Phytochemistry 142 (2017) 76–84

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Triterpenoids from *Cyclocarya paliurus* and their inhibitory effect on the secretion of apoliprotein B48 in Caco-2 cells

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ARTICLE INFO

Article history:
Received 3 April 2017
Received in revised form
19 June 2017
Accepted 25 June 2017

Keywords:
Cyclocarya paliurus
Juglandaceae
Triterpenoids
Cyclocarioside
Apoliprotein B48

ABSTRACT

Five previously undescribed compounds including two triterpenoid aglycones, 3β,23-dihydroxy-1,12-dioxo-olean-28-oic acid and 3β,23,27-trihydroxy-1-oxo-olean-12-ene-28-oic acid, and three triterpenoid glucosides cyclocarioside L-N, along with 17 known compounds were isolated from a CH_3Cl-soluble extract of the leaves of *Cyclocarya paliurus*. Two 27-nor-triterpenoid glycosides were isolated from the genus for the first time. Furthermore, the characterized compounds were tested for the inhibitory effects on apoliprotein B48 secretion in Caco-2 cells. Seven triterpenoid aglycones together with four triterpenoid saponins significantly decreased the apoliprotein B48 oversecretion induced by oleic acid in Caco-2 cells.

1. Introduction

Digestion and absorption of excessive lipids is a well-known contributor to the development of dyslipidemia, which is associated with metabolism syndrome including cardiovascular disease and diabetes mellitus (Karpe, 1999). Apolipoprotein B48 (apoB48) is critical for the intestinal absorption of dietary triglycerides and cholesterol and has been shown to be a useful marker of intestinally derived lipoprotein particles, especially chylomicron and chylomicron remnants (Otokozawa et al., 2009; Young, 1990). Both clinical observations and basic researches put insight that anomalous elevated plasma concentration of apoB48, especially in the fasting state, was positive correlated with an elevated risk of coronary artery disease (Mori et al., 2013; Valero et al., 2005).

Cyclocarya paliurus (Juglandaceae) (Batal) Iljinskaja (*C. paliurus*) is an edible and medicinal plant distributed in the highland of southern China (Shu et al., 1995b). Its leaves have been used to prevent and ameliorate dyslipidemia and diabetes in Chinese folkloric medicine. Previous studies reported that *C. paliurus* extract suppressed an increase in plasma triacylglycerol levels in lipid-loaded mice and ameliorate dyslipidemia in type 2 diabetic rats (Kurihara et al., 2003; Wang et al., 2013).

Triterpenoids have been extensively investigated due to various biological activities, such as anti-inflammatory, anti-tumor, anti-hyperglycemic as well as anti-hyperlipidemic effects (Alqahtani et al., 2013; Ghosh and Sil, 2013; Somova et al., 2003). For example, ginsenoside Rg3 and ursolic acid, the main constituents of red ginseng and Crataegii fructus, can inhibit the activities of pancreatic lipase and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and reduce the plasma levels of triglyceride

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http://dx.doi.org/10.1016/j.phytochem.2017.06.015
0031-9422/

and cholesterol in hyperlipidemic mice (Min et al., 2008). Triterpenic acids including oleanolic acid, ursolic acid, maslinic acid, corosolic acid, 23-hydroxyursolic acid, arjunolic acid and echinocystic acid are all reported to have inhibitory effect on cholesterol acyltransferase (ACAT) activity (Kim et al., 2005). Phytochemical investigations of the leaves of *C. paliurus* have resulted in the isolation of approximately 100 compounds, including polysaccharides, flavonoids, phenolic acids and triterpenoids (Shang et al., 2015). Up to date, pentacyclic and tetracyclic triterpenoid aglycones and glycosides, such as oleanane-, ursane- and dammarane-type triterpenoids, have been identified from the leaves of *C. paliurus* (Fu and Fang, 2009). Our previous findings demonstrated that ethanol extract of *C. paliurus* leaves was abundant in triterpenic acids and could mitigate dyslipidemia and down-regulate intestinal apoB48 secretion, indicating that triterpenoids might be the active components with biological activity (Ma et al., 2015).

In the present study, 5 previously undescribed and 17 known compounds were isolated from the chloroform extract of *C. paliurus* using column chromatography and Pre-HPLC methods (Fig. 1). Their structures were elucidated as 14 pentacyclic triterpenic acids and 8 tetracyclic triterpenoid glycosides based on the detailed spectroscopic analysis. Moreover, all the characterized compounds were evaluated for the inhibitory effects on apoB48 secretion of Caco-2 cells.

2. Results and discussion

2.1. Structural elucidation of the previously undescribed compounds

Compound **1** was obtained as a colorless powder. The molecular formula was determined to be $C_{30}H_{46}O_6$ on the basis of HR-ESI-MS (*m/z* 501.3218 $[M-H]^-$, calcd for $C_{30}H_{45}O_6$, 501.3216). The UV spectrum of **1** exhibited absorption maxima at 203 nm. The IR absorption bands at 3441, 2946 and 1696 cm^{-1} indicated the presence of hydroxyl groups, aliphatic C−H and carbonyl groups. In the ^1H NMR spectrum, six tertiary methyl groups at δ_H 1.26, 1.17, 1.10, 1.10, 1.01 and 0.93, an oxygen-bearing methylene group at δ_H 4.17 (1H, d, J = 10.7 Hz) and 3.73 (1H, d, J = 10.6 Hz), and an oxygenated methine group at δ_H 4.59 (1H, dd, J = 4.6, 11.8 Hz) were observed (Table 1). The ^{13}C and DEPT NMR spectra displayed 30 carbon resonances attributed to six methyl, ten methylene (one oxygenated), five methine (one oxygenated) and nine quaternary carbons (three carbonyls). Among them, two keto carbonyl groups were apparent from the signals at δ_C 213.0 and 211.6 as well as one carboxyl group from the signal at δ_C 181.1 (Table 1). Therefore, **1** was assigned as an olean-type triterpenoid. The spectroscopic data above were closely related to those of a known compound, 3β,23-dihydroxy-1-oxo-olean-12-en-28-oic acid (Okada et al., 2003). Instead of a double bond between C-12 and C-13 in the above known compound, compound **1** was established in the presence of a carbonyl group at C-12 according to the observed HMBC correlations from δ_C 211.6 (C-12) to δ_H 3.14 (H-11a), 2.25 (H-11b) and 3.22 (H-13) (Fig. 2). The configuration of the hydroxyl group at C-3 was deduced to be β oriented on the basis of the coupling constant of J = 11.8 Hz and an observed NOE effect between H-3 and H-5. The NOESY correlations of H-5/H-9, H-9/H-27, H-13/H-26 and H-18/H-26 suggested that the A/B, B/C and C/D rings were all trans-fused while the D/E ring was cis-fused (Fig. 3). Thus, the structure of **1** was established as 3β, 23-dihydroxy-1,12-dioxo-olean-28-oic acid.

