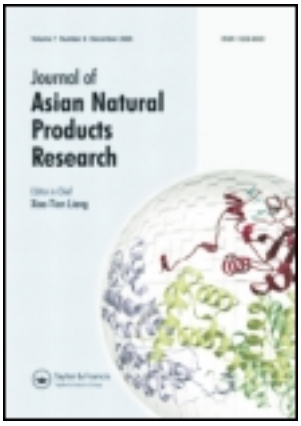

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Three new triterpene saponins from *Clematis chinensis*

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Three new triterpene saponins, clematochinenosides H–J (**1**–**3**), were isolated from the roots and rhizomes of *Clematis chinensis*. Their structures were elucidated on the basis of spectroscopic means including 1D and 2D NMR experiments and hydrolysis products.

Keywords: *Clematis chinensis*; triterpene saponins; clematochinenosides H–J

1. Introduction

The roots and rhizomes of *Clematis chinensis* Osbeck (Ranunculaceae) are used in a traditional Chinese herbal drug 'Weilingxian' that is commonly used as an antiinflammatory, antitumor, and analgesic agent [1]. Phytochemical research reveals that it is rich in triterpene saponins [2–8]. Our previous study showed that those saponins with isoferulic acid moiety showed inhibitory activity against the cyclooxygenase-1 and cyclooxygenase-2 enzymes [9]. In addition, monodesmosidic saponins exhibited cytotoxic activities against cultured tumor cells [10]. As a continual study on this plant, we report on the isolation and structural elucidation of three new saponins clematochinenosides H–J (Figure 1) from the roots and rhizomes of *C. chinensis*.

2. Results and discussion

Compound **1** was isolated as a white, amorphous powder. The HR-ESI-MS (positive-ion mode) experiment revealed a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 2007.8625, which is in agreement with the molecular formula $C_{88}H_{144}O_{49}$. The aglycone of **1** was identified as hederagenin in comparison with the ^1H and ^{13}C NMR data obtained by 2D NMR experiments with values of the literature (Table 1) [9]. The downfield chemical shift at δ_C 80.9 (Agly-3) and the upfield chemical shift at δ_C 176.5 (Agly-28) in the ^{13}C NMR spectrum of **1** indicated these compounds to be bisdesmosidic saponins. The ^1H NMR spectrum of **1** exhibited 10 anomeric proton resonances at δ 6.27 (1H, br s), 6.21 (1H, d, $J = 8.0$ Hz), 5.82 (1H, br s), 5.80 (1H, d, $J = 5.0$ Hz), 5.42 (1H, br s), 5.10 (1H, d, $J = 8.0$ Hz), 5.09 (1H, d, $J = 8.0$ Hz), 5.04 (1H, d, $J = 6.5$ Hz), 4.99 (1H, d, $J = 8.0$ Hz), and 4.90 (1H, d, $J = 8.0$ Hz), respectively (Table 2). The three proton doublets observed at δ 1.67 (3H, d, $J = 6.0$ Hz), 1.57 (3H, d, $J = 6.0$ Hz), and 1.50 (3H, d, $J = 6.0$ Hz) suggested the presence of three deoxyhexopyranosyl units in **1**. The monosaccharides obtained after acid hydrolysis of **1** were identified as glucose, rhamnose, arabinose, and ribose by thin-layer chromatography (TLC) in

