TRITERPENE GLYCOSIDES FROM *Lonicera.* ISOLATION AND STRUCTURAL DETERMINATION OF SEVEN GLYCOSIDES FROM FLOWER BUDS OF *Lonicera macranthoides*

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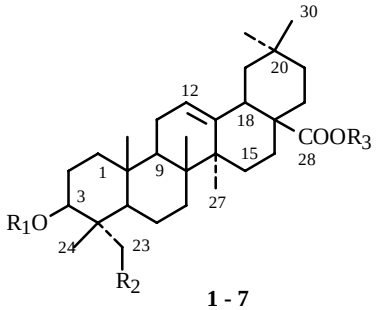
*The structures of seven triterpene glycosides (**1–7**), of which the 23-*O*-acetyl, 28-*O*-β-*D*-glucopyranosyl-(1→6)-*O*-β-*D*-glucopyranosyl ester of hederagenin 3-*O*-β-*D*-glucopyranosyl-(1→3)-*O*-α-*L*-rhamnopyranosyl-(1→2)-*O*-α-*L*-arabinopyranoside (**2**) was new, from the flower buds of* Lonicera macranthoides *were established using chemical and NMR spectroscopic methods.*

Key words: *Lonicera macranthoides* Hand.-Mazz., triterpene glycosides, hederagenin glycosides.

The dried flower buds of *Lonicera macranthoides* Hand.-Mazz., a plant of Lonicera in Caprifoliaceae, are commonly used in traditional Chinese medicine (TCM) in the southwest of China. *Lonicera macranthoides* Hand.-Mazz. was embodied in the PRC Pharmacopoeia (2005 edition) as a newly added species, which forms the item *Shanyinhua*, together with *Lonicera hypoglauca* Miq. and *Lonicera confuse* DC. It has antipyretic and detoxification properties and has been widely used to treat carbuncle and boil, toxin in blood, and fever and colds. Herein we report the structure of the seven triterpene glycosides from the dried flower buds of this plant.



1 - 7

	R_1	R_2	R_3
1:	β-D-Glc-(1→3)-α-L-Rha-(1→2)-α-L-Ara→	OH	β-D-Glc-(1→6)-β-D-Glc→
2:	β-D-Glc-(1→3)-α-L-Rha-(1→2)-α-L-Ara→	OAc	β-D-Glc-(1→6)-β-D-Glc→
3:	α-L-Rha-(1→2)-α-L-Ara→	OH	β-D-Glc-(1→6)-β-D-Glc→
4:	α-L-Rha-(1→2)-α-L-Ara→	OH	H
5:	β-D-Glc-(1→2)-α-L-Ara→	OH	H
6:	α-L-Ara→	OH	β-D-Glc→
7:	H	OH	β-D-Glc-(1→6)-β-D-Glc→

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TABLE 1. Chemical Shifts for ^{13}C Atoms of Aglycons of Glycosides **1**-**7** and Protons of the Aglycon of Glycoside **2** (δ, ppm, 0=TMS, C_5D_5N)

C atom	Compounds							
	1	**2**	**2** (^1H)	**3**	**4**	**5**	**6**	**7**
1	39.11	38.76		39.08	39.00	38.73	38.85	38.80
2	26.39	26.20		26.19	26.20	25.94	26.08	27.66
3	81.28	82.25	3.88 (dd)	81.13	81.08	82.26	81.95	73.48
4	43.61	42.48	-	43.53	43.53	43.51	43.51	42.85
5	47.65	48.63		47.78	47.76	47.93	47.66	48.63
6	18.18	18.44		18.23	18.16	18.22	18.21	18.59
7	32.83	32.94		32.86	33.24	33.24	32.84	32.86
8	39.97	39.97	-	39.97	39.78	39.76	39.99	39.91
9	48.24	48.43		48.24	48.18	47.14	48.21	48.17
10	36.93	36.95	-	36.93	36.91	36.93	39.98	37.21
11	23.42	23.42		23.43	23.85	23.85	23.42	23.35
12	122.81	122.85	5.39 (br.s)	122.95	122.61	122.50	122.95	122.94
13	144.17	144.17	-	144.14	144.80	144.80	144.13	144.21
14	42.18	42.10	-	42.20	42.16	42.16	42.16	42.15
15	28.35	28.25		28.34	28.36	28.34	28.29	28.28
16	23.87	23.84		23.89	23.69	23.78	23.68	23.81
17	47.08	47.11	-	47.09	46.65	46.67	47.01	47.02
18	41.71	41.77	3.17 (dd)	41.73	41.98	42.01	41.76	41.68
19	46.25	46.24		46.28	46.42	46.47	46.18	46.22
20	30.76	30.79	-	30.76	30.94	30.94	30.76	30.70
21	34.04	34.03		34.03	34.23	34.24	34.00	33.94
22	32.61	32.60		32.62	32.89	32.89	32.57	32.53
23	64.07	66.11	4.50	64.09	64.01	64.90	64.57	68.00
24	14.13	13.47	1.06 (s)	13.92	13.98	13.43	13.60	13.03
25	16.23	16.15	0.90 (s)	16.22	16.07	16.05	16.20	16.04
26	17.58	17.60	1.06 (s)	17.61	17.45	17.47	17.58	17.57
27	26.08	25.92	1.23 (s)	26.06	26.16	26.13	26.08	26.02
28	176.52	176.52	-	176.54	180.15	180.12	176.43	176.58
29	33.10	33.14	0.84 (s)	33.10	33.24	33.24	33.10	33.04
30	23.72	23.72	0.85 (s)	23.71	23.77	23.85	23.89	23.64

