New Triterpenoid Glycosides from the Roots of *Camellia oleifera* ABEL

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Five new triterpenoid saponins, oleiferosides I–M (**1**–**5**, resp.) were isolated from the roots of *Camellia oleifera* ABEL. Their structures were elucidated by a combination of 1D- and 2D-NMR spectroscopy, mass spectrometry, and chemical methods. All the compounds were identified as oleanane-type saponins with sugar moieties linked to C(3) of the aglycone. In addition, cytotoxic activities of these saponins were evaluated against four human tumor cell lines (A549, B16, BEL-7402, and MCF-7) by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) *in vitro* assay. All of the compounds showed significant cytotoxic activities against the tested cell lines.

Introduction. – The genus *Camellia* (Theaceae family) consists of 119 species, which are widely distributed in China, Japan, India, and many other South-East Asian countries [1]. As a woody crop, *C. oleifera* ABEL is widely cultivated in P. R. China, Japan, and Southeast Asia for producing cooking oil from the seeds [2]. The oil is rich in unsaturated fatty acids and comparable to olive oil [3]. Not only a crop, the plant is also used for treatment of diseases in folk medicine. Phytochemical studies led to the isolation of flavonoids, bibenzyl glycosides, and saponins from the seeds, seed cake, or flowers [4–7]. Pharmacological investigations revealed antioxidant, hepatoprotective, cytotoxic, and cell-injury-protective activities for these compounds [3–7]. The roots of *C. oleifera* have long been used as a traditional Chinese medicine for the treatment of common cold, ardent fever, urinary tract infection, nephritis, edema, and threatened abortion [8]. According to the Traditional Chinese Medicine, the roots, which are bitter and cool-natured, can invigorate lung and kidney, and can expel 'wind' and 'heat'. Significant cytotoxic activities against A549 human lung adenocarcinom, B16 mouse melanoma, BEL-7402 human hepatocarcinoma, and MCF-7 human breast carcinoma cell lines were found for the extract of the roots of *C. oleifera* in our previous investigation [9]. To date, 17 triterpenoids have been identified from the root extract, and many of them showed potent cytotoxic activities [8][9]. Here, we describe the isolation and structure elucidation of five new triterpenoid saponins, **1**–**5** (*Fig. 1*). Their cytotoxic activities against A549, B16, BEL-7402, and MCF-7 human tumor cell lines were evaluated by using MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay.

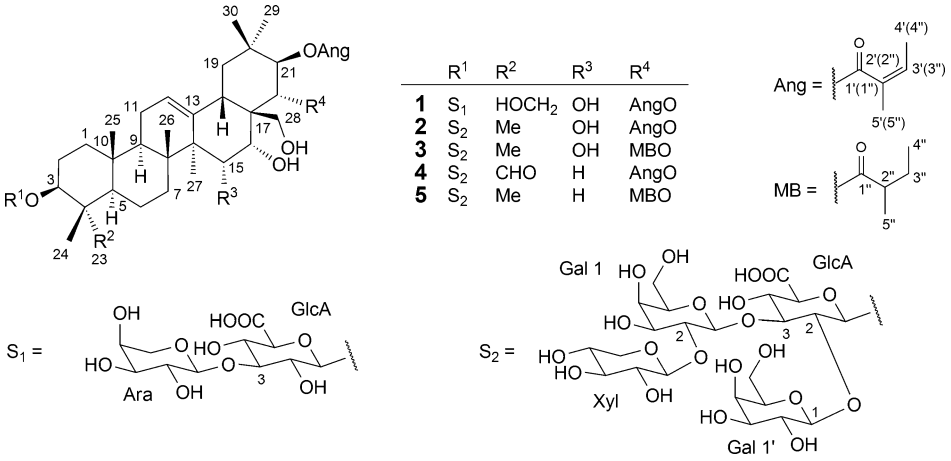

Fig. 1. *Structures of compounds* **1**–**5**

Results and Discussion. – Compound **1** was obtained as white amorphous powder. The negative-ion HR-ESI-mass spectrum of **1** exhibited a *quasi*-molecular-ion peak $[M-H]^-$ at *m*/*z* 993.5087, indicating the molecular formula of $C_{51}H_{78}O_{19}$. The IR spectrum showed the presence of OH (3456 cm^{-1}) and α,β-unsaturated ester groups (1601, 1603 cm^{-1}). The ^{13}C-NMR (*Table 1*) spectrum exhibited the resonances of 51 C-atoms, ascribable to ten Me, ten CH_2, twenty CH groups, and eleven quaternary C-atoms as revealed by the HSQC experiment. The ^{1}H-NMR (*Table 2*) spectrum showed six Me signals at δ(H) 0.70 (Me(24)), 0.87 (Me(29)), 0.99 (Me(25)), 1.00 (Me(26)), 1.09 (Me(30)), and 1.42 (Me(27)), two isolated CH_2–O signals at δ(H) 3.34 (*d*, *J* = 11.5, 1 H, CH_2(23)) and 3.64 (*d*, *J* = 11.5, 1 H, CH_2(23)), as well as δ(H) 3.05 (*d*, *J* = 12.0, 1 H, CH_2(28)) and 3.30 (*d*, *J* = 12.0, 1 H, CH_2(28)), five CH–O signals at δ(H) 3.76 (*d*, *J* = 4.5, H–C(15)), 3.55 (*d*, *J* = 4.5, H–C(16)), 3.65 (*dd*, *J* = 11.0, 4.5, H–C(3)), 5.58 (*d*, *J* = 10.5, H–C(22)) and 5.93 (*d*, *J* = 10.5, H–C(21)), and one olefinic H-atom signal at δ(H) 5.49 (*t*, *J* = 4.0, H–C(12)). Furthermore, the H-atom signals of two angeloyl (Ang) groups (δ(H) 6.05 (*dq*, *J* = 7.0, 1.5, H–C(3′), 21-*O*-Ang), 1.92 (*d*, *J* = 7.5, Me(4′), 21-*O*-Ang) and 1.82 (*s*, Me(5′), 21-*O*-Ang), and δ(H) 5.94 (*dq*, H–C(3″), *J* = 7.0, 1.5, 22-*O*-Ang), 1.90 (*d*, Me(4″), *J* = 7.5, 22-*O*-Ang) and 1.82 (*s*, Me(5″), 22-*O*-Ang)) were observed. The NMR data were similar to those reported for oleiferoside E [9], and the main difference was that two sp^3-C-atoms were replaced by olefinic C-atoms, which was indicated by the significant down-field shifts of the C(2″) signal of 22-*O*-Ang ($\Delta\delta$(C) +88.0 ppm) at δ(C) 129.6 and the C(3″) signal of 22-*O*-Ang ($\Delta\delta$(C) +112.5 ppm) at δ(C) 139.5. The analyses of the ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra revealed that an Ang group was attached at C(22) in compound **1**, instead of a 2-methylbutanoyl (MB) group in oleiferoside E. Therefore, compound **1** had a 21β,22α-bis(angeloyloxy)-3β,15α,16α,23α,28-pentahydroxyolean-12-ene type of aglycone. The locations of the two Ang groups at C(21) and C(22) were confirmed by the HMBC experiment (*Fig. 2*). The presence of a OH group at C(23) was confirmed by the correlations between CH_2(23) (δ(H) 3.34 and 3.64) and C(4) (δ(C) 44.1), and between CH_2(23) and C(24) (δ(C) 13.7) in the HMBC spectrum, as well as the correlation between H–C(3) (δ(H)

