

Article

New Triterpenoids with Cytotoxic Activity from *Actinidia Valvata*

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Abstract: Two new triterpenoids, 30-*O*- β -D-glucopyranosyloxy-2 α ,3 α ,24-trihydroxyurs-12,18-diene-28-oic acid *O*- β -D-glucopyranosyl ester (**1**) and 2 α ,3 β ,3,30-tetrahydroxyurs-12,18-diene-28-oic acid *O*- β -D-glucopyranosyl ester (**2**) were isolated from roots of *Actinidia valvata* Dunn. Their structures were elucidated by means of extensive spectroscopic studies. Both these two new compounds showed moderate cytotoxic activity *in vitro* against BEL-7402 and SMMC-7721 tumor cell line.

Keywords: *Actinidia valvata* Dunn; triterpenoid; cytotoxic activity

1. Introduction

Actinidia valvata Dunn is a shrub mainly growing in eastern China (Zhejiang and Jiangxi province) [1]. Its roots known as “mao ren shen” in traditional Chinese medicine exhibit anti-tumor and anti-inflammatory activity, and have been used for many years in the treatment of hepatoma, lung carcinoma and myeloma [2,3]. As we all know, hepatoma is very difficult to treat, and active components from medicinal herbs may be effective for research and development of new drugs [4].

In a previous study, we have carried out screening for cytotoxic activity of “mao ren shen”, and two new polyoxygenated triterpenoids, 2 β ,3 α ,6 α ,20 α ,24,30-hexahydroxyurs-12-en-28-oic acid and 2 β ,3 α ,20 β ,23,24,30-hexahydroxyurs-12-en-28-oic acid *O*- β -D-glucopyranosyl ester were separated [5]. In this paper, two new triterpenoid saponins with cytotoxic activity against BEL-7402 and SMMC-7721 tumor cell line are reported.

2. Results and Discussion

The roots of *Actinidia valvata* Dunn were extracted with 80% EtOH. The concentrated extract was suspended in H₂O and successively extracted with petroleum ether (60°–90°), AcOEt, and *n*-BuOH. The *n*-BuOH-soluble extract was repeatedly subjected to column chromatography to yield compound **1**, **2** (Figure 1). Both these compounds were triterpenoid saponins with 12,18-diene-urs skeleton. Their structures were elucidated by detailed spectroscopic analysis.

Compound **1** was a white amorphous powder, displayed positive *Liebermann–Burchard* test, was optically active with $[\alpha]_D^{25} = 5.89$ ($c = 0.1$, MeOH), and had the molecular formula C₄₂H₆₆O₁₆, with ten degrees of unsaturation, as determined according to a *pseudo*-molecular-ion peaks at 849.4240 ($[M + Na]^+$; calc. 849.4249) in the HR-ESI-MS.

¹³C-NMR (DEPT) spectra of compound **1** revealed 42 carbon signals, including five CH₃, two C=C bonds (tri-, four-substituted) and one C=O group. Assuming compound **1** has skeleton of urs-triterpenoid, the assignment of the two C=C bonds should be highly concerned. In ¹H-NMR spectra of compound **1**, five singlets at $\delta(H)$ 0.80–1.89 consisting of five CH₃, and proton signal at $\delta(H)$ 5.53 (br) was assigned to 12-position. In HMBC spectra, clear correlation of 19-CH₃ with two quaternary C-atoms at $\delta(C)$ 126.17 (*s*) and $\delta(C)$ 136.92 (*s*) was observed, and the correlation signals of 12-H with these two quaternary C-atoms were also observed. Thus, the two C=C bonds can be rightly assigned to 18-, 19-position, respectively. The HMBC correlation signal of 20-H with C-atom at $\delta(C)$ 69.68(*t*), was observed, then the oxygenation of 30-C may be deduced successfully. In NOSEY spectra, as the correlations of 2-H with 3-H, 2-H with 10-Me, 8-Me with 10-Me were observed, and by comparing with reference data [6,7], the configuration of 2 α -OH, 3 α -OH, and the 24-OH can be confirmed. ¹H-NMR and ¹³C-NMR data of glycon moiety of compound **1** indicated it featured two glucopyranosyls. The C-atom at $\delta(C)$ 95.82(*d*) and H (5.39, *d*, $J = 8.0$ Hz), C-atom at $\delta(C)$ 101.85(*d*) and H (4.20, *d*, $J = 8.0$ Hz) were assigned as anomeric C-atoms and protons, respectively. The linkage of two glucopyranosyls with aglycone maybe deduced by correlation of anomeric proton with glycosidated C-atoms in HMBC spectra [H (5.39, *d*, $J = 8.0$ Hz) to C=O, H (4.20, *d*, $J = 8.0$ Hz) to C-atom at 69.68(*t*)].