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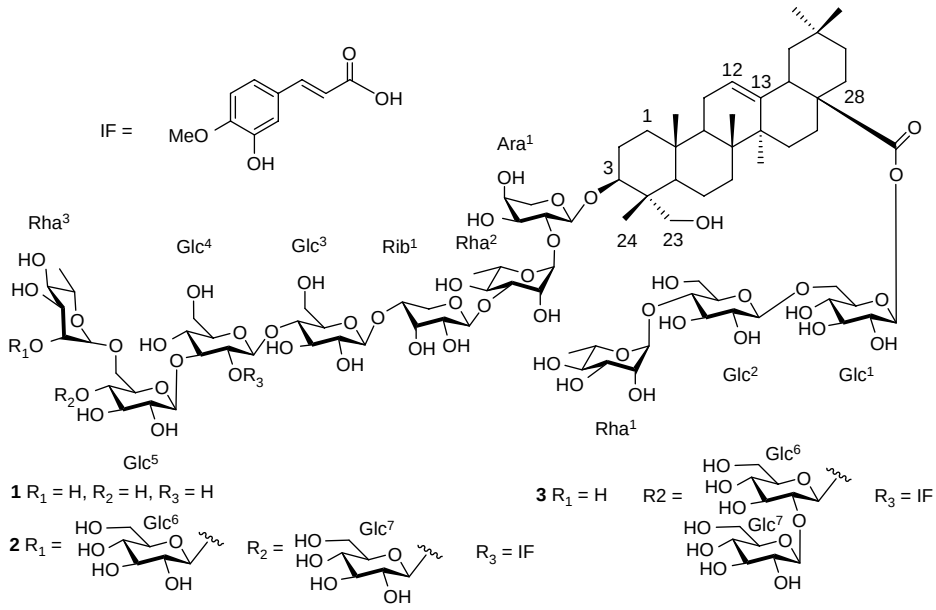

Figure 1. The structures of compounds **1**–**3**.

comparison with authentic samples. Mild alkaline hydrolysis of **1** afforded **1a**. The absolute configuration of the monosaccharides was determined to be D for glucose and ribose and L for rhamnose and arabinose by the GC analysis of chiral derivatives of the monosaccharides in the hydrolysate of each compound (see Section 3). The relatively large coupling constants (5.0–8.0 Hz) for the anomeric protons in the ^{1}H NMR spectrum (see Section 3) of **1** suggested that the arabinopyranosyl moiety has an α-configuration and the glucopyranosyl and ribopyranosyl moieties have a β-configuration. The α-configurations of the rhamnopyranosyl moieties were determined from the broad singlets observed for the anomeric protons. The large $^1J_{H-1,C-1}$ values of the rhamnosyl moieties (Rha^1, 172 Hz; Rha^2, 168 Hz; Rha^3, 170 Hz) confirmed that the anomeric protons were equatorial (α-pyranoid anomeric form) [11]. The spin–spin coupling system of individual monosaccharide units was identified by the analysis of 1D total correlation spectroscopy (1D TOCSY) and 2D NMR spectra. ^{1}H NMR spectral data of individual monosaccharide units were obtained by the selective irradiation of the anomeric protons or methyl groups of rhamnose units in a series of 1D TOCSY experiments. Analysis of the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) spectrum resulted in a sequential assignment of all proton resonances of the 10 monosaccharide units (see Table 2). In the heteronuclear single quantum coherence (HSQC) experiment, proton resonances were correlated with those of the corresponding carbons, and the associated anomeric protons were correlated with their respective carbon atoms from HSQC–TOCSY data, leading to unambiguous assignments of the carbons in each monosaccharide unit (see Table 3). Taking into account the known effects of O-glycosylation, **1** contains one L-arabinopyranosyl unit (Ara), one D-ribopyranosyl unit (Rib), three L-rhamnopyranosyl units (Rha), and five D-glucopyranosyl units (Glc). Sugar aglycone and sugar–sugar linkages were determined by HMBC and NOESY data analyses. In the HMBC spectrum, the anomeric proton signals at δ_H 6.21 (Glc^1-H-1), 4.99 (Glc^2-H-1),

Table 1. ^{13}C NMR spectral data for the aglycone moieties of **1**–**3** and **1a** (125 MHz, in C_5D_5N).