Table 1. *^{13}C-NMR Data* (125 MHz)[a]) *of Compounds* **1**–**5**. δ in ppm.

Position	δ(C)				
	1[b])	**2**[c])	**3**[c])	**4**[c])	**5**[c])
1	40.1	39.1	38.9	38.3	39.0
2	26.7	26.7	26.4	25.4	26.7
3	83.6	89.7	89.4	84.7	89.8
4	44.1	39.7	39.5	55.3	39.8
5	48.4	55.7	55.5	48.5	55.9
6	19.4	19.0	18.7	20.4	18.6
7	36.9	36.8	36.6	32.5	33.3
8	42.6	41.6	41.4	41.8	41.8
9	48.2	47.2	46.7	46.9	47.0
10	38.0	37.1	36.8	36.2	36.9
11	25.1	24.1	23.9	23.9	24.0
12	127.3	125.7	125.3	123.7	123.7
13	143.9	143.8	143.5	143.0	143.0
14	48.8	47.9	47.6	40.4	40.2
15	68.9	67.7	67.4	34.9	35.0
16	74.5	73.8	73.0	68.7	68.5
17	48.7	48.5	48.4	48.2	48.3
18	41.9	41.1	40.7	40.2	40.2
19	47.8	47.0	47.0	47.3	47.3
20	37.1	36.5	36.2	36.5	36.6
21	79.9	78.8	78.5	78.9	78.9
22	74.2	73.5	73.0	73.8	73.7
23	65.2	28.2	27.9	210.5	28.3
24	13.7	17.0	16.8	11.2	17.0
25	17.0	16.0	15.7	16.0	15.8
26	18.2	17.7	17.4	17.0	17.1
27	21.4	21.4	21.1	27.7	27.7
28	64.1	63.3	62.8	63.8	63.8
29	29.9	29.7	29.3	29.7	29.7
30	20.5	20.4	20.1	20.4	20.5
21-*O*-Ang					
1′	169.5	167.9	167.5	167.8	167.7
2′	129.6	129.1	128.6	129.1	128.9
3′	139.3	137.5	138.4	137.3	138.6
4′	16.3	16.1	15.9	16.0	16.2
5′	21.2	21.1	20.9	21.1	21.2
22-*O*-Ang					
1″	169.8	168.3		168.3	
2″	129.6	129.3		129.1	
3″	139.5	136.7		137.3	
4″	16.2	15.8		15.9	
5″	21.1	20.8		21.0	
22-*O*-MB					
1″			176.5		176.9
2″			41.4		41.8
3″			26.8		27.1
4″			11.7		12.1
5″			16.5		16.9

Table 1 (cont.)

Position	δ(C)				
	1[b])	**2**[c])	**3**[c])	**4**[c])	**5**[c])
Sugar units	GlcA	GlcA	GlcA	GlcA	GlcA
1	105.8	105.5	105.2	104.0	105.6
2	74.9	79.0	78.5	78.3	79.0
3	86.7	84.5	84.2	85.1	84.8
4	72.0	70.4	70.1	70.7	71.4
5	76.7	76.8	76.7	77.2	77.1
6	172.9	172.5	nd[d])	nd[d])	173.2
	Ara	Gal 1	Gal 1	Gal 1	Gal 1
1	105.8	101.9	101.6	101.8	101.9
2	74.8	83.6	83.4	84.1	83.8
3	73.1	75.1	74.8	75.1	75.1
4	69.9	71.5	71.2	71.0	70.1
5	67.7	76.8	76.2	77.1	77.0
6		62.1	61.8	62.0	62.0
		Xyl	Xyl	Xyl	Xyl
1		107.5	107.2	107.8	107.6
2		76.2	76.0	76.4	76.2
3		78.3	78.1	78.4	78.4
4		70.9	70.7	71.0	70.9
5		67.7	67.4	67.7	67.7
		Gal 1′	Gal 1′	Gal 1′	Gal 1′
1		103.2	102.9	103.0	103.2
2		74.0	73.7	73.9	74.0
3		75.2	75.0	75.5	75.2
4		70.4	70.1	70.0	70.4
5		76.4	76.2	76.5	76.5
6		62.3	62.0	62.3	62.3

[a]) The assignments were based on HSQC, HMBC, and NOSEY spectra. [b]) The NMR spectra were recorded in CD_3OD. [c]) The NMR spectra were recorded in (D_5)pyridine. [d]) nd, Not detected.

