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Five new triterpenoid saponins from the Roots of *Camellia oleifera* C. Abel with cytotoxic activities

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ABSTRACT

Five new triterpenoid saponins, oleiferosides P–T (**1–5**) were isolated from the EtOH extract of the roots of *Camellia oleifera* C. Abel. The structures of saponins **1–5** were elucidated on the basis of integrated spectroscopic techniques. All the compounds were characterized to be oleanane-type saponins with sugar moieties linked to the C-3 of the aglycone. By using the MTT assay, an *in vitro* analysis of the cytotoxic activities of these saponins on the human tumor cell lines (lung adenocarcinoma A549 cells, hepatic carcinoma SMMC-7721 cells and breast cancer MCF-7 cells). Among them, compound **4** showed a certain cytotoxic activity against all the tested cell lines.

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1. Introduction

Camellia oleifera C. Abel, which is mainly distributed in China, Japan, India and other South-East Asian countries, is the most representative and largest genus of the family Theaceae with more than 120 species recognized currently (Li et al., 2014; Zhao et al., 2011). And it has been grown as an oil crop in many countries including China, Brazil, Philippines, India and South Korea (Lee and Yen, 2006; Wang et al., 2013). The oil is rich in unsaturated fatty acids, which is comparable to the olive oil (Lee et al., 2007). Previous studies on constituents from the *Camellia* genus led to the isolation of different compounds such as flavonoids, bibenzyl glycosides and saponins from the seeds, seed cake or flowers (Chen et al., 2013; Chen et al., 2011; Sugimoto et al., 2009). Pharmacological investigations revealed that these aforementioned compounds exhibited antioxidant activity, hepatoprotective activity, cytotoxic activity and myocardial cell-injury-protective activity (Chen et al., 2013; Chen et al., 2011; Lee et al., 2007). The EtOH extract of the roots of *C. oleifera* C. Abel was successively subjected to column chromatography over D101 marcroporouse resins, silica gels and LX-2000 microreticular resin, and semi-preparative HPLC to give five new saponins, named oleiferosides P–T (**1–5**), the structures of the newly characterized saponins were elucidated by means of extensive spectroscopic analyses. Furthermore, the new compounds were screened for cytotoxity against three tumor cell lines (A549, SMCC-7721 and MCF-7 cells) by using 3-(4,5-dimethylthiazol)-2, 5-diphenyl tetrazoliumbromide (MTT) assay. Herein, we report on the isolation, structural elucidation and cytotoxity of the five new saponins.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The positive-ion HR-ESI–MS spectrum of **1** displayed an $[M+Na]^+$ peak at m/z 1101.5229 (calcd. 1101.5246), corresponding to a molecular formula of $C_{55}H_{82}O_{21}$. The IR spectrum showed the presence of a hydroxyl group (3456 cm^{-1}) and an α, β-unsaturated ester group (1601, 1603 cm^{-1}). The ^{13}C NMR (Table 1) spectrum showed the resonances of 55 carbons, ascribable to twelve methyl, nine methylene, twenty methine and fourteen quaternary carbons as revealed by the HSQC (Fig. 2) experiment. The ^1H NMR (Table 2) spectrum showed six methyl proton signals at δ_H 0.95 (Me-24), 0.92 (Me-25), 1.04 (Me-26), 1.96 (Me-27), 1.09 (Me-29) and 1.32 (Me-30), two isolated oxymethylene proton signals at δ_H 3.70 (1H, d, J = 11.5 Hz, H-23) and 4.32 (1H, d, J = 11.5 Hz, H-23), as well as δ_H 3.73 (1H, d, J = 12.0 Hz, H-28) and 3.89 (1H, d, J = 12.0 Hz, H-28), five isolated oxygenated methine proton signals at δ_H 5.67 (1H, d,

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Table 1
^{13}C NMR (125 MHz) spectroscopic data for compounds **1**–**5** in C_5D_5N.

