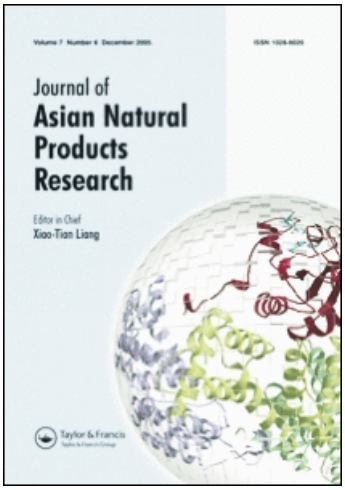

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Triterpenoid saponins from the stems of *Clematis parviloba*

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Sixteen triterpenoid saponins (**1**–**16**) were isolated from the stems of *Clematis parviloba*, including a new compound, parvilobaside A (**1**), which was established as 23-*O*-acetyl-hederagenin-3-*O*-β-D-ribopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabino-pyranoside on the basis of various spectroscopic techniques and chemical evidences. Among the isolated compounds, clematoside S (**2**) and α-hederin (**4**) showed moderate cytotoxic activities against four human tumor cell lines (HCT-8, Bel-7402, BGC-823, and A-2780) with IC_{50} values in the range of 1.44–6.86 μg/ml.

Keywords: *Clematis parviloba*; triterpenoid saponins; cytotoxic activity; parvilobaside A

1. Introduction

Clematis parviloba Gardn et. Champ (Ranunculaceae) is distributed in the southwest of China. Its stem has been used as a folk herbal drug for the treatment of rheumatism, arthralgia, and hydropsy as well as for its diuretic, galactopoietic, and emmenagogue effects [1]. Previous literature has shown the genus *Clematis* contain triterpenoid saponins, lignans, flavonoids, and alkaloids, however, chemical and biological work on this plant have not been reported until now [2]. Therefore, we carried out a phytochemical research on the stems of *C. parviloba*, and a new triterpenoid saponin, parvilobaside A (**1**), along with the 15 known triterpenoid saponins: clematoside S (**2**), saponin CP_4 (**3**), α-hederin (**4**), saponin CP_8 (**5**), saponin CP_{10} (**6**), kizuta saponin K_3 (**7**), clemastanoside D (**8**), kizuta saponin K_{10} (**9**), clematibetoside C (**10**), saponin PJ_3 (**11**), saponin PK (**12**), huzhangoside B (**13**), huzhangoside D (**14**), clematichinenoside C (**15**), and clematichinenoside B (**16**) (Figure 1) were isolated. The 15 known compounds were isolated from this plant for the first time. In addition, we also investigated the cytotoxic activities of some of these compounds against four human tumor cell lines.

2. Results and discussion

Parvilobaside A (**1**) was isolated as a white amorphous powder and gave positive reactions with Liebermann–Burchard and Molish reagents. Its molecular formula was established as $C_{48}H_{76}O_{17}$ with 11 degrees of unsaturation based on a quasi-molecular ion peak at *m/z* 947.4976 $[M + Na]^+$ in the HR-ESI-MS spectrum. The IR spectrum showed the presence of hydroxyl (3404 cm^{-1}) and carbonyl (1719 and 1692 cm^{-1}) groups. Acid hydrolysis of **1** with 1 M HCl gave arabinose, rhamnose, and ribose, which were identified

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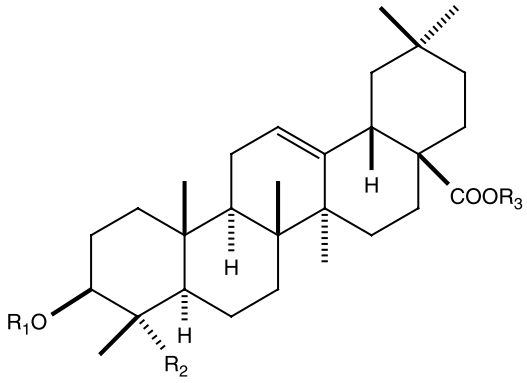

