Triterpenoid Saponins from the Roots of *Clematis uncinata*

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Eight new bisdesmosidic triterpenoid saponins, clematiunicinosides A–H (1–8), along with eleven known ones (9–19), were isolated from the roots of *Clematis uncinata*. Their structures were elucidated on the basis of spectroscopic analysis and chemical evidence. All the isolated saponins were tested for their cytotoxic activities on human caski cervical cancer (Caski) cells, and compounds 13, 17 and 19 exhibited inhibitory effect on Caski cells.

Key words triterpenoid saponin; *Clematis uncinata*; Ranunculaceae

The genus *Clematis*, belonging to the family Ranunculaceae, comprises of more than 300 species distributed throughout the world.[1)] Three plants of this genus (*Clematis chinensis* Osbeck, *C. hexapetala* Pall., and *C. mandshurica* Rupr.) are officially used as "Weilingxian," a traditional Chinese herbal drug, for the treatment of inflammation and cancer in China.[2)] Previous phytochemical investigations had demonstrated that triterpenoid saponins are the dominant constituents of this genus.[3)] Some of them showed significant anti-inflammatory and antitumor activities.[4–7)] During the course of our ongoing program to search for new saponins from traditional Chinese medicine,[8–12)] *Clematis uncinata* was selected as a subject for this study. The plant *Clematis uncinata* is mainly distributed in southern China, such as Guizhou, Zhejiang, Fujian, Guangdong and Guangxi provinces.[13)] The blank in chemical constituent, the potential medicinal importance and our interest in the chemistry of saponins prompted us to carry out a phytochemical investigation on this plant, which led to the isolation of eight new bisdesmosidic triterpenoid saponins, clematiunicinosides A–H (**1**–**8**), together with eleven known ones (**9**–**19**). In addition, the cytotoxic effects of the isolated triterpenoid saponins on Caski cells were evaluated. The present paper reports the isolation, structural elucidation and antiproliferative activities of these compounds.

Results and Discussion

The 70% (V/V) EtOH extract of the roots of *C. uncinata* was subjected to D101 macroporous resin column eluted successively with H_2O, 10% EtOH, 30% EtOH, 50% EtOH and 70% EtOH. The 50% EtOH and 70% EtOH eluates were further purified by repeated column chromatography over middle chromatogram isolated (MCI) gel, octadecyl silica (ODS), silica gel and preparative HPLC to afford eight new bisdesmosidic triterpenoid saponins (**1**–**8**), named as clematiunicinosides A–H, as well as eleven known ones (**9**–**19**).

Clematiunicinoside A (**1**) was obtained as an amorphous powder. It showed positive reactions to the Liebermann-Burchard and Molish tests, which suggested that **1** might be a triterpenoid glycoside. The molecular formula of **1** was established as $C_{70}H_{114}O_{34}$ on the basis of the quasi-molecular ion at *m/z* 1497.7111 $[M-H]^-$ (Calcd for $C_{70}H_{113}O_{34}$, 1497.7118) by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). The IR spectrum showed the presence of hydroxyl (3412 cm^{-1}) and carboxyl (1734 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** exhibited signals for seven angular methyl groups (δ_H 0.86, 0.88, 0.88, 1.07, 1.16, 1.24, 1.30, each 3H, s), one oxygenated methine [δ_H 3.40 (1H, dd, J=2.7, 11.5 Hz)], and one olefinic proton [δ_H 5.40 (1H, brs)]. In the ^{13}C-NMR spectrum of **1**, seventy carbon signals were observed including seven methyl carbons (δ_C 15.7, 17.1, 17.5, 23.7, 26.1, 28.2, 33.1), one oxygenated methine carbon (δ_C 88.7), two olefinic carbons (δ_C 122.9, 144.1), and one carbonyl carbon (δ_C 176.5). With the assistance of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), HSQC-total correlation spectroscopy (TOCSY), TOCSY and nuclear Overhauser effect spectroscopy (NOESY) experiments, all the ^{1}H- and ^{13}C-NMR signals of **1** were assigned and showed in Tables 1 and 2. The aglycone of **1** was identified as oleanolic acid by comparison of NMR data with those published in the literature.[14)]

Furthermore, the ^{1}H- and ^{13}C-NMR spectra of **1** exhibited seven sugar anomeric proton signals at δ_H 4.70 (1H, d, J=7.2 Hz), 4.99 (1H, d, J=8.0 Hz), 5.12 (1H, d, J=8.0 Hz), 5.84 (1H, brs), 5.95 (1H, d, J=4.0 Hz), 6.23 (1H, d, J=8.0 Hz) and 6.30 (1H, brs), corresponding to seven anomeric carbon signals at δ_C 105.2, 104.8, 106.8, 102.7, 104.6, 95.6 and 101.3, respectively (Tables 1, 2). Acid hydrolysis of **1** afforded D-glucose, D-ribose, L-arabinose and L-rhamnose, which were identified by HPLC analysis.[15)] The relative anomeric configurations of the sugar moieties were determined to be β for the D-glucose (J=8.0 Hz) and α for the L-arabinose (J=7.2 Hz) based on the $^3J_{H1\text{-}H2}$ coupling constants of their anomeric protons. The β configuration for D-ribose could be established by comparison of the NMR data with those reported in the literatures.[16,17)] The sequences and linkage positions of sugar moieties were subsequently deduced from an HMBC experiment. In the HMBC spectrum of **1**, the correlations between H-1 (δ_H 5.95) of D-ribose and C-3 (δ_C 81.1) of L-rhamnose^1, between H-1 (δ_H 6.30) of L-rhamnose^1 and C-2 (δ_C 75.8) of L-arabinose, between H-1 (δ_H 5.12) of D-glucose^3 and C-4 (δ_C 79.8) of L-arabinose, between H-1 (δ_H 4.70) of L-arabinose and C-3 (δ_C 88.7) of aglycone, between H-1 (δ_H 5.84) of L-rhamnose^2 and C-4 (δ_C 78.3) of D-glucose^2, between H-1 (δ_H

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Table 1. [1]H-NMR Data for the Sugar Moieties of **1**–**8** (400 MHz, in Pyridine-d_5, J in Hz)[a,b]