Compound **2** was isolated as a colorless powder. The molecular formula $C_{30}H_{46}O_6$ was deduced from HR-ESI-MS (*m/z* 520.3625 $[M+NH_4]^+$, calcd for $C_{30}H_{50}NO_6$, 520.3633). The UV spectrum of **2** exhibited absorption maxima at 204 nm. The IR absorption bands at 3441, 2945, 1696 and 1663 cm^{-1} indicated the presence of hydroxyl, aliphatic C−H, carbonyl and olefinic groups. The ^1H NMR spectrum of **2** exhibited the spectral features of a typical oleanane triterpene, including an olefinic proton at δ_H 5.86 (1H, t, J = 3.6 Hz), an oxygenated methine proton at δ_H 4.55 (1H, dd, J = 4.9, 11.8 Hz), two pairs of oxygenated methylene protons at δ_H 4.12 (1H, d, J = 10.7 Hz), 3.71 (1H, d, J = 10.5 Hz), 4.04 (1H, d, J = 12.0 Hz) and 3.93 (1H, d, J = 11.9 Hz), and five methyl singlets at δ_H 1.38, 1.20, 1.13, 1.01 and 0.87 (Table 1). In addition, the ^{13}C NMR spectrum displayed one keto carbonyl carbon at δ_C 213.5, one carboxylic carbon at δ_C 180.7, three oxygenated carbons at δ_C 73.2, 66.6 and 64.8 as well as two olefinic carbons at δ_C 128.4 and 139.8 (Table 1). These NMR data were quite similar to those of 3β,23-dihydroxy-1-oxo-olean-12-en-28-oic acid, except for an oxygenated methylene instead of a methyl group at C-27 (Okada et al., 2003). The HMBC correlations between H-27 (δ_H 4.04 and 3.93) and C-13 (δ_C 139.8), C-14 (δ_C 48.5), C-8 (δ_C 40.8) and C-15 (δ_C24.1) confirmed that the methylene was oxygenated and located at C-27 (Fig. 2). The relative configuration of compound **2** was determined by the NOESY correlations of H-3/H-5, H-5/H-9, H-9/H-27, H-18/H-30 and H-25/H-26 (Fig. 3). Therefore, compound **2** was elucidated as 3β,23,27-trihydroxy-1-oxo-olean-12-ene-28-oic acid.

Compound **15** was obtained as a colorless powder. Its molecular formula was established as $C_{38}H_{62}O_{12}$ by HR-ESI-MS (*m/z* 728.4607 $[M+NH_4]^+$, calcd for $C_{38}H_{66}NO_{12}$, 728.4580), suggesting 8 degrees of unsaturation. The UV spectrum of **15** exhibited absorption maxima at 203 nm. The IR absorption bands at 3439, 2929 and 1640 cm^{-1} indicated the presence of hydroxyl, aliphatic C−H and carbonyl groups, respectively. In the ^1H NMR spectrum, there were two anomeric protons at δ_H 5.53 (1H, br s) and 4.98 (1H, d, J = 7.8 Hz), two oxymethine protons at δ_H 4.43 (1H, ddd, J = 5.0, 10.5, 10.5 Hz) and 3.61 (1H, d, J = 2.1 Hz), and six methyl protons at δ_H 1.35, 1.27, 1.26, 1.04, 0.96 and 0.71 (Table 2). The ^{13}C NMR of 38 carbons resonances confirmed the aforementioned moieties (Table 2). Moreover, the ^{13}C NMR showed a carbonyl carbon at δ 176.9. These data suggested that **15** was a nor-triterpenoid glycoside with a dammarane-type aglycone and two sugar moieties. Comparing the NMR data of **15** with those of cyclocarioside I, **15** has a similar aglycone as those of cyclocarioside I, except for the C-17 side chain (Shu et al., 1995a). Five-membered lactone ring located at C-17 was proposed for the structure of compound **15** based on the HMBC correlations from H-23 (δ_H 2.64 and 2.50) to δ_C 176.9 (C-24), 89.7 (C-20), 32.5 (C-22) and H-22 (δ_H 1.97 and 1.82) to δ_C 176.9 (C-24), 89.7 (C-20), 49.9 (C-17), 29.7 (C-23) and 24.8 (C-21) (Fig. 2). Acid hydrolysis of **15** afforded L-arabinofuranose and D-quinovopyranose on the basis of HPLC by comparing with an authentic sugar sample and the attachments were confirmed by the HMBC correlations from δ_H 5.53 (H-1′, Ara) to δ_C 79.7 (C-3) and δ_H 4.98 (H-1″, Qui) to δ_C 76.3 (C-12), respectively. The β configuration of quinovopyranose was based on the coupling constant of the anomeric proton (J = 7.8 Hz), and α configuration of arabinofuranose was determined by the NOESY correlations of H-1'/H-3′ and H-3'/H-5'. Additionally, the H-3 and H-12 were present in β-orientation and α-orientation according to the coupling constant of the protons (J = 2.1 Hz and J = 10.5 Hz), respectively. The configuration at C-20 was determined to be *S* by a comparison of the ^{13}C NMR chemical shift data of analogous dammaranes (Zhao et al., 2015). Thus, compound **15** was deduced to be 20*S*,24-lactone-dammar-(3α,12β)- 12-*O*-β-D-quinovopyranosyl-3-*O*-α-L-arabinofuranoside, and named as cyclocarioside L.

Compound **16** was obtained as a colorless powder. Its HR-ESI-MS spectrum displayed an $[M+NH_4]^+$ molecular ion at *m/z* 775.4277 (calcd 770.4716), corresponding to a molecular formula of $C_{40}H_{68}NO_{13}$. The UV spectrum of **16** exhibited absorption maxima at 224 and 203 nm. The IR absorption bands at 3442, 2929 and

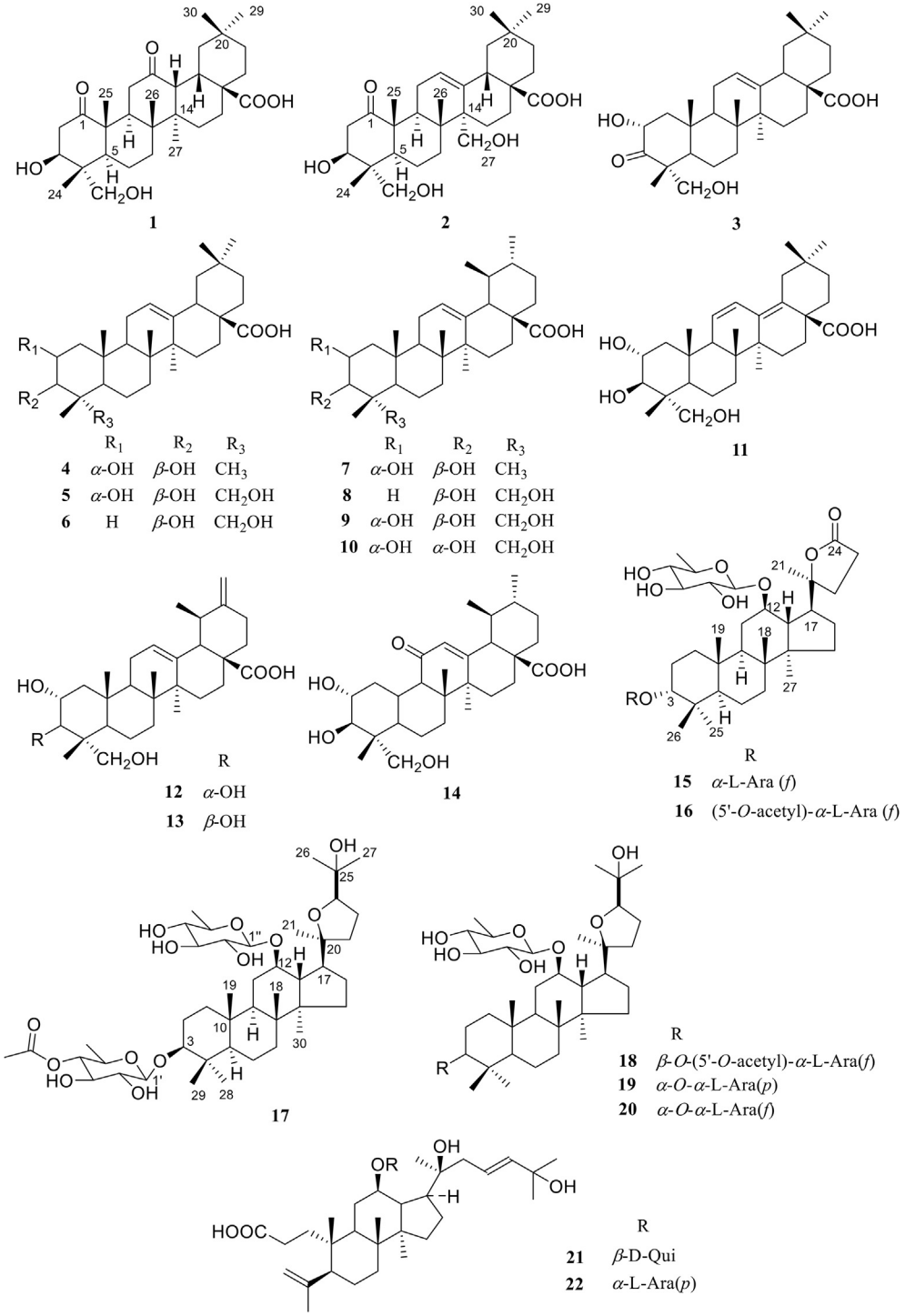

Fig. 1. Chemical structures of compounds **1**–**22**.