No.	1	2	3	4	5
1	39.4	38.7	38.5	38.8	38.5
2	26.5	26.0	25.8	25.9	25.8
3	81.8	81.2	80.6	80.6	80.5
4	43.9	43.3	43.2	43.2	43.2
5	47.1	47.1	46.7	47.2	46.9
6	18.8	18.3	17.7	18.3	17.7
7	35.5	35.0	32.5	36.6	32.5
8	42.4	41.5	39.8	41.5	39.8
9	47.3	46.7	45.8	45.7	45.7
10	37.3	36.7	36.4	36.7	36.4
11	24.4	23.8	23.6	23.8	23.6
12	128.1	127.6	124.9	126.1	125.9
13	141.2	140.6	140.6	142.2	140.6
14	47.7	48.0	40.9	47.6	40,9
15	69.9	69.3	30.8	66.4	30.5
16	72.0	71.1	71.3	71.7	71.9
17	48.4	46.5	47.0	47.4	47.5
18	40.7	40.0	39.3	40.4	39.3
19	47.1	45.9	46.9	46.9	47.0
20	36.6	36.0	35.8	35.9	35.9
21	78.2	77.8	77.9	77.8	77.8
22	71.7	71.0	72.2	71.3	72.3
23	64.5	63.8	63.8	63.9	63.8
24	14.2	13.5	13.4	13.5	13.4
25	16.8	16.2	16.0	16.2	16.0
26	18.1	17.5	16.6	17.5	16.6
27	21.4	20.8	26.8	20.4	26.8
28	63.0	62.1	63.3	63.1	63.2
29	30.0	29.2	29.2	29.2	29.2
30	20.1	19.5	19.5	19.5	19.5
21-O-Ang					
1′	168.2	167.4	167.3	167.2	166.9
2′	129.0	128.0	127.9	127.8	128.0
3′	139.1	139.5	139.1	140.0	137.8
4′	16.5	16.0	15.9	15.9	15.5
5′	21.6	20.9	20.8	20.8	20.6
22-O-Ang					
1″	167.7				167.5
2″	128.7				138.3
3″	138.9				128.2
4″	16.2				15.7
5″	21.4				20.7
22-O-MB					
1′		175.9	175.6	176.0	
2′		41.7	41.5	41.5	
3′		26.6	26.5	26.5	
4′		11.7	11.7	11.7	
5′		16.8	16.5	16.6	
15-O-AC					
1′	171.0	169.8			
2′	22.3	21.7			
16-O-AC					
1″	170.1	169.8	169.6	170.0	169.6
2″	22.0	21.7	21.8	22.0	21.8
Sugar	Glc-A	Glc-A	Glc-A	Glc-A	Glc-A
1	106.2	105.6	105.1	105.3	105.3
2	75.0	74.5	74.1	73.5	74.1
3	86.4	85.5	85.5	86.0	85.5
4	72.0	71.4	71.2	71.3	71.5
5	78.2	77.1	78.3	78.3	77.8
6	nd	172.7	nd	170.5	170.2
	Ara	Ara	Ara	Ara	Ara
1	106.1	105.6	105.1	105.3	105.3
2	73.2	72.6	72.3	72.3	72.3
3	75.0	74.3	74.0	74.1	74.1
4	69.7	69.1	68.9	68.9	68.9
5	67.6	67.0	66.8	66.8	66.7

Chemical shifts are in ppm, and the assignments were based on HSQC, HMBC, and NOSEY spectra.
nd = not detected.

J = 4.5 Hz, H-15), 5.89 (1H, d, J = 4.5 Hz, H-16), 4.33 (1H, dd, J = 11.0, 4.5 Hz, H-3), 6.32 (1H, d, J = 10.5 Hz, H-22) and 5.87 (1H, d, J = 10.5 Hz, H-21) and one olefinic proton signal at δ_H 5.62 (1H, brs, H-12). Furthermore, the proton signals of two angeloyl (Ang) groups at δ_H [6.00 (1H, dq, J = 7.0, 1.5 Hz, 21-O-Ang-3′), 2.05 (3H, d, J = 7.5 Hz, 21-O-Ang-4′), 2.03 (3H, s, 21-O-Ang-5′)] and δ_H [5.81 (1H, dq, J = 7.0, 1.5 Hz, 22-O-Ang-3″), 1.97 (3H, d, J = 7.5 Hz, 22-O-Ang-4″), 1.72 (3H, s, 22-O-Ang-5″)] were observed. And the same time the two acetoxy (AC) groups at δ_H 2.02 (3H, s, 15-O-AC-2′) and 2.53 (3H, s, 16-O-AC-2′) could also be observed. The ^1C NMR data indicated the presence of two acetoxy (AC) groups at δ_C 171.0 (15-O-AC-1′), 22.27 (15-O-AC-2′) and δ_C 170.5 (16-O-AC-1″), 21.98 (16-O-AC-2″), the other data were comparable to those reported for oleiferoside F published in the literature (Li et al., 2014), suggesting that