	R_1	R_2	R_3
1	Rib-(1→3)-Rha-(1→2)-Ara	CH_2OAc	H
2	Rib-(1→3)-Rha-(1→2)-Ara	CH_2OH	H
3	Rib-(1→3)-Rha-(1→2)-Ara	CH_3	H
4	Rha-(1→2)-Ara	CH_2OH	H
5	Glc-(1→4)-Rib-(1→3)-Rha-(1→2)-Ara	CH_2OH	H
6	Glc-(1→4)-Glc-(1→4)-Rib-(1→3)-Rha-(1→2)-Ara	CH_2OH	H
7	H	CH_2OH	Rha-(1→4)-Glc-(1→6)-Glc
8	Rib-(1→3)-Rha-(1→2)-Ara	CH_2OH	Glc
9	Ara	CH_2OH	Rha-(1→4)-Glc-(1→6)-Glc
10	Rib	CH_2OH	Rha-(1→4)-Glc-(1→6)-Glc
11	Rha-(1→2)-Ara	CH_3	Rha-(1→4)-Glc-(1→6)-Glc
12	Rha-(1→2)-Ara	CH_2OH	Rha-(1→4)-Glc-(1→6)-Glc
13	Rib-(1→3)-Rha-(1→2)-Ara	CH_3	Rha-(1→4)-Glc-(1→6)-Glc
14	Rib-(1→3)-Rha-(1→2)-Ara	CH_2OH	Rha-(1→4)-Glc-(1→6)-Glc
15	Glc-(1→4)-Rib-(1→3)-Rha-(1→2)-Ara	CH_3	Rha-(1→4)-Glc-(1→6)-Glc
16	Glc-(1→4)-Rib-(1→3)-Rha-(1→2)-Ara	CH_2OH	Rha-(1→4)-Glc-(1→6)-Glc

Figure 1. Structures of compounds **1**–**16**.

on TLC by comparison with authentic samples.

The ^{1}H NMR spectrum of **1** showed signals for six tertiary methyl groups at δ_H 0.86, 0.93, 0.98, 0.99, 1.08, and 1.29 (each s), a trisubstituted olefinic proton at δ_H 5.46 (br s), and a three-proton singlet at δ_H 2.07 (3H, s). In addition, the ^{1}H NMR spectrum revealed the presence of arabinose, rhamnose, and ribose moieties, with anomeric protons at δ_H 4.95 (d, $J = 7.0$ Hz), 6.19 (br s), and 6.01 (d, $J = 4.0$ Hz), respectively. The J values of these anomeric proton signals indicated that the glycosidic linkage of arabinose and rhamnose were α-configuration, and that of ribose was β-configuration [3]. The ^{13}C NMR spectrum of **1** revealed the presence of 30 carbon signals for an aglycone including six methyl carbon signals at δ_C 13.4, 15.9, 17.5, 23.8, 26.0, and 33.3, a pair of double-bond carbon signals at δ_C 122.5 and 144.9, an oxygen-bearing methine carbon at δ_C 81.9, and an oxygen-bearing methylene carbon at δ_C 65.9, which were typical of the hederagenin skeleton. The ^{13}C NMR spectral data also confirmed the presence of three sugar moieties with anomeric carbons at δ_C 104.8, 101.7, and 104.5, respectively [3]. The chemical shifts of C-3 and C-28 were observed at δ_C 81.9 and 180.2, respectively, implying that no sugar linkage was formed at the C-28 carboxyl group and that the triglycoside was attached to the C-3 hydroxyl group of the aglycone. In addition, the carbon

signals at δ_C 170.5 (CH_3CO) and 20.8 (CH_3CO) as well as the proton signal at δ_H 2.07 (3H, s) indicated the presence of an acetyl group.

The assignments of the ^1H and ^{13}C NMR signals of **1** were made by comparison with those of **2** [3], and were confirmed by $^1H-^1H$ COSY, HMQC, and HMBC spectral analysis. Comparison of the ^1H and ^{13}C NMR spectra of **1** and **2** revealed that the signals assignable to the aglycone and sugar moieties were similar except that **1** had an additional acetyl group, suggesting the same hederagenin-3-*O*-β-D-ribopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside moiety in **1** as in **2**. The connections of the aglycone and sugar residues were further confirmed from the HMBC correlations between Ara-H-1′ and C-3, Rha-H-1″ and Ara-C-2′, and Rib-H-1‴ and Rha-C-3″ (Figure 2).

The position of the acetyl group in **1** was determined to be at C-23 (δ_C 65.9) of the aglycone on the basis of the ^1H and ^{13}C NMR spectral data (Tables 1 and 2), which showed downfield shifts of the C_{23}-H signals (4.49 m; 4.56 m) as compared with those of **2**. In addition, the chemical shifts of C-23, 4, 3, 5, and 24 changed by + 1.7, − 1.1, + 0.7, + 0.7, and − 0.7 ppm as compared with those of **2**, respectively [4]. The HMBC spectrum provided further confirmation of the acetyl group position from the correlations between H-24 and C-3, C-23 (Figure 2). Therefore, the structure of compound **1** was elucidated as 23-*O*-acetyl-hederagenin-3-*O*-β-D-ribopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside, and named as parvilobaside A.

