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TRITERPENOID SAPONINS FROM *CLEMATIS CHINENSIS*

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Key Word Index—*Clematis chinensis*; Ranunculaceae; triterpenoid saponins; clematichinenosides, A and B.

Abstract—From the roots of *Clematis chinensis*, two new triterpenoid saponins, named clematichinenoside A and B, were isolated and their structures were elucidated as 3-*O*-*β*-D-ribopyranosyl-(1–3)-α-L-rhamnopyranosyl-(1–2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester and 3-*O*-*β*-D-glucopyranosyl-(1–4)-*β*-D-ribopyranosyl-(1–3)-α-L-rhamnopyranosyl-(1–2)-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl-(1–4)-*β*-D-glucopyranosyl-(1–6)-*β*-D-glucopyranosyl ester, respectively. In addition oleanolic acid, hederagenin and seven known saponins were obtained.

INTRODUCTION

Clematis chinensis Osbeck is a liana distributed widely in southern China. Its root is a source of the Chinese crude drug 'Wei-Ling-Xian' which has been recorded in Chinese Pharmacopoeia (1990 edn). It has commonly been used as an analgesic, diuretic and antiinflammatory agent [1]. Although a number of chemical constituents has been isolated from this plant, the identities of the bioactive compounds remain unknown. Two lactones, anemonin and protoanemonin, were obtained and considered as its antiinflammatory compounds, but recent pharmacological studies showed that such effects were very weak. As the plant roots are rich in saponins, the saponins have been systematically studied by Japanese chemists since 1979. As a result of their studies on the plant root, more than 20 prosapogenins were isolated from the alkaline hydrolysate of its crude saponins and among them six less polar prosapogenins were proved to be present in the root as genuine saponins by HPTLC detection [2–6]. However, these reported saponins and prosapogenins are all monodesmosides. To promote fundamental research on this Chinese crude drug, we have attempted to elucidate the chemical constituents of the roots of *Clematis chinensis*. In this paper, we report the isolation and structural elucidation of two new triterpenoid saponins named clematichinenoside A (**3**) and B (**10**), together with oleanolic acid (**1**), hederagenin (**5**) and seven known saponins (**2**, **4**, **6**–**9** and **11**). Saponins **6** and **8** are reported for the first time to be present in this plant. The existence of saponins **2**, **4**, **7**, **9** and **11** in the crude drug was determined on the basis of HPTLC detection.

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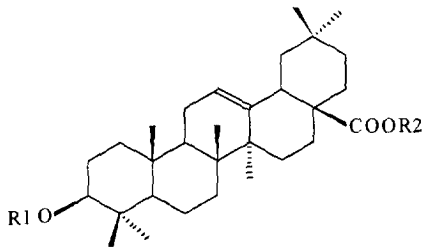
RESULTS AND DISCUSSION

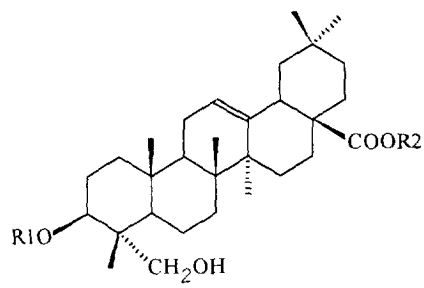
The butanol-soluble fraction obtained from ethanol extracts of the roots of *Clematis chinensis* was subjected to repeated chromatography on silica gel and RP-18 Lobar columns to give two triterpenoids **1** and **5**, and nine saponins **2**–**4** and **6**–**11**. Triterpenoids **1** and **5** were identified as oleanolic acid and hederagenin, respectively, by direct comparison with authentic samples.

Saponin **3**, an amorphous powder, mp 198–200°, showed positive reactions to Liebermann-Burchard and Molish tests. The FAB mass spectrum revealed a molecular ion peak at *m/z* 1051, corresponding to $[M\ (C_{52}H_{84}O_{20}) + Na]^+$. On acid hydrolysis (0.5 N H_2SO_4), **3** afforded oleanolic acid as the aglycone and arabinose, glucose, ribose and rhamnose as the sugar components, identified by co-TLC. In the ^{13}C NMR spectrum the signals due to the aglycone moiety were in good agreement with those of the 28-glycosyl ester of 3-*O*-glycosyl oleanolic acid (Table 1). Consequently **3** was considered as a bidesmoside.

