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SAPONINS FROM *CLEMATIS CHINENSIS*

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Key Word Index–*Clematis chinensis*; Ranunculaceae; Triterpenoid saponins; clematichinenoside C.

Abstract—From ethanol extracts of the roots of *Clematis chinensis*, a new saponin, named clematichinenoside C, was isolated and its structure was established as 3-*O*-β-D-glucopyranosyl-(1-4)-β-D-ribopyranosyl-(1-3)-α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1-4)-β-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl ester by spectroscopic and chemical means. In addition, one known saponin, huzhongoside B, was identified in the plant.

INTRODUCTION

The roots of *Clematis chinensis* is a source of Chinese crude drug "Wei-Ling-Xian" which has been used as an analgesic, diuretic, antitumor, antiinflammatory and insecticidal agent. It is a commonly-used traditional Chinese medicine and it has been recorded in Chinese Pharmacopoeia (1990 ed.). The previous phytochemical investigation has revealed that the plant roots are rich in saponins and more than 20 prosapogenins have been isolated from the alkaline hydrolysate of its crude saponins [1–5]. In order to elucidate chemical constituents of the roots, we re-examined its genuine saponins. In the preceding paper [6], we reported two new triterpenoid saponins named clematichinenoside A and B, along with nine known triterpenoids and saponins from the plant roots. During the course of our further investigation, another new saponin named clematichinenoside C (**1**), together with a known saponin, huzhongoside B (**2**), were obtained from the butanol-soluble fractions of the plant. This paper deals with the isolation and elucidation of **1** and **2**.

RESULTS AND DISCUSSION

The butanol-soluble fraction obtained from ethanol extracts was subjected to repeated chromatography on silica gel and RP-18 lobar column. Besides two triterpenoids and nine saponins reported in our previous paper [6], two other saponins **1** and **2** were separated.

Saponin **1**, an amorphous powder, mp: 219–221° had molecular formula $C_{70}H_{114}O_{34}$ (ESMS and ^{13}C NMR). It showed positive reactions to the Lieberman-Burchard and Molish tests. On acid hydrolysis (0.5N H_2SO_4), compound **1** afforded oleanolic acid as the aglycone and arabinose, glucose, ribose and rhamnose as sugar components (co-HPTLC). 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. The signals for C-3 at δ 88.50 and C-28 at δ 176.30 proved that **1** was a bidesmoside. On alkaline hydrolysis, compound **1** yielded a known saponin CP7 (**3**) (oleanolic acid 3-*O*-β-D-glucopyranosyl (1-4)-β-D-ribopyranosyl(1-3)-α-L-rhamnopyranosyl (1-2)-α-L-arabinopyranoside) which was identified by comparing its ^1H and ^{13}C NMR spectra with literature data. Besides, compound **1** also provided glucose and rhamnose (co-TLC) as C_{28} ester sugar units.

The ^1H NMR spectrum of **1** exhibited seven anomeric proton signals at δ 6.23 (*br*, *s*), 6.22 (*d*, $J = 8.1$), 5.83 (*br*, *s*), 5.82 (*d*, $J = 5.4$), 5.01 (*d*, $J = 7.9$), 4.98 (*d*, $J = 7.8$), 4.83 (*d*, $J = 5.9$) and two methyl signals of rhamnose units at δ 1.69 (*d*, $J = 6.1$) and 1.52 (*d*, $J = 6.0$), respectively. The proton system of each sugar unit was determined by a combined use of DQF-COSY and TOCSY experiments on a Bruker AMX 600 spectrometer. The chain of coupled protons could be pursued by a sequential "walk" via observed cross-peaks, starting from signals of the anomeric protons and rhamnose methyl. All correlation signals and sequences 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 of the sugar units. Considering the ^{13}C NMR glycosylation shift and comparing ^{13}C assignments in **1**

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with those of reference methyl glycosides, revealed the presence of a terminal α-L-rhamnopyranosyl unit, a terminal β-D-glucopyranosyl unit, a 2-substituted α-L-arabinopyranosyl unit, a 3-substituted α-L-rhamnopyranosyl unit, a 4-substituted β-D-ribopyranosyl unit, a 4-substituted β-D-glucopyranosyl unit and 6-substituted β-D-glucopyranosyl unit. The information concerning the sequence of the oligosaccharide chains and the linkage sites to the aglycone were obtained by spatial correlation between two protons in a NOESY experiment as well as by the scalar coupling between a carbon and a proton of the neighboring residue in the long-range $^{13}C-^{1}H$ correlation spectrum (HMBC). From the HMBC spectrum, the correlation peaks were