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Ara								
1	4.70 d (7.2)	4.92 d (6.0)	4.85 d (6.0)		4.82 d (5.5)	4.84 d (6.0)	4.84 d (6.0)	4.82 d (6.0)
2	4.53 dd (8.0, 6.0)	4.50 dd (8.0, 6.0)	4.56 dd (8.0, 6.0)		4.54 dd (8.0, 5.5)	4.57 dd (8.0, 6.0)	4.57 dd (8.0, 6.0)	4.53 dd (8.0, 6.0)
3	4.24	3.92	4.28		4.24	4.25	4.25	4.24
4	4.23	4.11	4.26		4.23	4.24	4.24	4.21
5a	4.36 brd (9.0)	4.33 brd (9.0)	4.30		4.29 brd (9.0)	4.30 brd (9.0)	4.30 brd (9.0)	4.28 brd (9.0)
5b	3.75 brd (9.0)	3.57 brd (9.0)	3.81 brd (10.4)		3.80 brd (9.0)	3.81 brd (9.0)	3.81 brd (9.0)	3.80 brd (9.0)
Xyl								
1				4.81 d (7.5)				
2				4.24				
3				4.19				
4				4.15				
5a				4.32				
5b				3.70 t (10.5)				
Rib								
1	5.95 d (4.0)	5.95 d (4.0)	5.95 d (4.4)	5.84 d (5.5)	5.80 d (5.5)	5.82 d (5.5)	5.82 d (6.0)	5.80 d (5.5)
2	4.33	4.31	4.31	4.12	4.07	4.09	4.10	4.08
3	4.52	4.51	4.55	4.71	4.60	4.62	4.66	4.65
4	4.17	4.17	4.18	4.38	4.30	4.15	4.31	4.32
5a	4.36	4.31	4.34	4.36	4.39	4.31	4.43	4.27
5b	4.18	4.15	4.17	4.31	4.27	4.29	4.30	4.08
Rha[1]								
1	6.30 brs	6.33 brs	6.28 brs	6.54 brs	6.23 brs	6.25 brs	6.25 brs	6.22 brs
2	4.90 brs	4.86 brs	4.91 brs	4.95	4.85 brs	4.87 brs	4.88 brs	4.85 brs
3	4.73	4.73	4.74	4.74	4.67	4.68	4.68	4.66
4	4.45	4.43	4.43	4.46	4.41	4.43	4.43	4.41
5	4.67	4.71	4.28	4.72	4.60	4.62	4.66	4.58
6	1.56 d (6.0)	1.56 d (6.0)	1.54 d (6.0)	1.52 d (6.0)	1.52 d (6.0)	1.53 d (6.0)	1.53 d (6.0)	1.52 d (6.0)
Rha[2]								
1	5.84 brs	5.82 brs	5.83 brs	5.84 brs	5.82 brs	5.85 brs	5.85 brs	5.83 brs
2	4.67 brs	4.66 brs	4.65 brs	4.66 brs	4.65 brs	4.67 brs	4.67 brs	4.65 brs
3	4.54 dd (9.5, 3.5)	4.53 dd (9.5, 3.5)	4.54	4.54 dd (9.5, 3.5)	4.53 dd (9.5, 3.5)	4.54 dd (9.5, 3.5)	4.55 dd (9.5, 3.5)	4.54 dd (9.5, 3.5)
4	4.32	4.31	4.32	4.33	4.32	4.33	4.33	4.33
5	4.95	4.94	4.94	4.93	4.93	4.95	4.95	4.94
6	1.69 d (6.0)	1.67 d (6.0)	1.70 d (6.0)	1.68 d (6.0)	1.68 d (6.0)	1.68 d (6.0)	1.68 d (6.0)	1.68 d (6.0)
Rha[3]								
1							5.45 brs	
2							4.65 brs	
3							4.55	
4							4.33	
5							4.25	

Table 1. Continued

Position	1	2	3	4	5	6	7	8
6							1.59 d (6.0)	
Glc[1]								
1	6.23 d (8.0)	6.21 d (8.0)	6.27 d (8.0)	5.00 d (8.0)	4.96 d (8.0)	4.96 d (7.5)	4.95 d (7.5)	4.89 d (7.5)
2	4.13	4.10	4.10	3.93 t (8.0)	3.88 t (8.0)	3.92 t (7.5)	3.91 t (7.5)	3.89 t (7.5)
3	4.21 t (9.0)	4.20 t (9.0)	4.20 t (9.0)	4.20	4.23	4.23	4.15	4.17
4	4.28	4.30	4.30	4.17	4.25	4.26	4.21	4.29
5	4.10	4.08	4.07	3.90 t (8.5)	3.91 t (8.5)	3.92 t (8.5)	3.86 t (8.5)	3.65 t (8.5)
6a	4.66 brd (10.0)	4.64 brd (10.0)	4.64 brd (10.0)	4.47	4.47	4.41	4.38	4.21
6b	4.31	4.30	4.33	4.31	4.27	4.29	4.30	4.16
Glc[2]								
1	4.99 d (8.0)	4.97 d (8.0)	4.98 d (7.6)	6.22 d (8.0)	5.15 d (7.4)	5.13 d (7.5)	5.11 d (8.0)	5.35 d (8.5)
2	3.93 t (8.0)	3.92 t (8.0)	3.92 t (7.6)	4.12	4.05 t (8.0)	4.08 t (7.5)	4.06 t (8.0)	5.77 t (8.5)
3	4.13	4.12	4.14	4.19	4.19	4.17	4.13	4.25
4	4.40 t (9.0)	4.38 t (9.0)	4.39	4.30	4.15	4.05	4.07	4.01
5	3.65 d (9.5)	3.63 d (9.5)	3.63 d (9.5)	4.09	3.99 t (8.5)	3.93 t (8.5)	3.92 t (8.5)	4.00
6a	4.18	4.18 t (9.0)	4.20	4.66 brd (10.0)	4.50	4.42	3.99	4.42
6b	4.09	4.06	4.08	4.31	4.26	4.18	4.65	4.50
Glc[3]								
1	5.12 d (8.0)	5.07 d (8.0)		4.98 d (8.0)	6.21 d (8.0)	6.23 d (8.0)	6.23 d (8.0)	6.22 d (8.0)
2	4.04 t (8.0)	4.01 t (8.0)		3.92 dd (9.0, 8.0)	4.11	4.08	4.13	4.13
3	4.20	4.16		4.13	4.22 t (9.0)	4.22 t (9.0)	4.21 t (9.0)	4.20 t (9.0)
4	4.22	4.23		4.38 t (9.0)	4.28	4.31	4.32	4.29
5	3.90 t (8.5)	3.87 t (8.5)		3.62 d (9.0)	4.08	4.10	4.10	4.07
6a	4.51	4.47		4.18	4.66 brd (10.5)	4.67 brd (10.5)	4.65 brd (10.5)	4.66 brd (10.5)
6b	4.37	4.34		4.07	4.31	4.32	4.24	4.31
Glc[4]								
1					4.98 d (8.0)	4.99 d (8.0)	4.99 d (8.0)	4.98 d (8.0)
2					3.92 t (8.0)	3.94 dd (9.0, 8.0)	3.93 dd (9.0, 8.0)	3.92 dd (9.0, 8.0)
3					4.13	4.15	4.15	4.14
4					4.38 t (9.0)	4.41 t (9.0)	4.41 t (9.0)	4.40 t (9.0)
5					3.64 d (9.0)	3.65 d (9.0)	3.65 d (9.0)	3.66 d (9.0)
6a					4.18	4.19	4.20	4.19
6b					4.07	4.09	4.09	4.05
Glc[5]								
1						5.27 d (7.5)	5.19 d (8.0)	4.98 d (8.0)
2						4.06 t (7.5)	4.03 t (8.0)	3.92
3						4.24	4.21	4.39
4						4.18	4.10	4.07
5						4.02	3.95	4.19 t (9.5)
6a						4.52	4.42	4.55
6b						4.29	4.15	4.23

a) Assignments were established by interpretation of the ^{1}H–^{1}H COSY, TOCSY, NOESY, HSQC, HMBC, and HSQC–TOCSY experiments. *b*) Overlapped signals are reported whitout designating multiplicity.