The ^1H NMR spectrum of **3** exhibited four anomeric proton signals at δ6.30 (*d*, $J = 8.2$), 4.84 (*d*, $J = 6.1$), 6.31 (*br s*), 5.96 (*d*, $J = 4.5$ Hz) and a methyl signal of a rhamnose unit at δ1.54 (*d*, $J = 6.2$ Hz), respectively. The proton system of each sugar unit was analysed by combined use of DQF-COSY and TOCSY experiments at 600 MHz. Starting from signals of anomeric protons and a methyl of rhamnose unit, the chain of coupled protons could be followed by a sequential 'walk' via observed cross-peaks. All correlation signals and the sequence of protons in each corresponding residue were then deduced. A ^1H–^{13}C one-bond chemical shift correlation experiment (HMQC) correlated all proton resonances with those of the corresponding carbons in each sugar



	R1	R2
1.	H	H
2.	-α-L-ara(2-1)-α-L-rha(3-1)-β-D-rib	H
3.	-α-L-ara(2-1)-α-L-rha(3-1)-β-D-rib	-β-D-glc
4.	-α-L-ara(2-1)-α-L-rha(3-1)-β-D-rib (4-1)-β-D-glc	H



	R1	R2
5.	H	H
6.	H	-β-D-glc(6-1)-β-D-glc(4-1)-α-L-rha
7.	-α-L-ara(2-1)-α-L-rha(3-1)-β-D-rib	H
8.	-α-L-ara(2-1)-α-L-rha(3-1)-β-D-rib	-β-D-glc(6-1)-β-D-glc(4-1)-α-L-rha
9.	-α-L-ara(2-1)-α-L-rha(3-1)-β-D-rib (4-1)-β-D-glc	H
10.	-α-L-ara(2-1)-α-L-rha(3-1)-β-D-rib (4-1)-β-D-glc	-β-D-glc(6-1)-β-D-glc(4-1)-α-L-rha
11.	-α-L-ara(2-1)-α-L-rha(3-1)-β-D-rib (4-1)-β-D-glc(4-1)-β-D-glc	H

unit. Comparison of ^{13}C assignments with those of reference methyl glycosides revealed the presence of a terminal ester β-D-glucopyranosyl unit, a terminal β-D-ribopyranosyl unit, a 3-substituted α-L-rhamnopyranosyl unit and a 2-substituted α-L-arabinopyranosyl unit. The proton and carbon signals of each sugar unit were assigned unambiguously as shown in Tables 2 and 3. The ^{13}C NMR signals due to C-28 of the genin moiety (δ176.4) and terminal ester β-D-glucopyranosyl unit indicated esterification of the carboxyl group with a glucose. The remaining three sugars must be present as a chain connected with the C-3 hydroxyl group. Information about the sequence of the oligosaccharide chain and the linkage sites to the aglycone was obtained by spatial correlation between two protons in a NOESY experiment as well by the scalar coupling between a carbon and a proton of the neighbouring residues in an HMBC experiment. The NOESY spectrum of **3** showed cross-peaks between signals at δ3.28 (H-3α of genin)/4.84 (H-1 of arabinose); 4.56 (H-2 of arabinose)/6.31 (H-1 of rhamnose) and 4.75 (H-3 of rhamnose)/5.96 (H-1 of ribose). Meanwhile the HMBC spectrum revealed cross-peaks between signals at δ176.4 (C-28 of genin)/ 6.30 (H-1 of glucose); 88.7 (C-3 of genin)/4.84 (H-1 of arabinose); 75.3 (C-2 of arabinose)/6.31 (H-1 of rhamnose) and 81.3 C-3 of rhamnose)/5.96 (H-1 of ribose). In some cases, the determination of configuration of the ribose unit in the polysaccharide was confusing. Except for interaction between H-1 (δ5.96) and H-2 (δ4.31) in the NOESY spectrum of **3**, there were no cross-peaks between the H-1 signal and other protons in the ribose unit. Compared with the oligosaccharide chain of other saponins found in this plant, β-D-ribopyranoside (with 1C form) was established. On the basis of the evidence (Fig. 1), the structure of saponin **3** was deduced to be 3-*O*-β-D-ribopyranosyl (1–3)-α-L-rhamnopyranosyl (1–2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester, which was named clematichinenoside A.