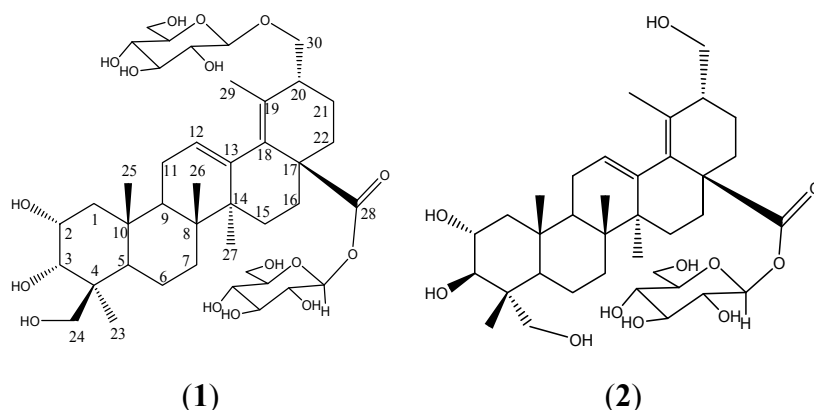
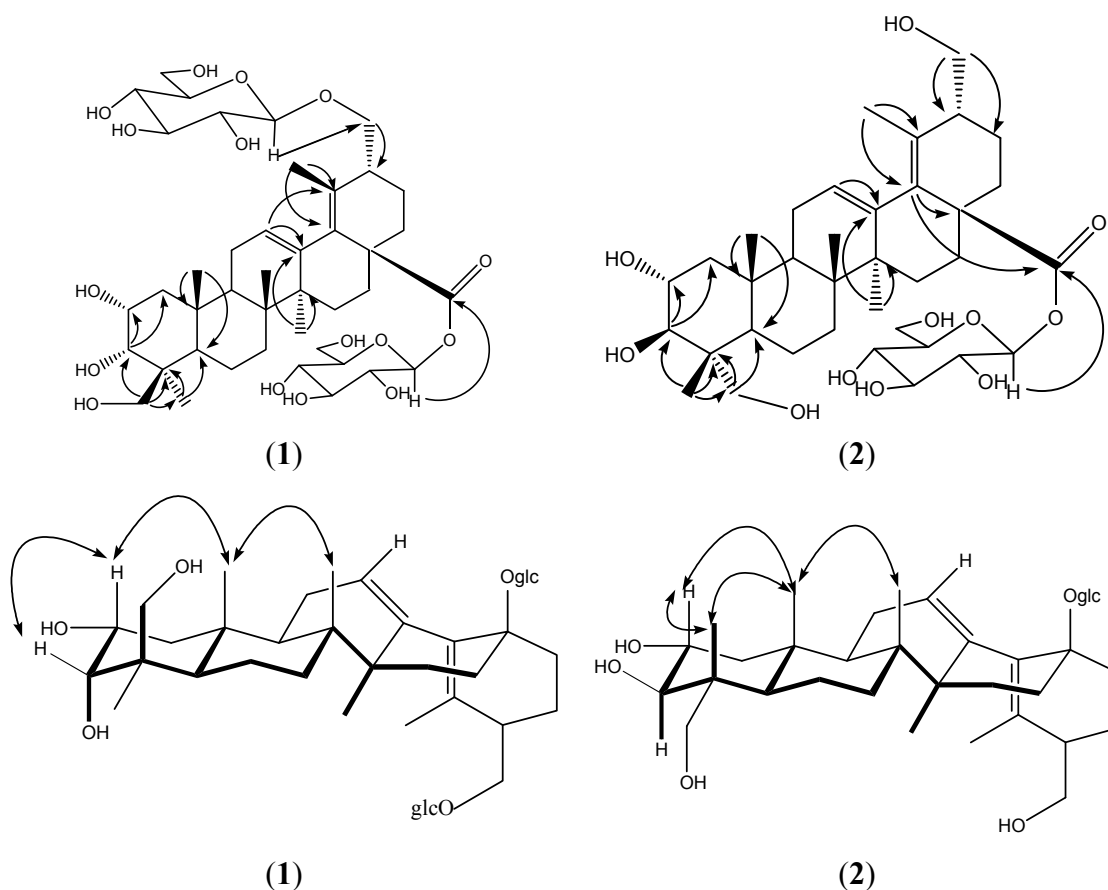
Basing on above analysis, in combination with the ¹H-, ¹³C-NMR spectra (Table 1), HMQC, HMBC, and NOSEY data (Figure 2), established the structure of compound **1** as 30-*O*- β -D-glucopyranosyloxy-2 α ,3 α ,24-trihydroxyurs-12,18-diene-28-oic acid *O*- β -D-glucopyranosyl ester (**1**).