Table 2. ^{13}C-NMR Data of **1**–**8** (100 MHz, in Pyridine-d_5)

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	38.9	39.0	39.0	39.0	38.9	39.0	39.0	38.9
2	26.7	26.4	26.7	26.9	26.6	26.6	26.6	26.6
3	88.7	81.0	88.8	88.6	88.7	88.7	88.7	88.7
4	39.5	43.5	39.6	39.6	39.5	39.6	39.6	39.5
5	56.1	47.7	56.1	56.1	56.0	56.0	56.0	55.8
6	18.6	18.1	18.5	18.6	18.5	18.5	18.5	18.5
7	33.1	32.7	33.2	33.0	33.0	33.1	32.9	33.0
8	39.9	39.8	39.8	39.9	39.9	39.9	39.9	39.8
9	48.1	48.1	48.1	48.0	48.0	48.1	48.1	48.0
10	37.0	36.8	37.1	37.0	37.0	37.0	37.0	37.0
11	23.8	23.8	23.8	23.7	23.8	23.8	23.8	23.7
12	122.9	122.9	122.8	122.8	122.8	122.8	122.8	122.8
13	144.1	144.0	144.4	144.1	144.1	144.1	144.1	144.1
14	42.1	42.1	42.5	42.1	42.1	42.1	42.1	42.1
15	28.3	28.2	28.8	28.2	28.2	28.3	28.3	28.2
16	23.4	23.3	27.0	23.3	23.3	23.4	23.4	23.3
17	47.0	47.0	47.5	47.0	47.0	47.0	47.0	47.0
18	41.7	41.6	41.8	41.6	41.6	41.6	41.6	41.6
19	46.2	46.1	41.4	46.2	46.2	46.2	46.2	46.2
20	30.7	30.7	35.7	30.7	30.7	30.7	30.7	30.7
21	34.0	33.9	73.4	34.0	34.0	34.0	34.0	34.0
22	32.5	32.5	39.7	32.5	32.5	32.6	32.5	32.5
23	28.2	63.8	28.2	28.1	28.2	28.2	28.2	28.1
24	17.1	14.1	17.2	17.2	17.1	17.1	17.1	17.1
25	15.7	16.1	15.7	15.7	15.6	15.6	15.6	15.6
26	17.5	17.5	17.6	17.4	17.4	17.5	17.5	17.4
27	26.1	26.0	25.7	26.1	26.0	26.1	26.1	26.0
28	176.5	176.5	176.5	176.5	176.5	176.5	176.5	176.5
29	33.1	33.0	28.4	33.1	33.1	33.1	33.1	33.1
30	23.7	23.6	25.0	23.6	23.7	23.7	23.7	23.6
Ara								
1	105.2	104.6	105.3		105.1	105.2	105.2	105.2
2	75.8	75.6	75.3		75.4	75.4	75.3	75.4
3	74.9	75.5	74.8		74.5	74.6	74.6	74.6
4	79.8	80.8	69.4		69.2	69.3	69.5	69.3
5	65.4	65.9	65.7		65.5	65.5	65.5	65.6
Xyl								
1				106.1				
2				77.1				
3				79.8				
4				71.5				
5				67.0				
Rib								
1	104.6	104.8	104.7	104.7	104.6	104.8	104.7	104.6
2	72.8	72.7	72.9	72.5	72.5	72.5	72.5	72.7
3	68.9	68.7	69.0	69.6	69.4	69.8	69.8	69.3
4	70.3	70.2	70.2	76.4	76.4	76.4	76.4	76.4
5	65.3	65.2	65.3	61.8	61.7	61.7	61.7	61.6
Rha[1]								
1	101.3	101.2	101.4	101.4	101.4	101.4	101.4	101.4
2	71.9	71.9	72.1	71.9	71.9	71.9	71.9	71.9
3	81.1	81.0	81.3	82.0	82.0	82.1	82.1	82.1
4	72.8	72.8	72.8	72.7	72.7	72.8	72.7	72.5
5	69.9	69.7	69.9	69.7	69.7	69.7	69.8	69.7
6	18.5	18.5	18.4	18.5	18.5	18.4	18.4	18.4
Rha[2]								
1	102.7	102.6	102.7	102.7	102.7	102.7	102.7	102.7
2	72.5	72.5	72.5	72.5	72.5	72.5	72.5	72.5
3	72.7	72.7	72.8	72.8	72.7	72.7	72.6	72.7

Table 2. Continued

Position	1	2	3	4	5	6	7	8
4	73.9	73.9	74.0	74.0	73.9	74.0	74.0	73.9
5	70.3	70.2	70.3	70.3	70.2	70.3	70.3	70.3
6	18.5	18.5	18.5	18.6	18.5	18.5	18.5	18.5
Rha[3]								
1							102.8	
2							72.0	
3							72.6	
4							73.9	
5							69.4	
6							18.6	
Glc[1]								
1	95.6	95.6	95.7	103.5	103.1	103.1	103.1	102.8
2	74.0	73.8	73.9	74.7	74.2	74.2	74.2	74.2
3	78.7	78.7	78.7	78.3	76.6	76.6	76.6	76.3
4	70.9	70.8	70.9	71.5	80.9	80.9	80.9	81.1
5	78.0	78.5	78.0	78.6	76.5	76.4	76.4	76.5
6	69.2	69.1	69.3	62.5	61.8	62.1	62.0	60.7
Glc[2]								
1	104.8	104.7	104.9	95.6	104.8	104.4	104.4	102.2
2	75.3	75.3	75.4	73.9	74.7	73.6	73.5	73.0
3	76.5	76.4	76.5	78.7	78.3	88.2	88.1	86.3
4	78.3	78.1	78.3	70.8	71.5	69.8	69.3	70.2
5	77.1	77.1	77.1	78.0	78.4	77.9	77.7	77.9
6	61.3	61.2	61.3	69.2	62.4	61.5	68.8	62.2
Glc[3]								
1	106.8	106.9		104.8	95.6	95.6	95.6	95.6
2	75.5	75.4		75.3	73.8	73.9	73.5	73.8
3	78.7	78.6		76.5	78.7	78.7	78.7	78.6
4	71.3	71.1		78.2	70.8	70.9	70.8	70.8
5	78.5	78.0		77.1	78.0	78.0	78.0	78.2
6	62.5	62.4		61.2	69.2	69.2	69.2	69.1
Glc[4]								
1					104.9	104.7	104.8	104.8
2					75.3	75.5	75.4	75.3
3					76.3	76.5	76.5	76.3
4					78.2	78.2	78.2	78.0
5					77.1	77.1	77.1	77.1
6					61.3	61.3	61.3	61.2
Glc[5]								
1						105.8	105.5	105.4
2						75.3	75.2	74.6
3						78.3	78.3	78.2
4						71.6	71.9	71.5
5						78.6	77.8	78.7
6						62.5	61.5	62.5

4.99) of D-glucose[2] and C-6 (δ_C 69.2) of D-glucose[1], as well as between H-1 (δ_H 6.23) of D-glucose[1] and C-28 (δ_C 176.5) of aglycone were observed. The above findings led to assignment of the sugar sequences and linkage positions of the two oligosaccharide chains were as shown in Fig. 1. Therefore, the structure of **1** was established as 3-*O*-*β*-D-ribopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→4)]-*α*-L-arabinopyranosyl olean-12-en-28-oic acid 28-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl ester.