Table 1. ^{13}C NMR data for genin moieties of saponins **2–4** and **6–11** in (C_5D_5N)

C	2	3	4	6	7	8	9	10	11
1	38.4	38.9	38.4	38.9	38.8	38.8	38.6	39.5	39.1
2	26.2	26.6	26.1	27.7	26.1	26.2	25.8	26.5	26.4
3	88.5	88.7	88.3	73.9	81.2	81.0	81.4	81.2	81.2
4	39.3	39.5	39.1	42.9	43.5	43.8	43.1	43.4	43.7
5	55.6	56.0	55.5	48.6	47.5	47.5	47.3	48.0	47.9
6	18.1	18.6	18.0	18.5	18.0	17.9	17.7	18.4	18.3
7	32.6	32.3	32.8	32.9	32.4	32.9	32.4	33.0	33.0
8	39.5	40.0	39.3	39.9	39.5	38.7	39.3	40.2	40.0
9	48.0	48.1	47.6	48.2	47.9	48.0	47.7	48.4	48.3
10	36.7	37.1	36.6	37.2	36.5	36.7	36.4	37.1	37.0
11	23.3	23.5	23.2	23.9	23.8	23.6	23.5	23.9	23.9
12	122.6	122.8	122.3	123.2	122.5	122.7	122.1	122.9	123.0
13	144.1	144.1	144.0	144.1	144.4	143.9	144.4	144.2	144.3
14	42.0	42.1	41.7	42.2	41.9	41.9	41.7	42.4	42.3
15	27.9	28.2	27.8	28.3	28.0	28.1	27.9	28.6	28.5
16	23.2	23.8	23.3	23.4	23.5	23.1	23.3	23.6	23.9
17	47.0	47.0	46.2	47.0	46.5	46.8	46.2	47.3	46.6
18	41.5	41.7	41.5	41.7	41.8	41.4	41.6	41.9	42.2
19	46.1	46.2	46.0	46.2	46.4	45.9	46.0	46.4	46.6
20	30.5	30.7	30.5	30.7	30.3	30.5	30.5	31.0	31.0
21	33.7	34.0	33.7	34.0	33.5	33.7	33.8	34.2	34.4
22	32.8	33.1	32.8	32.5	33.0	32.5	32.8	32.8	33.3
23	28.0	28.2	27.7	67.9	64.0	63.7	63.6	64.0	64.2
24	17.0	17.1	16.6	13.1	13.8	14.0	13.5	14.2	14.0
25	15.1	15.6	15.1	16.1	16.0	16.0	15.6	16.4	16.2
26	17.1	17.4	17.0	17.6	17.5	17.3	17.0	17.8	17.6
27	26.0	26.1	25.7	26.1	26.2	25.9	25.6	26.3	26.3
28	181.1	176.4	180.5	176.5	181.0	176.3		176.8	181.0
29	33.0	33.1	32.8	33.1	32.8	32.9	32.8	33.3	33.3
30	23.4	23.6	23.3	23.7	23.9	23.5	23.3	23.9	23.9

Saponin **10**, amorphous powder, mp 227–230° showed positive reactions to Liebermann-Burchard and Molish tests. The FAB mass spectrum revealed a molecular ion peak at *m/z* 1538 corresponding to $[M(C_{70}H_{114}O_{35}) + Na + H]^+$. On acid hydrolysis (0.5 N H_2SO_4) **10** afforded hederagenin as the aglycone and arabinose, glucose, ribose and rhamnose as the sugar components, identified by co-TLC. Thus, **10** was a bidesmoside. The carbon signals arising from the aglycone moiety were in good agreement with those of the 28-glycosyl ester of 3-*O*-glycosyl hederagenin (Table 1).

The ^1H NMR spectrum of **10** showed seven anomeric protons at δ5.83 (*br s*), 4.98 (*d*, J = 7.7 Hz, overlapped two protons), 5.06 (*d*, J = 6.5 Hz), 5.81 (*d*, J = 5.1 Hz), 6.21 (*d*, J = 8.1 Hz) and 6.28 (*br s*), and two methyls due to rhamnose units at δ1.54 (*d*, J = 6.0 Hz) and 1.68 (*d*, J = 6.1 Hz). On alkaline hydrolysis **10** yielded a prosapogenin identified as CP_8 (3-*O*-β-D-glucopyranosyl (1–4)-β-D-ribopyranosyl-(1–3)-α-L-rhamnopyranosyl-(1–2)-α-L-arabinopyranosyl hederagenin) [2] along with glucose and rhamnose as sugar components (co-TLC). Considering the above evidence and the molecular formula, **10** contained three glucose, two rhamnose, one arabinose and one ribose units, in which two glucose and one rhamnose units connected with the C-17 carboxyl, and the remaining units linked with the C-3 hydroxyl.