compound **1** had the 15α,16α-diacetoxy-21β, 22α-O-diangeloyloxy-3*β*,23,28-trihydroxyolean-12-ene type of aglycone. In the HMBC spectrum, the correclations were observed from H-21 at δ_H 5.87 to C-21-Ang-1′ at δ_C 168.2 and from H-22 at δ_H 6.32 to C-22-Ang-1′ at δ_C 167.7. It was proved that the two Ang groups were connected to C-21 and C-22 respectively. The locations of the two AC groups at C-15 and C-16 were also confirmed by the HMBC (Fig. 2) correlations between H-15 at δ_H 5.62 to C-15-O-AC-1′ at δ_C 171.0 and H-16 at δ_H 5.89 to C-16-O-AC-1′ at δ_C 170.5. A CH_2OH group was located at C-23, which was confirmed by the correlations between H-23 at δ_H 3.70, 4.32 and C-3 at δ_C 81.8, and between H-23 at δ_H 3.70, 4.32 and C-24 at δ_C 14.16 in the HMBC spectrum. The relative configuration of **1** was determined from the NOESY (Fig. 2) experiment. The NOEs from H-29 at δ_H 1.09 to H-22 at δ_H 6.32 and from H-30 at δ_H 1.32 to H-21 at δ_H 5.87 suggested that H-21 and H-22 are α- and β-oriented, which was confirmed that the two Ang groups at C-21 and C-22 were β- and α-oriented. The NOEs from H-18 at δ_H 1.09 to H-16 at δ_H 5.89 and from H-15 at δ_H 5.67 to H-16 at δ_H 5.89, indicated that H-18, H-15 and H-16 were co-facial and β-oriented, while the two AC groups were on the opposite side of the molecular plane and thus α-oriented. The anomeric proton signals at δ_H 5.18 (1H, d, J = 7.5 Hz, H-1 of glucuronic acid) and 5.29 (1H, d, J = 7.5 Hz, H-1 of arabinose), which showed the HSQC (Fig. 2) correlations to δ_C 106.2 (C-1 of glucuronic acid) and 106.1 (C-1 of arabinose) respectively, revealling the presence of two sugar residues. Acid hydrolysis of compound **1** and GC analysis confirmed one unit of D-glucuronic acid and one unit of L-arabinose. The HMBC correlations between δ_H 5.18 (H-1 of glucuronic acid) and δ_C 81.8 (C-3 of aglycone) and between δ_H 4.33 (H-3 of aglycone) and δ_C 106.2 (C-1 of glucuronic acid) suggested that the glycosidic chain attached to C-3 of the aglycone. The linkage of the glycosidic chain was determined by the HMBC correlations between δ_H 5.29 (H-1 of arabinose) and δ_C 86.4 (C-3 of glucuronic acid), and between δ_H 4.33 (H-3 of glucuronic acid) and δ_C 106.1 (C-1 of arabinose). The glucuronopyranosyl unit was in β-anomeric configuration based on the observation of the large $^3JH_{-1,H-2}$ coupling constant. The α-configuration of the arabinopyranosyl unit was confirmed by the NOESY correlations between δ_H 5.29 (H-1 of arabinose) and δ_H 4.20 (H-3 of arabinose). Based on the above analyses, the structure of **1** was established to be 15α,16α-diacetoxy-21β, 22α-*O*-diangeloyloxy-23,28-dihydroxyolean-12-ene 3β-*O*-α-L-arabinopyranosyl-(1 → 3)-β-D-glucuronopyranoside and named oleiferoside P, as shown in Fig. 1.

