Triterpenoids from the Roots of *Camellia oleifera* C.ABEL and Their Cytotoxic Activities

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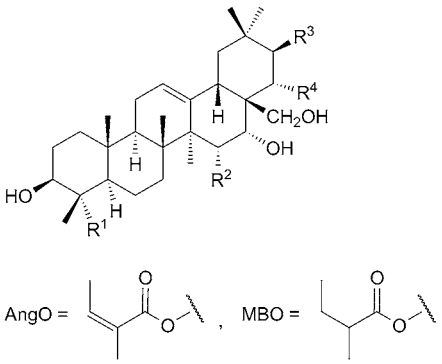
Three new triterpenoids, 21β,22α-diangeloyloxy-3β,15α,16α,28-tetrahydroxyolean-12-en-23-al (**1**), 21β-angeloyloxy-3β,15α,16α,28-tetrahydroxy-22α-(2-methylbutanoyloxy)olean-12-en-23-al (**2**), and 21β-angeloyloxy-3β,16α,28-trihydroxy-22α-(2-methylbutanoyloxy)olean-12-en-23-al (**3**), along with six known triterpenoids, were isolated from the roots of *Camellia oleifera* C.ABEL. The structures of compounds **1**–**3** were elucidated on the basis of spectroscopic analyses. Moreover, all compounds isolated were evaluated for their cytotoxic activities by MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay.

Introduction. – *Camellia oleifera* C.ABEL has been grown as an oil crop in many countries including China, Brazil, Philippines, India, and South Korea. The roots of *C. oleifera* is also well-known as traditional Chinese medicine used for treatment of common cold, bovillae, ardent fever, urinary tract infection, nephritis, edema, unpeaceful quickening, and threatened abortion [1]. Previous studies on constituents from the *Camellia* genus led to the isolation of different compounds such as flavones, triterpenoids, and its glycosides [2][3]. During the course of our investigation for cytotoxic agents from the EtOH extract of the roots of *C. oleifera*, three new triterpenoids, 21β,22α-diangeloyloxy-3β,15α,16α,28-tetrahydroxyolean-12-en-23-al (**1**), 21β-angeloyloxy-3β,15α,16α,28-tetrahydroxy-22α-(2-methylbutanoyloxy)olean-12-en-23-al (**2**), 21β-angeloyloxy-3β,16α,28-trihydroxy-22α-(2-methylbutanoyloxy)-olean-12-en-23-al (**3**), were isolated from the roots of *C. oleifera*, along with six known triterpenoids, 21β,22α-diangeloyloxy-3β,16α,28-trihydroxyolean-12-en-23-al (**4**) [4], 21β,22α-diangeloyloxy-3β,15α,16α,28-tetrahydroxyolean-12-ene (**5**) [5], 21β-angeloyloxy-3β,15α,16α,28-tetrahydroxy-22α-(2-methylbutanoyloxy)olean-12-ene (**6**) [6], 21β,22α-diangeloyloxy-3β,16α,28-trihydroxyolean-12-ene (**7**) [7], 21β-angeloyloxy-3β,16α,28-trihydroxy-22α-(2-methylbutanoyloxy)olean-12-ene (**8**) [8], and 22α-angeloyloxy-3β,15α,16α,28-tetrahydroxyolean-12-ene (**9**) [9] (*Fig. 1*). Herein, we report the isolation and structure elucidation of the new compounds **1**–**3**, as well as their cytotoxic activities.

Results and Discussion. – Compound **1** was isolated as a white powder. Its molecular formula was deduced as $C_{40}H_{60}O_9$ on the basis of the positive-ion-mode HR-ESI-MS, *m*/*z* 707.4113 ($[M+Na]^+$) and a comprehensive analysis of the NMR data.