Table 1. ^{1}H NMR data of saponins **1** and **2** (in C_5D_5N)

Number	[1] ^{1}H-NMR coupling		[2] ^{1}H-NMR coupling	
glc(G-1)				
1	6.22, *d*	8.1	6.22, *d*	8.1
2	4.19, *m*		4.10, *m*	
3	4.28, *m*		4.17, *m*	
4	4.31, *m*		4.17, *m*	
5	4.19, *m*		4.10, *m*	
6	4.66, *m*		4.65, *brd*	9.9
			4.34 *m*	
glc(G-2)				
1	4.98, *d*	7.8	5.16, *d*	
2	3.93, *m*		3.93, *dd*	8.0, 8.0
3	4.13, *m*		4.13, *m*	
4	4.40, *m*		4.40, *dd*	9.2, 9.2
5	3.65, *brd*	9.3	3.65, *brd*	9.4
6	4.07, *m*		4.06, *m*	
	4.30, *m*		4.20, *brd*	9.2
rha(R-1)				
1	5.83, *brs*		5.84, *brs*	
2	4.66, *brs*		4.66, *brs*	
3	4.53, *dd*	8.8, 2.9	4.53, *dd*	9.6, 2.6
4	4.30, *m*		4.31, *m*	
5	4.94, *dq*	9.2, 6.1	4.94, *dq*	9.4, 6.3
6	1.69, *d*	6.1	1.87, *d*	6.3
ara(A-1)				
1	4.83, *d*	5.9	4.82, *d*	6.1
2	4.55, *m*		4.57, *dd*	6.1, 6.1
3	4.23, *m*		4.25, *m*	
4	4.23, *m*		4.22, *m*	
5	3.81, *d*	10.5	3.79, *brd*	10.4
	4.31, *m*		4.28, *m*	
rha(R-2)				
1	6.23, *brs*		6.28, *brs*	
2	4.86, *brs*		4.90, *brs*	
3	4.66, *m*		4.74, *dd*	9.6, 3.1
4	4.41, *m*		4.41, *dd*	9.6, 9.4
5	4.60, *dq*	6.5, 6.0	4.62, *dq*	9.4, 6.2
6	1.52, *d*	6.0	1.53, *d*	6.2
rib(Ri-1)				
1	5.82, *d*	5.4	5.94, *d*	4.4
2	4.10, *m*		4.30, *m*	
3	4.68, *m*		4.30, *m*	
4	4.10, *m*		4.52, *m*	
5	4.30, *m*		4.30, *m*	
			4.15, *m*	
glc(G-3)				
1	5.01, *d*	7.9		
2	3.91, *m*			
3	4.18, *m*			
4	4.16, *m*			
5	3.91, *m*			
6	4.49, *d*	10.4		
	4.32, *m*			

observed between signals at δ 6.22 (H-1 of G-1)/δ 176.30 (C-28 of aglycone); δ 4.98 (H-1 of G-2)/δ 69.07 (C-6 of G-1); δ 5.83 (H-1 of R-1)/δ 78.26 (C-4 of G-2); δ 4.83 (H-1 of A-1)/δ 88.50 (C-3 of genin); δ 6.23 (H-1 of R-2)/δ 75.28 (C-2 of A-1); δ 5.82 (H-1 of Ri-1)/δ 82.01 (C-3 of R-2); δ 5.01 (H-1 of G-3)/δ 76.32 (C-4 of Ri-1). The above results were also supported by a 2D-NOESY experiment in which there were the following correlation peaks: δ 4.98 (H-1 of G-2)/δ 4.19, 4.66 (H-6 of G-1); δ 5.83 (H-1 of R-1)/δ 4.40 (H-4 of G-2); δ 4.83 (H-1 of A-1)/δ 3.65 (H-3 of genin); δ 6.23 (H-1 of R-2)/δ 4.55 (H-2 of A-1); δ 5.82 (H-1 of Ri-1)/δ 4.66 (H-3 of R-2); δ 5.01 (H-1 of G-3)/δ 4.10 (H-4 of Ri-1). Thus the structure of saponin **1** was identified as 3-*O*-β-D-glucopyranosyl-(1-4)-β-D-ribopyranosyl-(1-3)-α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranosyl ole-anolic acid 28-*O*-α-L-rhamnopyranosyl-(1-4)-β-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl ester, named clematichinenoside C.