Compound **2** was isolated and purified as white amorphous powder, and the molecular formula was determined as $[M + Na]^+$ at m/z 1103.5385 (calcd. 1119.5403), referring to a molecular formula of $C_{55}H_{84}O_{21}$. The IR spectrum showed absorption bands at 3454, 1605 and 1602 cm^{-1}, which were assignable to a hydroxyl and anα, β-unsaturated ketone group. Comparison to their ^1H and ^{13}C NMR data, **2** was similar to **1**, and the main differences in their NMR spectra arose from the significant upfield shifts of the C-2″ (−86.3 ppm) signal at δ_C 41.7 and C-3″ (−112.9 ppm) signal at δ_C 26.6. With the aid of the ^1H–^1H COSY, HSQC and HMBC spectra reveled that an Ang group was replaced to a 2-methylbutanoyl (MB) group, which was attached to C-22 in compound**2**. It was

Table 2
^{1}H NMR (125 MHz) spectroscopic data for compounds 1–5 in C_5D_5N.

	1	2	3	4	5
1	1.51 m, 0.97 m	1.48 m, 0.96 m	1.47 m, 0.90 m	1.51 m, 0.97 m	1.90 m, 1.41 m
2	2.28 m, 2.00 m	2.29 m, 1.98 m	2.28 m, 1.96 m	2.27 m, 1.99 m	2.27 m, 1.94 m
3	4.33 dd	4.34 dd	4.29 dd	4.28 dd	4.25 dd
	(10.0, 5.0)	(10.0, 5.0)	(10.0, 5.0)	(10.0, 5.0)	(10.0, 5.0)
5	1.74 m	1.52 m	1.79 m	1.84 m	1.75 m
6	1.70 m, 1.3 m	1.70 m, 1.31 m	1.71 m, 1.32 m	1.76 m, 1.38 m	1.69 m, 1.27 m
7	2.01 m, 1.31 m	2.00 m, 1.29 m	1.56 m, 1.15 m	2.18 m, 1.45 m	1.51 m, 1.10 m
9	1.80 m	1..80 m	2.50 m	2.72 m	2.86 m
11	1.95 m, 1.81 m	1.92 m, 1.78 m	1.91 m, 1.82 m	1.93 m, 1.84 m	1.89 m, 1.75 m
12	5.62 brs	5.60 brs	5.44 brs	5.53 brs	5.42 brs
15	5.67 brs	5.62 brs	1.60 m, 1.90 m	4.39 brs	1.59 m, 1.80 m
16	5.89 brs	5.84 brs	5.61 brs	5.82 brs	5.50 brs
18	3.23 m	3.22 m	3.05 m	3.08 m	3.06 m
19	2.78 m, 1.54 m	2.73 m, 1.52 m	2.65 m, 1.45 m	2.78 m, 1.47 m	2.63 m, 1.45 m
21	5.87 d (10.0)	5.79 d (10.0)	5.90 d (10.0)	5.83 d (10.0)	5.95 d (10.0)
22	6.32 d (10.0)	6.27 d (10.0)	6.23 d (10.0)	6.17 d (10.0)	6.25 d (10.0)
23	4.32 d (10.0)	4.33 d (10.0)	4.33 d (10.0)	4.28 d (10.0)	4.27 d (10.0)
	3.70 d (10.0)	3.69 d (10.0)	3.70 d (10.0)	3.65 d (10.0)	3.66 d(10.0)
24	0.95 s	0.94 s	0.94 s	0.94 s	0.90 s
25	0.92 s	0.90 s	0.91 s	0.95 s	0.87 s
26	1.04 s	1.02 s	0.83 s	1.06 s	0.79 s
27	1.96 s	1.70 s	1.45 s	1.76 s	1.40 s
28	3.89 d (10.0)	3.87 d (10.0)	3.68 d (10.0)	3.76 d (10.0)	3.60 d (10.0)
	3.73 d (10.0)	3.70 d (10.0)	3.53 d (10.0)	3.52 d (10.0)	3.43 d (10.0)
29	1.09 s	1.05 s	1.07 s	1.04 s	1.05 s
30	1.32 s	1.28 s	1.29 s	1.25 s	1.27 s
21-O-Ang					
3′	6.00 dq	6.07 dq	6.11 dq	6.04 dq	5.96 dq
	(7.0, 1.5)	(7.0, 1.5)	(7.0, 1.5)	(7.0, 1.5)	(7.0, 1.5)
4′	2.05 d (7.5)	2.10 d (7.5)	2.14 d (7.5)	2.09 d (7.5)	2.02 d (7.5)
5′	2.03 s	1.70 s	2.03 s	1.96 s	2.00 s
22-O-Ang					
3′′	5.81 dq				5.92 dq
	(7.0, 1.5)				(7.0, 1.5)
4′′	1.97 d (7.5)				2.04 d (7.5)
5′′	1.72 s				1.94 s
22-O-MB					
2′		2.45 m	2.50 m	2.53 m	
3′		1.71 m 1.43 m	1.62 m 1.49 m	1.81 m 1.49 m	
4′		0.88 t (7.5)	0.91 t (7.5)	0.88 t (7.5)	
5′		1.12 d (6.5)	1.20 d (6.5)	1.16 d (6.5)	
15-O-AC					
2′	2.02 s	1.99 s			
16-O-AC					
2′′	2.53 s	2.51 s	2.54 s	2.49 s	2.48 s
Sugar	Glc-A	Glc-A	Glc-A	Glc-A	Glc-A
1	5.18 d (10.0)	5.29 d (10.0)	5.19 d (10.0)	5.17 d (10.0)	5.11 d (10.0)
2	4.13 m	4.13 m	4.11 m	4.09 m	4.07 m
3	4.33 m	4.22 m	4.20 m	4.20 m	4.18 m
4	4.43 m	4.46 m	4.41 m	4.41 m	4.29 m
5	4.48 m	4.47 m	4.47 m	4.47 m	4.50 m
6	nd	nd	nd	nd	nd
	Ara	Ara	Ara	Ara	Ara
1	5.29 d (10.0)	5.19 d (10.0)	5.29 d (10.0)	5.27 d (10.0)	5.23 d (10.0)
2	4.53 m	4.54 m	4.55 m	4.50 m	4.52 m
3	4.20 m	4.20 m	4.20 m	4.17 m	4.15 m
4	4.33 m	4.32 m	4.33 m	4.29 m	4.27 m
5	4.43 dd	4.40 dd	4.42 dd	4.38 dd	4.39 dd
	(12.5, 3.0)	(12.5, 3.0)	(12.5, 3.0)	(12.5, 3.0)	(12.5, 3.0))
	3.82 dd	3.82 dd	3.81 dd	3.79 dd	3.77 dd
	(12.5, 2.0)	(12.5, 2.0)	(12.5, 2.0)	(12.5, 2.0)	(12.5, 2.0)

Chemical shifts are in ppm, and coupling constants (*J*) in Hz are given in parentheses. The assignments were based on HSQC, HMBC, and NOSEY spectra.

readily confirmed **2** had the same relative configuration from the NOESY experiment. Accordingly, compound **2** was formulated as 15α,16α-diacetoxy-21β-*O*-angeloyloxy-23,28-dihydroxyolean-12-ene 22α-*O*-(2-methylbutanoyloxy) olean 3β-*O*-α-L-arabino-pyranosyl-(1 →3)-β -D-glucuronopyranoside as shown in Fig. 1 and named oleiferoside Q.