3.65) and CH_2(23) (δ(H) 3.34 and 3.64) in the NOESY spectrum (*Fig. 2*). The anomeric H-atom signals at δ(H) 4.50 (*d*, *J* = 7.5, 1 H) and 4.50 (*d*, *J* = 7.5, 1 H), which showed the HSQC correlations to the signals at δ(C) 105.8 and 105.8, respectively, indicated the presence of two sugar residues. Acid hydrolysis of compound **1** and GC analysis revealed the presence of one D-glucuronic acid and one L-arabinose moieties. The HMBC correlations between δ(H) 4.50 (H–C(1) of glucuronic acid) and δ(C) 83.6 (C(3) of aglycone), and between δ(H) 3.65 (H–C(3) of aglycone) and δ(C) 105.8 (C(1) of glucuronic acid) (*Fig. 2*) suggested that the glycosidic chain was located at C(3) of the aglycone. The linkage of the glycosidic chain was determined by the HMBC correlations between δ(H) 4.50 (H–C(1) of arabinose) and δ(C) 86.7 (C(3) of glucuronic acid), and between δ(H) 3.56 (H–C(3) of glucuronic acid) and δ(H) 105.8 (C(1) of arabinose). The β-anomeric configuration of the glucuronopyranosyl unit was determined from the observation of the large ^3J(1,2) coupling constant, and the α-

Table 2. *^{1}H-NMR Data* (500 MHz)[a]) *for Compounds* **1**–**5**. δ in ppm, J in Hz.

Position	δ(H)				
	1[b])	**2**[c])	**3**[c])	**4**[c])	**5**[c])
1	1.64–1.69 (*m*), 0.98–1.01 (*m*)	1.44–1.52 (*m*), 0.93–0.98 (*m*)	1.45–1.51 (*m*), 0.90–0.97 (*m*)	1.43–1.53 (*m*), 0.92–0.12 (*m*)	1.42–1.50 (*m*), 0.86–0.95 (*m*)
2	1.86–1.90 (*m*), 1.73–1.79 (*m*)	2.29–2.34 (*m*), 1.90–1.96 (*m*)	2.26–2.30 (*m*), 1.86–1.94 (*m*)	2.18–2.26 (*m*), 1.86–1.93 (*m*)	2.23–2.30 (*m*), 1.85–1.95 (*m*)
3	3.65 (*dd*, J = 11.0, 4.5)	3.34 (*dd*, J = 11.0, 4.5)	3.29 (*dd*, J = 11.0, 4.5)	4.11 (*dd*, J = 11.0, 4.5)	3.35 (*dd*, J = 11.0, 4.5)
5	1.62–1.68 (*m*)	0.86–0.94 (*m*)	0.86–0.94 (*m*)	1.40–1.50 (*m*)	0.88 (br. *s*)
6	1.40–1.53 (*m*), 1.23–1.27 (*m*)	1.61–1.68 (*m*), 1.39–1.44 (*m*)	1.62–1.68 (*m*), 1.36–1.43 (*m*)	1.32–1.48 (*m*), 1.00–1.10 (*m*)	1.53–1.60 (*m*), 1.30–1.38 (*m*)
7	1.82–1.87 (*m*), 1.65–1.72 (*m*)	2.20–2.26 (*m*), 2.08–2.15 (*m*)	2.20–2.26 (*m*), 2.05–2.15 (*m*)	1.55–1.60 (*m*), 1.16–1.26 (*m*)	1.62–1.70 (*m*), 1.32–1.40 (*m*)
9	1.22–1.26 (*m*)	1.72–1.81 (*m*)	1.72–1.82 (*m*)	1.80–1.90 (*m*)	1.70–1.82 (*m*)
11	1.92–1.98 (*m*), 1.80–1.88 (*m*)	1.95–2.02 (*m*), 1.88–1.93 (*m*)	1.95–2.02 (*m*), 1.85–1.93 (*m*)	1.95–2.00 (*m*), 1.80–1.93 (*m*)	1.90–1.96 (*m*), 1.80–1.90 (*m*)
12	5.49 (*t*, J = 4.0)	5.61 (*t*, J = 4.0)	5.56 (*t*, J = 4.0)	5.49 (br. *s*)	5.49 (br. *s*)
15	3.76 (*d*, J = 4.5)	4.30 (br. *s*)	4.26 (br. *s*)	1.88 (br. *s*), 1.63 (br. *s*)	1.88–1.94 (*m*), 1.62–1.75 (*m*)
16	3.55 (*d*, J = 4.5)	4.52 (br. *s*)	4.45 (br. *s*)	4.55 (br. *s*)	4.56 (br. *s*)
18	2.59–2.66 (*m*)	3.10–3.15 (*m*)	3.13–3.20 (*m*)	3.12–3.18 (*m*)	3.10–3.21 (*m*)
19	2.60–2.63 (*m*), 1.17–1.23 (*m*)	3.15–3.21 (*m*), 1.44–1.56 (*m*)	3.13–3.18 (*m*), 1.48–1.57 (*m*)	3.12–3.20 (*m*), 1.42–1.55 (*m*)	3.12–3.20 (*m*), 1.43–1.55 (*m*)
21	5.93 (*d*, J = 10.5)	6.79 (*d*, J = 10.5)	6.71 (*d*, J = 10.5)	6.77 (*d*, J = 10.5)	6.73 (*d*, J = 10.5)
22	5.58 (*d*, J = 10.5)	6.40 (*d*, J = 10.5)	6.32 (*d*, J = 10.5)	6.39 (*d*, J = 10.5)	6.33 (*d*, J = 10.5)
23	3.64 (*d*, J = 11.5), 3.34 (*d*, J = 11.5)	1.36 (*s*)	1.32 (*s*)	10.0 (br. *s*)	1.40 (*s*)
24	0.70 (*s*)	1.23 (*s*)	1.19 (*s*)	1.55 (*s*)	1.23 (*s*)
25	0.99 (*s*)	0.93 (*s*)	0.88 (*s*)	0.90 (*s*)	0.90 (*s*)
26	1.00 (*s*)	1.10 (*s*)	1.06 (*s*)	0.90 (*s*)	0.95 (*s*)
27	1.42 (*s*)	1.94 (*s*)	1.90 (*s*)	1.88 (*s*)	1.92 (*s*)
28	3.30 (*d*, J = 12.0), 3.05 (*d*, J = 12.0)	3.85 (*d*, J = 10.0), 3.59 (*d*, J = 11.0)	3.82 (*d*, J = 10.0), 3.54 (*d*, J = 11.0)	3.48 (*d*, J = 11.0), 3.71 (*d*, J = 10.0)	3.49 (*d*, J = 11.0), 3.75 (*d*, J = 10.0)

Table 2 (cont.)