Clematiunicinoside B (**2**) was isolated as an amorphous powder. The HR-ESI-MS spectrum of **2** showed a quasi-molecular ion at *m/z* 1513.7051 $[M-H]^-$ (Calcd for $C_{70}H_{113}O_{35}$, 1513.7062), corresponding to the molecular formula $C_{70}H_{114}O_{35}$. Similar to **1**, the IR spectrum of compound **2** also showed the characteristic absorptions of hydroxyl (3410 cm^{-1}) and carboxyl (1733 cm^{-1}) groups. The ^{1}H-NMR data of **2** displayed the signals for six tertiary methyls (δ_H 0.85, 0.86, 0.94, 1.09, 1.14, 1.18, each 3H, s), one oxygenated methene [δ_H 3.91 (1H, t, *J*=8.5 Hz) and 4.27 (1H, overlapped)], one oxygenated methine [δ_H 4.23 (1H, overlapped)], one olefinic proton [δ_H 5.39 (1H, brs)], as well as seven anomeric protons [(δ_H 4.92 (1H, d, *J*=6.0 Hz), 4.97 (1H, d, *J*=8.0 Hz), 5.07 (1H, d, *J*=8.0 Hz), 5.82 (1H, brs), 5.95 (1H, d, *J*=4.0 Hz), 6.21 (1H, d, *J*=8.0 Hz) and 6.33 (1H, brs)]. In the ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT)

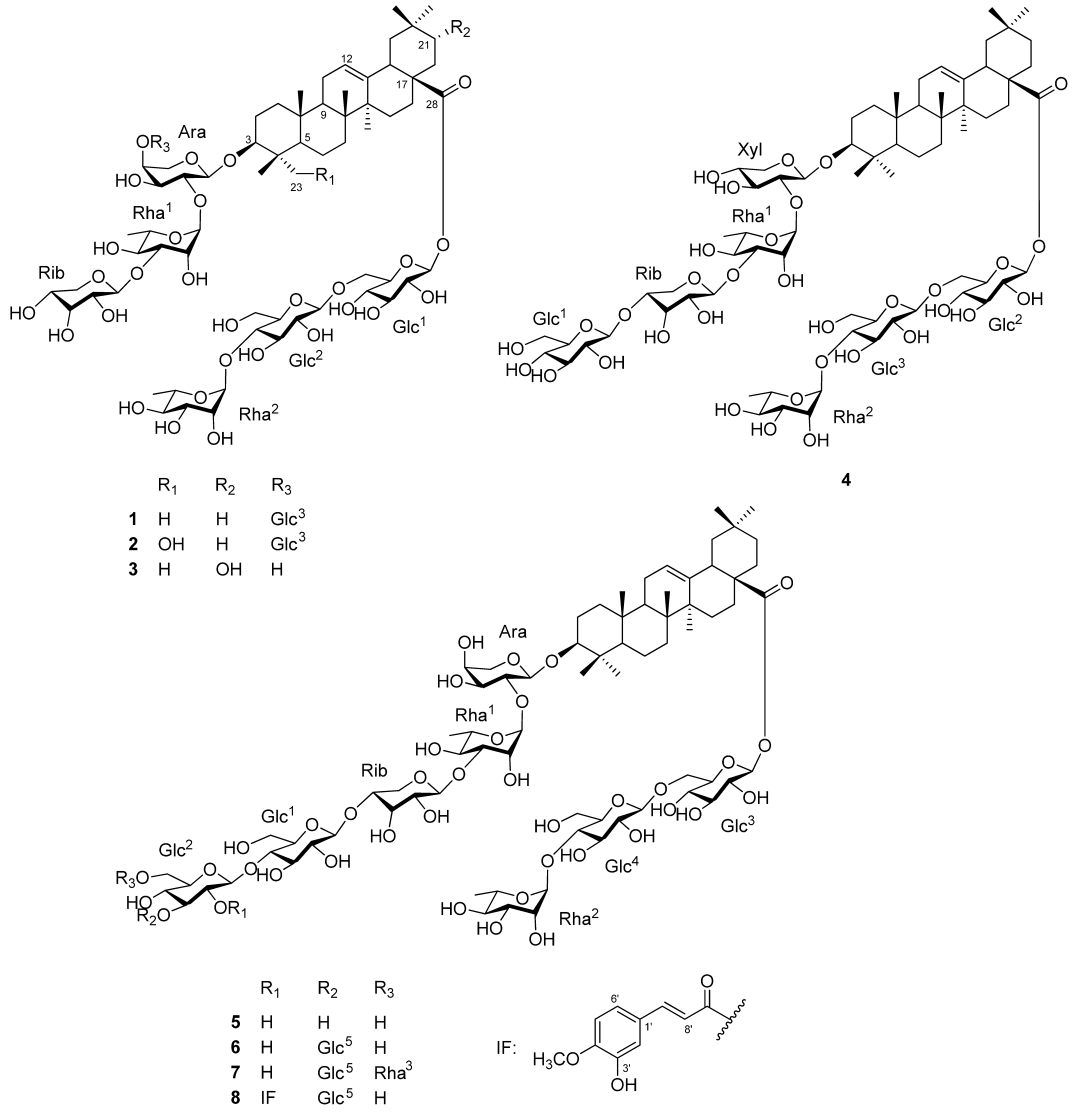

Fig. 1. Chemical Structures of Compounds **1**–**8**

spectra of **2**, seventy carbon signals were observed, which suggested that **2** was also a triterpenoid saponin with seven sugar units. A comprehensive analysis of the ^{1}H–^{1}H COSY, HSQC, HMBC, HSQC-TOCSY, TOCSY and NOESY spectra allowed the assignment of NMR data of **2** as shown in Tables 1 and 2. The NMR data of **2** assigned to the aglycone part were the same as those of hederagenin.[18] Acid hydrolysis of **2** also afforded D-glucose, D-ribose, L-arabinose and L-rhamnose. Comparison of the NMR data of the sugar units in **2** with those of **1** revealed that they were very similar, suggesting that **2** possessed the identical oligosaccharide chains to **1**. The linkage sequences and positions of these sugar units were confirmed by the HMBC experiment. Hence, the structure of **2** was established to be 3-*O*-*β*-D-ribopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→4)]-*α*-L-arabinopyranosyl hederagenin 28-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl ester.

Clematiunicinoside C (**3**) was obtained as an amorphous powder. The molecular formula of **3** was established as $C_{64}H_{104}O_{30}$ by its HR-ESI-MS (Found *m/z* 1351.6510 $[M-H]^-$; Calcd for $C_{64}H_{103}O_{30}$, 1351.6534). The ^{1}H- and ^{13}C-NMR spectra of **3** showed the signals corresponding to a triterpenoid saponin with six sugar units. The ^{1}H–^{1}H COSY, HSQC, HMBC, HSQC-TOCSY, TOCSY and NOESY spectra of **3** allowed the assignment of all proton and carbon signals (Tables 1, 2). The aglycone of **3** was identified to be 21*α*-hydroxyoleanolic acid by comparison of its NMR data with the literature values.[5] Acid hydrolysis of **3** also afforded D-glucose, D-ribose, L-arabinose and L-rhamnose. Comparison of the NMR data assigned to the sugar units of **3** with those of **2** indicated that **3** also possessed two oligosaccharide chains attached at C-3 and C-28 positions of the aglycone, respectively. The difference between them was the absence of the proton and carbon signals of the glucose[3] moiety linked to the C-4 position of arabinose in **3**. The sequences and linkages of the remained six sugar units could be deduced by an HMBC experiment. In the HMBC spectrum of **3**, correlation signals were observed between H-1 (δ_H 5.95) of D-ribose and C-3 (δ_C 81.3) of L-rhamnose[1], between H-1 (δ_H 6.28) of L-rhamnose[1] and C-2 (δ_C 75.3) of L-arabinose, between H-1 (δ_H 4.85) of L-arabinose and C-3 (δ_C 88.8) of aglycone, between H-1 (δ_H 5.83) of L-rhamnose[2] and C-4 (δ_C 78.3) of D-glucose[2], between H-1 (δ_H 4.98) of D-glucose[2] and C-6 (δ_C 69.3) of D-glucose[1], as well as between H-1 (δ_H 6.27) of D-glucose[1] and C-28 (δ_C

176.5) of aglycone. Thus, the structure of **3** was identified as 3-*O*-*β*-D-ribopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinopyranosyl 21*α*-hydroxyolean-12-en-28-oic acid 28-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl ester.