The compound **2** was obtained as white amorphous powder, displayed positive *Liebermann–Burchard* test, was optically active with $[\alpha]_D^{25} = 7.35$ ($c = 0.1$, MeOH), and had the molecular formula C₃₆H₅₆O₁₁ with nine degrees of unsaturation, as determined according to a *pseudo*-molecular-ion peaks at 687.3717 ($[M + Na]^+$; calc. 687.3720) in the HR-ESI-MS.

Table 1. ^1H and ^{13}C -NMR Data of 1 and 2 (in $\text{C}_5\text{D}_5\text{N}$). δ in ppm, J in Hz.

Position	1		2	
	$\delta(\text{C})^a$	$\delta(\text{H})^b$	$\delta(\text{C})^a$	$\delta(\text{H})^b$
1	42.81 (t)	1.31 (dd, $J=12,4.2$), 2.19 (dd, $J=12,4.2$)	48.03 (t)	1.30 (m), 2.29 (m)
2	66.98 (d)	3.88 (m)	68.93 (d)	4.25 (m)
3	74.90 (d)	3.70 (d, $J=2.0$)	78.18 (d)	4.19 (m)
4	45.36 (s)	—	43.61 (s)	—
5	49.94 (d)	1.79 (m)	48.31 (d)	1.73 (m)
6	19.15 (t)	1.33 (m)	18.41 (t)	1.04 (m), 1.15 (m)
7	33.23 (t)	1.28 (m), 1.30 (m)	33.86 (t)	1.31 (m), 1.75 (m)
8	40.67 (s)	—	39.80 (s)	—
9	48.96 (d)	1.71 (m)	48.03 (d)	1.83 (m)
10	39.07 (s)	—	38.31 (s)	—
11	24.25 (t)	1.74 (m), 1.93 (m)	23.64 (t)	1.74 (m), 2.01 (m)
12	129.19 (d)	5.53 (br)	127.98 (d)	5.60 (br)
13	137.97 (s)	—	137.53 (s)	—
14	44.69 (s)	—	43.90 (s)	—
15	29.09 (t)	1.09 (m), 1.88 (m)	28.58 (t)	1.06 (m), 2.25 (m)
16	24.72 (t)	1.74 (m), 1.98 (m)	32.97 (t)	1.79 (m), 1.98 (m)
17	48.83 (s)	—	47.25 (s)	—
18	126.17 (s)	—	129.59 (s)	—
19	136.92 (s)	—	131.27 (s)	—
20	51.45 (s)	3.30 (br)	50.74 (d)	3.55 (br)
21	24.67 (t)	1.97 (m)	23.87 (t)	1.99 (m)
22	35.19 (t)	1.28 (m), 1.53 (m)	24.41 (t)	2.06 (m)
23	22.56 (q)	1.07 (s)	66.52 (t)	3.66 (d, $J=10.2$), 4.16 (d, $J=10.2$)
24	65.72 (t)	3.36 (d, $J=8.0$), 3.68 (d, $J=8.0$)	14.42 (q)	1.03 (s)
25	17.86 (q)	0.94 (s)	17.95 (q)	1.08 (s)
26	18.21 (q)	0.85 (s)	18.27 (q)	1.15 (s)
27	23.17 (q)	1.89 (s)	22.23 (q)	0.97 (s)