Position	δ(H)				
	1[b])	**2**[c])	**3**[c])	**4**[c])	**5**[c])
29	0.87 (*s*)	1.19 (*s*)	1.15 (*s*)	1.18 (*s*)	1.17 (*s*)
30	1.09 (*s*)	1.42 (*s*)	1.36 (*s*)	1.42 (*s*)	1.40 (*s*)
21-*O*-Ang					
3′	6.05 (*dq*, *J* = 7.0, 1.5)	6.06 (*dq*, *J* = 7.5, 1.5)	6.13 (*dq*, *J* = 7.5, 1.5)	6.05 (*dq*, *J* = 7.5, 1.5)	6.15 (*dq*, *J* = 7.5, 1.5)
4′	1.92 (*d*, *J* = 7.5)	2.18 (*d*, *J* = 7.5)	2.22 (*d*, *J* = 7.5)	2.17 (*d*, *J* = 7.5)	2.24 (*d*, *J* = 7.5)
5′	1.82 (*s*)	2.10 (*s*)	2.09 (*s*)	2.09 (*s*)	2.13 (*s*)
22-*O*-Ang					
3″	5.94 (*dq*, *J* = 7.0, 1.5)	5.88 (*dq*, *J* = 7.5, 1.5)		5.98 (*dq*, *J* = 7.5, 1.5)	
4″	1.90 (*d*, *J* = 7.5)	2.06 (*d*, *J* = 7.5)		2.13 (*d*, *J* = 7.5)	
5″	1.82 (*s*)	1.84 (*s*)		1.99 (*s*)	
22-*O*-MB					
2″			2.12–2.21 (*m*)		2.26–2.38 (*m*)
3″			1.63–1.72 (*m*),		1.72–1.82 (*m*),
			1.28–1.35 (*m*)		1.35–1.45 (*m*)
4″			0.73 (*t*, *J* = 7.5)		0.88 (*t*, *J* = 7.5)
5″			1.07 (*d*, *J* = 6.5)		1.19 (*d*, *J* = 6.5)
GlcA					
1	4.50 (*d*, *J* = 7.5)	4.86 (*d*, *J* = 7.5)	4.82 (*d*, *J* = 6.5)	4.81 (*d*, *J* = 6.5)	4.91 (*d*, *J* = 6.5)
2	3.37–3.45 (*m*)	4.56–4.60 (*m*)	4.55–4.66 (*m*)	4.50–4.60 (*m*)	4.62–4.72 (*m*)
3	3.52–3.60 (*m*)	4.40–4.44 (*m*)	4.40–4.44 (*m*)	4.32–4.40 (*m*)	4.45–4.52 (*m*)
4	3.54–3.60 (*m*)	4.45–4.50 (*m*)	4.52–4.60 (m)	4.50–4.60 (*m*)	4.55–4.60 (*m*)
5	3.78–3.82 (*m*)	4.05–4.10 (*m*)	4.36–4.45 (m)	4.40–4.46 (*m*)	4.45–4.50 (*m*)
	Ara	Gal	Gal	Gal	Gal
1	4.50 (*d*, *J* = 7.5)	5.79 (*d*, *J* = 7.0)	5.78 (*d*, *J* = 7.0)	5.77 (*d*, *J* = 7.5)	5.78 (*d*, *J* = 7.5)
2	3.80–3.82 (*m*)	4.53–4.60 (*m*)	4.53–4.60 (*m*)	4.52–4.62 (*m*)	4.53–4.62 (*m*)
3	3.60–3.66 (*m*)	4.25–4.32 (*m*)	4.25–4.32 (*m*)	4.30–4.40 (*m*)	4.22–4.32 (*m*)
4	3.74–3.82 (*m*)	4.30–4.39 (*m*)	4.32–4.40 (*m*)	4.30–4.48 (*m*)	4.48–4.53 (*m*)
5	3.90 (*dd*, *J* = 12.5, 2.0),	4.39–4.48 (*m*),	4.38–4.46 (*m*),	4.10–4.18 (*m*),	4.06–4.13 (*m*),
	3.58 (*dd*, *J* = 12.5, 3.0)	4.05–4.12 (*m*)	4.33–4.42 (*m*)	4.35–4.42 (*m*)	4.35–4.48 (*m*)

Table 2 (cont.)

Position	δ(H)				
	1[b])	**2**[c])	**3**[c])	**4**[c])	**5**[c])
Xyl					
1		5.15 (*d*, *J* = 7.5)	5.12 (*d*, *J* = 7.5)	5.12 (*d*, *J* = 7.5)	5.15 (*d*, *J* = 7.5)
2		4.25–4.32 (*m*)	4.24–4.32 (*m*)	4.20–4.30 (*m*)	4.25–4.35 (*m*)
3		4.05–4.15 (*m*)	4.06–4.15 (*m*)	4.06–4.12 (*m*)	4.06–4.15 (*m*)
4		4.30–4.40 (*m*)	4.45–4.51 (*m*)	4.32–4.42 (*m*)	4.30–4.39 (*m*)
5		4.42–4.80 (*m*),	4.40–4.48 (*m*),	4.45–4.55 (*m*),	4.38–4.48 (*m*)
		3.52–3.58 (*m*)	3.52–3.58 (*m*)	3.52–3.60 (*m*)	3.50–3.56 (*m*)
Gal					
1		5.89 (*d*, *J* = 7.5)	5.89 (*d*, *J* = 7.5)	5.93 (*d*, *J* = 7.5)	5.93 (*d*, *J* = 7.5)
2		4.48–4.56 (*m*)	4.43–4.52 (*m*)	4.48–4.52 (*m*)	4.51–4.61 (*m*)
3		4.40–4.45 (*m*)	4.38–4.45 (*m*)	4.38–4.45 (*m*)	4.38–4.48 (*m*)
4		4.52–4.60 (*m*)	4.50–4.60 (*m*)	4.50–4.60 (*m*)	4.53–4.62 (*m*)
5		4.38–4.46 (*m*)	4.38–4.43 (*m*)	4.48–4.55 (*m*)	4.35–4.45 (*m*)
6		4.40–4.56 (*m*)	4.45–4.55 (*m*)	4.50–4.62 (*m*)	4.48–4.58 (*m*)

[a]) The assignments were based on HSQC, HMBC, and NOSEY spectra. [b]) The NMR spectra were recorded in CD_3OD. [c]) The NMR spectra were recorded in (D_5)pyridine.