1643 cm^{-1} assigned as hydroxyl, aliphatic C–H and carbonyl groups, respectively. The structure of **16** was deduced as a 27-nor-triterpenoid glycoside with a dammarane triterpenoid aglycone and two sugars according to the NMR data (Table 2), which were in good agreement with those of **15**, except for the presence of the acetyl group in **16**. In the HMBC spectrum, the correlations from H-5' (δ_H 4.80 and 4.60) to δ_C 171.1 indicated that the acetyl group was linked at C-5′ of the arabinofuranose. The linkage position of arabinofuranose at C-3 and the quinovopyranose at C-12 was confirmed by the HMBC correlations from δ_H 5.50 (H-1′) to δ_C 80.0 (C-3) and δ_H 4.97 (H-1″) to δ_C 76.3 (C-12). Therefore, compound **16** was deduced as 20*S*,24- lactone-dammar-(3α,12β)-12-*O*-β-D-quinovopyranosyl-3-*O*-α-(4′-*O*-acetyl)-L-arabinofuranoside, and named as cyclocarioside M.

Compound **17** was obtained as a colorless powder, having a molecular formula of $C_{44}H_{74}O_{13}$ as deduced from HR-ESI-MS (*m/z* 828.5473 $[M+NH_4]^+$, calcd for $C_{44}H_{78}NO_{13}$, 828.5468). The UV spectrum of **17** exhibited absorption maxima at 225 and 203 nm. The IR absorption bands at 3441 and 1642 cm^{-1} assigned as hydroxyl and carbonyl groups, respectively. The NMR data of **17**

Table 1
NMR data for compound **1** and **2** (δ in ppm, all measured in C_5D_5N, J in Hz).

Position	**1**			**2**		
	δ_C[b], type	δ_H[a] (J in Hz)	HMBC (H→C)	δ_C[c], type	δ_H[a] (J in Hz)	HMBC (H→C)
1	213.0, C			213.5, C		
2	45.5, CH_2	3.46, dd (11.8, 11.8) 2.80, dd (4.7, 11.4)	1, 3, 4, 10	45.6, CH_2	3.43, dd (11.9, 11.9) 2.81, dd (4.8, 11.8)	1, 3, 4, 10
3	73.3, CH	4.59, dd (4.6, 11.8)	4, 23, 24	73.2, CH	4.55, dd (4.9, 11.8)	24
4	44.4, C			44.1, C		
5	47.7, CH[d]	1.91, d (12.0)	3, 4, 6, 7, 10, 23, 25	47.8, CH	2.06, m[d]	4, 6, 7, 9, 10, 24, 25
6	18.4, CH_2	1.87, d (13.0), 1.63, m	5, 7, 10	18.5, CH_2	1.81, m[d], 1.59, m[d]	
7	31.8, CH_2	1.42, m[d], 1.20, m[d]	26	33.5, CH_2	2.07, m[d], 1.80, m[d]	4, 6, 10, 26
8	41.9, C			40.8, C		
9	42.4, CH	2.72, dd (3.8, 12.8)	10, 26	40.9, CH	3.18, dd (5.9, 10.9)	1, 8, 10, 11, 25, 26
10	52.7, C			52.9, C		
11	41.3, CH_2	3.14, dd (3.8, 16.4) 2.25, dd (13.6, 5.7)	9, 12	26.5, CH_2	2.86, dt (4.3, 18.7) 2.03, m[d]	9, 12, 13
12	211.6, C			128.4, CH	5.86, t (3.6)	9, 11, 13
13	53.0, CH	3.22, d (3.1)	12, 14, 18, 19, 27	139.8, C		
14	43.1, C			48.5, C		
15	28.7, CH_2	2.07, dt (5.0, 12.3), 1.06, br d (13.3)	14, 17	24.1, CH_2	2.16, d (9.3) 1.61, m[d]	8, 27
16	23.9, CH_2	1.96, m, 1.11, m	14, 15, 17, 18, 28	24.4, CH_2	2.16, d (9.3) 1.99, m[d]	27, 28
17	47.7, C[d]			47.1, C		
18	33.2, CH	3.36, br d (13.3)	13, 14, 17	42.5, CH	3.39, dd (4.1, 14.6)	12, 13, 16, 17
19	37.2, CH_2	2.31, d (12.7) 1.42, m[d]	17, 21, 29, 30	45.8, CH_2	1.80, m[d], 1.34, m[d]	18, 20, 30
20	31.4, C			31.3, C		
21	35.2, CH_2	1.42, m[d], 1.20, m[d]	29, 30	34.5, CH_2	1.42, m[d], 1.17, m[d]	20
22	34.1, CH_2	2.15, td (3.5,13.5) 1.75, d (13.4)	17, 21, 28	33.9, CH_2	2.07, m[d], 1.37, m[d]	20
23	65.9, CH_2	4.17, d (10.7) 3.72, d (10.6)	3, 4, 5, 24	66.6, CH_2	4.12, d (10.7), 3.71, d (10.5)	3, 4, 5, 24
24	13.9, CH_3	1.17, s	3, 4, 5, 23	14.1, CH_3	1.20, s	3, 4, 5, 23
25	15.4, CH_3	1.26, s	1, 5, 9, 10	16.4, CH_3	1.38, s	1, 5, 9, 10
26	16.9, CH_3	1.10, s	9, 27	19.8, CH_3	1.13, s	7, 8, 9, 14
27	21.1, CH_3	1.01, s	13, 14, 15	64.8, CH_2	4.04, d (12.0) 3.93, d (11.9)	8, 13, 14, 15
28	181.1, C			180.7, C		
29	23.9, CH_3	1.10, s	19, 20, 30	24.2, CH_3	1.01, s	19, 20, 30
30	34.0, CH_3	0.93, s	19, 20, 21, 30	33.6, CH_3	0.87, s	19, 20, 21, 30

[a] ^{1}H NMR spectra were measured at 600 MHz.
[b] ^{1}H NMR spectra were measured at 500 MHz.
[c] ^{13}C NMR spectra were measured at 125 MHz.
[d] Overlapped signals.