Saponin **2** was obtained as white powder, mp 224–225°, [α] −34.6 (pyridine). It was hydrolysed with acid to yield oleanolic acid, glucose, rhamnose, arabinose and ribose. Its ^{1}H and ^{13}C signals were unambiguously assigned by the ^{1}H NMR, ^{13}C NMR, DQF-COSY, TOCSY and HMQC spectra (see Tables 1 and 2). The sequencing of the sugar chains was clarified by using NOESY and HMBC experiments (see Fig. 1). The structure of saponin **2** was then established as 3-*O*-β-D-ribopyranosyl-(1-3)-α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranosyl oleanolic acid 28-O-α-L-rhamnopyranosyl-(1-4)-β-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl ester. It was identical with the known saponin huzhongoside B [7], but this is the first report of the compound in this plant.

EXPERIMENTAL

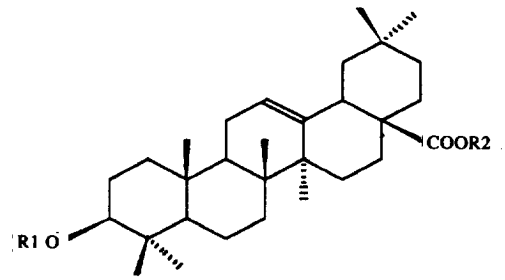
General. Mps: uncorr. ^{1}H, ^{13}C and 2D-NMR spectra were recorded on Bruker AMX-600 and AM-400 spectrometers. Optical rotations were measured with a JASCO DIP-181 polarimeter. Mass spectra were determined on a VG QUATTRO GC/MS/MS spectrometer. IR spectra were taken as KBr pellets on a PE 599B spectrometer. The solvents for the Sephadex LH-20 and

Table 2. ^{13}C-NMR data of saponins **1–3**

	Aglycone			Sugar			
C	**3**	**2**	**1**		**3**	**2**	**1**
				3-O-sugar			
1	38.4	38.5	38.7	ara 1	104.6	105.3	105.2
2	26.1	26.1	26.4	(A-1) 2	75.4	75.2	75.3
3	88.3	88.3	88.5	3	74.2	74.9	74.8
4	39.1	39.1	39.3	4	68.5	69.5	69.1
5	55.5	55.7	55.8	5	64.8	65.9	65.7
6	18.0	18.0	18.2	rha 1	101.1	101.3	101.3
7	32.8	32.5	32.9	(R-2) 2	71.4	77.0	71.9
8	39.3	39.4	39.6	3	81.5	81.2	82.0
9	47.6	47.6	47.8	4	72.3	72.8	72.7
10	36.6	36.6	36.8	5	69.4	69.5	69.7
11	23.2	23.2	23.4	6	18.0	18.4	18.4
12	122.3	122.3	122.6	rib 1	104.2	105.3	104.7
13	144.0	143.6	143.3	Ri-1) 2	72.0	72.5	72.5
14	41.7	41.6	41.8	3	68.8	70.2	69.4
15	27.8	27.7	28.0	4	76.1	69.1	76.3
16	23.3	23.2	23.1	5	61.3	65.2	61.7
17	46.2	46.6	46.8	glc 1	103.0		103.5
18	41.5	41.2	41.4	(G-3) 2	73.7		74.7
19	46.0	45.8	46.0	3	78.1		78.1
20	30.5	30.2	30.5	4	71.1		71.4
21	33.7	33.5	33.7	5	77.9		788.6
22	32.8	32.1	32.9	6	62.2		62.4
23	27.7	27.7	28.0	28-O-sugar			
24	16.6	16.6	16.9	glc 1		95.6	95.6
25	15.1	15.2	15.4	(G-1) 2		73.9	73.8
26	17.0	17.0	17.2	3		78.6	78.6
27	25.7	25.6	25.8	4		70.7	70.7
28		176.0	176.3	5		76.4	78.0
29	32.8	32.6	32.9	6		69.1	69.1
30	23.3	23.2	23.4	glc 1		104.8	104.8
				(G-2) 2		75.3	75.3
				3		76.4	76.4
				4		78.1	78.3

Lobar separations were different ratios of EtOH and H_2O and EtOH. The HPTLC solvents for the sugars were following two systems: (1) $CHCl_3$–MeOH–H_2O, 70:30:3 (2) Pyridine–*n*-butanol–H_2O, 6:4:1.

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.