Clematiunicinoside D (**4**) was obtained as an amorphous powder. The HR-ESI-MS spectrum of **4** exhibited a quasi-molecular ion at *m/z* 1497.7109 $[M-H]^-$ (Calcd for $C_{70}H_{113}O_{34}$, 1497.7113), consistent with the molecular formula $C_{70}H_{114}O_{34}$. The ^{1}H- and ^{13}C-NMR data of the aglycone part of **4** were closely similar to those of **1**, indicating that they possessed the same aglycone (oleanolic acid). Besides the signals assigned to the aglycone part, the NMR data of **4** showed the presence of seven anomeric protons [δ_H 4.81 (1H, d, J=7.5 Hz), 4.98 (1H, d, J=8.0 Hz), 5.00 (1H, d, J=8.0 Hz), 5.84 (1H, brs), 5.84 (1H, d, J=5.5 Hz), 6.22 (1H, d, J=8.0 Hz) and 6.54 (1H, brs)] as well as seven anomeric carbons (δ_C 95.6, 101.4, 102.7, 103.5, 104.7, 104.8, 106.1), which indicated that **4** also possessed seven sugar moieties. Acid hydrolysis of **4** afforded D-xylose, D-glucose, L-rhamnose and D-ribose by HPLC analysis.[15] The relative anomeric configurations of the sugar moieties were established based on the $^3J_{H1\text{-}H2}$ coupling constants of their anomeric protons as well as by comparison of the NMR data with those reported in the literatures.[4,16] In the HMBC spectrum of **4**, the correlations between H-1 (δ_H 5.00) of D-glucose^1 and C-4 (δ_C 76.4) of D-ribose, between H-1 (δ_H 5.84) of D-ribose and C-3 (δ_C 82.0) of L-rhamnose^1, between H-1 (δ_H 6.54) of L-rhamnose^1 and C-2 (δ_C 77.1) of D-xylose, between H-1 (δ_H 4.81) of D-xylose and C-3 (δ_C 88.6) of aglycone, between H-1 (δ_H 5.84) of L-rhamnose^2 and C-4 (δ_C 78.2) of D-glucose^3, between H-1 (δ_H 4.98) of D-glucose^3 and C-6 (δ_C 69.2) of D-glucose^2, and between H-1 (δ_H 6.22) of D-glucose^2 and C-28 (δ_C 176.5) of aglycone were observed. Based on the above results, the structure of **4** was elucidated as 3-*O*-*β*-D-glucopyranosyl(1→4)-*α*-D-ribopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl olean-12-en-28-oic acid 28-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl ester.

Clematiunicinoside E (**5**) was isolated as an amorphous powder. The molecular formula of **5** was determined as $C_{76}H_{124}O_{39}$ by HR-ESI-MS spectrum (*m/z* 1659.7601 $[M-H]^-$; Calcd for $C_{76}H_{123}O_{39}$, 1659.7641). Interpretation of the 2D-NMR spectra (^{1}H–^{1}H COSY, HSQC, HMBC, HSQC-TOCSY, TOCSY and NOESY) of **5** allowed the assignment of all proton and carbon signals as shown in Tables 1 and 2. The aglycone of **5** was also identified as oleanolic acid by comparison of the ^{1}H- and ^{13}C-NMR data with those of **1**. In addition, the NMR spectra of **5** exhibited eight anomeric proton and carbon resonances, which indicated the existence of eight sugar moieties in **5**. Acid hydrolysis of **5** also gave L-arabinose, D-glucose, D-ribose and L-rhamnose. The relative anomeric configurations of the sugar moieties were further determined based on the $^3J_{H1\text{-}H2}$ coupling constants of their anomeric protons as well as by comparison of the NMR data with those reported in the literatures.[4,16] Comparison of the NMR data assigned to the sugar units of **5** with those of **3** indicated that **5** possessed the same sugar chain at C-28 position of the aglycone. Different from **3**, the NMR spectra of **5** showed the presence of two additional glucose units [(δ_H 4.96 (1H, d, J=8.0 Hz) and 5.15 (1H, d, J=7.4 Hz), as well as δ_C 103.1 and 104.8]. The HMBC correlations between H-1 (δ_H 5.15) of D-glucose^2 and C-4 (δ_C 80.9) of D-glucose^1, and between H-1 (δ_H 4.96) of D-glucose^1 and C-4 (δ_C 76.4) of D-ribose indicated that the additional two glucose units were attached to C-4 position of glucose^1 and ribose, respectively. Based on the above evidence, **5** was identified as 3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-glucopyranosyl(1→4)-*β*-D-ribopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinopyranosyl olean-12-en-28-oic acid 28-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl ester.

The HR-ESI-MS spectrum of **6** showed a quasi-molecular ion at *m/z* 1821.8112 $[M-H]^-$ (Calcd for $C_{82}H_{133}O_{44}$, 1821.8170), corresponding to the molecular formula $C_{82}H_{134}O_{44}$, which was 162 mass units more than that of **5**. Acid hydrolysis of **6** afforded L-arabinose, D-glucose, D-ribose and L-rhamnose. Comparison of ^{1}H- and ^{13}C-NMR spectra of **6** with those of **5** suggested that they possessed the same oleanolic acid as aglycone and two oligosaccharide chains attached at C-3 and C-28 positions of the aglycone, respectively. The difference between them was the appearance of signals due to an additional glucose unit [δ_H 5.27 (1H, d, J=7.5 Hz); δ_C 105.8] in **6**. The location of the additional glucose moiety was deduced by the HMBC correlation between H-1 (δ_H 5.27) of the additional glucose moiety (D-glucose^5) and C-3 (δ_C 88.2) of D-glucose^2. Thus, the structure of **6** was established as 3-*O*-*β*-D-glucopyranosyl(1→3)-*β*-D-glucopyranosyl(1→4)-*β*-D-glucopyranosyl(1→4)-*β*-D-ribopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinopyranosyl olean-12-en-28-oic acid 28-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl ester.

The molecular formula of **7** was determined as $C_{88}H_{144}O_{48}$ on the basis of the quasi-molecular ion at *m/z* 1967.8811 $[M-H]^-$ (Calcd for $C_{88}H_{143}O_{48}$, 1967.8749) by its HR-ESI-MS spectrum. Similar to **6**, acid hydrolysis of **7** afforded L-arabinose, D-glucose, D-ribose and L-rhamnose. The NMR data of **7** were very similar to those of **6**, except for the presence of signals corresponding to an additional rhamnose unit. In the HMBC spectrum of **7**, the correlation between H-1 (δ_H 5.45) of the additional rhamnose moiety and C-6 (δ_C 68.8) of D-glucose^2 was observed, which indicated that the additional rhamnose unit (L-rhamnose^3) was attached to the C-6 position of D-glucose^2. Therefore, the structure of **7** was determined as 3-*O*-*β*-D-glucopyranosyl(1→3)-[*α*-L-rhamnopyranosyl(1→6)]-*β*-D-glucopyranosyl(1→4)-*β*-D-glucopyranosyl(1→4)-*β*-D-ribopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinopyranosyl olean-12-en-28-oic acid 28-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl ester.