Glycosides **1** and **3** were assigned as the 28-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl ester of hederagenin 3-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside (macranthoidin A) and the 28-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl ester of hederagenin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside (dipsacoside B), respectively, by TLC with authentic samples and direct comparison of the spectral data [1].

Glycosides **4** and **5** were identified as hederagenin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside and hederagenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside respectively, which were isolated from *Lonicera japonica* Thunb. Previously [2]. Glycoside **6** was elucidated as the 28-*O*-*β*-D-glucopyranosyl ester of hederagenin 3-*O*-*α*-L-arabinopyranoside (HN-Saponin F), which was previously reported from *Hedera nepalensis* K. Koch [3]. Glycoside **7** was assigned as the 28-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl ester of hederagenin (Dipsacussaponin A), which was previously isolated from *Hedera rhombea* Bean. [4]. Glycosides **4**–**7** were identified by comparison of the ESI-MS, ^{13}C NMR, melting point (mp), and optical rotation data ($[\alpha]_D$) with reference data. They were all isolated for the first time from *Lonicera macranthoides* Hand.-Mazz.

TABLE 2. Chemical Shifts for ^{13}C Atoms of Carbohydrates of Glycosides, **1 - 7** (δ, ppm, 0=TMS, C_5D_5N) and Protons of the Carbohydrates of Glycoside **2** (δ, ppm, 0=TMS, C_5D_5N)

C atom	Compound							
	1	**2**	**2** (^1H)	**3**	**4**	**5**	**6**	**7**
C_3-O-								
Ara-1	104.87	105.33	4.84 (d)	104.20	104.37	103.92	106.65	
2	75.52	75.82	4.46 (dd)	75.96	75.86	81.35	73.14	
3	74.99	74.48	4.43 (dd)	74.17	74.70	73.63	74.74	
4	69.53	69.52	4.59 (m)	69.76	69.71	68.27	69.62	
5	66.33	66.25	4.27 (m)	65.41	65.66	64.96	66.97	
			3.76 (d)					
Rha-1	101.49	101.73	6.13 (s)	101.71	101.69			
2	71.78	71.60	4.97 (s)	72.35	72.39			
3	83.11	83.57	4.78 (dd)	72.59	72.57			
4	73.04	73.03	4.43 (t)	73.96	74.17			
5	69.79	69.86	4.69 (d)	69.14	69.32			
6	18.54	18.54	1.56 (d)	18.54	18.55			
Glc-1	108.89	106.86	5.52 (d)			105.95		
2	75.94	76.19	4.08 (t)			76.25		
3	78.41	78.44	4.25 (m)			78.29		
4	71.63	71.54	4.15 (m)			71.40		
5	78.43	78.36	3.95 (t)			78.29		
6	62.64	62.63	4.45 (dd)			62.54		
			4.33 (m)					
C_{23}-$COCH_3$		170.62						
-$COCH_3$		20.84	2.00					
C_{28}–O-								
Glc-1	95.70	95.74	6.22 (d)	95.70			95.76	95.69
2	73.96	73.94	4.08 (t)	73.96			74.17	73.88
3	78.77	78.80	4.18 (m)	78.44			79.32	78.75
4	71.09	71.04	4.27 (m)	71.09			71.17	70.99
5	78.59	78.49	4.12 (m)	78.01			78.93	77.98
6	69.72	69.53	4.69 (dd)	69.53			62.26	69.45
			4.31 (d)					
Glc-1	105.30	105.34	5.0 (d)	105.31				105.33
2	75.20	75.22	3.96 (t)	75.20				75.17
3	78.54	78.49	4.13 (m)	78.77				78.40
4	71.69	71.60	4.16 (m)	71.63				71.54
5	78.00	78.03	3.85 (t)	78.44				78.45
6	62.73	62.72	4.43 (dd)	62.74				62.65
			4.20 (m)					