(Table 2) revealed that it was a dammarane triterpenoid glycoside with two sugars and were quite similar to cyclocarioside B, except for the signals of the glycosyl unit (Jiang et al., 2006). The acetyl group were observed in **17** and its linkage at C-4′ of quinovopyranose was determined by the correlations from δ_H 5.25 (H-4′) to δ_C 170.8 (C=O), 70.6 (C-5′) and 18.5 (C-6′) in the HMBC spectrum. The long-range correlations from δ_H 4.71 (H-1′) to δ_C 82.0 (C-3) and δ_H 5.00 (H-1″) to δ_C 77.9 (C-12) implied the linkage of two quinovoses at C-3 and C-12. Furthermore, the large coupling constant of the anomeric protons confirmed the β relative configurations. The configuration at C-20 and C-24 was determined to be *S* and *R* based on comparison to the ^{13}C NMR chemical shift data of analogous expoxydammaranes (Jiang et al., 2006). The α-orientation of H-3 and H-12 was clarified by the NOESY correlations between δ_H 3.66 (H-3) and δ_H 1.03 (H-28) as well as δ_H 4.38 (H-12) and δ_H 0.61 (H-30). Thereby, compound **17** was determined as (20*S*,24*R*)-epoxydammarane (3β,12β)-25-hydroxyl-12-*O*-β-D- quinovopyranosyl-3-*O*-(4′-*O*-acetyl)-β-D-quinovopyranoside, and named as cyclocarioside N.

The 17 known triterpenoids were identified as cyclocaric acid B (**3**) (Cui and Li, 2012), maslinic acid (**4**) (Zhang et al., 2006), arjunolic acid (**5**) (Cui and Li, 2012), hederagenin (**6**) (Kizu and Tomimori, 1982), 2α-hydroxyursolic acid (**7**) (Sashida et al., 1992), 3β,23-dihydroxy-12-ene-28-ursolic acid (**8**) (Xie et al., 2007), 2α,3β,23-trihydroxy-12-ene-28-ursolic acid (**9**) (Meng et al., 2009), 2*a*,3*a*,23-trihydroxyurs-12-en-28-oic acid (**10**) (Xiao et al., 2013), 2*a*,3β,23- trihydroxyoleana-11,13(18)-dien-28-oic acid (**11**) (Mahato and Nandy, 1990), 2*a*,3*a*,23-trihydroxyurs- 12,20(30)-dien-28-oic acid (**12**) (Xiao et al., 2013), actinidic acid (**13**) (Xiao et al., 2013), 2α,3β,23-trihydroxyurs-11- oxo-12-ene-28-oic acid (**14**) (Zhao et al., 2007), cylocarioside H (**18**) (Li et al., 2012), cyclocarioside J (**19**) (Liu et al., 2014), cyclocarioside I (**20**) (Shu et al., 1995a), pterocaryoside A (**21**) (Kennelly et al., 1995) and pterocaryoside B (**22**) (Kennelly et al., 1995) by comparing their spectroscopic data with those reported in the literature.

2.2. The inhibitory effect on the secretion of apoB48 in Caco-2 cells

Overconsumption of exogenous cholesterol and triglyceride was proved to be associated with dyslipidemia. Apolipoprotein B48 (apoB48) is the constitutive protein involved in dietary lipid absorption, and a high level of apoB48 in plasma elevates cardiovascular risk factors (Otokozawa et al., 2009). Caco-2, derived from a human colorectal carcinoma, is an intestinal cell line as a common research model for investigating intestinal-derived lipoprotein synthesis and secretion (Levy et al., 1995). In the current study, the isolated triterpenoids were tested for inhibitory effect on apoB48 oversecretion in Caco-2 cell model.

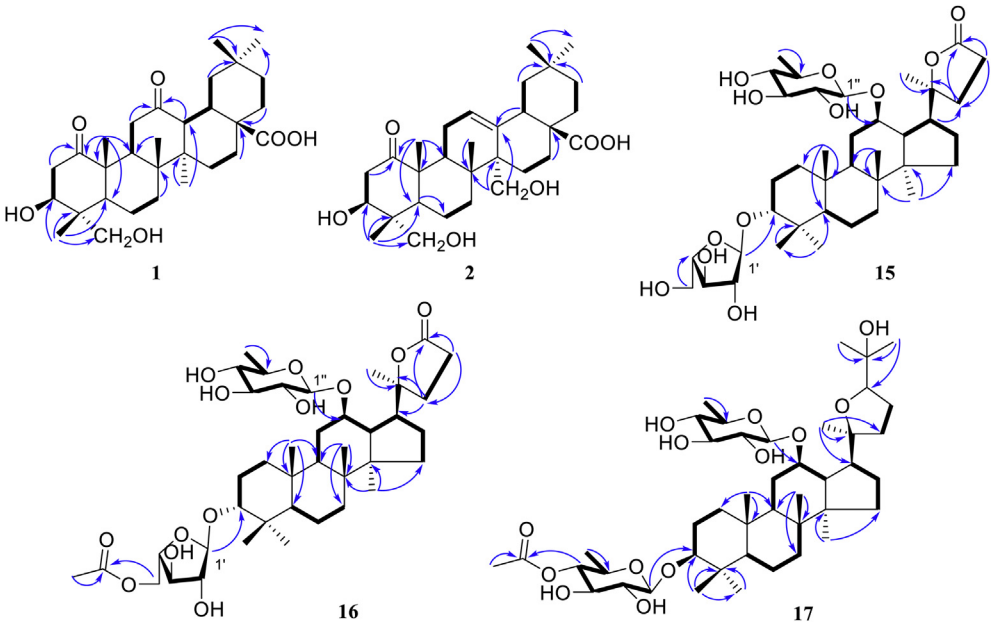

Fig. 2. Selected key COSY (bold lines) and HMBC (→) correlations of **1**, **2**, **15**, **16** and **17**.

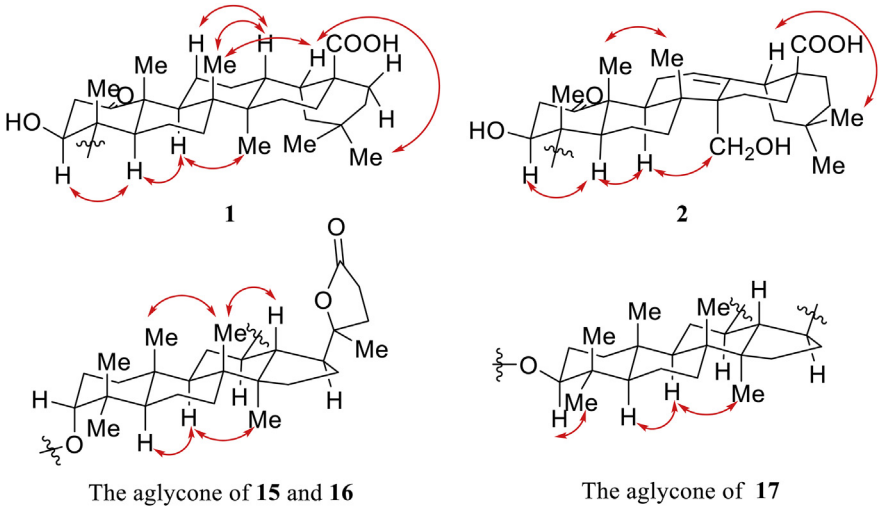

Fig. 3. Selected key NOESY (↔) correlations of **1**, **2** and the aglycons of **15**, **16** and **17**.

Our results showed that the apoB48 levels in the medium were significantly increased ($p < 0.05$) when cells were incubated with oleic acid (Fig. 4). Compared with the control group, most of the triterpenoids declined the apoB48 oversecretion induced by oleic acid. Especially, the treatments of seven pentacyclic triterpenoids **1**, **3**, **5, 6, 8**, **9** and **10**, together with four triterpenoid saponins **17**, **19**, **21** and **22** significantly decreased the apoB48 oversecretion ($p < 0.05$, Fig. 4). As a positive control, statin showed inhibitory effect on the apoB48 secretion, in accordance with the previous report (Pal et al., 2005).