R1

1. –α–L–ara(2–1)–α–L–rha(3–1)–β–D–rib (4–1)–β–D–glc
2. –α–L–ara(2–1)–α–L–rha(3–1)–β–D–rib
3. –α–L–ara(2–1)–α–L–rha(3–1)–β–D–rib (4–1)–β–D–glc

R2

–β–D–glc(6–1)–β–D–glc(4–1)–α–L–rha

–β–D–glc(6–1)–β–D–glc(4–1)–α–L–rha

H

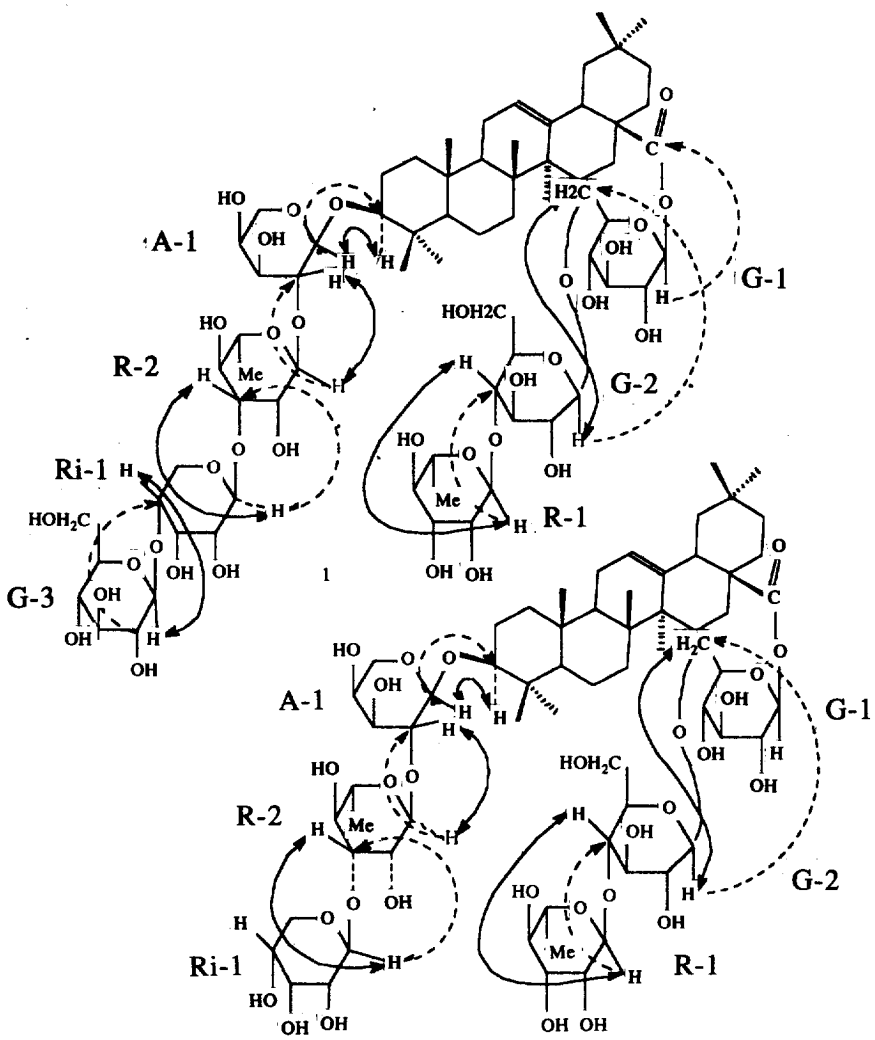


Fig. 1. NOE correlation (in actual line) and ^{13}C–^{1}H long-range correlation (in dotted line) of **1** and **2**.

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 in H_2O (10, 30, 70, 90%). The EtOH–H_2O (7:3) eluent was subjected to repeated Sephadex and RP-18 Lobar column chromatography to give **1** (400 mg) and **2** (300 mg).

Clematichinensoide C **1**. Amorphous powder, mp: 219–221°. $[\alpha]_D$ −34.61° (*c* 0.67, pyridine), ESMS *m/z*: 1520 $[M + Na - H]^+$, IR: γ_{max}^{KBr} cm^{-1}: 3400 (br), 2940, 1730, 1640 and 1070. ^1H and ^{13}C NMR data: see Tables 1 and 2 and Fig. 1.

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

Alkaline hydrolysis of **1**. Saponin **1** was hydrolysed with 2% KOH aq. for 1 hr. The reaction mixture 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 column chromatography to give **3**. The aq. layer was detected by HPTLC to give sugar components.

Huzhongoside B **2**. Physical constants and spectroscopic data were identical with literature data. ^1H and ^{13}C NMR data: see Tables 1 and 2 and Fig. 1.

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