28	178.07 (s)	—	176.24 (s)	—
29	17.44 (q)	1.68 (s)	16.83 (q)	1.75 (s)
30	69.68 (t)	4.09 (d, $J=20.0$), 4.45 (d, $J=20.0$)	62.20 (t)	4.38 (d, $J=10.2$)
28-glc-1	95.82 (d)	5.39 (d, 8)	95.82 (d)	5.60 (d, $J=8.0$)
2	73.75 (d)	3.31 (m)	74.19 (d)	3.31 (m)
3	77.91 (d)	3.33 (m)	78.84 (d)	3.33 (m)
4	71.46 (d)	3.33 (m)	71.12 (d)	3.33 (m)
5	78.54 (d)	3.38 (m)	79.25 (d)	3.38 (m)
6	62.49 (t)	3.64 (dd, $J=12.0, 1.8$), 3.77 (dd, $J=12.0, 1.8$)	62.99 (t)	3.65 (dd, $J=12.0, 1.8$), 3.78 (dd, $J=12.0, 1.8$)
30-glc-1	101.85 (d)	4.20 (d, $J=8.0$)		
2	74.53 (d)	3.31 (m)		
3	77.83 (d)	3.33 (m)		
4	70.97 (d)	3.33 (m)		
5	77.95 (d)	3.38 (m)		
6	62.30 (t)	3.63 (dd, $J=12.0, 1.8$), 3.76 (dd, $J=12.0, 1.8$)		

^a Recorded at 150 MHz, multiplicity by DEPT; ^b Recorded at 600 MHz.

Figure 1. Structures of compound **1** and **2**.**Figure 2.** Key HMBC ($\rightarrow$) and NOESY ($\leftrightarrow$) correlations of compound **1** and **2**.

Comparing the ^1H -NMR and ^{13}C -NMR (Table 1) spectra, compound **2** has only a 28-glucopyranosyl. The configuration of $2\alpha\text{-OH}$, $3\beta\text{-OH}$, and 23-OH can be confirmed by analyzing the correlations of NOESY spectra and comparing with reference data [6,7]. In combination with the ^1H -, ^{13}C -NMR spectra (Table 1), HMQC, HMBC, and NOESY data (Figure 2), established the structure of compound **2** as $2\alpha,3\beta,23,30$ -tetrahydroyurs-12, 18-diene-28-oic acid O - β -D-glucopyranosyl ester (**2**).

Compounds **1** and **2** showed moderate *in vitro* cytotoxic activity against BEL-7402 (IC_{50} value of 92.2 and 89.7 $\mu\text{g/mL}$, resp.) and SMMC-7721 (IC_{50} value of 58.1 and 89.7 $\mu\text{g/mL}$, resp.), as determined by classical MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) colorimetric assay.