	R^1	R^2	R^3	R^4
1	CHO	OH	AngO	AngO
2	CHO	OH	AngO	MBO
3	CHO	H	AngO	MBO
4	CHO	H	AngO	AngO
5	Me	OH	AngO	AngO
6	Me	OH	AngO	MBO
7	Me	H	AngO	AngO
8	Me	H	AngO	MBO
9	Me	OH	H	MBO

Fig. 1. *Structures of compounds* **1**–**9**

The IR spectrum indicated the presence of OH groups (3452 cm^{-1}), an CHO group (2818, 2719, 1739 cm^{-1}), and two α,β-unsaturated ester C=O groups (1605 and 1602 cm^{-1}). The ^{13}C-NMR spectrum (*Table 1*) exhibited resonances for 40 C-atoms, substitution patterns of which were deduced from DEPT and HSQC experiments as ten Me, seven CH_2, and eleven CH groups, and twelve quaternary C-atoms. It showed signals of five O–CH C-atoms at δ(C) 72.1, 67.6, 72.0, 78.5, and 73.4, corresponding to C(3), C(15), C(16), C(21), and C(22), respectively, as well as one OCH_2 signal at δ(C) 63.2, corresponding to C(28) and one CHO signal at δ(C) 208.4, corresponding to C(23). The ^{1}H-NMR spectrum (*Table 1*) displayed six signals at δ(H) 1.48 (*s*, 3 H), 1.09 (*s*, 3 H), 1.08 (*s*, 3 H), 1.01 (*s*, 3 H), 0.98 (*s*, 3 H), and 0.92 (*s*, 3 H), which were assigned to six tertiary Me groups. An olefinic H-atom signal at δ(H) 5.51 (*t*, $J = 4.0$, H–C(12)), a CH signal at δ(H) 3.70 (*dd*, $J = 11.0$, 4.5, H–C(3)), an CHO signal at δ(H) 9.42 (*s*, H–C(23)), and signals of a CH_2–O group at δ(H) 3.30, 2.99 (2*d*, $J = 12.0$, CH_2(28)) suggested that compound **1** belongs to the olean-12-ene-type pentacyclic triterpene family. Its ^{1}H-NMR spectrum also exhibited signals of two angeloyl (=2-methylbut-2-enoyl; Ang) groups at δ(H) (6.05 (*dq*, $J = 7.0$, 1.5, H–C(3′), 21-*O*-Ang), 1.94 (*d*, $J = 7.0$, Me(4′), 21-*O*-Ang), 1.83 (*d*, $J = 1.5$, Me(5′), 21-*O*-Ang)), and 6.08 (*dq*, $J = 7.0$, 1.5, 1 H, 22-*O*-Ang-3″), 1.94 (*d*, $J = 7.0$, Me(4″), 22-*O*-Ang), 1.83 (*d*, $J = 1.5$, Me(5″), 22-*O*-Ang)). The two AngO groups were located at C(21) and C(22) on the basis of the downfield shifts of H–C(21) at δ(H) 5.87 (*d*, $J = 10.5$) and H–C(22) at δ(H) 5.44 (*d*, $J = 10.5$), which was supported by the correlation between δ(H) 5.87 (*d*, $J = 10.5$, H–C(21)) and δ(C) 168.9 (C(1′)21-*O*-Ang), as well as the correlation between δ(H) 5.44 (*d*, $J = 10.5$, H–C(22)) and δ(C) 169.8 (C(1″)22-*O*-Ang) in the HMBC spectrum (*Fig. 2*).

The relative configuration of compound **1** was established from its NOESY spectrum (*Fig. 3*). The cross-peaks between H–C(21) (δ(H) 5.87) and Me(29) (δ(H) 0.92), as well as those between H–C(22) (5.44) and Me(30) (1.09), and CH_2(28) (3.30 and 2.99), suggested that H–C(21) and H–C(22) are α- and β-oriented, respectively. The H–C(15) (δ(H) 3.76) correlated with CH_2(28) (3.30 and 2.99) and H–C(18) (2.65), indicating that the 15-OH group is α-configured. In addition, the small coupling constant (J(15,16) = 4.5) suggested that H–C(15) is axial and H–C(16) is equatorial.