The other 15 known compounds (**2**–**16**) were identified as clematoside S (**2**) [3], saponin CP_4 (**3**) [5], α-hederin (**4**) [6], saponin CP_8 (**5**) [5], saponin CP_{10} (**6**) [5], kizuta saponin K_3 (**7**) [5], clemastanoside D (**8**) [7], kizuta saponin K_{10} (**9**) [8], clematibetoside C (**10**) [9], saponin PJ_3 (**11**) [6], saponin PK (**12**) [6], huzhangoside B (**13**) [7], huzhangoside D (**14**) [7], clematichinenoside C (**15**) [10], and clematichinenoside B (**16**) [5], respectively, by comparison of their physical and spectroscopic data with those reported in the literature.

Compounds **2**, **4**, and **13**–**15** were tested for their cytotoxic activities *in vitro* against human HCT-8, Bel-7402, BGC-823, A-549,

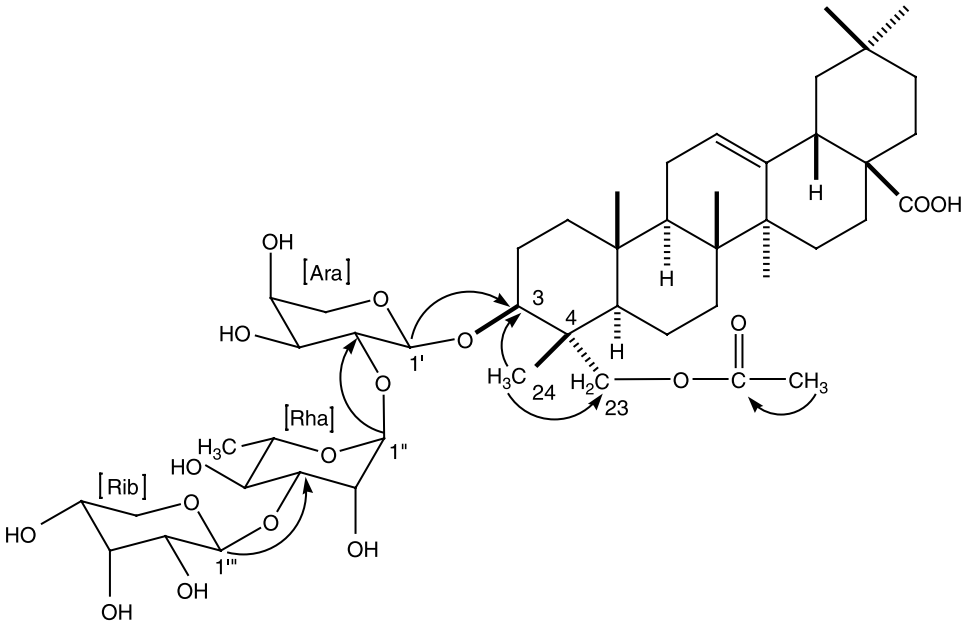

Figure 2. Key HMBC (H → C) correlations of compound **1**.

Table 1. ^{1}H NMR spectral data of compound **1** (500 MHz, C_5D_5N).

No.	**1**, δ_H (*J* in Hz)	No.	**1**, δ_H (*J* in Hz)
1	1.50 (m), 0.98 (overlap)	3-Ara-1	4.95 (d, 7.0)
2	2.20 (m), 1.92 (m)	2	4.55 (m)
3	3.94 (m)	3	4.22 (m)
5	1.28 (overlap)	4	4.21 (m)
6	1.50 (m), 1.28 (overlap)	5	4.33 (m), 3.81 (br d, 12.0)
7	2.04 (m), 1.81 (m)	Rha-1	6.19 (br s)
9	1.71 (m)	2	4.92 (br s)
11	1.91 (2H, m)	3	4.74 (dd, 9.0, 2.5)
12	5.46 (br s)	4	4.42 (m)
15	2.13 (m), 1.15 (m)	5	4.60 (m)
16	2.12 (m), 1.93 (m)	6	1.57 (3H, d, 6.0)
18	3.29 (br d, 14.0)	Rib-1	6.01 (d, 4.0)
19	1.77 (m), 1.27 (m)	2	4.29 (m)
21	1.44 (m), 1.19 (m)	3	4.50 (m)
22	1.52 (m), 1.27 (m)	4	4.16 (m)
23	4.56 (m), 4.49 (m)	5	4.38 (m), 4.24 (m)
24	1.08 (3H, s)		
25	0.86 (3H, s)		
26	0.98 (3H, s)		
27	1.29 (3H, s)		
29	0.93 (3H, s)		
30	0.99 (3H, s)		
CH_3CO	2.07 (3H, s)		

Table 2. ^{13}C NMR spectral data of compounds **1** and **2** (125 MHz, C_5D_5N).