Analysis of the structures and their activities of compounds **1**–**14** led to the following findings: (a) the compounds with inhibitory effect on the apoB48 secretion all had a hydroxyl group at C-23; (b) **4** and **7** with methyl groups at C-23 showed no inhibitory activity; (c) **11**–**13** with two double bonds and **14** with α, β-unsaturated ketone had no effect on apoB48 secretion; (d) compounds **5**, **6**, **8** and **9** had the same substituents and replacement positions, while **8** and **9** with ursane-type showed better inhibitory activity on apoB48 secretion than **5** and **6** with oleanane-type. The above results suggested that the hydroxyl group at C-23 might be essential for their activities. Under same conditions, ursane-type may be more active skeleton than oleanane-type. Furthermore, double bonds and α, β-unsaturated ketone may be responsible for the activity decrease.

Among compounds **15**–**22**, compounds **17** and **19** with pyranoses showed stronger inhibitory effects on the apoB48 oversecretion than **18** and **20** with furanoses, indicating that the glycosyl moiety may have different impact on their inhibitory activity.

3. Conclusion

5 previously undescribed compounds and 17 known compounds were isolated and identified from the chloroform extract of

Table 2
NMR data for compound **15**–**17** (δ in ppm, all measured in C_5D_5N, J in Hz).

Position	**15**		**16**		**17**	
	δ_C[b], type	δ_H[a]	δ_C[b], type	δ_H[a]	δ_C[b], type	δ_H[a]
1	36.0, CH_2	2.99, m, 1.81, m[c]	36.0, CH_2	2.99, m, 1.81, m[c]	36.0, CH_2	3.14, br d (13.5), 2.10, m
2	21.7, CH_2	1.81, m[c], 1.70, m	21.1, CH_2	1.81, m[c], 1.66, m[c]	22.5, CH_2	1.95, m[c], 1.92, m[c]
3	79.7, CH	3.61, d (2.1)	80.0, CH	3.56, br s	82.0, CH	3.66, m[c]
4	38.4, C		38.3, C		38.4, C	
5	51.3, CH	1.56, m[c]	51.3, CH	1.54, m[c]	51.4, CH	1.65, m[c]
6	18.7, CH_2	1.56, m[c], 1.48, m[c]	18.6, CH_2	1.51, m[c], 1.50, m[c]	18.7, CH_2	1.58, m[c], 1.50, m[c]
7	36.7, CH_2	1.52, m[c], 1.16, m	36.7, CH_2	1.53, m[c], 1.14, m	36.8, CH_2	1.50, m[c], 1.20, m
8	41.8, C		41.7, C		41.9, C	
9	54.3, CH	1.83, m[c]	54.3, CH	1.84, m[c]	54.3, CH	1.92, m[c]
10	40.4, C		40.3, C		40.3, C	
11	34.0, CH_2	2.64, m, 1.56, m[c]	33.9, CH_2	2.64, m[c], 1.55, m[c]	34.9, CH_2	2.86, dd (3.7, 12.2) 1.50, m[c]
12	76.3, CH	4.43, ddd (5.0, 10.5, 10.5)	76.3, CH	4.43, ddd, (5.2, 10.7, 10.7)	77.9, CH	4.38, ddd (4.7, 10.7, 10.7)
13	42.1, CH	1.74, m[c]	42.0, CH	1.74, m[c]	41.7, CH	1.88, m[c]
14	50.7, C		50.6, C		50.5, C	
15	31.6, CH_2	1.40, m, 1.01, m	31.6, CH_2	1.39, m, 1.00, m[c]	31.9, CH_2	1.43, m[c], 0.99, m
16	25.8, CH_2	1.74, m[c], 1.43, m	25.8, CH_2	1.74, m[c], 1.44, m	27.1, CH_2	1.84, m[c], 1.75, m[c]
17	49.9, CH	1.91, m	49.9, CH	1.90, m[c]	49.6, CH	1.84, m[c]
18	17.4, CH_3	1.04, s	17.3, CH_3	1.03, s	17.4, CH_3	1.10, s
19	17.0, CH_3	1.35, s	17.0, CH_3	1.33, s	17.09, CH_3	1.43, s
20	89.7, C		89.6, C		86.8, C	
21	24.8, CH_3	1.26, s	24.7, CH_3	1.25, s	24.9, CH_3	1.17, s
22	32.5, CH_2	1.97, m, 1.82, m[c]	32.5, CH_2	1.95, m 1.82, m[c]	34.5, CH_3	1.75, m, 1.55, m[c]
23	29.7, CH_2	2.64, m[c], 2.50, m	29.7, CH_2	2.64, m[c], 2.49, m	26.7, CH_2	2.03, m, 1.98, m[c]
24	176.9, C		176.9, C		84.6, CH	3.94, m[c]
25	23.3, CH_3	0.96, s	23.3, CH_3	0.95, s	71.6, C	
26	30.3, CH_3	1.27, s	30.2, CH_3	1.25, s	26.5, CH_3	1.44, s
27	16.8, CH_3	0.71, s	16.8, CH_3	0.70, s	28.0, CH_3	1.38, s
28					23.6, CH_3	1.03, s
29					30.2, CH_3	1.27, s
30					17.11, CH_3	0.61, s
1′	106.8, CH	5.53, br s	107.0, CH	5.50, d (1.4)	102.0, CH	4.71, d (7.8)
2′	84.2, CH	4.84, m	82.0, CH	4.84, dd, (1.6, 4.0)	75.9, CH	3.99, t (8.4)
3′	79.8, CH	4.80, dd (6.3, 7.8)	80.2, CH	4.60, m	76.1, CH	4.16, t (9.0)
4′	86.5, CH	4.72, m	84.5, CH	4.73, ddd (3.1, 7.0, 7.0)	77.7, CH	5.25, t (9.5)
5′	63.4, CH_2	4.35, dd (3.0, 12.0), 4.25, dd (4.6, 11.8)	65.5, CH_2	4.80, dd (3.1, 11.7), 4.60, m	70.6, CH	3.66, m[c]
6′					18.5, CH_3	1.34, d (6.2)
$CH_3\underline{C}$=O			171.1, C		170.8, C	
$\underline{C}H_3C$=O			21.1, CH_3	1.97, s	21.4, CH_3	2.04, s
1″	101.7, CH	4.98, d (7.8)	101.7, CH	4.97, d (7.7)	102.3, CH	5.00, d (7.8)
2″	75.9, CH	3.95, dd (8.0, 8.5)	75.9, CH	3.94, dd (7.2, 8.8)	75.9, CH	3.93, m[c]
3″	78.7, CH	4.14, t (8.8)	78.7, CH	4.14, t (8.9)	78.8, CH	4.14, t (8.8)
4″	77.3, CH	3.68, t (9.0)	77.3, CH	3.68, t (9.0)	77.2, CH	3.66, m[c]
5″	73.2, CH	3.76, m	73.2, CH	3.77, m	73.2, CH	3.80, m
6″	19.0, CH_3	1.61, d (6.0)	19.0, CH_3	1.60, d (6.0)	18.9, CH_3	1.61, d (6.0)

[a] ^{1}H NMR spectra were measured at 500 MHz.
[b] ^{13}C NMR spectra were measured at 125 MHz.
[c] Overlapped signals.

C. paliurus. 27-nor-triterpenoid glycosides (**15** and **16**) were first isolated from the genus. All the characterized compounds were evaluated for the inhibitory effects on apoB48 secretion of Caco-2 cells. Among them, seven pentacyclic triterpenoids and four triterpenoid saponins exerted to significantly decrease the apoB48 secretion. In conclusion, triterpenoids are major and functional constituents in *C. paliurus* contributing to improve lipid disorders. This present study is beneficial for understanding the chemical composition and biological effects of *C. paliurus* as the popular and functional tea in China.