Table 1. *^{1}H- and ^{13}C-NMR* (500 and 125 MHz, resp.) *Data of Compounds* **1**–**3**. In $CDCl_3$; δ in ppm, J in Hz. For atom numbering, *cf. Fig. 2*.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.69–1.72 (*m*), 1.08–1.10 (*m*)	39.1	1.71–1.74 (*m*), 1.05–1.09 (*m*)	38.3	1.75–1.76 (*m*), 1.09–1.13 (*m*)	38.2
2	1.71–1.75 (*m*), 1.10–1.25 (*m*)	26.4	1.71–1.73 (*m*), 1.68–1.70 (*m*)	26.1	1.71–1.74 (*m*), 1.64–1.66 (*m*)	26.2
3	3.70 (*dd*, *J* = 11.0, 4.5)	72.1	3.70 (*dd*, *J* = 11.0, 4.5)	71.7	3.82 (*dd*, *J* = 11.0, 4.5)	71.9
4		56.2		55.1		55.2
5	0.78 (*d*, *J* = 10.5)	48.5	0.78 (*d*, *J* = 10.5)	48.2	0.78 (*d*, *J* = 10.5)	48.2
6	1.42–1.61 (*m*), 1.10–1.25 (*m*)	20.6	1.53–1.57 (*m*), 1.14–1.26 (*m*)	20.9	1.57–1.59 (*m*), 1.15–1.26 (*m*)	20.7
7	1.74–1.77 (*m*), 1.63–1.67 (*m*)	35.7	1.75–1.77 (*m*), 1.63–1.66 (*m*)	35.8	1.71–1.74 (*m*), 1.59–1.61 (*m*)	32.2
8		40.7		41.3		40.1
9	1.26 (*d*, *J* = 12.5)	47.3	1.26 (*d*, *J* = 12.5)	47.1	1.26 (*d*, *J* = 12.5)	46.5
10		36.5		35.9		35.9
11	1.92–1.95 (*m*), 1.41–1.63 (*m*)	24.2	1.86–1.93 (*m*), 1.42–1.53 (*m*)	23.6	1.86–1.99 (*m*), 1.42–1.53 (*m*)	23.5
12	5.51 (*t*, *J* = 4.0)	126.3	5.53 (*t*, *J* = 4.0)	125.9	5.46 (*t*, *J* = 4.0)	124.4
13		142.5		141.6		140.9
14		48.1		47.9		41.2
15	3.76 (*d*, *J* = 4.5)	67.6	3.72 (*d*, *J* = 4.5)	67.5	1.66–1.68 (*m*), 1.35–1.40 (*m*)	33.6
16	3.78 (*d*, *J* = 4.5)	72	3.74 (*d*, *J* = 4.5)	73.4	3.94 (*t*, *J* = 4.0)	69.6
17		47.7		46.6		47.8
18	2.65 (*dd*, *J* = 14.0, 4.0)	40.7	2.71 (*dd*, *J* = 14.0, 4.0)	39.8	2.72 (*dd*, *J* = 14.0, 4.0)	39.3
19	1.98–2.60 (*m*), 1.75–1.77 (*m*)	47.3	2.52–2.90 (*m*), 1.75–1.78 (*m*)	45.9	2.52–2.90 (*m*), 1.75–1.77 (*m*)	46.4
20		36.3		35.3		35.8
21	5.87 (*d*, *J* = 10.5)	78.5	5.76 (*d*, *J* = 10.5)	77.3	5.82 (*d*, *J* = 10.5)	77.3
22	5.44 (*d*, *J* = 10.5)	73.4	5.32 (*d*, *J* = 10.5)	73.3	5.41 (*d*, *J* = 10.5)	73.2
23	9.42 (*s*)	208.4	9.41 (*s*)	207	9.42 (*s*)	207.1
24	1.08 (*s*)	9.3	1.08 (*s*)	9	1.08 (*s*)	9
25	1.01 (*s*)	16.4	1.00 (*s*)	15.8	1.01 (*s*)	15.9
26	0.98 (*s*)	17.6	0.98 (*s*)	17.2	0.92 (*s*)	16.8
27	1.48 (*s*)	21.4	1.42 (*s*)	20.3	1.48 (*s*)	27.1
28	3.30 (*d*, *J* = 12.0), 2.99 (*d*, *J* = 12.0)	63.2	3.28 (*d*, *J* = 12.0), 2.93 (*d*, *J* = 12.0)	62.6	3.26 (*d*, *J* = 12.0), 2.98 (*d*, *J* = 12.0)	63.6
29	0.92 (*s*)	29.5	0.92 (*s*)	28.9	0.94 (*s*)	29
30	1.09 (*s*)	20	1.09 (*s*)	19.5	1.09 (*s*)	19.6
1′		168.9		168.9		167.4
2′		127.8		127.8		127.4
3′	6.05 (*dq*, *J* = 7.0, 1.5)	140.2	6.13 (*dq*, *J* = 7.0, 1.5)	140.2	6.01 (*dq*, *J* = 7.0, 1.5)	139.2
4′	1.94 (*d*, *J* = 7.0)	16.1	1.93 (*d*, *J* = 7.0)	16.1	1.96 (*d*, *J* = 7.0)	15.9
5′	1.83 (*d*, *J* = 1.5)	20.9	1.83 (*d*, *J* = 1.5)	20.9	1.86 (*d*, *J* = 1.5)	20.7
1″		169.8		178.7		178.8
2″		128.4	2.33–2.55 (*m*, 1 H)	41.3	2.33–2.36 (*m*, 1 H)	41.1
3″	6.08 (*dq*, *J* = 7.0, 1.5)	139.1	1.59–1.62 (*m*), 1.15–1.26 (*m*)	26.6	1.61–1.64 (*m*), 1.15–1.26 (*m*)	26.7
4″	1.94 (*d*, *J* = 7.0)	16.1	0.86 (*t*, *J* = 7.5)	11.8	0.86 (*t*, *J* = 7.5)	11.8
5″	1.83 (*d*, *J* = 1.5)	20.9	1.05 (*d*, *J* = 6.7)	16.4	1.04 (*d*, *J* = 6.7)	16.5