All proton signals of each sugar unit were assigned by combined use of DQF-COSY and TOCSY experiments in the above described manner. Due to the coincidence of two anomeric proton signals at δ4.98 we had little difficulty in distinguishing between these two sugar units. However, we found two isolated one-proton signals at δ3.66 and 4.39 which correlated with an anomeric proton signal at δ4.98 in the TOCSY spectrum. Further studies on NOESY and DQF-COSY spectra showed the signals at δ3.66 and 4.39 should be H-5 and H-4 of the same glucose unit. Consequently, a TOCSY spectrum of **10** was further studied to discover the relayed correlations among protons more than four bonds away. As a result, the correlations from H-5 to H-1 [H-5 (3.66) → H-4 (4.39) → H-3 (4.13) → H-2 (3.91) → H-1 (4.98)] and from H-5 to H-6 [H-5 (3.66) → H-6 (4.07, 4.20)] were clearly displayed. After arranging one glucose unit, another sugar unit could easily be assigned starting from the coincident anomeric proton signal by various spectral analyses. Therefore all correlation signals and the sequence of protons in each corresponding residue were deduced, as well as all corresponding carbons which were assigned from a HMQC spectrum. Comparison of the ^{13}C assignments with those of reference methyl glycosides revealed the presence of a terminal β-D-glucopyranosyl unit, a terminal α-L-rhamnopyranosyl unit, a 2-sub-

Table 2. ^{13}C NMR data for sugar moieties of saponins **2–4** and **6–11** (in C_5D_5N)

Sugar	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**	**11**
3-*O*-sugar									
ara 1	105.1	105.4	104.6		104.5	104.6	104.4	104.6	104.2
2	75.6	75.3	75.4		75.4	75.3	75.7	75.3	75.9
3	74.5	75.0	74.2		75.0	74.9	74.7	75.1	74.3
4	69.0	69.5	68.5		69.6	68.7	69.1	69.6	69.9
5	65.2	66.0	64.8		66.1	66.3	65.8	66.4	65.8
rha 1	101.4	101.4	101.1		101.3	101.1	101.4	101.3	101.6
2	71.9	72.1	71.4		71.9	71.8	71.8	71.8	71.6
3	81.2	81.3	81.5		81.2	80.9	81.0	82.0	82.0
4	72.7	72.8	72.3		72.7	72.5	72.7	72.7	72.7
5	70.1	69.9	69.4		69.6	69.7	69.4	69.0	96.4
6	18.4	18.5	18.0		18.4	18.3	18.3	18.4	18.5
rib 1	104.5	104.6	104.2		104.5	104.6	103.4	104.7	103.1
2	72.7	72.8	72.0		72.7	72.5	72.3	72.5	72.5
3	70.1	69.0	68.8		70.2	70.1	69.7	69.7	69.4
4	69.0	70.3	76.1		68.7	69.6	76.5	76.4	76.4
5	65.2	65.3	61.3		65.2	65.0	61.8	61.7	61.7
gle 1			103.0				104.5	103.4	104.9
2			73.7				74.8	75.3	74.6
3			78.1				78.5	78.2	76.6
4			71.1				71.5	71.4	81.0
5			77.9				78.3	78.6	76.7
6			62.2				62.5	62.4	62.0
									104.6
									74.8
									78.5
									71.9
									78.3
									62.5
28-*O*-sugar									
glc 1		95.8		95.8		95.4		95.5	
2		74.1		73.9		73.7		73.8	
3		78.9		78.7		77.9		78.6	
4		71.0		70.9		70.6		70.7	
5		79.4		78.0		76.9		77.9	
6		62.1		69.2		68.9		69.0	
glc 1				104.9		104.5		104.7	
2				75.3		75.1		74.6	
3				76.5		76.3		76.3	
4				78.3		78.5		78.0	
5				72.2		77.8		77.1	
6				61.3		61.0		61.1	
rha 1				102.7		102.5		102.6	
2				72.6		72.5		72.4	
3				72.8		72.3		72.7	
4				74.0		73.6		73.9	
5				70.3		70.1		70.2	
6				18.5		18.3		18.4	

stituted α-L-arabinopyranosyl unit, a 3-substituted α-L-rhamnopyranosyl unit, a 4-substituted β-D-glucopyranosyl unit, a 4-substituted β-D-ribopyranosyl unit, a 6-substituted β-D-glucopyranosyl unit. The proton and carbon signals were assigned as presented in Tables 2 and 3.