No.	**1**	**2**	No.	**1**	**2**
1	38.7	39.1	25	15.9	16.1
2	26.1	26.4	26	17.5	17.5
3	81.9 (+0.7)	81.2	27	26.0	26.2
4	42.5 (−1.1)	43.6	28	180.2	180.1
5	48.6 (+0.7)	47.9	29	33.3	33.3
6	18.4	18.2	30	23.8	23.9
7	33.3	32.9	CH_3CO	170.5	
8	39.8	39.8	CH_3CO	20.8	
9	48.4	48.2	3-Ara-1	104.8	104.6
10	36.9	36.9	2	75.8	75.5
11	23.8	23.9	3	74.2	75.1
12	122.5	122.6	4	69.3	69.7
13	144.9	144.8	5	65.8	66.2
14	42.1	42.2	Rha-1	101.7	101.5
15	28.2	28.4	2	71.8	72.0
16	23.7	23.8	3	81.0	81.3
17	46.7	46.7	4	72.9	72.8
18	42.0	42.0	5	70.0	69.8
19	46.4	46.5	6	18.5	18.4
20	31.0	31.0	Rib-1	104.5	104.7
21	34.2	34.3	2	72.9	72.9
22	33.0	33.3	3	68.9	68.8
23	65.9 (+1.7)	64.1	4	70.3	70.3
24	13.4 (−0.7)	14.1	5	65.3	65.3

and A-2780 cell lines. Compounds **2** and **4** showed moderate cytotoxic activities against human HCT-8, Bel-7402, BGC-823, and A-2780 cell lines with IC_{50} values in the range of 1.44–6.86 μg/ml, while compounds **13**–**15** were almost inactive against these human cell lines (Table 3). The result indicated that the activities were related with the number and position of the sugar moieties.

3. Experimental

3.1 General experimental procedures

Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. The IR spectra were obtained on a Nicolet 5700 instrument with Centaurus FT-IR Microscope. NMR spectra were measured in pyridine-d_5 on a Bruker AM-500 spectrometer, using TMS as the internal standard. ESI-MS data were recorded on a Q-Trap LC/MS/MS with turbo ion spray source. HR-ESI-MS data were obtained on an ACCUTOF CS (GEOL) instrument. Precoated silica gel GF_{254} plates (Qingdao Haiyang Chem. Co., Qingdao, China) were employed for TLC. Spots were visualized by spraying 10% H_2SO_4 in 95% EtOH followed by heating. For column chromatography, silica gel (Qingdao Haiyang Chem. Co., Qingdao, China), reversed-phase C_{18} silica gel (Merck, Darmstadt, Germany), and Sephadex LH-20 (Pharmacia, New Market, NJ, USA) were used. The medium pressure liquid chromatography (MPLC) was performed on a system equipped with a Büchi pump and Büchi columns. The HPLC was performed on a Waters Delta Prep HPLC system and Waters Nova-Pak HR C18 (6 μm, 7.8 × 300 mm) semi-preparative column were used.

3.2 Plant material

The stems of *C. parviloba* were collected in Yunnan Province, China, in 1997, and authenticated by Prof. Yulin Lin (Institute of Medicinal Plant Development). A voucher specimen is deposited in the Natural Medicine Research Center of the Institute of Medicinal Plant Development, China.

3.3 Extraction and isolation

The air-dried stems of *C. parviloba* (9.5 kg) were ground and extracted three times with 95% EtOH under reflux. The combined extract was concentrated under reduced pressure to yield 450 g of residue, which was suspended in water and extracted successively with petroleum ether (60–90°C), $CHCl_3$, EtOAc, and *n*-BuOH.

The $CHCl_3$ extract (50 g) was chromatographed over a silica gel column eluted with petroleum ether–EtOAc–MeOH (9:1:0–0:0:1) to yield 14 combined fractions (A1–A14). Fractions A9 (500 mg) and A11 (7 g) were subjected to silica gel columns eluted with $CHCl_3$–MeOH (1:0–0:1) and then purified by Sephadex LH-20 columns eluted with $CHCl_3$–MeOH (1:1) to give compounds **4** (50 mg) and **2** (2 g), respectively. Fraction A12 (2 g) was subjected to MPLC over silica

Table 3. IC_{50} values of compounds **2**, **4**, **13**–**15** in MTT assay.