Compound **3** was obtained as white amorphous powder. The positive-ion HR-ESI–MS spectrum of **3** showed an $[M+Na]^+$ peak at *m*/*z* 1045.5349(calcd. 1045.5348), referring to a molecular formula of $C_{53}H_{82}O_{19}$. The IR spectrum showed absorption bands at 3456, 1604 and 1602 cm^{-1}, which were in accordance with a hydroxyl and an α, β-unsaturated ketone groups. Comparison to their ^{1}H and ^{13}C NMR data, **3** was closely similar to **2**, and the main differences in their NMR spectra were accounted by the absence of the AC group (δ_C 170.3, C-15-O-AC-1′, δ_H 1.99 s; δ_C 21.3, 15-O-AC-2′) in **2** and presences of one methylene group (δ_H 1.60 m, δ_H 1.90 m; δ_C 30.8, C-15) in **3**. The assignment was confirmed by the observation of HMBC correlations between C-15 at δ_C 30.8 and Me-27 at δ_H 1.45 s. Thus, the structure of compound **3** was assigned to be 16α-acetoxy-21β-*O*-angeloyloxy-23,28-diahydroxyolean-12-ene 22α-

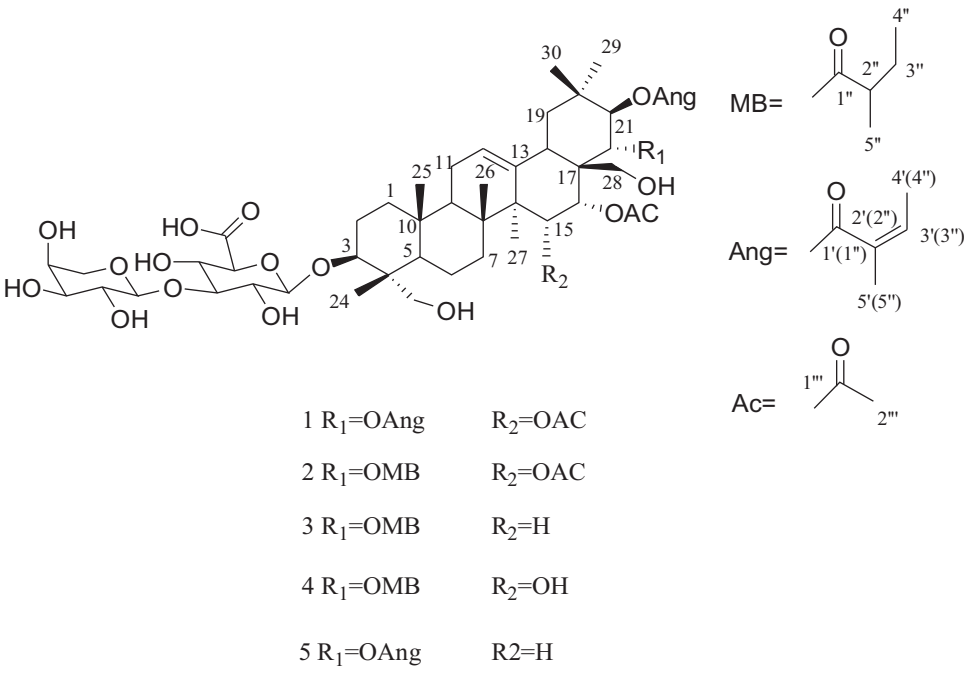

Fig. 1. The structures of new triterpenoid saponins from the Roots of *Camellia oleifera* C. Abel.

O-(2-methylbutanoyloxy) olean 3β-*O*-α-L-arabinopyranosyl-(1→3)-β -D-glucuronopyranoside as shown in Fig. 1 and named oleiferoside R.

Compound **4** was obtained as white amorphous powder. Its molecular formula of $C_{53}H_{82}O_{20}$ was determined by the observation of the $[M+Na]^+$ ion peak at *m*/*z* 1061.5247 (calcd. 1061.5297) in the HR–ESI–MS spectrum. The IR spectrum showed absorption bands at 3455, 1603 and 1601 cm^{-1}, which were in accordance with a hydroxyl and anα, β-unsaturated ketone group. Comparison to their ^1H and ^{13}C NMR data, **4** was closely similar to **2**, except for disappearance of a series of signals of the AC group (δ_C 170.3, C-15-O-AC-1′, δ_H 1.99 s; δ_C 21.3, 15-O-AC-2′) and presence of a hydroxyl group linked to C-15 of **4**. The assignment was confirmed by the observation of HMBC correlations between C-15 at δ_H 4.39 and Me-27 at δ_C 20.4 and the only one AC group linked to C-16 by the observation of HMBC correlations between H-16 at δ_H 5.82 and C-16-O-AC-1′ at δ_C 170.0. Thus, the structure of compound **4** was assigned to be 16α-acetoxy-21β -*O*-angeloyloxy-15*α*, 23, 28-trihydroxyolean-12-ene 22*α*-*O*-(2-methylbutanoyloxy) olean 3β-*O*-α-L-arabinopyranosyl-(1→3)- β-D-glucuronopyranoside as shown in Fig. 1 and named oleiferoside S.