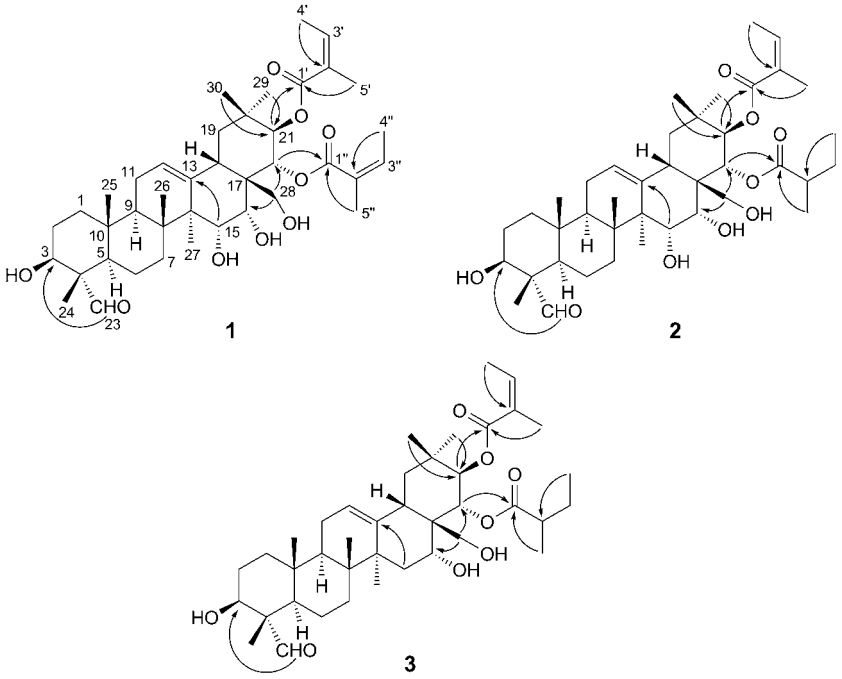


Fig. 2. *Key HMBCs of compounds* **1**–**3**

The CHO group was established at position 23, which was confirmed by the correlation between H–C(23) at δ(H) 9.42 (*s*, 1 H) and C(3) at δ(C) 72.1 in the HMBC spectrum (*Fig. 2*), and the correlation between H–C(3) at δ(H) 3.70 (*dd*, $J = 11.0, 4.5$) and H–C(23) at δ(H) 9.42 (*s*, 1 H), as well as the correlation between Me(24) at δ(H) 1.08 (*s*) and Me(25) at δ(H) 1.01 (*s*) in the NOESY spectrum (*Fig. 3*). Thus, the structure of compound **1** was elucidated as 21β,22α-diangeloyloxy-3β,15α,16α,28-tetrahydroxyolean-12-en-23-al.