3. Experimental Section

3.1. General

Silica-gel plates (Sinopharm Chemical Reagent Co., Ltd.) were used for TLC analysis. mp: WRS-1A micro-melting-point apparatus; uncorrected. Optical rotations: JASCO P-1300 spectropolarimeter. IR: Spectra: BRUKER VECTOR-22 spectrophotometer; in cm^{-1} . ^1H -, ^{13}C -, 2D-NMR spectra: BRUKER AVANCE 600 spectrometer; chemical shifts δ in ppm rel. to $(\text{CH}_3)_4\text{Si}$, coupling constant J in Hz. ESI-MS: Finnigan LCQ mass spectrometer; in m/z . HR-ESI-MS: Q-Tof micro YA019 mass spectrometer.

3.2. Material

The roots of *Actinidia valvata* Dunn were collected in Changshan County, Zhejiang Province, China, in October 2006, and identified by Zheng Han-Chen, Department of pharmacognosy, School of pharmacy, Second military medical university. A voucher specimen (No. 20061005) was deposited at Department of pharmacognosy, School of pharmacy, Second military medical university.

3.3. Extraction and Isolation

The powdered plant material of roots of *Actinidia valvata* Dunn 30 kg was refluxed with 8 times of 80% EtOH solution for 3 times, 1.5 h each time. The extract was concentrated under reduced pressure to brown syrup, which was partitioned between H_2O and petroleum ether (PE), AcOEt, and BuOH, successively. The *n*-BuOH soluble fraction (280.6 g) was subjected to column chromatography (CC) on silica gel (SiO_2), eluting with $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (10:1:0.1 to 2:1 0.1) to afford 9 fraction 1–9. Fraction 5 were repeatedly subjected to CC (Pharmadex LH-20 and RP C-18) to yield compound **1** (10.4 mg) and **2** (14.6 mg).

30-*O*- β -D-glucopyranosyloxy-2 α ,3 α ,24-trihydroxyurs-12, 18-diene-28-oic acid *O*- β -D-glucopyranosyl ester (**1**): white amorphous powder, mp 140°–142°, $[\alpha]_{\text{D}}^{25} = 5.89$ ($c = 0.1$, MeOH). IR (KBr): 3432, 2920, 2852, 1641, 1380, 1038. ^1H -NMR (600 MHz, $\text{C}_5\text{D}_5\text{N}$) and ^{13}C -NMR (150 MHz, $\text{C}_5\text{D}_5\text{N}$): Table 1. ESI-MS: 849.48 ($[M + \text{Na}]^+$), HR-ESI-MS: 849.4240 ($[M + \text{Na}]^+$, $\text{C}_{42}\text{H}_{66}\text{N}_a\text{O}_{16}^+$, calc. 849.4249).

2 α ,3 β ,23,30-tetrahydroxyurs-12, 18-diene-28-oic acid *O*- β -D-glucopyranosyl ester (**2**): white amorphous powder. mp 220° (carbonification), $[\alpha]_{\text{D}}^{25} = 7.35$ ($c = 0.1$, MeOH). IR (KBr): 3448, 2963, 1681, 1644, 1381, 1278, 1080. ^1H -NMR (600 MHz, $\text{C}_5\text{D}_5\text{N}$) and ^{13}C -NMR (150 MHz, $\text{C}_5\text{D}_5\text{N}$): Table 1. ESI-MS: 687.89 ($[M + \text{Na}]^+$), HR-ESI-MS: 687.3717 ($[M + \text{Na}]^+$, $\text{C}_{36}\text{H}_{56}\text{N}_a\text{O}_{11}^+$, calc. 687.3720).

4. Conclusions

In the present research, two new triterpenoids, 30-*O*- β -D-glucopyranosyloxy-2 α ,3 α ,24-trihydroxyurs-12, 18-diene-28-oic acid *O*- β -D-glucopyranosyl ester and 2 α ,3 β ,23,30-tetrahydroxyurs-12, 18-diene-28-oic acid *O*- β -D-glucopyranosyl ester were isolated from roots of *Actinidia valvata* Dunn, and their structures were elucidated by means of extensive spectroscopic studies. Both these new compounds showed moderate cytotoxic activity *in vitro* against BEL-7402 and SMMC-7721 tumor cell line.

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