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THE FIRST CHLOROGENIC ACID ESTER SAPONIN FROM *Lonicera macranthoides*

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An original triterpenoid saponin, named lonimacranthoide I, was isolated from the flower buds of Lonicera macranthoides *(Caprifoliaceae). It has hederagenin as aglycone. Lonimacranthoide I is a rare chlorogenic acid ester acylated at C-23 of hederagenin. The structure of the saponin was established based on chemical and spectral methods.*

Keywords: *Lonicera macranthoides*, Caprifoliaceae, chlorogenic acid ester saponin, lonimacranthoide I.

The dried flower buds of *Lonicera macranthoides* Hand.-Mazz. (Caprifoliaceae) are commonly used in traditional Chinese medicine in the southwest of China. A number of compounds, such as caffeoylquinic acid derivatives, flavonoids, and saponins, have been reported from this species [1–8]. Herein we report the isolation and structural elucidation of the new saponin named lonimacranthoide I. Lonimacranthoide I is a rare chlorogenic acid ester acylated at C-23 of hederagenin.

Lonimacranthoide I was obtained as an amorphous powder. The molecular formula was established to be $C_{81}H_{122}O_{40}$ by its positive HR-ESI-MS data for the $[M + Na]^+$ at *m/z* 1757.7274 (calcd *m/z* 1757.7404) and DEPT NMR spectra. The spectral features and physicochemical properties revealed **1** to be a triterpenoid saponin. The NMR spectrum indicated that the skeleton of **1** is hederagenin [5–8], identified by co-TLC after total acid hydrolysis of **1**.

The chemical shifts of C-3 (δ 81.7) and C-28 (176.5) revealed that **1** was a bisdesmosidic glycoside [5–8]. The ^1H and ^{13}C NMR spectra of **1** exhibited six sugar anomeric protons at δ 4.82 (1H, d, J = 6.6 Hz), 4.99 (1H, d, J = 7.6 Hz), 5.15 (1H, d, J = 7.7 Hz), 5.58 (1H, d, J = 7.1 Hz), 6.20 (1H, d, J = 8.4 Hz), and 6.23 (1H, br.s) and sugar anomeric carbons at δ 95.6, 101.5, 105.0, 105.3, 105.4, and 106.6 (see Table 1).

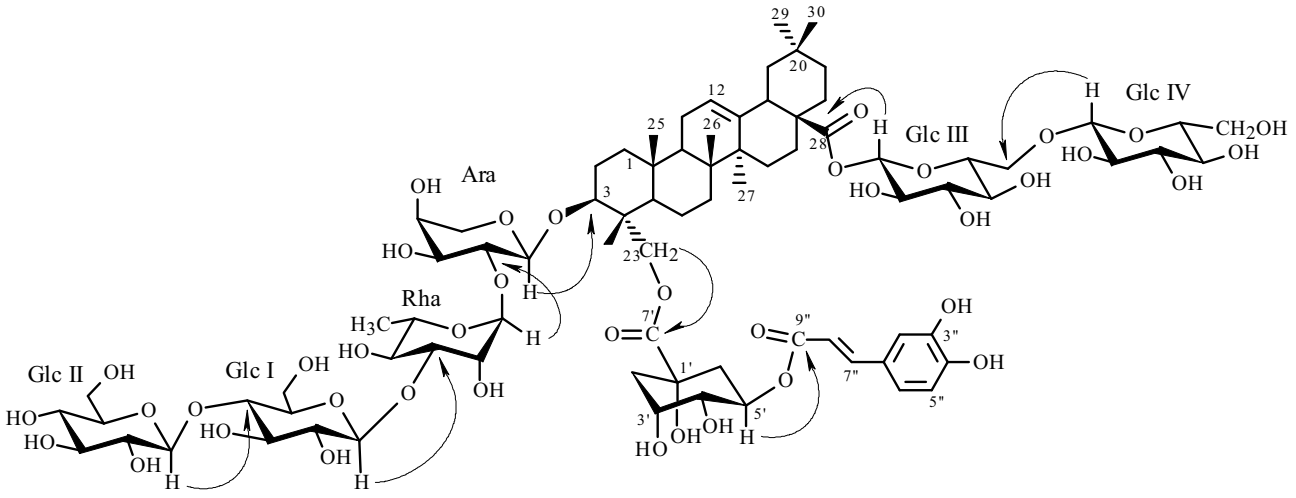

Fig. 1. Key HMBC correlations of **1** (from H to C).