Compound **2** was also obtained as a white powder. Its molecular formula was deduced as $C_{40}H_{62}O_9$ on the basis of the positive-ion-mode HR-ESI-MS (*m*/*z* 709.4270 ($[M + Na]^+$)) and the comprehensive analysis of the NMR data. The IR spectrum indicated the presence of OH groups (3475 cm^{-1}), an CHO group (2820, 2719, 1742 cm^{-1}), a C=O group (1720 cm^{-1}), and an α,β-unsaturated ester C=O group (1600 cm^{-1}). Compound **2** has a similar structure as compound **1**, which was confirmed by comparison of their ^{1}H- and ^{13}C-NMR spectra, the main differences arising from the signals at δ(C) 178.7, 41.3, 26.6, 11.8, and 16.4 due to a 2-methylbutanoyl (MB) group in compound **2**. The angeloyl group is located at C(21) on the basis of the downfield shifts of C(21) at δ(C) 77.3, which was supported by the correlation between δ(H) 5.76 (*d*, $J = 10.5$, H–C(21)) and δ(C) 168.9 (C(1′), 21-*O*-Ang) in the HMBC spectrum (*Fig. 2*). On the other hand, the 2-MBO group was established at C(22) on the basis of the downfield shift of C(22) at δ(C) 73.3, which was supported by the correlation between δ(H) 5.32 (*d*, $J = 10.5$, H–C(22)) and δ(C) 178.7 (C(1″), 22-*O*-MB) in the HMBC spectrum (*Fig. 2*).

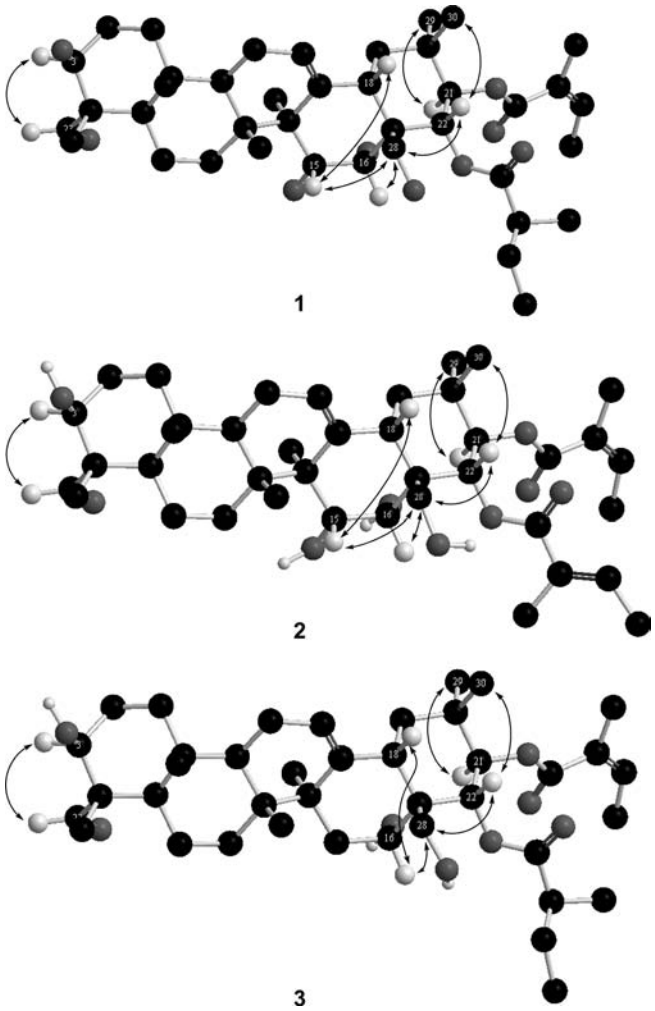


Fig. 3. *Key NOESY correlations of compounds* **1**–**3**

The relative configuration of compound **2** was further substantiated by its NOESY spectrum (*Fig. 3*). The cross-peaks between H–C(21) (δ(H) 5.76) and Me(29) (0.92), as well as between H–C(22) (5.32) and Me(30) (1.09), and CH_2(28) (3.28 and 2.93), indicated that H–C(21) and H–C(22) are α- and β-oriented, respectively. The H–C(15) (δ(H) 3.72) correlated with CH_2(28) (3.28 and 2.93), indicating that the 15-OH group is α-configured. In addition, the small coupling constant (J(15,16) = 4.5) suggested that H–C(15) is axial and H–C(16) is equatorial. Thus, the structure of compound **2** was elucidated as 21β-angeloyloxy-3β,15α,16α,28-tetrahydroxy-22α-(2-methylbutanoyloxy)olean-12-en-23-al.