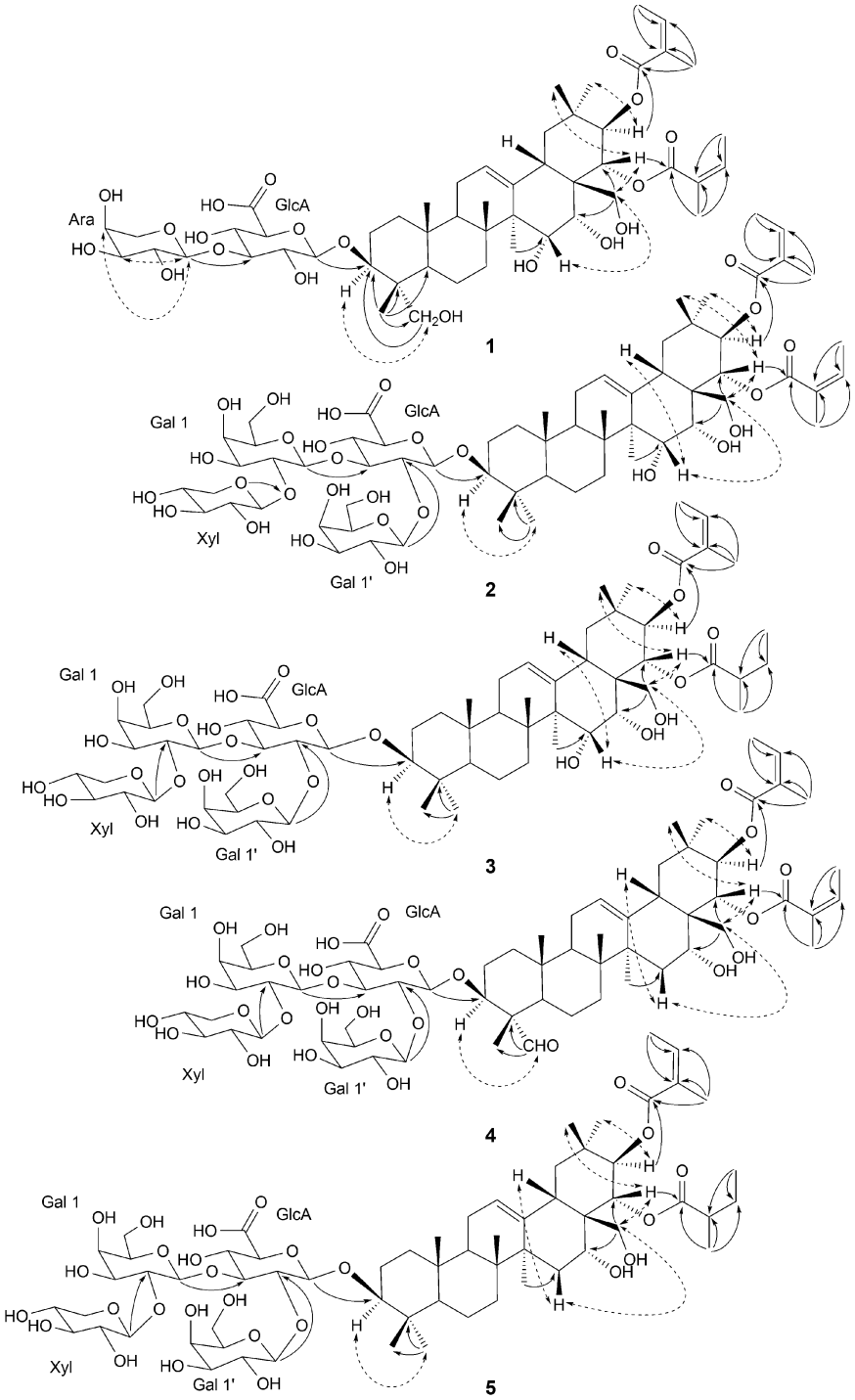

Fig. 2. *Key HMBCs* (H → C) *and NOESY* (H ⇠⇢ H) *correlations of compounds* **1**–**5**

anomeric configuration of arabinopyranosyl unit was assigned according to the NOESY correlations between δ(H) 4.50 (H–C(1) of arabinose) and δ(H) 3.65 (H–C(3) of arabinose) (*Fig. 2*). On the basis of the above analysis, the structure of **1** was elucidated as (3β,15α,16α,21β,22α)-15,16,23,28-tetrahydroxy-21,22-bis{[(2*Z*)-2-methylbut-2-enoyl]oxy}olean-12-en-3-yl 3-*O*-α-L-arabinopyranosyl-β-D-glucopyranosiduronic acid, and named as oleiferoside I.