No.	**1**	**1a**	**2**	**3**	No.	**1**	**1a**	**2**	**3**	No.	**1**	**1a**	**2**	**3**
1	39.0	39.0	39.1	39.1	11	23.8	24.0	23.6	23.8	21	33.9	34.3	33.9	33.9
2	26.3	26.3	26.3	26.4	12	122.9	123.0	122.9	123.0	22	32.5	33.4	32.7	32.5
3	80.9	80.9	80.9	80.9	13	144.0	145.1	144.0	144.0	23	63.7	63.7	63.8	63.8
4	43.5	43.5	43.6	43.5	14	42.0	42.0	42.1	42.0	24	14.1	16.5	14.1	14.1
5	47.6	47.6	47.6	47.6	15	28.2	28.2	28.2	28.2	25	16.1	15.7	16.1	16.1
6	18.0	18.0	18.0	18.1	16	23.3	23.6	23.3	23.3	26	17.5	16.5	17.5	17.5
7	32.6	32.6	32.7	32.6	17	46.9	46.7	47.0	47.0	27	26.3	20.8	26.3	26.4
8	39.8	39.8	39.9	39.9	18	41.6	42.2	41.6	41.6	28	176.5	180.1	176.5	176.5
9	48.1	48.1	48.2	48.2	19	46.1	46.7	46.1	46.1	29	33.0	33.0	33.0	33.0
10	36.8	36.8	36.8	36.8	20	30.6	31.1	30.7	30.7	30	23.6	23.6	23.6	23.6

5.82 (Rha^1-H-1), 5.04 (Ara^1-H-1), 6.27 (Rha^2-H-1), 5.80 (Rib^1-H-1), 4.90 (Glc^3-H-1), 5.10 (Glc^4-H-1), 5.09 (Glc^5-H-1), and 5.42 (Rha^3-H-1) showed cross-peaks with the carbon signals at δ_C 176.5 (Agly-C-28), 69.1 (Glc^1-C-6), 78.3 (Glc^2-C-4), 80.9 (Agly-C-3), 75.3 (Ara^1-C-2), 81.9 (Rha^2-C-3), 76.2 (Rib^1-C-4), 81.0 (Glc^3-C-4), 88.9 (Glc^4-C-3), and 68.8 (Glc^5-C-6), respectively. These signals provide ample evidence to determine the linkages between the sugars, and the sugar and the aglycone. From the above evidence, the structure of **1** was established as 3-*O*-α-L-rhamnopyranosyl-(1 → 6)-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-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside, which has been named clematochinenoside H.

Compound **2** was isolated as a white, amorphous powder. The HR-ESI-MS (positive-ion mode) experiment revealed a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 2508.0181, in agreement with the molecular formula $C_{110}H_{172}O_{62}$. The ^{1}H NMR spectrum of **2** showed signals for 12 anomeric protons, along with signals for 3 deoxyhexopyranosyl units. The spectroscopic properties of **2** were closely related to those of **1**. When the ^{13}C NMR spectrum of **2** was compared with that of **1**, two sets of additional 12 signals corresponding to two terminal β-D-glucopyranosyl groups appeared, and two HMBC correlations were observed between the signals at δ 5.31 (Glc^6-H-1) and δ 82.0 (Rha^3-C-2), and δ 4.90 (Glc^7-H-1) and δ 72.0 (Glc^5-C-4). Further analysis of the NMR spectral data of **2** revealed the presence of a 3-hydroxy-4-methoxycinnamoyl group (isoferuloyl) (Table 4). The typical protons of the isoferuloyl moiety resonated at δ_H 7.52 (1H, d, $J = 2.0$ Hz), 7.09 (1H, dd, $J = 8.5, 2.0$ Hz), 6.90 (1H, d, $J = 16.0$ Hz), 8.10 (1H, d, $J = 16.0$ Hz), 6.86 (1H, d, $J = 8.5$ Hz), and 3.73 (3H, s) and δ_C 116.4,

Table 2. ^{1}H NMR spectral data for the sugar moieties of compounds **1**–**3** (500 MHz, in C_5D_5N).