Compound **3** was also obtained as a white powder. Its molecular formula was deduced as $C_{40}H_{62}O_8$ on the basis of the positive-ion-mode HR-ESI-MS (m/z 693.4316

($[M+Na]^+$)) and the comprehensive analysis of the NMR data. The IR spectrum indicated the presence of OH groups (3450 cm^{-1}), an CHO group (2820, 2720, 1738 cm^{-1}), a C=O group (1722 cm^{-1}), and an α,β-unsaturated ester C=O groups (1603 cm^{-1}). Compound **3** had a similar structure as compound **4**, which was confirmed by comparison of their ^1H- and ^{13}C-NMR spectra, the main difference again arising from the presence of a 2-MB group in compound **3**. The AngO group was placed at C(21) on the basis of the downfield shifts of C(21) at δ(C) 77.3, which was supported by the correlation between δ(H) 5.82 (*d*, $J=10.5$, H–C(21)) and δ(C) 167.4 (C(1′), 21-*O*-Ang) in the HMBC spectrum (*Fig. 2*). The 2-MBO group was established at C(22) on the basis of the downfield shifts of C(22) at δ(C) 73.2, which was supported by the correlation between δ(H) 5.41 (*d*, $J=10.5$, H–C(22)) and δ(C) 178.8 (C(1″), 22-*O*-MB-1″) in the HMBC spectrum (*Fig. 2*).

The relative configuration of compound **3** was further substantiated by its NOESY spectrum (*Fig. 3*). The cross-peaks between H–C(21) (δ(H) 5.82) and Me(29) (0.94), as well as between H–C(22) (5.41) and Me(30) (1.09), and CH_2(28) (3.26 and 2.98), suggested that H–C(21) and H–C(22) are α- and β-oriented, respectively. The H–C(16) (δ(H) 3.94) correlated with CH_2(28) (3.26 and 2.98) and H–C(18) (2.72), indicating that the 16-OH group is α-configured. Thus, the structure of compound **3** was elucidated as 21β-angeloyloxy-3β,16α,28-trihydroxy-22α-(2-methylbutanoyloxy)olean-12-en-23-al.

Cytotoxic activities of the isolated compounds **1**–**9** were tested by MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay *in vitro* and expressed as IC_{50} values. They all exhibited moderate cytotoxic activities against human HeLa, SMMC-7721, and HL-60 tumor cell lines, with IC_{50} values ranging from 25.77 to 44.38 μM (*Table 2*). Preliminary analysis of the structure–activity relationship of these natural triterpenoids revealed that compounds **1**, **4**, and **7** with 21β,22α-diangeloyloxy groups, exhibited slightly increased cytotoxic activities. Our results suggested that triterpenoids might be, at least in part, responsible for the proposed therapeutic effect of the roots of *C. oleifera*.

Table 2. In vitro *Cytotoxic Activities of Triterpenoids* **1**–**9** *from the Roots of* Camellia oleifera

Compound	IC_{50} [μM]		
	HeLa	SMMC-7721	HL-60
1	25.77 ± 2.35	28.55 ± 2.18	27.02 ± 2.36
2	32.53 ± 2.20	31.09 ± 2.85	31.24 ± 2.72
3	33.17 ± 2.62	36.26 ± 1.98	38.37 ± 2.56
4	27.48 ± 2.82	27.40 ± 2.19	26.85 ± 3.02
5	40.70 ± 2.76	44.38 ± 3.35	40.72 ± 2.89
6	34.95 ± 3.18	38.57 ± 1.88	36.92 ± 2.72
7	26.72 ± 2.13	25.94 ± 2.64	26.83 ± 2.30
8	30.08 ± 2.52	30.18 ± 2.16	32.33 ± 2.88
9	32.39 ± 2.84	27.93 ± 1.96	30.62 ± 2.66
Norcantharidin	4.16 ± 0.37	5.23 ± 0.42	3.45 ± 0.26

We are grateful to Prof. *Xue-Dong Yang* at the Department of Pharmaceutical Science and Technology, Tianjin University, for the HR-ESI-MS recording, and Mrs. *Li-Ping Shi* at the

Instrumentation Center of Shanghai Institute of Organic Chemisty, Chinese Academy of Science for the NMR recordings. This work was funded by the *Priority Academic Program Development of Jiangsu Higher Education Institutions* (*PAPD*).