Clematiunicinoside H (**8**) showed a molecular formula $C_{92}H_{142}O_{47}$ by its HR-ESI-MS spectrum at *m/z* 1997.8654 $[M-H]^-$ (Calcd for $C_{92}H_{141}O_{47}$, 1997.8649). The UV spectrum of **8** showed the absorptions maxima at 244, 296 and 326 nm. The IR spectrum implied the presence of hydroxyl (3413 cm^{-1}) and carboxyl (1715 cm^{-1}) groups as well as aromatic ring (1633, 1450 cm^{-1}). Acid hydrolysis of **8** afforded D-glucose, L-rhamnose, L-arabinose and D-ribose, which were identified by HPLC analysis.[15] With the aid of 2D-NMR experiments (^{1}H–^{1}H COSY, HSQC, HMBC, HSQC-TOCSY, TOCSY and NOESY), all proton and carbon resonances in **8** were assigned as shown in Tables 1 and 2. Comparison of the ^{1}H- and ^{13}C-NMR data of **8** with those of **6** revealed that they were similar except that **8** displayed proton and carbon

Table 3. Cytotoxic Activities of Compounds **1**–**19**, Oleanolic Acid and Hederagenin against Caski Cervical Cancer Cell by the MTT Assay[a)]

Compounds	IC_{50} (μM)	Cell survival rate (% of blank, at concentration of 80 μM)	Compounds	IC_{50} (μM)	Cell survival rate (% of blank, at concentration of 80 μM)
1	>80	100.62±1.01	**12**	>80	107.33±3.21
2	>80	93.38±1.76	**13**	9.05±3.61	
3	>80	93.45±2.45	**14**	>80	97.27±3.70
4	>80	95.38±1.72	**15**	>80	72.84±4.13
5	>80	96.01±3.19	**16**	>80	85.71±5.05
6	>80	95.58±2.22	**17**	26.07±1.47	
7	>80	77.76±1.49	**18**	>80	75.51±5.50
8	>80	101.24±1.46	**19**	2.97±1.07	
9	>80	77.24±2.34	Oleanolic acid	>80	90.73±2.39
10	>80	98.31±2.91	Hederagenin	>80	83.57±1.82
11	>80	95.63±0.98	Cisplatin	3.37±0.77	

a) Data was expressed as means±S.E. (n=3).

signals due to an additional isoferuloyl moiety [δ_H 3.75 (3H, s), 6.89 (1H, d, J=8.0 Hz), 6.90 (1H, d, J=16.0 Hz), 7.09 (1H, dd, J=2.0, 8.0 Hz), 7.54 (1H, d, J=2.0 Hz) and 8.10 (1H, d, J=16.0 Hz); δ_C 55.9, 112.1, 115.4, 116.4, 121.5, 128.6, 145.8, 148.4, 150.9 and 166.8].[4)] The location of the isoferuloyl moiety could be deduced by an HMBC experiment. Hence, in the HMBC spectrum, the correlation between H-2 (δ_H 5.77) of D-glucose[2] and the carbonyl carbon (δ_C 166.8) of isoferuloyl moiety was observed, suggesting that the isoferuloyl moiety was attached at the C-2 position of D-glucose[2]. Based on the above evidence, the structure of **8** was established as 3-*O*-β-D-glucopyranosyl(1→3)-[(2-*O*-isoferuloyl)-β-D-glucopyranosyl]-(1→4)-β-D-glucopyranosyl(1→4)-β-D-ribopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl olean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl ester.

Compounds **9**–**19** were identified as clematernoside E (**9**), huzhangoside D (**10**), clematiganoside A (**11**), huzhangoside B (**12**), huzhangoside C (**13**), clematichinenoside C (**14**), clemastanoside D (**15**), hederasaponin B (**16**), CP_7 (**17**), CP_6 (**18**) and CP_4 (**19**), respectively, by comparison of their spectral data with literature values.[16,17,19–23)]

The cytotoxic activities of all the saponins, as well as their aglycones oleanolic acid and hederagenin against Caski cells were evaluated in this study. Aglycones and most of the saponins did not show obvious effects under the higher concentration of 80 μM with the exception of compounds **13**, **17** and **19** (Table 3). Compounds **13**, **17** and **19** displayed potent effect with IC_{50} values of 9.05±3.61 μM, 26.07±1.47 μM and 2.97±1.07 μM, respectively. Herein, cisplatin was used as a positive control with IC_{50} value of 3.37±0.77 μM. These results suggested that some saponins isolated from *Clematis uncinata* might show better antitumor activities on Caski cells than their aglycones. Considering the insufficient samples size of our current investigation, the structure–activity relationship of these compounds was still an open question, which need to be solved in the further study.

Experimental

Optical rotations were measured on a JASCO P-1020 digital polarimeter in a 0.1 dm length cell at room temperature. UV spectra were obtained on a JASCO-V-550 UV/VIS spectrophotometer. IR spectra were recorded on a JASCO FT/IR-480 plus infrared spectrometer. HR-ESI-MS data were obtained on an Agilent 6210 ESI/time-of-flight (TOF) or a Waters Xevo-G2 QTOF mass spectrometer. ^{1}H-, ^{13}C-, and 2D-NMR experiments were performed on Bruker AV-400 or Bruker AV-500 spectrometer using pyridine-d_5 as solvent with tetramethylsilane (TMS) as internal standard. Analytical HPLC was performed on an Agilent 1260 chromatography equipped with a G1311C pump, a G1315D photodiode array detector and a Cosmosil 5C_{18}-MS-II Waters column (4.6×250 mm, 5 μm, Nacalai Tesque Inc., Kyoto, Japan). Preparative HPLC was carried on an Agilent 1260 chromatography equipped with a G1310B pump, a G1365D detector and a Cosmosil 5C_{18}-MS-II Waters column (20×250 mm, 5 μm, Nacalai Tesque Inc., Kyoto, Japan). Column chromatographies were performed on silica gel (300–400 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China), D101 macroporous resin (Tianjin Chemical Co., Ltd., Tianjin, China), MCI gel (75–150 μm, Mitsubishi Chemical Co., Tokyo, Japan), and ODS (40–64 μm, Merck, Darmstadt, Germany) columns. TLC was performed using precoated silica gel GF_{254} plates (Yantai Chemical Industry Research Institute, Yantai, China).

Plant Material The roots of *C. uncinata* were collected in Tianmu Mountain, Zhejiang province of P.R. China, in August of 2008 and authenticated by Prof. Guang-xiong Zhou (Institute of Traditional Chinese Medicine & Natural Products, Jinan University). A voucher specimen (No. 2008082301) was deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, P. R. China.

Extraction and Isolation The air-dried roots of *C. uncinata* (2.0 kg) were pulverized and extracted with 70% (v/v) EtOH under reflux (2×4 L) twice (1.5 h each). The solution was concentrated under vacuum to yield a crude extract (258 g). The EtOH extract was redissolved in water and then subjected to D101 macroporous resin column eluted successively with H_2O, 10% EtOH, 30% EtOH, 50% EtOH, and 70% EtOH.