No.	**1**	**2**	**3**	No.	**1**	**2**	**3**
Glc[1]				Glc[3]			
1	6.21 d (8.0)	6.21 d (8.0)	6.20 d (7.5)	1	4.90 d (8.0)	4.87 d (8.0)	4.88 d (7.5)
2	4.08 dd (9.0, 8.0)	4.08 dd (9.0, 8.0)	4.08 dd (9.0, 7.5)	2	3.87 dd (9.0, 8.0)	3.86 dd (9.0, 8.0)	3.86 dd (9.0, 7.5)
3	4.20 dd (9.0, 9.0)	4.19 dd (9.0, 9.0)	4.19 dd (9.0, 9.0)	3	4.15 m	4.12 m	4.12 m
4	4.28 m	4.28 m	4.28 m	4	4.27 m	4.26 m	4.25 m
5	4.07 m	4.07 m	4.07 m	5	3.64 m	3.64 m	3.64 m
6a/b	4.66 m/4.31 m	4.66 m/4.31 m	4.64 m/4.30 m	6a/b	4.23 m/4.14 m	4.23 m/4.14 m	4.22 m/4.13 m
Glc[2]				Glc[4]			
1	4.99 d (8.0)	4.98 d (8.0)	4.97 d (8.0)	1	5.10 d (8.0)	5.29 d (8.0)	5.28 d (8.0)
2	3.92 dd (9.0, 8.0)	3.91 dd (9.0, 8.0)	3.91 dd (9.0, 8.0)	2	4.06 dd (9.0, 8.0)	5.71 dd (9.0, 8.0)	5.70 dd (9.0, 8.0)
3	4.14 d (9.0)	4.19 d (9.0)	4.19 d (9.0)	3	4.01 m	4.13 m	4.13 m
4	4.40 (9.0)	4.41 (9.0)	4.40 (9.0)	4	4.05 m	4.04 m	4.03 m
5	3.66 m	3.62 m	3.62 m	5	3.86 m	3.64 br d (10.0)	3.63 br d (9.5)
6a/b	4.19 m/4.05 m	4.16 m/4.07 m	4.15 m/4.06 m	6a/b	4.22 m/4.17 m	4.22 m/3.94 m	4.22 m/3.94 m
Rha[1]				Glc[5]			
1	5.82 br s	5.83 br s	5.82 br s	1	5.09 d (8.0)	4.88 d (8.5)	4.88 d (8.0)
2	4.64 br s	4.64 br s	4.64 br s	2	3.91 dd (9.0, 8.0)	3.87 dd (9.0, 8.5)	3.87 dd (9.0, 8.0)
3	4.54 dd (9.0, 3.5)	4.53 dd (9.0, 3.5)	4.53 dd (9.0, 3.5)	3	4.07 dd (9.0, 9.0)	4.06 dd (9.0, 9.0)	4.06 dd (9.0, 9.0)
4	4.33 dd (9.0, 9.0)	4.32 dd (9.0, 9.0)	4.32 dd (9.0, 9.0)	4	3.89 dd (9.0, 9.0)	3.82 dd (9.0, 9.0)	3.82 dd (9.0, 9.0)
5	4.93 m	4.92 m	4.92 m	5	4.06 m	4.06 m	4.06 m
6	1.67 d (6.0)	1.67 d (6.0)	1.66 d (6.0)	6a/b	4.48 m/3.78 m	4.53 m/3.69 m	4.53 m/3.69 m
Ara[1]				Rha[3]			
1	5.04 d (6.5)	5.05 d (6.5)	5.04 d (6.5)	1	5.42 br s	5.62 br s	5.42 br s
2	4.56 dd (8.0, 6.5)	4.54 dd (8.0, 6.5)	4.54 dd (8.0, 6.5)	2	4.65 br s	4.83 br d	4.63 br d
3	3.99 dd (8.0, 4.0)	4.01 dd (8.0, 4.0)	4.01 dd (8.0, 4.0)	3	4.56 m	4.54 m	4.53 m
4	4.11 m	4.10 m	4.10 m	4	4.17 dd (9.0, 9.0)	4.16 dd (9.0, 9.0)	4.16 dd (9.0, 9.0)
5a/b	4.22 m/3.64 m	4.23 m/3.67 m	4.21 m/3.66 m	5	4.82 m	4.25 m	4.79 m
Rha[2]				6	1.57 d (6.0)	1.55 d (6.0)	1.56 d (6.0)
1	6.27 br s	6.23 br s	6.22 br s	Glc[6]			
2	4.85 br s	4.83 br s	4.83 br s	1		5.31 d (8.0)	4.84 d (8.0)
3	4.64 m	4.67 m	4.66 m	2		4.07 m	4.23 m
4	4.39 dd (9.0, 9.0)	4.39 dd (9.0, 9.0)	4.39 dd (9.0, 9.0)	3		4.19 m	4.03 m

