield shift. The HMBC correlations from Me(23) to C(3) and C(24), as well as NOESY cross-peaks between H–C(23) and H–C(5) were observed, which supported a 23-OH group. Finally, the upfield shift of the C(24) signal confirmed the 3β-configuration, a 3α-OH group being expected to cause a higher shift for C(24) [10].

The resonance at δ(H) 3.65 (*t*), coupled with δ(C) 77.7 (*d*, C(3)), indicated that the second OH group was located at C(3), and its β-orientation was inferred from the coupling constant ($J = 7.5$ Hz).

The C=C bond in compound **1** was established to be located between C(12) and C(13), as confirmed by HMBC correlations from H–C(9), H–C(11), and H–C(18) to C(12), and from H–C(9), H–C(11), H–C(5), and H–C(18) to C(13), as well as by ^{1}H,^{1}H-COSY correlations between H–C(11) and H–C(12). From all these data, compound **1** was identified as (3β)-olean-12-ene-3,23-diol.

Compound **2**, isolated in crystalline form (m.p. 219–221°), showed a positive *Liebermann–Burchard* reaction. Its molecular formula was determined as $C_{30}H_{48}O_2$ by FT-ICR-MS (*m*/*z* at 441.3730 ($[M+H]^+$, calc. 441.3727)) and NMR spectroscopy. The IR spectrum of **2** suggested the presence of OH, C=O, and C=C groups (3456, 1742, and 1635 cm^{-1}, resp.). The assignments of all ^{1}H- and ^{13}C-NMR signals were successfully carried out with ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Table 2*).

The ^{1}H-NMR spectrum of **2** showed signals corresponding to one vinyl H-atom at δ(H) 5.63 (*t*, $J = 5.0$ Hz, H–C(12)) and eight Me *singlets* at 0.83 (Me(28)), 0.86 (Me(25) and Me(29)), 0.88 (Me(30)), 1.02 (Me(26)), 1.20 (Me(27)), 1.34 (Me(23)), and 1.37 (Me(24)). The ^{13}C-NMR and DEPT spectra of **2** showed a C=O signal at δ(C) 219.6, and olefinic resonances at 121.9 and 144.8. In addition, nine CH_2 and four CH groups were found, as well as six quaternary C-atoms. These data indicated that **2** was also an olean-12-ene triterpene [10].

The keto group was positioned at C(3) by considering the lowfield-shifted ^{13}C-NMR signals of C(2), C(3), and C(4), and the HMBC correlations from H–C(1), H–C(5), Me(23), and Me(24) to C(3), respectively. From the signal observed at δ(H)

Table 1. *NMR Data of Compound* **1**. In $CDCl_3$ at 500/125 MHz, resp.; δ in ppm, J in Hz.

Position	^{13}C	^1H	$^1H,^1H$-COSY	HMBC
1	38.5 (*t*)	1.01, 1.68		C(3), C(5), C(10), C(25)
2	27.4 (*t*)	1.65	H–C(3)	C(1), C(3)
3	77.7 (*d*)	3.65 (*t*, $J = 7.5$)	H–C(2)	C(2), C(4), C(24)
4	42.1 (*s*)			
5	50.0 (*d*)	0.84	H–C(6)	C(7), C(24), C(25)
6	18.8 (*t*)	1.42, 1.50	H–C(5)	C(5), C(7), C(8)
7	32.7 (*t*)	1.35, 1.55		C(5), C(6), C(26)
8	40.0 (*s*)			
9	47.9 (*d*)	1.60		C(7), C(8), C(10), C(12), C(25), C(26)
10	37.1 (*s*)			
11	23.7 (*t*)	1.93	H–C(12)	C(8), C(10), C(12), C(13)
12	121.9 (*d*)	5.18 (*t*, $J = 4.8$)	H–C(11)	C(9), C(11), C(18)
13	145.4 (*s*)			
14	42.0 (*s*)			
15	27.1 (*t*)	0.78, 2.01		C(13), C(16), C(17)
16	26.4 (*t*)	1.01, 1.73		C(14), C(15), C(17), C(28)
17	32.7 (*s*)			
18	47.5 (*d*)	2.00		C(12), C(13), C(14), C(28)
19	47.1 (*t*)	1.02, 1.68		C(13), C(17), C(18), C(21), C(30)
20	31.3 (*s*)			
21	35.0 (*t*)	1.08, 1.38		C(17), C(19), C(20), C(22)
22	37.4 (*t*)	1.25, 1.46		C(17), C(18), C(20), C(21)
23	72.5 (*t*)	3.44 (*d*, $J = 10.0$)		C(3), C(24)
		3.76 (*d*, $J = 10.0$)		
24	11.6 (*q*)	0.91 (*s*)		C(3), C(4), C(5)
25	16.1 (*q*)	0.99 (*s*)		C(1), C(5), C(9), C(10)
26	17.1 (*q*)	0.97 (*s*)		C(7), C(8), C(9), C(14)
27	26.2 (*q*)	1.13 (*s*)		C(8), C(13), C(14), C(16)
28	28.6 (*q*)	0.83 (*s*)		C(16), C(17), C(18), C(22)
29	33.6 (*q*)	0.87 (*s*)		C(19), C(20), C(21), C(30)
30	23.9 (*q*)	0.87 (*s*)		C(19), C(20), C(21), C(29)