Glycoside **2** has the molecular formula $C_{61}H_{98}O_{28}$, determined by ESI (+)-MS (1301 $[M+Na]^+$) and ESI (–)-MS (1277 $[M-H]^+$) and confirmed by ^1H NMR and ^{13}C NMR data. The ^1H NMR and ^{13}C NMR spectra of **2** were very similar to those of **1**. However, signals for the acetyl groups were observed in the ^1H NMR spectrum at δ 2.00 (3H s), as well as signals at δ 170.6 and 20.8 ppm from the ^{13}C NMR spectrum. Comparison of the ^{13}C NMR spectra of **2** with those of **1** showed that the acetyl group was attached to C-23 (δ 66.1), since the chemical shifts of C-23, C-4, C-3, C-5, and C-24 changed for +2.0, –1.1, +1.0, +1.0, and –0.7 ppm, respectively [5]. The HMBC spectrum provided further confirmation of this acetyl group from the correlation between H-23 (δ 4.50) and C (δ 170.6) of an acetyl group. Total acid hydrolysis of **2** produced arabinose, glucose, rhamnose, and the aglycon, which had the same chromatographic mobility as hederagenin. Moreover, in the ESI (+)-MS, fragments at *m/z* 1139 $[M+Na\text{-glc}]^+$, 993 $[M+Na\text{-glc-rha}]^+$, 861 $[M+Na\text{-glc-rha-ara}]^+$, and 772 $[M+Na\text{-3glc-Ac}]^+$, and in the ESI (–)-MS, fragments at *m/z* 953 $[M-H\text{-2glc}]^-$ and 910 $[M-H\text{-2glc-Ac}]^-$, were seen.

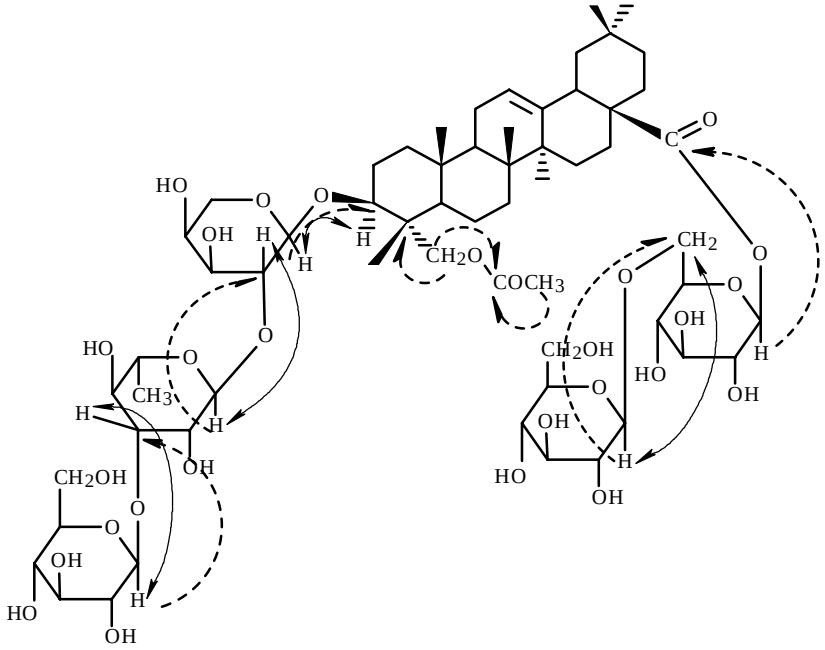

Fig. 1. Key ROESY correlations (H ⟷ H) and Key HMBC correlations (H ⟶ C) of **2**.

Consequently, the structure of **2** was assigned as the 23-*O*-acetyl, 28-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl ester of hederagenin 3-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside. The structure of **2** was also confirmed by ROESY and HMBC spectra; see Fig. 1.