Table 2 – *continued*

No.	1	2	3	No.	1	2	3
5	4.62 m	4.64 m	4.63 m	4		4.26 m	4.13 m
6	1.50 d (6.0)	1.50 d (6.0)	1.50 d (6.0)	5		3.93 m	4.04 m
Rib[1]				6a/b		4.45 m/4.34 m	4.24 m/4.13 m
1	5.80 d (5.0)	5.81 d (5.0)	5.80 d (5.0)	Glc[7]			
2	4.09 m	4.07 m	4.06 m	1		4.90 d (8.0)	5.22 d (8.0)
3	4.62 m	4.64 m	4.64 m	2		4.02 m	3.90 m
4	4.31 m	4.29 m	4.29 m	3		4.11 m	4.17 m
5a/b	4.29 m/4.29 m	4.28 m/4.28 m	4.28 m/4.28 m	4		4.14 m	4.21 m
				5		4.01 m	3.98 m
				6a/b		5.05 m/4.62 m	4.42 m/4.30 m

121.4, 112.1, 145.6, 115.4, 150.9, 148.4, 128.6, 166.7, and 55.8. The correlation observed between Glc[4]-H-2 at δ 5.71 and the carbonyl carbon at δ 166.7 in the HMBC spectrum indicated that the isoferuloyl moiety is linked to the sugar chain on Glc[4]-C-2 (δ 73.2). Accordingly, the structure of **2** was determined as 3-*O*-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 4)]-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-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (clematochinenoside I).

The molecular formula of **3** was determined as $C_{110}H_{172}O_{62}$ from the pseudomolecular ion peak at *m/z* 2508.0181 $[M + Na]^+$ in the HR-ESI-MS. Acid hydrolysis of **3** with 2 N aqueous CF_3COOH gave hederagenin, isoferulic acid, L-arabinose, D-glucose, L-rhamnose, and D-ribose. Comparison of the NMR data for **3** and **2** revealed that the compounds are similar except for the linkage site of the terminal glucopyranosyl group (Glc[7]) in the sugar chain, which is connected to Glc[6]-C-2 in **3** instead of Rha[3]-C-2 in **2** according to HMBC correlations observed between Glc[7]-H-1 at δ 5.22 and Glc[6]-C-2 at δ 84.4. The proton and carbon signals were assigned unambiguously using ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, 1D TOCSY, HSQC, HMBC, and nuclear overhauser effect spectroscopy (NOESY) NMR experiments. Therefore, the structure of **3** was proposed as 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4) [α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranosyl-(1 → 3)-[(2-*O*-isoferuloyl)-β-D-glucopyranosyl]-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-ribopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyrano-

Table 3. ^{13}C NMR spectral data for the sugar moieties of compounds **1**–**3** and **1a** (125 MHz, in C_5D_5N).