The sequencing of the sugar chains was clarified using NOESY and HMBC experiments. The NOESY spectrum of **10** showed cross-peaks between signals at δ4.27 (H-3α of genin)/5.06 (H-1 of arabinose); 4.55 (H-2 of arabinose)/6.28 (H-1 of rhamnose); 4.68 (H-3 of rhamnose)/5.81 (H-1 of ribose); 4.35 (H-4 of ribose)/4.98 (H-1 of glucose); 4.30, 4.66 (H_2-6 of glucose)/4.98 (H-1 of glucose) and 4.39 (H-4 of glucose)/5.83 (H-1 of rhamnose). The above results were supported by an HMBC experiment in which the following cross-peaks were observed: δ176.4 (C-28 of genin)/6.21 (H-1 of glucose); 4.30, 4.66 (H_2-6 of glucose)/104.7 (C-1 of glucose); 78.0 (C-4 of

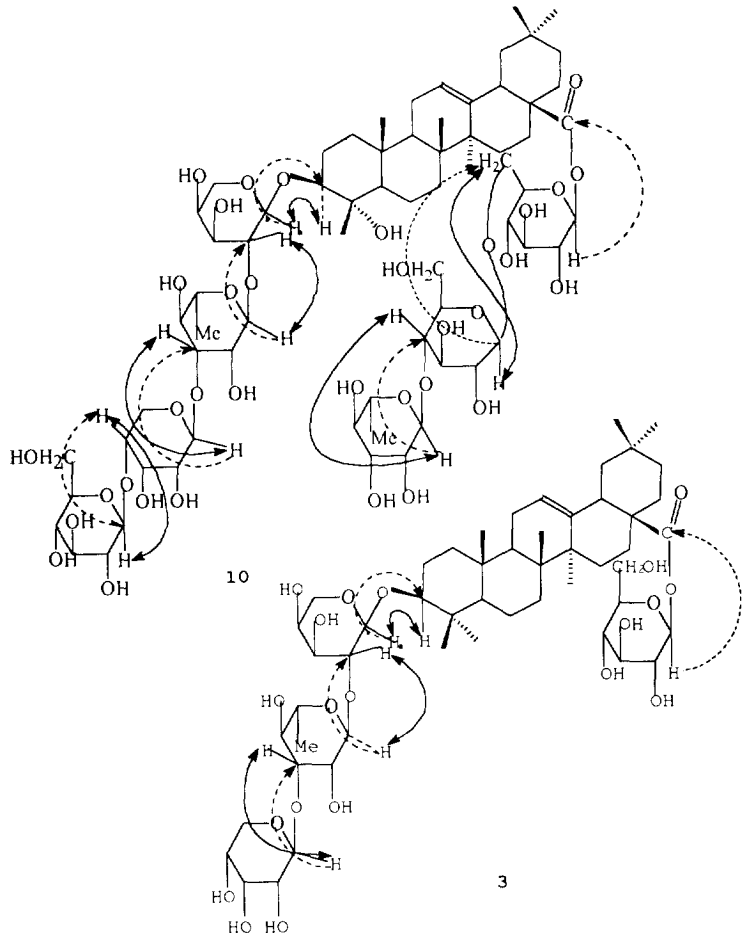


Fig. 1. NOE correlation (in actual line) and ^{13}C–^{1}H long range correlation (in dotted line) of **3** and **10**.

glucose)/5.83 (H-1 of rhamnose); 80.9 (C-3 of genin)/5.06 (H-1 of arabinose); 4.55 (H-2 of arabinose)/101.3 (C-1 of rhamnose); 82.0 (C-3 of rhamnose)/5.81 (H-1 of ribose) and 4.35 (H-4 of ribose)/103.4 (C-1 of glucose). On the basis of the above evidence (Fig. 1), the structure of saponin **10** was elucidated as 3-*O*-β-D-glucopyranosyl-(1–4)-β-D-ribopyranosyl-(1–3)-α-L-rhamnopyranosyl-(1–2)-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl-(1–4)-β-D-glucopyranosyl-(1–6)-β-D-glucopyranosyl ester named clematichinenoside B.