The 50% EtOH eluate (74 g) was subjected to a MCI gel column eluted with gradient mixtures of MeOH–H_2O (3 : 7, 5 : 5, 7 : 3, v/v) to give three fractions (Fractions 1–3). Fraction 1 (5 g) was separated by ODS column (5×40 cm) with MeOH–H_2O (15 : 85, 20 : 80, 25 : 75, 30 : 70, 35 : 65, 40 : 60, 50 : 50) as eluent to afford three subfractions (1A–C). Subfraction 1A (0.4 g) was isolated by preparative HPLC (CH_3CN–H_2O, 30 : 70, 6.0 mL/min) to yield compounds **2** (19 mg,

t_R=17.8 min) and **3** (34 mg, t_R=20.1 min). Subfraction 1B (0.8 g) was also isolated by preparative HPLC (CH_3CN–H_2O, 30 : 70, 6.0 mL/min) to yield compounds **10** (35 mg, t_R=25.5 min) and **11** (42 mg, t_R=33.3 min). Fraction 2 (12 g) was separated by ODS column eluted with gradient mixtures of MeOH–H_2O (25 : 75, 30 : 70, 35 : 65, 40 : 60, 50 : 50, 60 : 40) to afford six subfractions (2A–F). Subfraction 2A (1.4 g) was separated by preparative HPLC on a reversed-phase C_{18} column using CH_3CN–H_2O (30 : 70, 6.0 mL/min) as eluent to yield compounds **7** (40 mg, t_R=52.1 min), **8** (36 mg, t_R=57.2 min), and **9** (32 mg, t_R=63.6 min). Subfraction 2C (2.9 g) was also separated by preparative HPLC (CH_3CN–H_2O, 32 : 68, 6.0 mL/min) to yield compounds **4** (27 mg, t_R=42.3 min), **5** (31 mg, t_R=38.4 min), and **6** (36 mg, t_R=45.2 min), respectively. Subfraction 2E (2.7 g) was isolated successively by preparative HPLC (CH_3OH–H_2O, 68 : 32, 6.0 mL/min) to yield compounds **1** (34 mg, t_R=32.5 min) and **14** (58 mg, t_R=27.2 min). Subfraction 2F (1.6 g) was isolated by preparative HPLC (CH_3OH–H_2O, 68 : 32, 6.0 mL/min) to yield compounds **12** (160 mg, t_R=35.5 min) and **13** (65 mg, t_R=38.2 min). Fraction 3 (7 g) was isolated by ODS column using MeOH–H_2O (30 : 70, 35 : 65, 40 : 60, 45 : 55, 50 : 50, 60 : 40) as eluent to give three subfractions (3A–C). Subfractions 3A (1.2 g) and 3B (4.3 g) were respectively isolated by preparative HPLC (CH_3CN–H_2O, 35 : 65, 6.0 mL/min) to yield compounds **15** (24 mg, t_R=52.3 min) and **16** (33 mg, t_R=45.6 min).

The 70% EtOH eluate (23 g) was separated by silica gel column (15×60 cm) using gradient mixtures of $CHCl_3$–CH_3OH–H_2O (9 : 1 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5) as eluent to give five fractions (Fractions A–E). Fraction A (0.7 g) was isolated by preparative HPLC (CH_3CN–0.05% TFA, 45 : 55, 6.0 mL/min) to yield compound **19** (42 mg, t_R=25.4 min). Fraction C (1.5 g) was also isolated by preparative HPLC (CH_3CN–0.05% TFA, 45 : 55, 6.0 mL/min) to yield compounds **17** (18 mg, t_R=25.1 min) and **18** (16 mg, t_R=28.1 min), respectively.

Clematiunicinoside A (**1**): Amorphous powder; $[\alpha]_D^{20}$ −46.6 (c=0.10, MeOH); IR ν_{max} (KBr) cm^{-1}: 3412, 2936, 1734, 1642, 1065; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.86 (3H, s, 25-CH_3), 0.88 (3H, s, 30-CH_3), 0.88 (3H, s, 29-CH_3), 1.07 (3H, s, 26-CH_3), 1.16 (3H, s, 24-CH_3), 1.24 (3H, s, 27-CH_3), 1.30 (3H, s, 23-CH_3), 3.40 (1H, dd, J=2.7, 11.5 Hz, 3-H), 5.40 (1H, brs, 12-H). ^{1}H-NMR data of sugar moieties and ^{13}C-NMR data, see Tables 1 and 2; HR-ESI-MS m/z: 1497.7111 $[M-H]^-$ (Calcd for $C_{70}H_{113}O_{34}$, 1497.7118).

Clematiunicinoside B (**2**): Amorphous powder; $[\alpha]_D^{20}$ −35.6 (c=0.13, MeOH); IR ν_{max} (KBr) cm^{-1}: 3410, 2936, 1733, 1642, 1064; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.85 (3H, s, 29-CH_3), 0.86 (3H, s, 30-CH_3), 0.94 (3H, s, 25-CH_3), 1.09 (3H, s, 26-CH_3), 1.14 (3H, s, 24-CH_3), 1.18 (3H, s, 27-CH_3), 4.23 (1H, overlapped, 3-H), 3.91 (1H, t, J=8.5 Hz, 23-H_b), 4.27 (1H, overlapped, 23-H_a), 5.39 (1H, brs, 12-H); ^{1}H-NMR data of sugar moieties and ^{13}C-NMR data, see Tables 1 and 2; HR-ESI-MS m/z: 1513.7051 $[M-H]^-$ (Calcd for $C_{70}H_{113}O_{35}$, 1513.7062).

Clematiunicinoside C (**3**): Amorphous powder; $[\alpha]_D^{20}$ −40.5 (c=0.10, MeOH); IR ν_{max} (KBr) cm^{-1}: 3411, 2936, 1737, 1643, 1385, 1063; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.90 (3H, s, 25-CH_3), 1.04 (3H, s, 30-CH_3), 1.12 (3H, s, 26-CH_3), 1.15 (3H, s, 24-CH_3), 1.21 (3H, s, 29-CH_3), 1.29 (3H, s, 23-CH_3), 1.37 (3H, s, 27-CH_3), 3.30 (1H, dd, J=4.0, 11.6 Hz, 3-H), 3.68 (1H, brs, 21-H), 5.50 (1H, brs, 12-H); ^{1}H-NMR data of sugar moieties and ^{13}C-NMR data, see Tables 1 and 2; HR-ESI-MS m/z: 1351.6510 $[M-H]^-$ (Calcd for $C_{64}H_{103}O_{30}$, 1351.6534).

Clematiunicinoside D (**4**): Amorphous powder; $[\alpha]_D^{20}$ −54.6 (c=0.10, MeOH); IR ν_{max} (KBr) cm^{-1}: IR (KBr) ν_{max} 3412, 2937, 1644, 1067; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.86 (3H, s, 25-CH_3), 0.88 (3H, s, 30-CH_3), 0.88 (3H, s, 29-CH_3), 1.05 (3H, s, 26-CH_3), 1.20 (3H, s, 24-CH_3), 1.23 (3H, s, 27-CH_3), 1.34 (3H, s, 23-CH_3), 3.30 (1H, dd, J=4.0, 11.5 Hz, 3-H), 5.38 (1H, brs, 12-H); ^{1}H-NMR data of sugar moieties and ^{13}C-NMR data, see Tables 1 and 2; HR-ESI-MS m/z: 1497.7109 $[M-H]^-$ (Calcd for $C_{70}H_{113}O_{34}$, 1497.7113).