3.94 (*td*, $J = 10.4$, 4.5 Hz, H–C(6)), which was correlated with δ(C) 68.0 (C(6)), and from the $^1H,^1H$-COSY correlation between δ(H) 3.94 and 1.65 (H–C(5)), the OH group in **2** was positioned at C(6). The chemical shift of H–C(6) and the observed coupling constants (*Table 2*) pointed to α-configuration. This was consistent with deshielded resonances for C(5), C(6), and C(7) (δ(C) 59.0, 68.0, 43.4, resp.) compared to the known 6β-isomer (δ(C) 56.1, 67.5, 45.1) [11]. Finally, the structure and absolute configuration of **2** were unequivocally confirmed by single-crystal X-ray diffraction[1]), as shown in the *Figure*.

The known compounds (3β,22α)-3,22-dihydroxyolean-12-en-29-oic acid, β-amyrin, wilforlide A, wilforilide B, and β-amyrin palmitate were identified by comparison with literature data [6–9].

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[1]) Crystallographic data (excluding structure factors) have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication number CCDC-257358. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: data_request@ccdc.cam.ac.uk), or *via* the internet (http://www.ccdc.cam.ac.uk/products/csd/request).

Table 2. *NMR Data of Compound* **2**. In $CDCl_3$ at 500/125 MHz, resp.; δ in ppm, J in Hz.

Position	^{13}C	^{1}H	$^{1}H,^{1}H$-COSY	HMBC
1	39.2 (*t*)	1.63, 1.80		C(3), C(5), C(10), C(25)
2	33.3 (*t*)	2.28, 2.68		C(1), C(3)
3	219.6 (*s*)			
4	47.4 (*s*)			
5	59.0 (*d*)	1.65	H−C(6)	C(4), C(6), C(10),C(25)
6	68.0 (*d*)	3.94 (*td*, $J = 10.4, 4.5$)	H−C(5), C(7)	C(5), C(7), C(8), C(10)
7	43.4 (*t*)	1.63	H−C(6)	C(5), C(6), C(8), C(9), C(14), C(26)
8	42.3 (*s*)			
9	47.5 (*d*)	2.00		C(8), C(10), C(11), C(12), C(13), C(25), C(26)
10	38.3 (*s*)			
11	23.9 (*t*)	1.93	H−C(12)	C(9), C(10), C(12), C(13), C(25), C(26)
12	121.9 (*d*)	5.23	H−C(11)	C(9), C(11), C(14), C(18)
13	144.8 (*s*)			
14	41.0 (*s*)			
15	26.3 (*t*)	1.90		C(13), C(27)
16	27.2 (*t*)	2.02		C(15), C(17), C(28)
17	32.8 (*s*)			
18	45.9 (*d*)	1.68		C(12), C(13), C(19)
19	47.0 (*t*)	1.62		C(13), C(17), C(18), C(20), C(21), C(30)
20	31.3 (*s*)			
21	34.9 (*t*)	1.15, 1.41		C(17), C(19), C(20), C(22)
22	37.3 (*t*)	1.30, 1.46		C(16), C(17), C(20), C(28)
23	32.0 (*q*)	1.34 (*s*)		C(3), C(4), C(5), C(24)
24	20.4 (*q*)	1.37 (*s*)		C(3), C(4), C(5), C(23)
25	17.5 (*q*)	1.02 (*s*)		C(1), C(5), C(9), C(10)
26	17.5 (*q*)	1.02 (*s*)		C(7), C(8), C(9), C(14)
27	26.0 (*q*)	1.20 (*s*)		C(8), C(13), C(14), C(15)
28	28.7 (*q*)	0.83 (*s*)		C(16), C(17), C(18), C(22)
29	33.5 (*q*)	0.86 (*s*)		C(19), C(20), C(21), C(30)
30	23.9 (*q*)	0.88 (*s*)		C(19), C(20), C(21), C(29)