Compound **5** was obtained as white amorphous powder. The positive-ion HR-ESI–MS spectrum of **5** showed an $[M+Na]^+$ peak at *m*/*z* 1043.5184 (calcd. 1043.5192), referring to a molecular formula of $C_{53}H_{80}O_{19}$. The IR spectrum showed absorption bands at 3456, 1604 and 1602 cm^{-1}, which were in accordance with a hydroxyl and an α,β-unsaturated ester groups. Comparison to their ^1H and ^{13}C NMR data, **5** was closely similar to **1**, and the main differences in their NMR spectra were accounted by the absence of the AC group (δ_C 171.0, C-15-O-AC-1′, δ_H 2.02 s; δ_C 22.3, 15-O-AC-2′) in **1** and presence of an methylene group (δ_H 1.59 m, δ_H 1.80 m; δ_C 30.5, C-15) in **5**. The assignment was confirmed by the observation of HMBC correlations between C-15 at δ_C 30.5 and Me-27 at δ_H 1.40 s, and the only one AC group linked to C-16 by the observation of HMBC correlations between H-16 at δ_H 5.50 and C-16-O-AC-1′ at δ_C 169.6. Therefore, the structure of compound **5** was assigned to be 16α-acetoxy-21β,22α-*O*-diangeloyloxy-23,28-dihydroxyolean-12-ene 3β-*O*-α-L-arabinopyranosyl-(1→3)-β -D-glucuronopyranoside as shown in Fig. 1 and named oleiferoside T.

In conclusion, five isolated new triterpenoid saponins from the roots of *C. oleifera C.* Abel (P–U) and their structures were determined. Preliminary cytotoxicity screening revealed that oleiferoside S (compound **4**) exhibited a certain cytotoxicity against A549, SMCC-7721 and MCF-7 cells shown in Table 2.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. IR spectra were determined on a PerkinElmer 983 G spectrometer. NMR spectra were recorded on a Varian Inova 500 spectrometer in C_5D_5N using tetramethylsilane (TMS) as the internal standard. HR–ESI–MS spectra were taken on a Micromass Q-TOF2 spectrometer. HPLC analysis and purification were carried out on a SB-C18 semipreparative HPLC column (250 × 9.4 mm i.d., 5 μm, Agilent Corp. Palo Alto, CA, USA) with a Shimadzu HPLC system composed of a LC-20AT pump with a SPD-20A detector (Shimadzu Corp., Kyoto, Japan), and the wavelength for detection was 203 nm. Medium pressure liquid chromatography (MPLC) purification was performed on a Büchi Flash Chromatography system composed of a C-650 pump with a flash column (460 mm × 26 mm i.d., Büchi Corp., Flawil, Switzerland). Silica gel (200–300 mesh) for column chromatography and precoated silica gel TLC plates were purchased from Qingdao Marine Chemical Factory. Compounds on TLC were colored by 10% sulfuric acid alcohol solution. Sephadex LH-20 for column chromatography was obtained from GE Corp. GC was carried out on a GC-14C (Shimadzu Corp.) with a flame ionization detector (FID). D101 macroporous resin and LX-2000 mircroreticular resin (a type of phenol modified polystyrene-base anion exchange resin) for column chromatography were acquired from Xi'an Sunresin New Material Co. Ltd..

3.2. Plant material

The roots of *C. oleifera* C. Abel were collected in Qichun, Hubei province of China in November 2011, and identified by Professor Xiao-Ran Li at Soochow University. A voucher sample (No. 11-11-