4. Experimental section

4.1. General experimental procedures

Melting points were obtained using a X-4 micro melting point apparatus (Taike, Beijing, CN) and optical rotations were measured in methanol using an JASCY-1020 polarimeter. A Shimadzu UV-2500PC spectrophotometer and Nicolet Impact 410 spectrophotometer were used for recording the UV spectra (Methanol) and IR spectra (KBr pellets), respectively. 1D and 2D NMR spectra were recorded on a Bruker Avance-300 (Bruker, Switzerland, 300 MHz for ^{1}H NMR, 75 MHz for ^{13}C NMR), Bruker Avance-500 (Bruker, Switzerland, 500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) and Bruker Avance-600 (Bruker, Switzerland, 600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR) NMR spectrometer with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals, and coupling constants are expressed in hertz. ESI-MS and HR-ESI-MS were performed on a HP-1100 LC/EST and Synapt™ Q-TOF mass spectrometer (Waters, Milford, America), respectively. Agilent 1260 Infinity equipped with UV and Alltech 3300 ELSD detector was used to analyse the samples and Alliance semi preparative HPLC with COSMOSIL 5C18-AR-II (250 mm × 20 mm, 5 μm) used for further purification. Column chromatography were performed with silica gel (100–200 and 200–300 mesh, Qing-dao Marine Chemical Group Co., Shandong, CN), ODS RP-18 gel (40–63 mm, Merck, Darmstadt, Germany), Sephadex LH-20

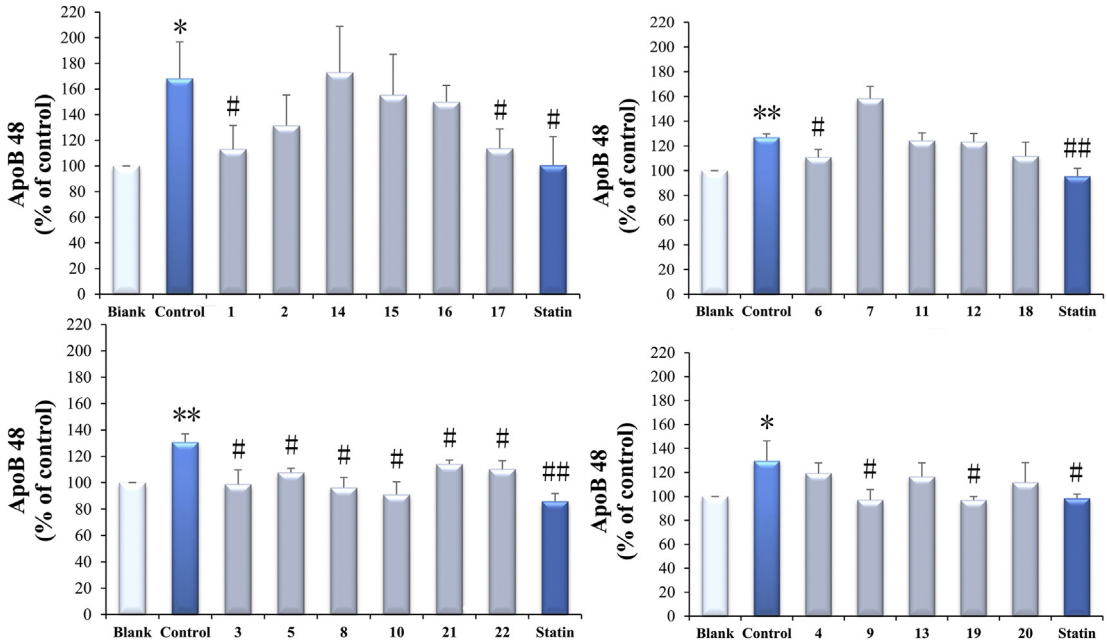

Fig. 4. Inhibitory effect of triterpenoids from *C. paliurus* on apoB48 secretion of Caco-2 cells. ApoB48 concentrations are expressed as a percentage of control, mean ± S.D. (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$ represents significant difference as compared with the data of blank group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ represents significant difference as compared with the data of control group.

(Pharmacia, Uppsala, Sweden). D-Quinovose, L-Arabinose, pyridine (Reagent Plus, ≥ 99%), L-cysteine methyl ester hydrochloride, and isothiocyanate were purchased from Sigma (St. Louis, MO, America). Methanol and acetonitrile (HPLC-grade) was purchased from TEDIA. All other chemicals are analytically pure. The fractions were monitored by TLC and 1% vanillin in H_2SO_4 was used as the spraying reagent to visualize spots.

4.2. Plant material

The leaves of *Cyclocarya paliurus* (Batal) Iljinskaja (Juglandaceae) were collected in October 2010 in the campus of the Nanjing Forestry University, China (GPS coordinates: 118.822414, 32.085054), and were authenticated by Prof. Minjian Qin (China Pharmaceutical University). A voucher herbarium specimen (No. L20100033) has been deposited in Department of Natural Medicinal Chemistry of the university.

4.3. Extraction and isolation

Air-dried leaves of *C. paliurus* (48.5 kg) were extracted three times with 80% ethanol under reflux and evaporated to afford a crude extract (8.3 kg). The extract was suspended in water and partitioned with chloroform to yield a chloroform-soluble extract (3.6 kg). The chloroform extract (460 g) was subjected to a reduced pressure chromatography and eluted with gradient mixtures of CH_3Cl-MeOH (100:0, 100:3, 100:5, 5:1 and 0:100) to yield 5 fractions (Fr.1 - 5).

Fr.2 (210 g) was chromatographed over a silica gel column and eluted with gradient mixtures of CH_3Cl-MeOH (100:3 → 0:100) to obtained 4 subfractions (Fr.2a - 2d). Fr.2a was subjected to a silica gel column and eluted with gradient mixtures of CH_3Cl-MeOH (100:03 → 1:1), Sephadex LH-20 column eluted with CH_3Cl-MeOH (1:1) and followed by semi-preparative HPLC (MeOH-H_2O, 80:20) together with recrystallization to obtain **3** (15 mg), **4** (10 mg), **6** (700 mg), **7** (35 mg), **8** (900 mg), **10** (600 mg) and **12** (20 mg). Fr.2b was fractionated by silica gel column and eluted with CH_3Cl-MeOH (100:3 → 1:1) to yield 5 subfractions (Fr.2b-1 - Fr.2b-5). Fr.2b-2 was subjected to Sephadex LH-20 column and eluted with CH_3Cl-MeOH (1:1), followed by ODS column eluted with MeOH-H_2O (50:50 → 100:0) to obtain **5** (1000 mg), **9** (4 mg), **11** (15 mg) and **13** (30 mg). Fr.2b-3 subjected to column chromatography on ODS with MeOH-H_2O (50:50 → 100:0) and purified by semi-preparative HPLC (MeOH-H_2O, 73:27) to give **1** (5 mg) and **2** (2 mg). **14** (3 mg) was obtained from Fr.2c using Sephadex LH-20 column (CH_3Cl-MeOH, 1:1), followed by ODS column (MeOH-H_2O, 50:50 → 100:0) and semi-preparative HPLC (MeOH-H_2O, 65:35).