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TABLE 1. ^{1}H and ^{13}C NMR Spectral Data for the Aglycone and Sugar Moieties of **1** in Pyridine-d_5

Aglycone				Sugar moieties			
C atom	δ_C	δ_H	HMBC (H→C)		δ_C	δ_H	HMBC (H→C)
1	38.6	1.01 (m), 1.47 (m)		Ara [b] (at aglycone C-3)			
2	26.0	1.83 (m), 2.10 (m)		1	105.0	4.82 (d, J = 6.6)	aglycone-C-3
3	81.7	3.97 (m)		2	75.1	4.45(m)	
4	42.2			3	74.7	4.11 (m)	
5	48.5	1.45 (m)		4	69.5	4.00 (m)	
6	18.5	1.48 (m), 1.68 (m)		5	66.6	3.69 (m), 4.20 (m)	
7	32.8	1.37 (m), 1.60 (m)		Rha[c] (at Ara C-2)			
8	39.9			1	101.5	6.23 (s)	Ara-C-2,
9	48.2	1.60 (m)					Rha-C-3,5
10	36.7			2	71.6	4.95 (s)	
11	23.4	1.89 (m), 2.00 (m)		3	84.1	4.85 (m)	
12	122.7	5.36 (br.s)		4	73.1	4.48 (m)	
13	144.2			5	69.7	4.69 (m)	
14	41.8			6	18.5	1.58 (d, J = 6.1)	Rha-C-4,5
15	28.2	1.25 (m), 2.29 (m)		Glc[d] I (at Rha C-3)			
16	23.7	1.74 (m), 1.83 (m)		1	106.6	5.58 (d, J = 7.1)	Rha C-3
17	47.1			2	75.7	4.11 (m)	
18	42.5	3.14 (m)		3	76.7	4.36 (m)	
19	46.2	1.20 (m), 1.71 (m)		4	81.3	4.38 (m)	
20	30.7			5	76.3	4.21 (m)	
21	33.8	1.04 (m), 1.26 (m)		6	61.9	4.43 (m), 4.61 (m)	
22	32.5	1.71 (m), 1.84 (m)		Glc[d] II (at Glc I C-4)			
23	66.5	4.40 (m), 4.80 (m)	Chlorogenic acyl C-7′	1	105.3	5.15 (d, J = 7.7)	Glc I-C-4
24	13.2	1.06 (s)	C-3, 4, 5, 23	2	74.9	3.94 (m)	
25	16.0	0.87 (s)	C-26	3	78.7	4.17 (m)	
26	17.5	1.02 (s)	C-8, 9, 14	4	71.5	4.10 (m)	
27	26.1	1.32 (s)	C-8, 14	5	78.3	3.84–4.18 (m)	
28	176.5			6	62.4	4.20 (m), 4.41 (m)	
29	33.0	0.84 (s)	C-19, 30	Glc[d] III (at aglycone C-28)			
30	23.7	0.82 (s)	C-19, 20, 29	1	95.6	6.20 (d, J = 8.4)	aglycone-C-28
				2	73.8	4.06 (m)	
				3	78.0	4.11 (m)	
				4	71.0	4.28 (m)	
				5	77.9	4.08 (m)	
				6	69.4	4.30 (m), 4.65 (m)	
				Glc[d] IV (at Glc III C-6)			
				1	105.4	4.99 (d, J = 7.6)	Glc III-C-6
				2	74.7	3.96 (m)	
				3	78.3	3.84–4.18 (m)	
				4	71.5	4.16 (m)	
				5	78.3	3.84–4.18 (m)	
				6	62.7	4.30 (m), 4.42 (m)	

[a]Values in parentheses are ^{1}H–^{1}H splittings in cases where these are clearly resolved.
[b]Ara = α-L-arabinopyranose; [c]Rha = α-L-rhamnopyranose; [d]Glc = β-D-glucopyranose.