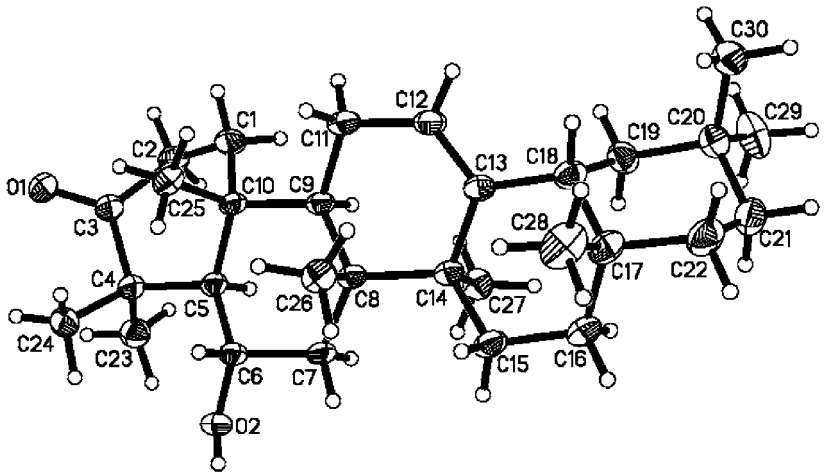

Figure. *Single-crystal X-ray structure of compound* **2**

The new compounds **1** and **2** were tested for *in vitro* antitumor activity against human cervical squamous carcinoma (Hela) cells. Compound **1** showed moderate antitumor activity, with an IC_{50} value of 28.9 μg/ml relative to 5.6 μg/ml for cisplatin used as a positive control.

Experimental Part

General. TLC: Silica-gel plates (*GF_{254}*, *Qingdao Haiyang Chemical Co. Ltd.*, Qingdao, China); detection by spraying with 10% H_2SO_4 in EtOH, followed by heating at 105°. Column chromatography (CC): silica gel (200–300 mesh). Melting points (m.p.): X_4 micro melting-point apparatus; uncorrected. IR Spectra: *Nicolet NEXUS-470* FT-IR spectrometer, KBr pellets; in cm^{-1}.^{1}H- and ^{13}C-NMR Spectra: *Bruker Avance DRX-500* spectrometer, at 500/125 MHz, resp.; δ in ppm rel. to Me_4Si as internal standard. FT-ICR-MS: *Bruker Apex III* spectrometer; in *m*/*z*. ESI-MS: *Bruker Esquire-3000^{plus}* spectrometer.

Plant Material. The stalks of *Celastrus hypoleucus* (OLIV.) WARB were collected in Jiujiang, Jiangxi province, P. R. China, in September 2002. A voucher specimen was deposited at the Herbarium of the Jiujiang Forestry Institute (JJF), Jiangxi province, China, and identified by Prof. *Hong-xiang Sun* (Zhejiang University, Hangzhou, China).

Extraction and Isolation. Shade-dried powder (10 kg) of the stalks of *C. hypoleucus* was extracted at r.t. with MeOH (3×), with occasional stirring. After filtration and evaporation *in vacuo*, a gummy residue (514 g) was obtained, which was taken up in H_2O and extracted with petroleum ether (PE; 4 × 3 l) and AcOEt (4 × 3 l).

a) The PE extract (103 g) was adsorbed on SiO_2 (100 g) and subjected to CC (1 kg SiO_2; PE/AcOEt 10:0 → 0:10): 16 main fractions (Fr.). From *Fr.1* and *2*, β-amyrin and celastrol were obtained, resp. *Fr.* 3 (3 g) was rechromatographed (60 g SiO_2; PE) to afford β-sitosterol. *Fr. 8* (4 g) was rechromatographed (80 g SiO_2; PE/acetone 5:1) to afford **2** (31.2 mg). *Fr. 12* was rechromatographed (SiO_2; PE/acetone 3:1) to afford wilforilide B.

b) The original AcOEt extract (202 g) was adsorbed on SiO_2 (200 g) and chromatographed (3 kg SiO_2; $CHCl_3$/MeOH 10:0 → 0:10): 15 main fractions. *Fr. 4* afforded a solid material, which was recrystallized from acetone to give pure **1** (20.8 mg). *Fr. 8* (3 g) was rechromatographed (60 g SiO_2; PE/AcOEt 3:1) to afford β-amyrin and wilforlide A.

(3β)-Olean-12-ene-3,23-diol (**1**). Colorless powder. M.p. 206–208°. IR (KBr): 3458, 1629. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 441 ($[M-H]^-$), 465 ($[M+Na]^+$). FT-ICR-MS: 443.3888 ($[M+H]^+$, $C_{30}H_{51}O_2^+$, calc. 443.3884).

(6α)-6-Hydroxyolean-12-en-3-one (**2**). Colorless needles. M.p. 219–221°. IR (KBr): 3456, 1742, 1635. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 439 ($[M-H]^-$), 463 ($[M+Na]^+$). FT-ICR-MS: 441.3730 ($[M+H]^+$, $C_{30}H_{48}O_2^+$; calc. 441.3727).

Antitumor Assay. The cytotoxicities of **1** and **2** towards human cervical squamous carcinoma (Hela) cell were tested as follows. The Hela cells were cultured at 37° under a humidified atmosphere of 5% CO_2 in *RPMI-1640* medium supplemented with 10% fetal calf serum, and dispersed in replicate 96-well plates (5×10^4 cells/well) for 48 h. Compounds **1** and **2** (1.25–200 μg/ml) or cisplatin (pos. control) were then added. After 48 h of exposure to the toxins, cell viability was determined by the MTT[2]) colorimetric assay [12] by measuring the absorbance at λ_{max} 570 nm with an ELISA reader. Each test was performed in triplicate ($n=3$).

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[2]) MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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