Fr.3 (100 g) was chromatographed over a silica gel column and eluted with CH_3Cl-MeOH (100:5, 7:1, 5:1, 1:1, 0:100) to yield 5 subfractions (Fr.3a - Fr.3e). Fr.3b was fractioned by ODS column (MeOH-H_2O, 40:60 → 100:0) to obtain 4 subfractions (Fr.3b-1 - Fr.3b-4). Fr.3b-1 was purified by a silica gel column (CH_3Cl-MeOH, 20:1) to obtain **17** (18 mg). **18** (60 mg), **19** (25 mg) and **20** (35 mg) were isolated from Fr.3b-2 by Sephadex LH-20 column (CH_3Cl-MeOH, 1:1) and a silica gel column (CH_3Cl-MeOH, 10:1). Fr.3b-3 was isolated using a silica gel column (CH_3Cl-MeOH, 100:5 → 0:100) and semi-preparative HPLC (MeOH-H_2O, 80:20) to yield **15** (10 mg) and **16** (5 mg). Fr.3c was separated by ODS column (MeOH-H_2O, 60:40), silica gel column (CH_3Cl-MeOH, 10:1) and semi-preparative HPLC (CH_3CN-H_2O, 50:50) to yield **21** (60 mg) and **22** (40 mg).

4.3.1. 3β,23-dihydroxy-1,12-dioxo-olean-28-oic acid (**1**)

Colorless amorphous powder; $[\alpha]_D^{27}$ +44°(*c* 0.095 MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.40) nm; IR (KBr) ν_{max} 3441, 2946, 1696, 1050 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS *m/z* 501.3218 $[M-H]^-$ (calcd for $C_{30}H_{45}O_6$, 501.3216).

4.3.2. 3β,23,27-trihydroxy-1-oxo-olean-12-ene-28-oic acid (**2**)

Colorless amorphous powder; $[\alpha]_D^{27}$ +73.6° (*c* 0.063, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.70) nm; IR (KBr) ν_{max} 3441, 2945, 1696, 1633 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS *m/z* 520.3625 $[M+NH_4]^+$ (calcd for $C_{30}H_{50}NO_6$, 520.3633).

4.3.3. Cyclocarioside L (**15**)

Colorless amorphous powder; $[\alpha]_D^{27}$ −17.6° (*c* 0.081, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.77) nm; IR (KBr) ν_{max} 3439, 2929, 1640, 1070 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 728.4607 $[M+NH_4]^+$ (calcd for $C_{38}H_{66}NO_{12}$, 728.4580).

4.3.4. Cyclocarioside M (**16**)

Colorless amorphous powder; $[\alpha]_D^{27}$ −12.3° (*c* 0.104, MeOH); UV (MeOH) λ_{max} (log ε) 224 (3.10), 203 (3.35) nm; IR (KBr) ν_{max} 3442, 2929, 1643, 1068 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 775.4277 $[M+NH_4]^+$ (calcd for $C_{40}H_{68}NO_{13}$, 770.4716).

4.3.5. Cyclocarioside N (**17**)

Colorless amorphous powder; $[\alpha]_D^{27}$ −8.1° (*c* 0.086, MeOH); UV (MeOH) λ_{max} (log ε) 225 (3.17), 203 (3.35) nm; IR (KBr) ν_{max} 3441, 1642, 1071 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 828.5473 $[M+NH_4]^+$ (calcd for $C_{44}H_{78}NO_{13}$, 828.5468).

4.4. Acid hydrolysis of compounds **15**–**17**

Compounds **15**–**17** (each 1 mg) were hydrolyzed in 2 M HCl under reflux in a boiling water bath for 2 h. The reaction mixture was neutralized by $AgCO_3$ and extracted with $CHCl_3$ (3 × 3 mL). The aqueous layer was concentrated and dried to obtain the monosaccharide fraction. The residue was dissolved in pyridine (1 mL) containing 2 mg of L-cysteine methyl ester hydrochloride and heated at 60 °C for 1 h. Then o-torylisothiocyanate (2 mg) was added and heated at 60 °C for another 1 h. The reaction mixture was analyzed by RP-HPLC under the following conditions: column, Grace Alltima C18 column (20 × 250 mm, 4.6 μm); solvent, 25% CH_3CN in 50 mM H_3PO_4 for 40 min; flow rate, 0.8 mL/min; detector, UV; wavelength, 250 nm; temperature, 35 °C; injection volume, 10 μL. The retention times of the sugar derivatives for **15**–**17** are 21.21 and 32.18 min, respectively, consistent with those of derivatives of standard L-arabinose and D-quinovose (Huang et al., 2014; Wang et al., 2014).

4.5. Bioassay for the inhibitory effects on apoB48 oversecretion

The Caco-2 cells were seeded in 6-well transwell plates with polycarbonate microporous membranes (0.4 μm pore size, inserts of 24.0 mm diameter) at a density of 4×10^4–5×10^5/apical well. The cultural condition was previously reported with a little modification (Pal et al., 2005). The medium in both apical and basolateral wells of the blank group (Blank) contained DMEM with 0.1% bovine serum albumin, while the control group (Control) and the treatment groups contained DMEM with bovine serum albumin/oleic acid (4/1). The cells in treatment groups were treated with tested compounds (10 μM) or statin (10 μM) and incubated for 24 h. The medium in the basolateral well was collected and stored at −80 °C. The medium were tested for apoB48 quantification by Elisa kit (Sakai et al., 2003). Measurements were performed in triplicate and are representative of three independent experiments. The data were evaluated using the SPSS software package (Version 19.0, SPSS Inc., Chicago, IL, USA). Statistical significance was performed using one-way analysis of variance (ANOVA). The results were regarded as significantly different at $p < 0.05$.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgments

The authors thank Prof. Minjian Qin (China Pharmaceutical University) for authenticating the plant material. This work was partially sponsored by grants from the National Natural Science Foundation of China (No. 81503316, 31270673), the Natural Science Foundation of Jiangsu Province (No. BK20161460, BK20160926), College Students Innovation Project for the R&D of Novel Drugs (No. J1030830), the Priority Academic Program Development of Jiangsu Higher Educational Institutions.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.phytochem.2017.06.015.

References

Alqahtani, A., Hamid, K., Kam, A., Wong, K.H., Abdelhak, Z., Razmovski-Naumovski, V., Chan, K., Li, K.M., Groundwater, P.W., Li, G.Q., 2013. The pentacyclic triterpenoids in herbal medicines and their pharmacological activities in diabetes and aiabetic complications. Curr. Med. Chem. 20, 908–931.

Cui, B.S., Li, S., 2012. Chemical constituents from leaves of *Cyclocarya paliurus*. Chin. Tradit. Herb. Drugs 43, 2132–2136.

Fu, X.X., Fang, S.Z., 2009. Secondary metbolites and its physiological function of *Cyclocarya paliurus*. Anhui Agric. Sci. Technol. 37, 13612–13614.

Ghosh, J., Sil, P.C., 2013. Arjunolic acid: a new multifunctional therapeutic promise of alternative medicine. Biochimie 95, 1098–1109.

Huang, X.J., Tang, J.Q., Li, M.M., Liu, Q., Li, Y.L., Fan, C.L., Pei, H., Zhao, H.N., Wang, Y., Ye, W.C., 2014. Triterpenoid saponins from the rhizomes of *Anemone flaccida* and their inhibitory activities on LPS-induced NO production in macrophage RAW264. 7 cells. J. Asian Nat. Prod. Res. 16, 910–921.

Jiang, Z.Y., Zhang, X.M., Zhou, J., Qiu, S.X., Chen, J.J., 2006. Two new triterpenoid glycosides from *Cyclocarya paliurus*. J. Asian Nat. Prod. Res. 9, 93–98.

Karpe, F., 1999. Postprandial lipoprotein metabolism and atherosclerosis. J. Intern. Med. 246, 341–355.

Kennelly, E.J., Cai, L., Long, L., Shamon, L., Zaw, K., Zhou, B.N., Pezzuto, J.M., Kinghorn, A.D., 1995. Novel highly sweet secodammarane glycosides from *Pterocarya puliurus*. J. Agric. Food Chem. 43, 2602–2607.