The monosaccharides were identified as arabinose, rhamnose, and glucose (ratio 1:1:4) by GC analysis with authentic monosaccharides and a combination of 2D NMR experiments. The β-anomeric configurations of the glucose units were determined by their $J_{1,2}$ (7.1–8.4 Hz), the α-anomeric configuration of the arabinose was determined by the $J_{1,2}$ = 6.6 Hz, and the α-anomeric configuration of the rhamnose was determined by H-1 (δ 6.23)/C-3 (δ 84.1) and H-1(6.23)/C-5(69.7) correlation peaks in the HMBC spectrum. The sequence of the sugar linkages connected to C-3 of the aglycone was deduced from the following HMBC correlations: H-1 (5.15) of Glc II with C-4 (81.3) of Glc I, H-1 (5.58) of Glc I with C-3 (84.1) of Rha, H-1 (6.23) of Rha with C-2 (75.1) of Ara, and H-1 (4.82) of Ara with C-3 (81.7) of aglycone (see Fig. 1). The second bisdesmosidic part at C-28 was established by the following HMBC information: the correlations between H-1 (4.99) of Glc IV and C-6 (69.4) of Glc III, and H-1 (6.20) of Glc III and C-28 (176.5) of the aglycone (see Fig. 1).

TABLE 2. ^{1}H and ^{13}C NMR Spectral Data for C-23-*O*-Chlorogenic Acyl of Compound **1** and Chlorogenic Acid in Pyridine-d_5

C atom	**1**			Chlorogenic acid	
	δ_C	δ_H	HMBC	δ_H	δ_C
1′	72.9				76.0
2′	38.3	2.68 (m), 2.91 (m)		2.68 (m), 2.93 (m)	38.8
3′	71.0	4.65 (m)		4.76 (m)	71.2
4′	71.6	4.17 (m)		4.30 (dd, J = 3.1, 8.3)	73.6
5′	71.5	6.21 (m)		6.20 (m)	72.3
6′	38.5	2.70 (m), 2.91 (m)		2..71 (m), 2.91 (m)	39.3
7′	174.2				177.2
1″	126.7				126.9
2″	115.8	7.54 (d, J = 2.3)	C-3″	7.46 (d, J = 2.1)	115.5
3″	145.9				145.7
4″	147.5				147.6
5″	116.9	7.15 (d, J = 8.3)	C-4″, 6″	7.13 (d, J = 8.1)	116.7
6″	122.1	7.01 (dd, J = 2.3, 8.3)		7.03 (dd, J = 2.1, 8.1)	121.9
7″	145.9	7.91 (d, J = 15.9)	C-6″, 8″, 9″	7.95 (d, J = 15.9)	145.9
8″	115.6	6.50 (d, J = 15.9)	C-1″	6.55 (d, J = 15.9)	115.8
9″	166.7				167.2

Besides the signals due to the aglycone and component sugars, the ^{1}H NMR spectra of **1** also showed two protons of a double bond in the *trans*-configuration [6.50 (1H, d, J = 15.9 Hz, H-8″), 7.91 (1H, d, J = 15.9 Hz, H-7″)], three aromatic protons of a 1,3,4-trisubstituted benzene [7.01 (1H, dd, J = 2.3, 8.3 Hz, H-6″), 7.15 (1H, d, J = 8.3 Hz, H-5″), and 7.54 (1H, d, J = 2.3 Hz, H-2″)], three oxygenated methine protons [4.65 (1H, m, H-3′), 4.17 (1H, m, H-4′), and 6.21 (1H, m, H-5′)], and four methylene protons [2.68, 2.91 (2H, m, H-2′) and 2.70, 2.91 (2H, m, H-6′)]; the ^{13}C NMR spectra of **1** also showed 16 carbon signals. The above NMR data (see Table 2) were the same as those of a chlorogenic acid moiety, indicating its presence. The molecular formula of **1** also confirmed this. Comparison of the ^{13}C NMR spectra of **1** with those of macranthoidin B [5] showed that the chlorogenate group was attached to C-23 (δ 66.5) of the aglycone, since the chemical shifts of C-23, C-4, C-3, C-5, and C-24 changed for +2.4, –1.1, +0.5, +0.2, and –1.0 ppm, respectively (see Table 1) [7, 9]. The HMBC spectrum provided further confirmation of this chlorogenic acyl group from the correlation between H-23 (δ 4.40, 4.80) of the aglycone and C-7′ (δ 174.2) of the chlorogenic acyl group.