Saponins **2** and **4** were hydrolysed with mineral acid to yield oleanolic acid (**1**) as a common aglycone. For sugar components, **2** afforded arabinose, rhamnose, ribose and **4** afforded additional glucose besides the three sugars in **2** (TLC). In the ^{13}C NMR spectra of **2** and **4** (Tables 1 and 2) the C-3 signals at δ88.5 and 88.3, and C-28 signals at 181.1 and 180.5, respectively, indicated that C-28 was free and C-3 was connected with sugar chains. By comparison with reported data **2** and **4** were identified with known saponins CP_4 (3-*O*-β-D-ribopyranosyl-(1–3)-α-L-rhamnopyranosyl-(1–2)-α-L-arabinopyranosyl oleanolic acid) and CP_7 (3-*O*-β-D-glucopyranosyl-(1–4)-β-D-ribopyranosyl-(1–3)-α-L-rhamnopyranosyl-(1–2)-α-L-arabinopyranosyl oleanolic acid), respectively.

Saponins **6**–**9** and **11** provided hederagenin (**5**) as a common aglycone on acid hydrolysis. From consideration of C-3 and C-28 chemical shifts in ^{13}C NMR spectra, **6** was a 28-glycosyl ester, **7**, **9** and **10** were 3-*O*-glycosides and **8** was a 28-glycosyl ester of the 3-*O*-glycoside, respectively. On alkaline hydrolysis **8** yielded **7**, and **10** yielded **9**, identified by co-TLC, IR and NMR. By comparison of their chemical and spectroscopic data (^{1}H and ^{13}C NMR) with the literature, **6** was identified as kizuta saponin K3 (hederagenin-28-*O*-α-L-rhamnopyranosyl-(1–4)-β-D-glucopyranosyl-(1–6)-β-D-glucopyranosyl ester) [7], **7** as saponin CP_6 (3-*O*-β-D-ribopyranosyl-(1–3)-α-L-rhamnopyranosyl-(1–2)-α-L-arabinopyranosyl hederagenin) [2, 8], **8** as huzhangoside D (3-*O*-β-D-ribopyranosyl-(1–3)-α-L-rhamnopyranosyl-(1–2)-α-L-arabinopyranosyl hederagenin 28-α-L-rhamnopyranosyl-(1–4)-β-D-glucopyranosyl-(1–6)-β-D-glucopyranosyl ester) [8], **9** as saponin CP_8 (3-*O*-β-D-glucopyranosyl-(1–4)-β-D-ribopyranosyl-(1–3)-α-L-rhamnopyranosyl-(1–2)-α-L-arabinopyranosyl hederagenin) [2] and **11** as

Table 3. ^{1}H NMR data of saponins **3** and **10** (in C_5D_5N)

Number	**3** ^{1}H NMR	Coupling	**10** ^{1}H NMR	Coupling
3-*O*-sugar				
ara 1	4.84 *d*	6.10	5.06 *d*	6.5
2	4.58 *dd*	6.1, 6.4	4.55 *dd*	6.5, 7.0
3	4.26 *dd*	6.4, 3.6	4.01 *dd*	7.0, 3.0
4	4.22 *br s*		4.14 *m*	
5	3.80 *br d*	10.4	3.67 *br d*	10.9
	4.33 *m*		4.24 *br d*	10.9
rha 1	6.31 *br s*		6.28 *br s*	
2	4.91 *br s*		4.84 *br s*	
3	4.75 *dd*	9.5, 3.0	4.68 *m*	
4	4.43 *t*	9.5	4.40 *t*	9.2
5	4.63 *dq*	9.5, 6.2	4.68 *m*	
6	1.54 *d*	6.2	1.54 *d*	6.0
rib 1	5.96 *d*	4.5	5.81 *d*	5.1
2	4.31 *m*		4.09 *m*	
3	4.51 *br s*		4.67 *m*	
4	4.17 *br s*		4.35 *m*	
5	4.15 *br d*	8.10	4.24 *br d*	10.9
	4.35 *br d*	8.10	4.30 *br d*	10.9
glc 1			4.98 *d*	7.7
2			3.91 *dd*	7.7, 8.8
3			4.20 *t*	8.8
4			4.20 *t*	8.8
5			3.90 *m*	
6			4.77 *br d*	9.9
			4.32 *br d*	9.9
28-*O*-sugar				
glc 1	6.30 *d*	8.2	6.21 *d*	8.1
2	4.21 *br d*	8.5	4.12 *m*	
3	4.30 *m*		4.18 *m*	
4	4.37 *m*		4.30 *m*	
5	4.06 *m*		4.12 *m*	
6	4.46 *dd*	12.0, 2.2	4.30 *m*	
	4.41 *dd*	12.0, 4.2	4.66 *m*	
glc 1			4.98 *d*	7.7
2			3.91 *dd*	7.7, 8.3
3			4.13 *m*	
4			4.39 *dd*	9.3, 9.3
5			3.66 *br d*	9.3
6			4.20 *br d*	9.0
			4.07 *m*	
rha 1			5.83 *br s*	
2			4.66 *br s*	
3			4.53 *dd*	9.0, 3.1
4			4.31 *t*	9.0
5			4.94 *dq*	9.0, 6.1
6			1.68 *d*	6.1