No.	**1**	**1a**	**2**	**3**	No.	**1**	**1a**	**2**	**3**	No.	**1**	**1a**	**2**	**3**
Glc[1]					Rha[2]					Glc[5]				
1	95.6		95.6	95.6	1	101.3	101.3	101.4	101.4	1	105.8	105.8	105.8	105.2
2	73.8		73.8	73.8	2	71.8	71.9	71.9	71.9	2	75.2	75.1	74.4	73.9
3	78.6		78.6	78.7	3	81.9	81.9	82.0	82.1	3	78.3	78.2	78.2	76.4
4	70.7		70.7	70.8	4	72.7	72.7	72.8	72.7	4	72.0	72.0	72.0	82.0
5	78.0		78.0	78.0	5	69.6	69.7	69.6	69.8	5	76.4	76.4	76.4	74.8
6	69.1		69.1	69.2	6	18.4	18.4	18.4	18.4	6	68.8	68.8	68.8	68.0
Glc[2]					Rib[1]					Rha[3]				
1	104.7		104.8	104.8	1	104.6	104.6	104.6	104.7	1	102.8	102.8	101.3	102.7
2	75.3		75.3	75.3	2	72.6	72.5	72.5	72.5	2	71.9	71.8	82.0	71.9
3	76.3		76.4	76.5	3	69.6	69.6	69.5	69.6	3	72.6	72.6	72.6	72.7
4	78.3		78.2	78.2	4	76.2	76.3	76.3	76.5	4	74.0	74.0	74.7	74.1
5	77.1		77.1	77.1	5	61.5	61.5	61.6	61.5	5	69.9	69.8	69.7	69.9
6	61.2		61.2	61.2						6	18.6	18.6	18.5	18.6
Rha[1]					Glc[3]					Glc[6]				
1	102.6		102.6	102.7	1	102.8	102.8	103.1	102.9	1			107.2	102.4
2	72.5		72.4	72.6	2	74.3	74.2	74.2	74.2	2			75.9	84.4
3	72.7		72.7	72.8	3	76.4	76.4	76.6	76.5	3			78.3	78.1
4	73.9		73.9	74.0	4	81.0	81.0	80.9	81.1	4			71.2	71.1
5	70.2		70.2	70.3	5	76.3	76.3	76.4	76.2	5			78.6	78.8
6	18.5		18.5	18.5	6	60.6	60.6	61.9	60.6	6			62.4	62.3
Ara[1]					Glc[4]					Glc[7]				
1	104.6	104.6	104.6	104.7	1	104.3	104.3	104.3	102.2	1			105.1	106.7
2	75.3	75.2	75.3	75.3	2	73.2	73.2	73.2	72.7	2			74.7	76.0
3	75.2	75.2	75.2	75.2	3	88.9	88.8	88.9	86.5	3			78.3	78.1
4	69.7	69.7	69.7	69.7	4	69.4	69.3	69.4	70.1	4			71.5	71.6
5	66.3	66.3	66.3	66.4	5	77.7	77.7	77.7	77.8	5			78.4	78.2
					6	61.4	61.3	61.4	62.0	6			62.5	63.0

Table 4. ^{1}H NMR and ^{13}C NMR spectral data for the isoferuloyl moieties of compounds **2** and **3** (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR, in C_5D_5N).

No.	**2**		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C
1		166.7		166.6
2	6.90 d (16.0)	116.4	6.90 d (16.0)	116.4
3	8.10 d (16.0)	145.6	8.11 d (16.0)	145.6
4		128.6		128.6
5	7.52 d (2.0)	115.4	7.53 d (2.0)	115.4
6		148.4		148.4
7		150.9		150.9
8	6.86 d (8.5)	112.1	6.88 d (8.5)	112.1
9	7.09 dd (8.5 2.0)	121.4	7.09 dd (8.5 2.0)	121.4
OCH_3	3.73 s	55.8	3.73 s	55.8