Clematiunicinoside E (**5**): Amorphous powder; $[\alpha]_D^{20}$ −47.0 (c=0.10, MeOH); IR ν_{max} (KBr) cm^{-1}: 3413, 2936, 1643, 1065; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.84 (3H, s, 25-CH_3), 0.87 (3H, s, 30-CH_3), 0.88 (3H, s, 29-CH_3), 1.06 (3H, s, 26-CH_3), 1.12 (3H, s, 24-CH_3), 1.23 (3H, s, 27-CH_3), 1.26 (3H, s, 23-CH_3), 3.26 (1H, dd, J=3.5, 11.5 Hz, 3-H), 5.39 (1H, brs, 12-H); ^{1}H-NMR data of sugar moieties and ^{13}C-NMR data, see Tables 1 and 2; HR-ESI-MS m/z: 1659.7601 $[M-H]^-$ (Calcd for $C_{76}H_{123}O_{39}$, 1659.7641).

Clematiunicinoside F (**6**): Amorphous powder; $[\alpha]_D^{20}$ −37.5 (c=0.10, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400, 2935, 1644, 1067; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.86 (3H, s, 25-CH_3), 0.88 (3H, s, 30-CH_3), 0.88 (3H, s, 29-CH_3), 1.07 (3H, s, 26-CH_3), 1.13 (3H, s, 24-CH_3), 1.24 (3H, s, 27-CH_3), 1.28 (3H, s, 23-CH_3), 3.27 (1H, dd, J=3.5, 11.0 Hz, 3-H), 5.39 (1H, brs, 12-H); ^{1}H-NMR data of sugar moieties and ^{13}C-NMR data see, Tables 1 and 2; HR-ESI-MS m/z: 1821.8112 $[M-H]^-$ (Calcd for $C_{82}H_{133}O_{44}$, 1821.8170).

Clematiunicinoside G (**7**): Amorphous powder; $[\alpha]_D^{20}$ −41.75 (c=0.10, MeOH); IR ν_{max} (KBr) cm^{-1}: 3414, 2935, 1735, 1643, 1064; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.86 (3H, s, 25-CH_3), 0.89 (3H, s, 30-CH_3), 0.89 (3H, s, 29-CH_3), 1.07 (3H, s, 26-CH_3), 1.13 (3H, s, 24-CH_3), 1.24 (3H, s, 27-CH_3), 1.28 (3H, s, 23-CH_3), 3.27 (1H, dd, J=3.5, 11.5 Hz, 3-H), 5.40 (1H, brs, 12-H); ^{1}H-NMR data of sugar moieties and ^{13}C-NMR data, see Tables 1 and 2; HR-ESI-MS m/z: 1967.8811 $[M-H]^-$ (Calcd for $C_{88}H_{143}O_{48}$, 1967.8749).

Clematiunicinoside H (**8**): Amorphous powder; $[\alpha]_D^{20}$ −52.8 (c=0.16, MeOH); UV λ_{max} (MeOH) nm (logε): 244 (3.59), 296 (3.92), 326 (4.05); IR ν_{max} (KBr) cm^{-1}: 3413, 2936, 1715, 1633, 1450, 1264, 1064; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.85 (3H, s, 25-CH_3), 0.88 (3H, s, 30-CH_3), 0.88 (3H, s, 29-CH_3), 1.06 (3H, s, 26-CH_3), 1.12 (3H, s, 24-CH_3), 1.24 (3H, s, 27-CH_3), 1.26 (3H, s, 23-CH_3), 3.26 (1H, dd, J=3.0, 11.0 Hz, 3-H), 3.75 (3H, s, OCH_3), 5.39 (1H, brs, 12-H), 6.89 (1H, d, J=8.0 Hz, 5′-H), 6.90 (1H, d, J=16.0 Hz, 8′-H), 7.09 (1H, dd, J=2.0, 8.0 Hz, 6′-H), 7.54 (1H, d, J=2.0 Hz, 2′-H), 8.10 (1H, d, J=16.0 Hz, 7′-H); ^{13}C-NMR data of isoferuloyl moiety (pyridine-d_5, 100 MHz) δ: 55.9 (OCH_3), 112.1 (C-5′), 115.4 (C-2′), 116.4 (C-8′), 121.5 (C-6′), 128.6 (C-1′), 145.8 (C-7′), 148.4 (C-4′), 150.9 (C-3′), 166.8 (C-9′); ^{1}H-NMR data of sugar moieties, and ^{13}C-NMR data of aglycone and sugar moieties, see Tables 1 and 2; HR-ESI-MS m/z: 1997.8654 $[M-H]^-$ (Calcd for $C_{92}H_{141}O_{47}$, 1997.8649).

Acid Hydrolysis of Compounds 1–8 and Determination of the Absolute Configurations of Monosaccharides Each solution of compounds **1**–**8** (each 10 mg) in 2 mol/L HCl (10 mL) was heated in water bath (80°C) for 4 h. The solution was evaporated with a stream of N_2. Each residue was dissolved in pyridine (2.0 mL), and stirred with L-cysteine methyl

ester hydrochloride (10 mg) for 1 h at 60°C, and then *O*-tolyl isothiocyanate (15 μL) was added to the mixture and heated at 60°C for another 1 h. The reaction mixtures were analyzed by HPLC and detected at 250 nm. Analytical HPLC was performed on a Cosmosil 5C_{18}-MS-II column (4.6×250 mm, 5 μm) at 20°C using CH_3CN–0.05% CH_3COOH (25 : 75, 0.8 mL/min) as the solvent. Peaks were detected with a G1315D photodiode array detector. The absolute configurations of the monosaccharides were confirmed to be D-glucose, D-xylose, D-ribose, L-arabinose and L-rhamnose by comparison of the retention times of monosaccharide derivatives with those of standard samples: D-glucose (t_R 19.98 min), L-glucose (t_R 18.29 min), D-xylose (t_R 23.41 min), L-xylose (t_R 21.81 min), D-ribose (t_R 24.12 min), L-ribose (t_R 16.49 min), D-arabinose (t_R 24.31 min), L-arabinose (t_R 22.43 min), L-rhamnose (t_R 33.82 min), and D-rhamnose (using D-cysteine methyl ester and L-rhamnose, t_R 17.82 min), respectively.

Cytotoxic Assay The cytotoxicity activities of the compounds on Caski cells were tested with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described in the previous report.[24] Briefly, compounds **1**–**19**, oleanolic acid and hederagenin (Guangzhou Qiyun Biotechnology Co., Ltd., Guangzhou, China) were dissolved in dimethyl sulfoxide (DMSO) and diluted in the culture medium to the concentrations ranging from 0.3125–80 μM. Caski cells (6×10^3 cells/well) were cultured in 96-well plates for 24 h. The medium was replaced by the medium with the different concentrations of compounds and cultured for another 48 h. MTT solution was then added in the medium for coloration. The absorption value was recorded by the Microplate reader (Thermo, U.S.A.) at a wavelength of 570 nm. Cell viability was calculated according to the following formula: cell viability (%)=OD (compound)/OD (control)×100. IC_{50} values were obtained from cell viability plots fitted with a sigmoidal dose-response function with variable slope using Origin 8 software. Cisplatin was used as a positive control.

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