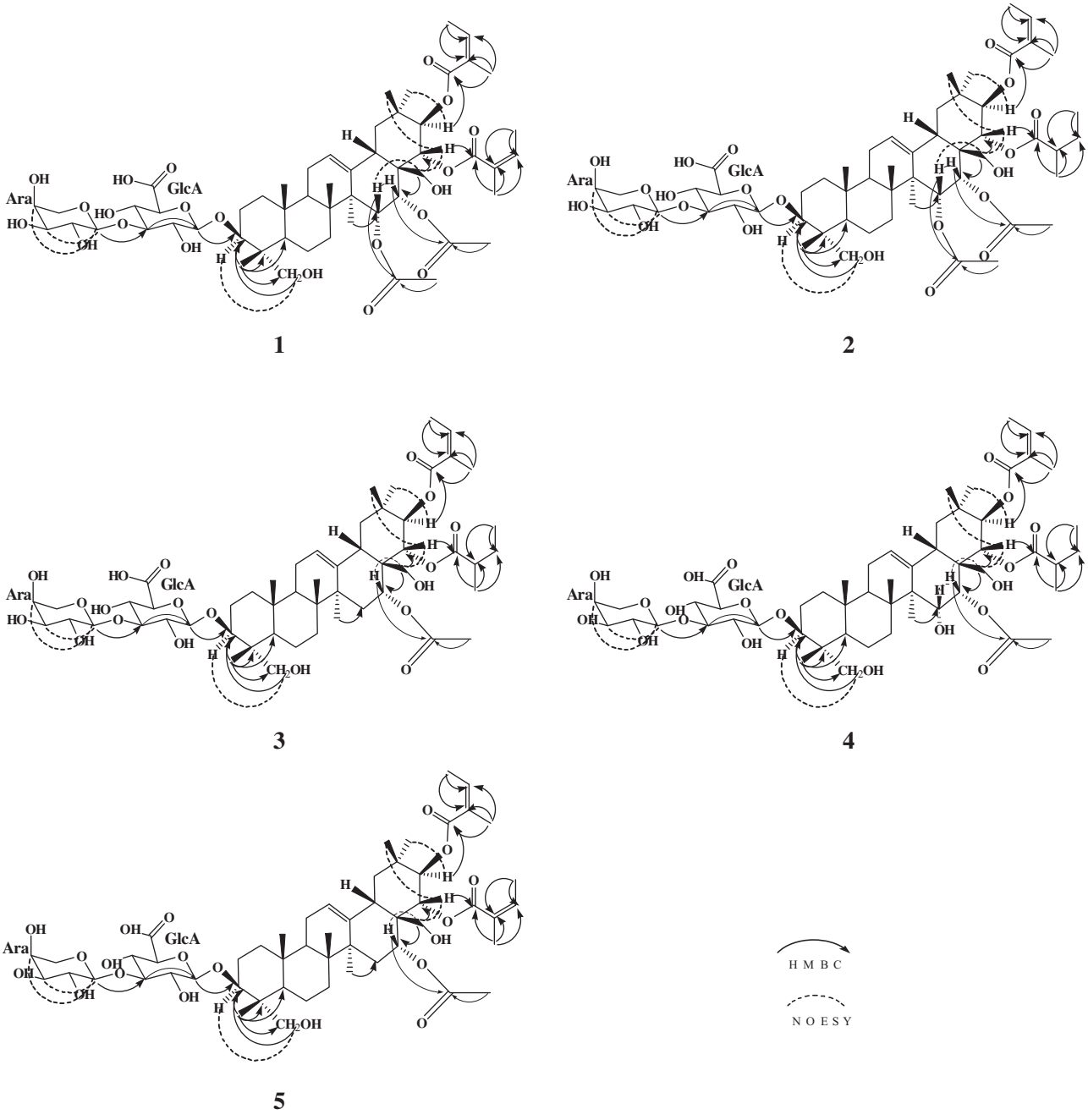

Fig. 2. Key HMBC and NOE correlations for compounds **1–5**.

06-01) was deposited in the herbarium of the College of Pharmacy, Soochow University.

3.3. Extraction and isolation

The dried plant material (10 kg) were crushed with a grinder into fine debris, then extracted twice with 50% EtOH (100 L) at 80 °C under reflux. After removing the solvent under reduced pressure to yield the combined extracts (0.45 kg), which was then dissolved in distilled water and passed through a D101 macroporous resin column (i.d. 30 cm × 200 cm, Xi'an Sunresin New Materials Co. Ltd.), using a sequential mixture of EtOH-H_2O (0%, 30%, 60%, 80%, each 50 L). The 60% EtOH eluate (80 g) was further applied to a LX-2000 mircroreticular resin column (26 × 200 cm; Xi'an Sunresin New Materials Co. Ltd.), eluted with a gradient of aqueous MeOH (MeOH–H_2O 0%, 40%, 70%, and 100%, each 40 L). The 70% MeOH fraction (25.2 g) was separated on a silica gel (200–300 mesh) column (30 × 18 cm), eluted with a gradient of $CHCl_3$–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 (80:20) eluate (4.5 g) was chromatographed by MPLC over ODS, eluted with MeOH–H_2O (50:50, 60:40, 70:30, 80:20, 90:10, 100:0, each 500 mL) at a flow rate of 20.0 mL/min to afford 6 fractions. Fraction 2 (80 mg) was separated by semi-preparative HPLC over an ODS column, eluted with MeOH–H_2O (70:30) to yield compound **1** (16 mg, *t*R 58.7 min), **2** (14 mg, *t*R 56.4 min) and **4** (12 mg, *t*R 50.2 min). In a similar way, fraction 4 (62 mg) was purified by semi-preparative HPLC, eluted with MeOH–H_2O (68:32) to yield compound **3** (16 mg, *t*R 70.7 min) and **5** (18 mg, *t*R 75.0 min). All the semi-preparative HPLC separations were

conducted at a flow rate of 2 mL/min and the detection wavelength was 203 nm. The purities of the isolated saponins were >95% by HPLC with ELSD detection.

3.4. Oleiferoside P (1)

(15α,16α-diacetoxy-21β, 22α-*O*-diangeloyloxy-23, 28-dihydroxyolean-12-ene, 3β-*O*-α-L-arabinopyranosyl-(1→3)-β-D-glucuronopyranoside): White amorphous powder; $[\alpha]^{20}_{D}$ + 6.4 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 206 (3.26), 254 (4.22) nm; IR ν_{max} (KBr): 3456, 2925, 1603, 1601, 1161, 1042 cm^{-1}; HR-ESI–MS $[M+Na]^+$ *m/z* 1101.5229 (calcd. for $C_{55}H_{82}O_{21}Na$, 1101.5246); ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data are given in Tables 1 and 2.

3.5. Oleiferoside Q (2)

(15α,16α-diacetoxy-21β-*O*-angeloyloxy-23,28-dihydroxyolean-12-ene 22α-*O*-(2-methylbutanoyloxy) olean 3β-*O*-α-L-arabinopyranosyl-(1→3)-β-D-glucuronopyranoside): White amorphous powder; $[\alpha]^{20}_{D}$ + 5.3 (c 0.11, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.23), 254 (3.84) nm; IR ν_{max} (KBr): 3454, 2986, 1605, 1602, 1180, 1046 cm^{-1}; HR–ESI–MS $[M+Na]^+$ *m/z* 1103.5385 (calcd. for $C_{55}H_{84}O_{21}Na$, 1103.5403); ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data are given in Tables 1 and 2.