syl-(1 → 6)-β-D-glucopyranoside (clematochinenoside J).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a PerkinElmer 243B digital polarimeter (PerkinElmer, Waltham, MA, USA). UV spectra were obtained on a TU-1901 spectrometer (Puxi, Beijing, China). IR spectra were recorded on an Avater-360 spectrometer (Thermo Scientific, Waltham, MA, USA). NMR spectra were recorded on a Bruker INOVA 500 spectrometer (Bruker, Billerica, MA, USA), with tetramethylsilane as an internal standard. HR-ESI-MS was measured on a Bruker APEX IV FT-MS (7.0 T) mass spectrometer (Bruker) in the positive-ion mode. HPLC was performed on an octadecylsilanized column (Agilent Technologies 250 × 10 mm i.d., 5 μm, Santa Clara, CA, USA) with an Alltech evaporative light scattering detector (Grace, Dearfield, IL, USA). GC was carried out on an Agilent 6890N gas chromatograph (Agilent Technologies). Column chromatography was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), D101 porous polymer resin (Tianjin Chemical Industry Co., Ltd, Tianjin, China), and C18 silica gel (150–200 mesh, Merck, Darmstadt, Germany; performed by applying a N_2 pressure of 0.5 MPa).

3.2 Plant materials

The roots and rhizomes of *C. chinensis* were collected in September 2007 in Shaoguan, Guangdong Province, China. The identification of the plant was performed by one of the authors (P.-F.T.). A voucher specimen (CC 200712) is maintained in the Herbarium of Peking University Modern Research Center for Traditional Chinese Medicine.

3.3 Extraction and isolation

The dried roots and rhizomes (2 kg) of *C. chinensis* were extracted with 50% EtOH. After removing the solvent, the residue (400 g) was suspended in H_2O and extracted with *n*-BuOH. The *n*-BuOH extract (130 g) was subjected to D101 porous polymer resin column chromatography and eluted with H_2O and 30% and 80% EtOH, successively. The fraction eluted with 30% EtOH (120 g) was then subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH (4:1, 1:1) to afford fractions 1 and 2 (31 and 15 g, respectively). Fraction 2 (15 g) was

subjected to C_{18} silica gel column chromatography and eluted with MeOH–H_2O in a gradient of MeOH (MeOH–H_2O, 15:85–60:40%) to afford subfractions 2-1 to 2-4 (2.5, 3.2, 1.7, and 3.2 g, respectively). Subfraction 2-2 (1.0 g) was isolated by preparative HPLC (MeCN–H_2O, 28:72, 2.0 ml/min) to yield compounds **1** (52 mg). Subfraction 2-4 (1.5 g) was isolated by preparative HPLC (MeCN–H_2O, 30:70, 2.0 ml/min) to yield compounds **2** (31 mg) and **3** (37 mg).

3.3.1 *Clematochinenoside H (**1**)*

White amorphous solid, $[\alpha]_D^{20}$ −32 (*c* 1.0, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3422, 2927, 1730, and 1632; ^1H NMR (C_5D_5N, 500 MHz): δ 1.14 (3H, s, Me-27), 1.08 (3H, s, Me-24), 1.06 (3H, s, Me-26), 0.93 (3H, s, Me-25), 0.85 (3H, s, Me-29), 0.84 (3H, s, Me-30), 4.24 (1H, dd, $J = 12.0$, 5.0 Hz, H-3), 3.85 (1H, d, $J = 12.0$ Hz, H-23a), 4.21 (1H, d, $J = 12.0$ Hz, H-23b), 5.36 (1H, br s, H-12); ^1H NMR spectral data for sugar moieties, see Table 2; ^{13}C NMR spectral data, see Tables 1 and 3. HR-ESI-MS: *m/z* 2007.8625 $[M + Na]^+$ (calcd for $C_{88}H_{144}O_{49}Na$, 2007.8668).