Experimental Part

General. TLC: Precoated silica gel plates (SiO_2; *Qingdao Marine Chemical Factory*, Qingdao, China); visualized by 10% H_2SO_4 alcohol soln. Column chromatography (CC): SiO_2 (400–600 mesh; *Qingdao Marine Chemical Factory*, Qingdao, China). Medium pressure liquid chromatography (MPLC): ODS column (460 mm × 26 mm i.d., *Büchi Borosilikat 4.6*, CH-Flawil). Semi-prep. HPLC: ODS column (250 × 20 mm i.d., 5 μm *PRC-ODS* column; *Shimadzu Co., Ltd.*) with a *Waters 2996* detector; flow rate, 2 ml/min, and the wavelength for detection, 254 nm. Optical rotation: *Perkin-Elmer model 241* polarimeter. IR Spectra: *Perkin-Elmer 983G* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR spectra: *Varian Inova 500* spectrometer; in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Micromass Q-TOF 2* spectrometer; in *m*/*z*.

Plant Material. The roots of *Camellia oleifera* C.ABEL were collected in Hubei Province of China in November 2006, and identified by *X.-R. L.* of our college. A voucher sample (No. 06-11-06-01) is deposited with the Herbarium of the College of Pharmacy, Soochow University.

Extraction and Isolation. The dried plant material (10 kg) was percolated with 150 l of EtOH. The solvent was subsequently dried under reduced pressure to give the residue, which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble fraction was further partioned between petroleum ether (PE) and 90% MeOH. The 90% MeOH fraction (26 g) was then subjected to MPLC (SiO_2; PE/AcOEt 10:90, 15:85, 20:80, 25:75, 30:70, 40:60, 50:50, 100:0, each 500 ml, at 5 ml/min) to afford eight fractions, *Frs. 1–8*. *Fr. 2* (500–1000 ml, 303 mg) was isolated by semi-prep. HPLC (*PRC-ODS*; MeOH/H_2O 90:10, at 2 ml/min) to yield compounds *21β,22α-diangeloyloxy-3β,16α,28-trihydroxyolean-12-ene* (**7**; 146 mg; t_R 27.5 min), and *21β-angeloyloxy-3β,16α,28-trihydroxy-22α-(2-methylbutanoyloxy)olean-12-ene* (**8**; 125 mg; t_R 29.0 min), *Fr. 3* (1000–1500 ml, 186 mg) was submitted to semi-prep. HPLC (*PRC-ODS*; MeOH/H_2O 90:10, at 2 ml/min) to give *21β-angeloyloxy-3β,16α,28-trihydroxy-22α-(2-methylbutanoyloxy)olean-12-en-23-al* (**3**; 21 mg; t_R 24.0 min) and *21β,22α-diangeloyloxy-3β,16α,28-trihydroxyolean-12-en-23-al* (**4**; 136 mg; t_R 21.5 min). *Fr. 4* (1500–2000 ml, 155 mg) was subjected to semi-prep. HPLC (*PRC-ODS* column; MeOH/H_2O 85:15, at 2 ml/min) to yield *22α-angeloyloxy-3β,15α,16α,28-tetrahydroxyolean-12-ene* (**9**; 111 mg; t_R 25.0 min). *Fr. 5* (2000–2500 ml, 274 mg) was separated by to semi-prep. HPLC (*RC-ODS*; MeOH/H_2O 85:15, at 2 ml/min) to afford *22α-diangeloyloxy-3β,15α,16α,28-tetrahydroxyolean-12-ene* (**5**; 168 mg; t_R 17.0 min) and *21β-angeloyloxy-3β,15α,16α,28-tetrahydroxy-22α-(2-methylbutanoyloxy)olean-12-ene* (**6**; 26 mg; t_R 19.0 min). *Fr. 5* (2500–3000 ml, 61 mg) was purified by semi-prep. HPLC (*PRC-ODS*; MeOH/H_2O 80:20, at 2 ml/min) to yield *21β,22α-diangeloyloxy-3β,15α,16α,28-tetrahydroxyolean-12-en-23-al* (**1**; 35 mg; t_R 25.5 min) and *21β-angeloyloxy-3β,15α,16α,28-tetrahydroxy-22α-(2-methylbutanoyloxy)olean-12-en-23-al* (**2**; 12 mg; t_R 27.0 min). The purity of the compounds was determined by anal. HPLC with PDA detection and ranged from 92% (for **2**) to 95% (for **5**).