3.6. Oleiferoside R (3)

(16α-acetoxy-21β-*O*-angeloyloxy-23,28-diahydroxyolean-12-ene 22α-*O*-(2-methylbutanoyloxy) olean 3β-*O*-α-L-arabinopyranosyl-(1→3)-β-D-glucuronopyranoside): white amorphous powder; $[\alpha]\ ^{20}_{D}$ + 2.1 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 208 (4.28), 254 (3.85) nm; IR ν_{max} (KBr): 3456, 2934, 1604, 1602, 1183, 1048 cm^{-1}; HR–ESI–MS $[M+Na]^+$ *m/z* 1045.5349 (calcd. for $C_{53}H_{82}O_{19}Na$, 1045.5348); ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data are given in Tables 1 and 2.

3.7. Oleiferoside S (4)

(16α-acetoxy-21β-*O*-angeloyloxy-15α, 23,28-trihydroxyolean-12-ene, 22α-*O*-(2-methylbutanoyloxy) olean 3β-*O*-α-L-arabinopyranosyl-(1→3)-β-D-glucuronopyranoside): white amorphous powder; $[\alpha]^{20}_{D}$ + 2.5 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.29), 254 (2.82) nm; IR ν_{max} (KBr): 3455, 2732, 1603, 1601, 1182, 1046 cm^{-1}; HR-ESI–MS $[M+Na]^+$ *m/z* 1061.5247 (calcd. for $C_{53}H_{82}O_{20}Na$, 1061.5297); ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data are given in Tables 1 and 2.

3.8. Oleiferoside T (5)

(16α-acetoxy-21β,22α-*O*-diangeloyloxy-23, 28-dihydroxyolean-12-ene, 3β-*O*-α-L-arabinopyranosyl-(1→3)-β -D-glucuronopyranoside): white amorphous powder; $[\alpha]^{20}_{D}$ + 5.6 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 210 (4.18), 254 (4.34) nm; IR ν_{max}(KBr): 3456, 2963, 1604, 1602, 1168, 1040 cm^{-1}; HR–ESI–MS $[M+Na]^+$ 1043.5184 (calcd. for $C_{53}H_{80}O_{19}Na$, 1043.5192); ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data are given in Tables 1 and 2.

3.9. General acid hydrolysis of the compounds 1–5

The acid hydrolysis and sugar analysis of the five new compounds were performed as previously describe (Li et al., 2014). A solution of each saponin (2 mg) dissolved in 2 N HCl (dioxane–H_2O, 1:1, 2.0 mL) was heated to 80 °C for 4 h. The reaction mixture was extracted with $CHCl_3$, and the aqueous layer was evaporated to give a mixture of monosaccharides. Each residue was dissolved in dry pyridine (1 mL), then L-cysteine methyl ester hydrochloride in dry pyridine (0.06 M, 2 mL) was added. After heating at 60 °C for 2 h, the solvent was evaporated under N_2, and 0.2 mL trimethylsilylimidazole was added. Then the mixture was heated at 60 °C for another 2 h and partitioned between n-hexane and water. The organic layer was investigated by GC under the following conditions: FID detector with a temperature of 280 °C; injector temperature 250 °C; initial temperature 160 °C, then raised to 280 °C at 5 °C/min, final temperature maintained for 10 min; carrier gas N_2 (2.0 kg/cm^3). Under these conditions, the following sugar units of each compound was identified by comparison with authentic sample: t_R (min) 19.770 min (D-glucuronic acid), 33.284 min (L-arabinos).

Table 3
In vitro cytotoxic activities of compounds **1–5** (IC_{50} values in μM).

	Cell line IC_{50} (μM)		
	A549	SMMC-7721	MCF-7
1	>100	>100	>100
2	>100	>100	>100
3	93.91 ± 2.63	97.54 ± 1.43	86.78 ± 3.48
4	25.96 ± 0.32	34.85 ± 0.71	25.52 ± 0.82
5	83.73 ± 1.71	88.42 ± 1.57	78.33 ± 2.78
Norcantharidin	4.16 ± 0.28	7.93 ± 0.46	5.17 ± 0.38

IC_{50} is means ± standard deviation of five new triterpenoid saponins.

4. Antiumoral cytotoxic bioassays

The viability of the cells after treated with the isolated saponins against A549, SMCC-7721 and MCF-7 human tumor cell lines (Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) was evaluated using the MTT assay performed as previously reported (Wang et al., 2008). Cells were plated into 96-well flat-bottomed cultured plates at a concentration of 1×10^5 cells per well in fresh culture medium. After 24 h, the test solutions were applied to the cells in different final concentrations at 6.25, 12.5, 25, 50, 100 μM. After 24 h, the MTT solution was added to the wells and the plates were incubated at 37 °C for 4 h. After the medium was removed, 100 μL of DMSO was added to each well. The plants were gently against until the color reaction was uniform and the OD_{570} was determined using a microplate reader. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the vehicle in the MTT assay(Zhang et al., 2015). The IC_{50} values (50% inhibitory concentration) of compounds **1–5** are shown in Table 3.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2015.08.005.

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