Compound **2** was obtained as white amorphous powder. Its molecular formula of $C_{63}H_{98}O_{28}$ was deduced from the negative-ion-mode peak $[M-H]^-$ at *m*/*z* 1301.6198 in its HR-ESI mass spectrum. The IR spectrum indicated the presence of OH (3453 cm^{-1}) and α,β-unsaturated ester groups (1602, 1604 cm^{-1}). The aglycone structure of **2** was similar to that of **1**, and the main difference arose from the significant chemical-shift change of C(23), which was not hydroxylated in **2**. The assignment was confirmed by the observation of HMBC correlations between C(23) at (δ(C) 28.2) and Me(24) (δ(H) 1.23). Thus, the aglycone of **2** was elucidated as 21β,22α-bis(angeloyloxy)-3β,15α,16α,28-tetrahydroxyolean-12-ene. The ^{1}H-NMR spectrum of **2** indicated the presence of four sugar residues, which were identified as one D-glucuronic acid, two D-galactoses, and one D-xylose by the acid hydrolysis and GC analysis. The ^{1}H- and ^{13}C-NMR data for the sugar units of **2** were the same as those of oleiferoside A [9], suggesting that the glycosidic chain was β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl. The β-anomeric configurations of the four sugar units were deduced from the observation of their large ^3J(1,2) coupling constants. Therefore, the structure of **2** was elucidated as (3β,15α,16α,21β,22α)-15,16,28-trihydroxy-21,22-bis{[(2*Z*)-2-methylbut-2-enoyl]oxy}olean-12-en-3-yl β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-β-D-glucopyranosiduronic acid, and named as oleiferoside J.

Compound **3** was obtained as white amorphous powder. Its molecular formula, $C_{63}H_{100}O_{28}$, was determined by the *quasi*-molecular-ion peak $[M-H]^-$ at *m*/*z* 1303.6311 in its HR-ESI mass spectrum. The IR spectrum indicated the presence of OH (3452 cm^{-1}), C=O (1720 cm^{-1}), and α,β-unsaturated ester groups (1600 cm^{-1}). The NMR spectra data of **3** were closely similar to those of **2**, except that two olefinic C-atoms were replaced by sp^3-C-atoms, which suggested that a MB instead of a Ang group was attached at C(22). The assignment was supported by the HMBC correlations between δ(H) 6.32 (H–C(22)) and δ(C) 176.5 (C(1″) of MB), between δ(H) 0.73 (H–C(4″) of MB) and δ(C) 41.4 (C(2″) of MB) and 26.8 (C(3″) of MB), and between δ(H) 1.07 (H–C(5″) of MB) and δ(C) 176.5 (C(1″) of MB), 41.4 (C(2″) of MB), and 26.8 (C(3″) of MB). Thus, the structure of **3** was determined as (3β,15α,16α,21β,22α)-15,16,28-trihydroxy-22-[(2-methylbutanoyl)oxy]-21-{[(2*Z*)-2-methylbut-2-enoyl]oxy}-olean-12-en-3-yl β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-β-D-glucopyranosiduronic acid, and named as oleiferoside K.

Compound **4** was obtained as white amorphous powder. Its molecular formula, $C_{63}H_{96}O_{28}$, was deduced from the negative-ion-mode peak $[M-H]^-$ at *m*/*z* 1299.5965 in its HR-ESI mass spectrum. The IR spectrum indicated the presence of OH (3452 cm^{-1}) and α,β-unsaturated ester groups (1602, 1604 cm^{-1}). The NMR spectra data of **4** were similar to those of **2**, and one major difference was the significant chemical-shift change of the signals of C(15) ($\Delta\delta$(C) −32.8 ppm) at δ(C) 34.9, of C(14) ($\Delta\delta$(C) −7.5 ppm) at δ(C) 40.4, and of C(16) ($\Delta\delta$(C) −5.1 ppm) at δ(C) 68.7,

due to the presence of a CH_2(15) group in compound **4** rather than a CH(15) group in compound **2**. Another difference was the significant chemical-shift change of C(23), which was part of an aldehyde group in compound **4**. The assignment was confirmed by the observation of HMBC correlations between C(23) (δ(C) 210.5) and Me(24) (δ(H) 1.55). Therefore, the structure of **4** was elucidated as (3β,16α,21β,22α)-16,28-dihydroxy-21,22-bis{[(2*Z*)-2-methylbut-2-enoyl]oxy}-23-oxoolean-12-en-3-yl β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-β-D-glucopyranosiduronic acid, and named as oleiferoside L.

Compound **5** was obtained as white amorphous powder. The negative-ion-mode HR-ESI-MS of **5** exhibited a *quasi*-molecular-ion peak $[M-H]^-$ at *m*/*z* 1287.6391, indicating the molecular formula $C_{63}H_{100}O_{27}$. The IR spectrum showed the signals of OH (3450 cm^{-1}), C=O (1720 cm^{-1}), and α,β-unsaturated ester group (1600 cm^{-1}). The NMR data of **5** were similar to those of **3**, and the main differences arose from the significant upfield shifts of the signals of C(15) ($\Delta\delta$(C) – 32.4 ppm) at δ(C) 35.0, of C(14) ($\Delta\delta$(C) – 7.4 ppm) at δ(C) 40.2, and of C(16) ($\Delta\delta$(C) – 4.5 ppm) at δ(C) 68.5, suggesting that the H–C(15) in **3** was replaced by a CH_2(15) group in **5**. Moreover, the aglycone structure of **5** was also confirmed by comparing its spectroscopic data with those of 21β-(angeloyloxy)-22α-(2-methylbutanoyloxy)-3β,16α,28-trihydroxyolean-12-ene [8] and aesculioside IVc [10]. Therefore, the structure of **5** was elucidated as (3β,16α,21β,22α)-16,28-dihydroxy-22-[(2-methylbutanoyl)oxy]-21-{[(2*Z*)-2-methylbut-2-enoyl]oxy}olean-12-en-3-yl β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)]-β-D-glucopyranosiduronic acid, and named as oleiferoside M.

Bioactivities. Cytotoxic activities of these saponins against four human tumor cell lines (A549, B16, BEL-7402, and MCF-7) were evaluated by using the MTT *in vitro* assay. All compounds had significant cytotoxic activity against A549 (human lung adenocarcinoma, B16 (mouse melanoma), BEL-7402 (human hepatocarcinoma), and MCF-7 (human breast carcinoma) (*Table 3*).

A variety of triterpenoid saponins have been isolated from *Camellia* plants, and *C. sinensis* and *C. sinensis* var. *assamica* are the two species mostly investigated [1]. The present report on five new triterpenoid saponins from *C. oleifera* and their cytotoxic activities may enrich our knowledge about the constituents of the genus *Camellia* from a chemotaxonomic perspective.