Kim, D.H., Han, K.M., Chung, I.S., Kim, D.K., Kim, S.H., Kwon, B.M., Jeong, T.S., Park, M.H., Ahn, E.M., Baek, N.L., 2005. Triterpenoids from the flower of *Campsis grandiflora* K. Schum. as human acyl-CoA: cholesterol acyltransferase inhibitors. Arch. Pharm. Res. 28, 550–556.

Kizu, H., Tomimori, T., 1982. Studies on the constituents of Clematis species. V. on the saponins of the root of *Clematis chinensis* Osbeck. Chem. Pharm. Bull. 30, 3340–3346.

Kurihara, H., Asami, S., Shibata, H., Fukami, H., Tanaka, T., 2003. Hypolipemic effect of *Cyclocarya paliurus* (Batal) Iljinskaja in lipid-loaded mice. Bio. Pharm. Bull. 26, 383–385.

Levy, E., Mehran, M., Seidman, E., 1995. Caco-2 cells as a model for intestinal lipoprotein synthesis and secretion. FASEB J. 9, 626–635.

Li, S., Cui, B., Liu, Q., Tang, L., Yang, Y., Jin, X., Shen, Z., 2012. New triterpenoids from the leaves of *Cyclocarya paliurus*. Planta Med. 78, 290–296.

Liu, Y., Zhang, M.Q., Li, X.L., Xu, T.H., Xie, S.X., Xu, Y.J., Xu, D.M., 2014. Study on chemical constituents of *Cyclocarya paliurus*. J. Asian Nat. Prod. Res. 16, 206–209.

Ma, Y., Jiang, C., Yao, N., Li, Y., Wang, Q., Fang, S., Shang, X., Zhao, M., Che, C., Ni, Y., 2015. Antihyperlipidemic effect of *Cyclocarya paliurus* (Batal.) Iljinskaja extract and inhibition of apolipoprotein B48 overproduction in hyperlipidemic mice. J. Ethnopharmacol. 166, 286–296.

Mahato, S.B., Nandy, A.K., 1990. Determination of structure and stereochemistry of tomentosic acid by X-Ray crystallography. A novel mechanism for transformation of arjungenin to tomentosic acid. J. Chem. Soc. Perk. T. 2 (8), 1445–1450.

Meng, L., Huang, C., Liu, H., Chen, X., 2009. Studies on the chemical constituents of ethyl acetate extract from the roots of *Actinidia chrysantha*. J. Chin. Med. Mater. 32, 1544–1546.

Min, S.W., Jung, S.H., Cho, K.H., Kim, D.H., 2008. Antihyperlipidemic effects of Red Ginseng, Crataegii Fructus and their main constituents ginsenoside Rg3 and ursolic acid in mice. Biomol. Ther. 16, 364–369.

Mori, K., Ishida, T., Yasuda, T., Monguchi, T., Sasaki, M., Kondo, K., Hasokawa, M., Nakajima, H., Haraguchi, Y., Sun, L., Shinohara, M., Toh, R., Nishimura, K., Hirata, K., 2013. Fasting serum concentration of apolipoprotein B48 represents residual risks in patients with new-onset and chronic coronary artery disease. Clin. Chim. Acta 421, 51–56.

Okada, Y., Omae, A., Okuyama, T., 2003. A new triterpenoid isolated from *Lagerstronemia speciosa* (L.) PERS. Chem. Pharm. Bull. 51, 452–454.

Otokozawa, S., Ai, M., Diffenderfer, M.R., Asztalos, B.F., Tanaka, A., Lamon-Fava, S., Schaefer, E.J., 2009. Fasting and postprandial apolipoprotein B-48 levels in healthy, obese, and hyperlipidemic subjects. Metabolism 58, 1536–1542.

Pal, S., Ho, S.S., Takechi, R., 2005. Red wine polyphenolics suppress the secretion of ApoB48 from human intestinal CaCo-2 cells. J. Agric. Food Chem. 53, 2767–2772.

Sakai, N., Uchida, Y., Ohashi, K., Hibuse, T., Saika, Y., Tomari, Y., Kihara, S., Hiraoka, H., Nakamura, T., Ito, S., 2003. Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA. J. Lipid Res. 44, 1256–1262.

Sashida, Y., Ogawa, K., Mori, N., Yamanouchi, T., 1992. Triterpenoids from the fruit galls of *Actinidia polygama*. Phytochemistry 31, 2801–2804.

Shang, X.L., Wu, Z.F., Yin, Z.Q., Zhang, J., Liu, Z.J., Fang, S.Z., 2015. Simultaneous determination of flavonoids and triterpenoids in *Cyclocarya paliurus* leaves using high-performance liquid chromatography. Afr. J. Tradit. Complem. 12, 125–134.

Shu, R., Xu, C., Li, L., 1995a. Studies on the sweet principles from the leaves of *Cyclocarya paliurus* (Batal.) Iljinsk. Acata Pharm. Sin. 30, 757–761.

Shu, R.G., Xu, C.R., Li, L.N., Yu, Z.L., 1995b. Cyclocariosides II and III: two seco-dammarane triterpenoid saponins from *Cyclocarya paliurus*. Planta Med. 61, 551–553.

Somova, L.O., Nadar, A., Rammanan, P., Shode, F.O., 2003. Cardiovascular, anti-hyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. Phytomedicine 10, 115–121.

Valero, R., Lorec, A.M., Paganelli, F., Beliard, S., Atlan, C., Lairon, D., Vialettes, B., Portugal, H., 2005. Fasting apoprotein B-48 level and coronary artery disease in a population without frank fasting hypertriglyceridemia. Metabolism 54, 1442–1447.

Wang, C.Q., Wang, Y., Wang, W.J., Wang, L., Ye, W.C., 2014. New oleanane saponins from Schefflera kwangsiensis. Phytochem. Lett. 10, 268–271.

Wang, Q., Jiang, C., Fang, S., Wang, J., Ji, Y., Shang, X., Ni, Y., Yin, Z., Zhang, J., 2013. Antihyperglycemic, antihyperlipidemic and antioxidant effects of ethanol and aqueous extracts of *Cyclocarya paliurus* leaves in type 2 diabetic rats. J. Ethnopharmacol. 150, 1119–1127.

Xiao, S.J., He, D.H., Ding, L.S., Zhou, Y., Chen, F., 2013. Triterpenes from aerial parts of *Clematoclethra scandens* subsp. actinidioides. China J. Chin. Mater. Med. 38, 358–361.

Xie, G.B., Zhou, S.X., Lei, L.D., Tu, P.F., 2007. Studies on triterpenoid constituents in the leaf of *Ilex pernyi*. China J. Chin. Mater. Med. 32, 1890–1892.

Young, S., 1990. Recent progress in understanding apolipoprotein B. Circulation 82, 1574–1594.

Zhang, Y.H., Hu, J., Shi, R.B., Guo, Y.J., Dong, Y., 2006. Studies on the chemical constituents of *Schizonepeta tenuifolia* Briq. China J. Chin. Mater. Med. 31, 1118–1119.

Zhao, L.X., Park, H.G., Jew, S.S., Lee, M.K., Kim, Y.C., Thapa, P., Karki, R., Jahng, Y., Jeong, B.S., Lee, E.S., 2007. Modification of C11, C28, C2,3,23 or C2,23,28 functional groups on asiatic acid and evaluation of hepatoprotective effects. Bull. Kor. Chem. Soc. 28, 970–976.

Zhao, X.R., Huo, X.K., Dong, P.P., Wang, C., Huang, S.S., Zhang, B.J., Zhang, H.L., Deng, S., Liu, K.X., Ma, X.C., 2015. Inhibitory effects of highly oxygenated lanostane derivatives from the fungus *Ganoderma lucidum* on P-glycoprotein and alpha-glucosidase. J. Nat. Prod. 78, 1868–1876.