On the basis of the above evidence, glycoside **2** was assigned to be a new compound named 23-*O*-acetyl, 28-*O*-*β*-D-glucopyranosyl-(1→6)-O-*β*-D-glucopyranosyl ester of hederagenin 3-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside (**2**).

EXPERIMENTAL

General Methods. ^{1}H NMR and ^{13}C NMR, ROESY, HMQC, and HMBC spectra: Bruker spectrometers operating at 500 MHz; ESI-MS: Agilent 1100 LC/MSD SL; JASCO P-1020 optical rotation apparatus.

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

Extraction and Purification. The dried buds (38.0 kg) were extracted with hot ethanol for three times. After removal of ethanol, the water suspension was re-extracted with petroleum ether and EtOAc, and the obtained aqueous portion was passed through MCI gel HP-20 and eluted with water, 50% EtOH, and 90% EtOH. The 50% fraction (30.0 g) was repeatedly 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 compounds **1** (280 mg), **2** (15 mg), and **3** (80 mg). The 90% fraction (10.0 g) was repeatedly chromatographed on silica gel columns using a gradient of $CHCl_3$–MeOH–H_2O (10:1:0.1→17:3:0.2→4:1:0.1) to give compounds **4** (10 mg), **5** (9 mg), **6** (10 mg), and **7** (20 mg). All these products were further purified by RP-C_{18} (YMC; 12 nm) and Sephadex LH-20 (Amersham Biosciences) using an MeOH-H_2O solvent system.

Acid Hydrolysis of Compound 2. The sample (5 mg) was hydrolyzed by heating in 1N H_2SO_4 (aq.) for 1 hour. The reaction mixture was neutralized and then extracted with Et_2O. The Et_2O layer was concentrated to dryness to give the aglycon. The aqueous layer was detected by HPTLC to give the sugar components.

Glycoside 1, $C_{59}H_{96}O_{27}$, white amorphous powder, mp 228–230°C (MeOH), $[\alpha]_D^{25.9}$ –9.6° (*c* 0.125; MeOH), ESI-MS *m/z*: 1259 $[M+Na]^+$; for ^{13}C NMR, see Tables 1 and 2.

Glycoside 2, $C_{61}H_{98}O_{28}$, white amorphous powder, mp 225–227° (MeOH), $[\alpha]_D^{25.9}$ –3.7° (*c* 0.125; MeOH), ESI (+)-MS *m/z*: 1301 $[M+Na]^+$, ESI (–)-MS: 1277 $[M-H]^-$, IR bands (KBr, ν_{max}, cm^{-1}): 3500–3000 (OH), 1720 (COOR), 1630

(C=C), 1090–1030 (C-O), 1750, 1250 (CH_3COO). ^1H NMR and ^{13}C NMR as well as HSQC, HMBC, and ROESY; for spectral data see Tables 1 and 2 and Fig. 1.

Glycoside 3, $C_{53}H_{86}O_{22}$, white amorphous powder, mp 232–234°C (MeOH), $[\alpha]_D^{25.9}$ –7.6° (*c* 0.5; MeOH), ESI-MS *m/z*: 1097 $[M+Na]^+$; for ^{13}C NMR, see Tables 1 and 2.

Glycoside 4, $C_{41}H_{66}O_{12}$, white amorphous powder, mp 228–230°C (MeOH), $[\alpha]_D^{25.9}$ –6.4° (*c* 0.125; MeOH), ESI-MS *m/z*: 773 $[M+Na]^+$; for ^{13}C NMR, see Tables 1 and 2.

Glycoside 5, $C_{41}H_{66}O_{13}$, white amorphous powder, mp 248–250°C (MeOH), $[\alpha]_D^{25.9}$ +8.5° (*c* 0.125; MeOH), ESI-MS *m/z*: 789 $[M+Na]^+$; for ^{13}C NMR, see Tables 1 and 2.

Glycoside 6, $C_{41}H_{66}O_{13}$, white amorphous powder, mp 204–206°C (MeOH), $[\alpha]_D^{25.9}$ +35.5° (*c* 0.125; MeOH), ESI-MS *m/z*: 789 $[M+Na]^+$; for ^{13}C NMR, see Tables 1 and 2.

Glycoside 7, $C_{42}H_{68}O_{14}$, white amorphous powder, mp 186–188°C (MeOH), $[\alpha]_D^{25.9}$ +14.8° (*c* 0.125; MeOH), ESI-MS *m/z*: 819 $[M+Na]^+$; for ^{13}C NMR, see Tables 1 and 2.

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