	IC_{50} (μg/ml)				
Compound	HCT-8	Bel-7402	BGC-823	A-549	A-2780
2	3.20	4.98	>10	>10	1.44
4	2.94	5.99	6.86	>10	1.81
13	>10	>10	>10	>10	>10
14	>10	>10	>10	>10	>10
15	>10	>10	>10	>10	>10

gel eluted with $CHCl_3$–MeOH–H_2O (77:23:2.5) to give compounds **5** (150 mg) and **6** (200 mg).

The EtOAc extract (25 g) was chromatographed over a silica gel column eluted with $CHCl_3$–MeOH (1:0–0:1) to yield 20 combined fractions (B1–B20). Fraction B13 (200 mg) was subjected to a reversed-phase C_{18} silica gel column eluted with MeOH–H_2O (4:6–1:0), and then purified by preparative HPLC with 75% MeOH affording compounds **1** (15 mg) and **3** (10 mg).

The *n*-BuOH extract (200 g) was subjected to a macroporous resin (AB-8) column and eluted with H_2O, 10, 30, 50, and 90% EtOH, respectively. The 50% EtOH fraction (110 g) was subjected to a silica gel column eluted with $CHCl_3$–MeOH–H_2O (9:1:0.1–0:1:1) to yield 16 combined fractions (C1–C16). Fraction C9 (300 mg) was subjected to a reversed-phase C_{18} silica gel column eluted with MeOH–H_2O (4:6–1:0), and then purified by preparative HPLC with 60% MeOH affording compounds **7** (30 mg) and **8** (20 mg). Fraction C10 (2 g) was subjected to MPLC over reversed-phase C_{18} silica gel eluted with MeOH–H_2O (55:45–80:20) to give 10 fractions (C10-1–C10-10). Further purification of fraction C10-3 (150 mg) by preparative HPLC with 50% MeOH afforded compounds **9** (20 mg) and **10** (20 mg); and purification of fraction C10-8 (80 mg) by preparative HPLC with 30% ACN afforded compound **11** (20 mg). Fraction C11 (2 g) was subjected to MPLC over silica gel eluted with $CHCl_3$–MeOH–H_2O (8:2:0.2–0:1:1), and then subjected to a reversed-phase C_{18} silica gel column eluted with MeOH–H_2O (6:4–7:3) to give compound **13** (300 mg). Fraction C12 (45 g) was chromatographed over a silica gel column eluted with $CHCl_3$–MeOH–H_2O (8:2:0.2–0:1:1) to give five fractions (C12-1–C12-5). Fractions C12-2 (200 mg) and C12-3 (15 g) were subjected to reversed-phase C_{18} silica gel columns eluted with MeOH–H_2O (4:6–8:2) to give compounds **12** (15 mg) and **14** (5 g), respectively. Fraction C13 (3 g) was subjected to a reversed-phase C_{18} silica gel column eluted with MeOH–H_2O (4:6–0:1) to give two fractions. Each fraction was subjected to MPLC over silica gel eluted with $CHCl_3$–MeOH–H_2O (7:3:0.5) to afford compounds **15** (50 mg) and **16** (200 mg), respectively.

3.3.1 *Paviloboside A (**1**)*

White amorphous powder; mp 208–210°C, $[\alpha]_D^{20}$ −60.6 ($c = 0.16$, MeOH). IR ν_{max} (cm^{-1}): 3404, 2942, 1719, 1692, 1458, 1385, 1367, 1243, 1132, 1051, 988, 921, 783, and 645. ^1H and ^{13}C NMR spectral data are listed in Tables 1 and 2. ESI-MS (*m/z*): 947 $[M+Na]^+$; HR-ESI-MS (*m/z*): 947.4976 $[M+Na]^+$ (calcd for $C_{48}H_{76}O_{17}Na$, 947.4980).

3.4 *Acid hydrolysis*

A solution of **1** (3 mg) in 1 M HCl–MeOH (2 ml) was heated at 110°C for 2 h. After removing the HCl and MeOH, the reaction mixture was dissolved in H_2O (10 ml) and extracted with $CHCl_3$ (2 × 10 ml). The sapogenin was detected in the $CHCl_3$ layer by TLC. The H_2O layer was concentrated and subjected to co-TLC analysis with authentic samples of L-arabinose, L-rhamnose, and D-ribose, and developed with $CHCl_3$–*n*-BuOH–MeOH–HOAc–H_2O (17:10:7:2:3). Detection was carried out with 5% α-naphthol in 10% sulfuric acid–ethanol solution.

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