saponin CP_{10} (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 hederagenin) [4].

Saponins **6** and **8** were first isolated from this plant. Saponins **2**, **4**, **7**, **9** and **11** were previously found in this plant by HPTLC detection, but the presence of these known saponins was confirmed by column separation in our work.

EXPERIMENTAL

General. Mps: uncorr. ^{1}H, ^{13}C and 2D NMR spectra were recorded on Bruker AMX-600 and AM-400 spectrometers. Optical rotations were measured with a JASCO DIP-181 polarimeter. Mass spectra were determined on a VG QUATTRO GC/MS/MS spectrometer. IR spectra were taken as KBr pellets on a PE 599B spectrometer.

Plant material. The roots of *Clematis chinensis* Osbeck were collected in Fan-Chang County, Anhui province of eastern China, and identified by Ms Qian Bixuan of the herbarium of our institute.

Extraction and isolation of saponins. The dried and powered roots (7 kg) were percolated with 95% EtOH. The EtOH soln was evapd *in vacuo* to give concentrates, which were extracted with petrol, $CHCl_3$, EtOAc and *n*-BuOH, respectively. The *n*-BuOH extracts were chromatographed on a highly porous polymer eluted with a stepwise increase of EtOH content in H_2O (10, 30, 70, 90%). The 70% EtOH eluent was subjected to repeated Sephadex and RP-18 Lobar column chromatography to give **1** (25 mg), **2** (14 mg), **3** (11 mg) **4** (34 mg), **5** (8 mg), **6** (9 mg), **7** (15 mg), **8** (150 mg), **9** (40 mg), **10** (260 mg) and **11** (19 mg).

Clematichinenoside A (**3**). Amorphous powder, FAB-MS (*m/z*): 1051 $[M(C_{52}H_{84}O_{20}) + Na]^+$. Mp: 198–200°. $[\alpha]_D$ − 35.5° (pyridine; *c* 0.14). IR ν_{max}^{KBr} cm^{-1}: 3400 (*br*), 2940, 1730, 1640, 1070. ^{1}H, ^{13}C NMR data: see Tables 1–3.

Clematichinenoside B (**10**). Amorphous powder, FAB-MS (*m/z*): 1538 $[M(C_{70}H_{114}O_{35}) + Na + H]^+$. Mp 227–230°. $[\alpha]_D$ − 26.8° (pyridine; *c* 0.35), IR ν_{max}^{KBr} cm^{-1}: 3400 (*br*), 2940, 1730, 1640, 1070. ^{1}H and ^{13}C NMR (in pyridine-d_5) data: see Tables 1–3.

Saponins **2**, **4**, **6–9** *and* **11**. Physical constants and spectroscopic data were identical with the lit. data. ^{13}C NMR data: see Tables 1 and 2.

Acid hydrolysis of saponins. Each saponin was hydrolysed by heating in 0.5 N H_2SO_4 aq. for 1 hr. The reaction mixt. was neutralized and then extracted with Et_2O. The Et_2O layer was concd to dryness to give the corresponding aglycone. The aq. layer was detected by HPTLC to give sugar components.

Alkaline hydrolysis of saponins **8** *and* **10**. Each saponin was hydrolysed with 2% KOH aq. for 1 hr. The reaction mixt. was neutralized with HCl soln, and then extracted with *n*-BuOH. The *n*-BuOH was concd *in vacuo* to dryness and subjected to RP-18 CC in which **8** gave **7**, and **10** gave **9**, respectively. The aq. layer was analysed by HPTLC to give sugar components.

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