Table 3. In vitro *Cytotoxic Activities of Compounds* **1**–**5**

Compound	Cell line IC_{50} [μM]			
	A549	B16	7402	MCF-7
1	23.45 ± 0.28	20.46 ± 0.65	15.71 ± 1.72	17.22 ± 2.42
2	11.37 ± 0.14	13.15 ± 0.95	12.02 ± 0.16	11.95 ± 0.45
3	29.73 ± 1.48	19.47 ± 1.51	28.12 ± 1.85	14.00 ± 1.92
4	10.63 ± 0.07	13.14 ± 0.24	12.73 ± 0.84	11.95 ± 1.20
5	12.03 ± 0.24	10.81 ± 0.53	12.59 ± 0.16	12.76 ± 0.42
Norcantharidin	4.16 ± 0.28	3.93 ± 0.22	3.45 ± 0.26	5.17 ± 0.38

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Experimental Part

General. TLC: Pre-coated silica-gel plates (SiO_2; *Qingdao Marine Chemical Factory*); visualization with 10% H_2SO_4 alcohol soln. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Factory*) and *Sephadex LH-20* (*GE Healthcare Corp.*, Beijing, P. R. China). GC/MS: *GC-14C* (*Shimadzu Corp.*) with a flame ionization detector (FID). HPLC: *Agilent Corp. SB-C18* semi-prep. HPLC column (250 × 9.4 mm i.d., 5 μm; *Agilent Corp.*, Palo Alto, CA, USA) on a *Shimadzu* HPLC system composed of a *LC-20AT* pump with a *SPD-20A* detector (*Shimadzu Corp.*, Kyoto, Japan); the wavelength for detection, 203 nm. Medium pressure liquid chromatography (MPLC): *Büchi* flash chromatography system composed of a *C-650* pump with a flash column (460 mm × 26 mm i.d., *Büchi* Corp., CH-Flawil) and ODS (*Merck KGaA*, DE-Darmstadt). Specific rotations: *Perkin–Elmer model 241* polarimeter (*PerkinElmer Inc.*, Waltham, MA, USA). IR Spectra: *Perkin–Elmer 983 G* spectrometer (*PerkinElmer Inc.*, Waltham, MA, USA). ^{1}H-, ^{13}C-, and 2D-NMR spectra: *Varian Inova 500* spectrometer (*Varian Inc.*, Palo Alto, CA, USA) in (D_5)pyridine or CD_3OD; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Micromass Q-TOF2* spectrometer (*Waters Corp.*, London, UK); in *m*/*z*.

Plant Material. The roots of *C. oleifera* ABEL were collected in Qichun, Hubei province of P. R. China in November 2011, and identified by Prof. *Xiao-Ran Li* at Soochow University. A voucher sample (No. 11-11-06-01) was deposited with the herbarium of the College of Pharmacy, Soochow University.

Extraction and Isolation. The air-dried roots (9.3 kg) of *C. oleifera* were crushed with a grinder into fine debris, and extracted twice with 70% EtOH (2 × 50 l) at 80° under reflux. The solvent was subsequently removed under reduced pressure to yield a residue (0.45 kg), which was then dissolved in dist. H_2O and passed through a *D101* macroporous resin column (i.d. 30 cm × 200 cm; *Xi'an Sunresin New Materials Co. Ltd.*). The column was eluted with gradient aq. EtOH (0, 30, 60, and 80%; each 40 l). The 60% EtOH eluate (42 g) was further vacuum chromatographed (SiO_2 (60–100 mesh; 30 × 18 cm); $CHCl_3$/MeOH 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 0:100; each 6.0 l). The $CHCl_3$/MeOH 60:40 eluate (4.5 g) was subjected to MPLC (ODS; MeOH/H_2O 50:50, 60:40, 70:30, 80:20, 90:10, 100:0; each 500 ml; flow rate, 20.0 ml/min) to afford six fractions. *Fr. 3* (500–1000 ml, 68 mg) was separated by semi-prep. HPLC (ODS; MeOH/H_2O 70:30) to yield compounds **5** (t_R 50.7 min; 12 mg), **2** (t_R 56.4 min; 16 mg), and **3** (t_R 58.2 min; 12 mg). In a similar way, *Fr. 5* (125 mg) was separated by semi-prep. HPLC (MeOH/H_2O 60:40) to give compound **1** (t_R 66.5 min; 12 mg). *Fr. 4* was purified by semi-prep. HPLC (MeOH/H_2O 65:35) to yield compound **4** (t_R 70.7 min; 16 mg). The purities of the isolated saponins were > 93% (determined by ^{1}H-NMR).

Oleiferoside I (= *(3β,15α,16α,21β,22α)-15,16,23,28-Tetrahydroxy-21,22-bis{[(2Z)-2-methylbut-2-enoyl]oxy}olean-12-en-3-yl 3*-O-α-L-*Arabinopyranosyl-β*-D-*glucopyranosiduronic Acid*; **1**). White amorphous powder. $[\alpha]_D^{20} = +6.6$ ($c = 0.11$, MeOH). UV (MeOH): 206 (4.26), 254 (4.22). IR (KBr): 3456 (OH), 2963, 2925, 2850, 1603 (C=C–C=O), 1601 (C=C–C=O), 1460, 1372, 1161, 1042. ^{1}H- (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): *Tables 2* and *1*, resp. HR-ESI-MS: 993.5087 ($[M-H]^-$, $C_{51}H_{77}O_{19}^-$; calc. 993.5059).

Oleiferoside J (= *(3β,15α,16α,21β,22α)-15,16,28-Trihydroxy-21,22-bis{[(2Z)-2-methylbut-2-enoyl]-oxy}olean-12-en-3-yl β*-D-*Galactopyranosyl-(1 → 2)-[β*-D-*xylopyranosyl-(1 → 2)-β*-D-*galactopyranosyl-(1 → 3)]-β*-D-*glucopyranosiduronic Acid*; **2**). White amorphous powder. $[\alpha]_D^{20} = +1.2$ ($c = 0.10$, MeOH). UV (MeOH): 209 (4.25), 254 (3.84). IR (KBr): 3453 (OH), 2960, 2918, 2850, 1604 (C=C–C=O), 1602 (C=C–C=O), 1460, 1374,1180, 1046. ^{1}H- (500 MHz, (D_5)pyridine) and ^{13}C-NMR (125 MHz, (D_5)pyridine): *Tables 2* and *1*, resp. HR-ESI-MS: 1301.6198 ($[M-H]^-$, $C_{63}H_{97}O_{28}^-$; calc. 1301.6166).