(3β,15α,16α,21β,22α)-3,15,16,28-Tetrahydroxy-23-oxoolean-12-ene-21,22-diyl (2Z,2′Z)-Bis(2-methylbut-2-enoate) (=*21β,22α-Diangeloyloxy-3β,15α,16α,28-tetrahydroxyolean-12-en-23-al*; **1**). $[\alpha]_D^{25} = +16.32$ ($c = 0.18$, MeOH). IR (KBr): 3452, 2962, 2925, 2855, 2818, 2719, 1739, 1605, 1602, 1462, 1378, 1185, 1040. ^1H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS (pos.): 707.4113 ($[M+Na]^+$, $C_{40}H_{60}NaO_9^+$; calc. 707.4135).

(3β,15α,16α,21β,22α)-3,15,16,28-Tetrahydroxy-22-[(2-methylbutanoyl)oxy]-23-oxoolean-12-en-21-yl (2Z)-2-Methylbut-2-enoate (=*21β-Angeloyl-3β,15α,16α,28-tetrahydroxy-22α-(2-methylbutanoyloxy)-olean-12-en-23-al*; **2**). $[\alpha]_D^{25} = +18.16$ ($c = 0.16$, MeOH). IR (KBr): 3475, 2960, 2922, 2853, 2820, 2719, 1742, 1720, 1600, 1460, 1375, 1168, 1047. ^1H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS (pos.): 709.4270 ($[M+Na]^+$, $C_{40}H_{62}NaO_9^+$; calc. 709.4292).

(3β,16α,21β,22α)-3,16,28-Trihydroxy-22-[(2-methylbutanoyl)oxy]-23-oxoolean-12-en-21-yl (2Z)-2-Methylbut-2-enoate (=*21β-Angeloyloxy-3β,16α,28-trihydroxy-22α-(2-methylbutanoyloxy)olean-12-en-*

23-al; **3**). $[\alpha]_D^{25} = +13.85$ ($c = 0.15$, MeOH). IR (KBr): 3450, 2958, 2920, 2850, 2820, 2720, 1738, 1722, 1603, 1462, 1381, 1176, 1038. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS (pos.): 693.4316 ($[M + Na]^+$, $C_{40}H_{62}NaO_8^+$; calc. 693.4342).

Cytotoxic Activity. To evaluate the cytotoxic activities of the triterpenoids from roots of *C. oleifera* against human HeLa, SMMC-7721, and HL-60 tumor cell lines (Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences), MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay was performed [10]. The amount of formazan was determined by photometry at 570 nm. Cells were placed into 96-well flat-bottomed cultured plates at a concentration of 2×10^5 cells per well in complete RPMI 1640 culture medium. Twenty-four hours after placing, the medium containing foetal calf serum was removed, and test solns. were added to cells at concentrations of 2.5, 5, 10, 20, 50, and 100 μM. After incubation with drugs for 24 h, MTT soln. was added to the wells, and the plates were incubated at 37° for 4 h. The active control group was treated with norcantharidin (purity > 99.0% as determined by HPLC; *Nanjing Zelang Medical Technology Co., Ltd.*). The amount of formazan was determined by photometry at 540 nm. Results were expressed as percentage of the absorbance in control cells compared to that in the drug-treated cells. The IC_{50} values (50% inhibitory concentrations) of compounds **1**–**9** were compiled in *Table 2*.

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