3.3.2 *Clematochinenoside I (**2**)*

White amorphous solid, $[\alpha]_D^{20}$ −58 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 243 (2.34), 295 (sh), 324 (4.28) nm; IR (KBr) ν_{max} (cm^{-1}): 3402, 2931, 1727, and 1632; ^1H NMR (C_5D_5N, 500 MHz): δ 1.15 (3H, s, Me-27), 1.10 (3H, s, Me-24), 1.07 (3H, s, Me-26), 0.93 (3H, s, Me-25), 0.85 (3H, s, Me-29), 0.84 (3H, s, Me-30), 4.26 (1H, dd, $J = 12.0$, 5.0 Hz, H-3), 3.89 (1H, d, $J = 12.0$ Hz, H-23a), 4.22 (1H, d, $J = 12.0$ Hz, H-23b), 5.37 (1H, br s, H-12); ^1H NMR spectral data for sugar moieties, see Table 2; ^{13}C NMR spectral data, see Tables 1 and 3; ^1H NMR and ^{13}C NMR spectral data for isoferuloyl moiety, see Table 4. HR-ESI-MS: *m/z* 2508.0181 $[M + Na]^+$ (calcd for $C_{110}H_{172}O_{49}Na$, 2508.0198).

3.3.3 *Clematochinenoside J (**3**)*

White amorphous solid, $[\alpha]_D^{20}$ −55 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 243 (2.33), 295 (sh), 324 (4.28) nm; IR(KBr) ν_{max} (cm^{-1}): 3402, 2932, 1726, and 1630; ^1H NMR (C_5D_5N, 500 MHz): δ 1.15 (3H, s, Me-27), 1.10 (3H, s, Me-24), 1.07 (3H, s, Me-26), 0.93 (3H, s, Me-25), 0.85 (3H, s, Me-29), 0.84 (3H, s, Me-30), 4.26 (1H, dd, $J = 12.0$, 5.0 Hz, H-3), 3.89 (1H, d, $J = 12.0$ Hz, H-23a), 4.22 (1H, d, $J = 12.0$ Hz, H-23b), 5.37 (1H, br s, H-12); ^1H NMR spectral data for sugar moieties, see Table 2; ^{13}C NMR spectral data, see Tables 1 and 3; ^1H NMR and ^{13}C NMR spectral data for isoferuloyl moiety, see Table 4. HR-ESI-MS: *m/z* 2508.0181 $[M + Na]^+$ (calcd for $C_{110}H_{172}O_{49}Na$, 2508.0198).

3.4 *Acid hydrolysis*

Each compound (5 mg) was hydrolyzed with 2 N aqueous CF_3COOH (10 ml) at 110°C for 2 h. The reaction mixture was diluted with H_2O (20 ml) and extracted with EtOAc (3 × 10 ml). The combined EtOAc extract was evaporated under reduced pressure and analyzed by TLC. Hederagenin was detected as a product of **1**–**3**, and isoferulic acid as a product of **2** and **3**. The aqueous layer was repeatedly evaporated with MeOH under vacuum until the solvent was completely removed. The residue was dissolved in anhydrous pyridine (100 μl) and then mixed with a pyridine solution of L-cysteine methyl ester hydrochloride (100 μl). After warming at 60°C for 1 h, hexamethyldisilazane (100 μl) and trimethylsilyl chloride (40 μl) were added, and the mixture was warmed at 60°C for another 30 min. The mixture was filtered through a 0.45 μm membrane to remove the precipitate and analyzed by GC. Separations were carried out on a

HP-5 column (28 m × 0.32 mm). Highly pure helium was employed as carrier gas (1.0 ml/min flow rate), and the flame ionization detector was operated at 260°C (column temp 180°C). The retention times of the monosaccharide derivatives were as follows: L-Rha, 5.41 min; D-Glc, 11.63 min; D-Rib, 5.21 min; L-Ara, 4.97 min.

3.5 Alkaline hydrolysis

Pure compound **1** (15 mg) was refluxed in 5% KOH solution (pH 12–13) at 90°C for 1 h. The reaction mixtures were neutralized with 5% HCl solution and then concentrated to dryness. The residues were extracted with *n*-BuOH, and the organic layers of pure compounds were analyzed by NMR spectroscopy. Hydrolysis of **1** afforded **1a**.

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