Oleiferoside K (= *(3β,15α,16α,21β,22α)-15,16,28-Trihydroxy-22-[(2-methylbutanoyl)oxy]-21-{[(2Z)-2-methylbut-2-enoyl]oxy}olean-12-en-3-yl β*-D-*Galactopyranosyl-(1 → 2)-[β*-D-*xylopyranosyl-(1 → 2)-β*-D-*galactopyranosyl-(1 → 3)]-β*-D-*glucopyranosiduronic Acid*; **3**). White amorphous powder.

$[\alpha]_D^{20} = +1.0$ ($c = 0.10$, MeOH). UV (MeOH): 210 (4.26), 254 (3.85). IR (KBr): 3452 (OH), 2960, 2916, 2852, 1720 (C=O), 1600 (C=C–C=O), 1460, 1376,1183, 1048. ^{1}H- (500 MHz, (D_5)pyridine) and ^{13}C-NMR (125 MHz, (D_5)pyridine): *Tables 2* and *1*, resp. HR-ESI-MS: 1303.6311 ($[M-H]^-$, $C_{63}H_{99}O_{28}^-$; calc. 1303.6323).

Oleiferoside L (*=(3β,16α,21β,22α)-16,28-Dihydroxy-21,22-bis{[(2Z)-2-methylbut-2-enoyl]oxy}-23-oxoolean-12-en-3-yl β-*D*-Galactopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 2)-β-*D*-galactopyranosyl-(1 → 3)]-β-*D*-glucopyranosiduronic Acid*; **4**). White amorphous powder. $[\alpha]_D^{20} = -2.6$ ($c = 0.10$, MeOH). UV (MeOH): 208 (4.29), 254 (3.82). IR (KBr): 3452 (OH), 2960, 2912, 2856, 1604 (C=C–C=O), 1602 (C=C–C=O), 1462, 1379, 1182, 1046. ^{1}H- (500 MHz, (D_5)pyridine) and ^{13}C-NMR (125 MHz, (D_5)pyridine): *Tables 2* and *1*, resp. HR-ESI-MS: 1299.5965 ($[M-H]^-$, $C_{63}H_{95}O_{28}^-$; calc. 1299.6010).

Oleiferoside M (*=(3β,16α,21β,22α)-16,28-Dihydroxy-22-[(2-methylbutanoyl)oxy]-21-{[(2Z)-2-methylbut-2-enoyl]oxy}olean-12-en-3-yl β-*D*-Galactopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 2)-β-*D*-galactopyranosyl-(1 → 3)]-β-*D*-glucopyranosiduronic Acid*; **5**). White amorphous powder. $[\alpha]_D^{20} = +4.6$ ($c = 0.12$, MeOH). UV (MeOH): 211 (4.18), 254 (4.34). IR (KBr): 3450 (OH), 2963, 2920, 2850, 1720 (C=O), 1600 (C=C–C=O), 1441, 1382, 1168, 1040. ^{1}H- (500 MHz, (D_5)pyridine) and ^{13}C-NMR (125 MHz, (D_5)pyridine): *Tables 2* and *1*, resp. HR-ESI-MS: 1287.6391 ($[M-H]^-$, $C_{63}H_{99}O_{27}^-$; calc. 1287.6374).

Acid Hydrolysis and Sugar Analysis of Compounds **1**–**5**. Each saponin (2 mg) was dissolved in 2N HCl (2 ml) and stirred at 80° for 4 h. The mixture was extracted with $CHCl_3$, and the aq. layer was evaporated to give a mixture of monosaccharides. The residue was dissolved in anh. pyridine (1 ml), followed by the addition of 2 mg of L-cysteine methyl ester hydrochloride (*Tokyo Chemical Industry*, 99%). After heating at 60° for 2 h, the solvent was eliminated under N_2, and 0.2 ml 1-trimethylsilyl-1*H*-imidazole (*Tokyo Chemical Industry*, 99%) was added. Then, the mixture was heated at 60° for another 2 h, and partitioned between hexane and H_2O. The org. layer was investigated by GC under the following conditions: cap. column, *HP-5* (30 m × 0.25 mm × 0.25 μm; *Dikma*); FID detector with a temp. of 280°; injection temp., 250°; initial temp., 160°, then raised to 280° at 5°/min, final temp., maintained for 10 min; carrier gas, N_2. The standard sugars experienced the same reaction and GC conditions. The retention times (t_R) of D-glucuronic acid, D-galactose, D-xylose, and L-arabinose were 19.770, 25.643, 32.257, and 33.284 min, resp.

Cytotoxicity Assay. The MTT colorimetric assay was performed to evaluate the cytotoxic activities of the isolated saponins against A549, B16, BEL-7402, and MCF-7 human tumor cell lines (Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences). Cells were placed into 96-well flat-bottomed culture plates at a concentration of 2×10^5 cells per well in complete RPMI 1640 culture medium. After 24 h, the medium containing fetal calf serum was removed, and the test solns. were applied to the cells in different final concentrations of 0.625, 1.25, 2.5, 5, 10, 20, 50, 100 μM. After 24 h, MTT soln. was added to the wells, and the plates were incubated at 37° for 4 h. The positive control group was treated with norcantharidin (purity higher than 99.0% as determined by HPLC; *Nanjing Zelang Medical Technology Co., Ltd.*, Nanjing, China). The amount of formazan was determined by photometry at 570 nm. The results were expressed as the percentage of absorbance in the control cells in comparison to that in the drug-treated cells. The IC_{50} values (50% inhibitory concentration) of compounds **1**–**5** are compiled in *Table 3*. The experiment was performed in triplicate for each sample.

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