On the basis of the above evidence, lonimacranthoide I (**1**) was found to be a new compound and established as 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl hederagenin 23-*O*-chlorogenic acyl-28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**1**).

EXPERIMENTAL

General Methods. UV spectra, Shimadzu UV-2501PC; IR spectra, IMPACT 400 (KBr); NMR spectra, Bruker AV-500; HR-ESI-MS spectra, Agilent 1100 LC/MSD TOF Mass Spectrometer; GC were carried out on a Shimadzu GC-2010 gas chromatograph, with a DB-1 capillary column (30 m × 0.25 mm) and an FID detector operated at 270°C (column temperature: initial temperature 150°C for 2 min and rising 5°C/min to final 200°C), 3.0 mL/min N_2 as carrier gas.

Plant Material. The flower buds of *Lonicera macranthoides* Hand.-Mazz., collected from Hunan province of P. R. China in 2003, were taxonomically identifided by Prof. Chang-Qi Yuan. A voucher specimen was deposited in Nanjing Botanical Garden Mem. Sun Yat-Sen, Nanjing, Jiangsu, China.

Extraction and Purification. The dried buds (38.0 kg) were extracted with hot 95% ethanol three times. After removal of ethanol, the residual water suspension was re-extracted with petroleum ether and EtOAc, and the obtained aqueous portion was passed through Diaion HP-20 and eluted with water, 50% EtOH, and 90% EtOH. The 50% EtOH fraction (30.0 g) was chromatographed on silica gel columns using a gradient of $CHCl_3$–MeOH–H_2O (17:3:0.2→4:1:0.1→7:3:0.5 →3:3:0.5) to give nine fractions (A–I). Fraction I (2.0 g) was repeatedly chromatographed on RP-C_{18} (YMC; 12 nm) and Sephadex LH-20 (Amersham Biosciences) columns using an MeOH–H_2O solvent system to give compound **1** (20 mg).

Acid Hydrolysis of 1. Lonimacranthoide I (5 mg) was heated in 1 mL of 1 M HCl (dioxane–H_2O, 1:1) at 80°C for 3 h in a water bath. The dioxane was removed, and the solution was extracted with EtOAc (1 mL × 3). The EtOAc portion was washed with water. The aglycone was obtained after removal of EtOAc. The aglycone was tested by TLC with an authentic sample. The aqueous solution from the acid hydrolysis of saponin was neutralized by passing through an Amberlite MB-3 resin column eluted with water, then concentrated and dried. The dried sugar mixture was dissolved in pyridine (0.5 mL), and then treated with hexamethyldisilazane (0.2 mL) and trimethylchlorosilane (0.1 mL) at room temperature for 6 h. After centrifugation, the above fraction was analyzed by GC analysis with authentic monosaccharides.

Lonimacranthoide I (1). $C_{81}H_{122}O_{40}$. White amorphous powder. UV (MeOH, λ_{max}, nm) (log ε): 332 (3.9). IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 2910 (CH), 1720 (C=O, ester), 1627 (unconjugated C=C), 1600 (conjugated C=C), 1440 (conjugated C=C), 1060 (C-O-C). ^1H and ^{13}C NMR, see Tables 1 and 2. HR-ESI-MS (positive mode): *m/z* 1757.7274 $[M + Na]^+$ (calcd 1757.7404).

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