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

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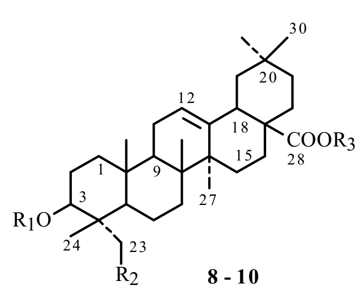
*Two new triterpenoid saponins, lonimacranthoide II (**8**) and III (**9**), were isolated from the flower buds of* Lonicera macranthoides *Hand.-Mazz. (Caprifoliaceae), as well as one known saponin **10**. The structures of the saponins were established based on chemical and spectral methods.*

Key words: *Lonicera macranthoides* Hand.-Mazz., Caprifoliaceae, triterpene glycosides, hederagenin glycoside, oleanolic acid glycosides.

We have previously reported the structures of seven triterpene glycosides (**1–7**) from dried flower buds of *Lonicera macranthoides* Hand.-Mazz. [1]. Through further study, we obtained two new triterpenoid saponins, lonimacranthoide II (**8**) and III (**9**), and one known saponin **10** from the plant.

Lonimacranthoide II (**8**) has the molecular formula $C_{65}H_{106}O_{31}$, determined from its positive HR-ESI-MS data as well as DEPT spectra. The spectral features and physicochemical properties suggest that **8** is a triterpenoid saponin. The seven methyl groups [δ 0.86 (s, 3 × CH_3), 1.06, 1.11, 1.23 and 1.26] and one trisubstituted olefinic proton (δ 5.39, br.s) observed in the ^{1}H NMR spectrum coupled with the information from the ^{13}C NMR spectrum (seven tertiary methyl carbons at δ 15.7, 17.2, 17.5, 23.7, 26.1, 28.2 and 33.0 and two olefinic carbons at δ 122.9 and 144.2) indicate that the aglycone possesses an olean-12-ene skeleton (see Tables 1 and 3). The ^{1}H and ^{13}C NMR spectra of **8** exhibited six sugar anomeric protons at δ 4.79 (1H, d, J = 6.0 Hz), 4.99 (1H, d, J = 7.8 Hz), 5.15 (1H, d, J = 7.7 Hz), 5.39 (1H, d, J = 7.9 Hz), 6.15 (1H, br.s), and 6.20 (1H, d, J = 8.1 Hz) and sugar anomeric carbons at δ 95.6, 101.5, 105.1, 105.3, 105.3, and 106.5 (see Tables 2 and 3).



8 - 10

8: R_1 = β-D-Glc-(1→4)-β-D-Glc-(1→3)-α-L-Rha-(1→2)-α-L-Ara→, R_2 = H, R_3 = β-D-Glc-(1→6)-β-D-Glc→

9: R_1 = β-D-Glc-(1→4)-β-D-Glc-(1→3)-α-L-Rha-(1→2)-α-L-Ara→, R_2 = OH, R_3 = β-D-Glc→

10: R_1 = β-D-Glc-(1→3)-α-L-Rha-(1→2)-α-L-Ara→, R_2 = H, R_3 = β-D-Glc-(1→6)-β-D-Glc→

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TABLE 1. Chemical Shifts for ^{13}C Atoms of Aglycons of Glycosides **8**, **9**, and **10** (δ, ppm, 0 = TMS, C_5D_5N)

C atom	Compound			C atom	Compound		
	8	**9**	**10**		**8**	**9**	**10**
1	38.9	38.6	40.0	16	23.8	23.9	23.8
2	26.6	26.0	26.6	17	47.1	47.0	47.1
3	88.7	81.2	88.8	18	41.7	41.7	41.7
4	39.6	43.6	39.6	19	46.3	46.2	46.3
5	56.0	47.7	56.1	20	30.8	30.8	30.7
6	18.6	18.2	18.5	21	34.0	34.0	34.0
7	32.6	32.8	32.6	22	33.1	32.6	33.1
8	39.9	40.0	39.9	23	28.2	64.1	28.2
9	48.1	48.2	48.1	24	17.2	14.1	17.1
10	37.1	36.9	37.1	25	15.7	16.2	15.6
11	23.4	23.4	23.4	26	17.5	17.5	17.5
12	122.9	123.0	122.9	27	26.1	26.1	26.1
13	144.2	144.2	144.2	28	176.5	176.4	176.5
14	42.2	42.2	42.2	29	33.0	33.1	33.1
15	28.3	28.3	28.3	30	23.7	23.7	23.7

TABLE 2. Chemical Shifts for ^{13}C Atoms of Carbohydrates of Glycosides **8**, **9**, and **10** (δ, ppm, 0 = TMS, C_5D_5N)

C atom	Compound			C atom	Compound		
	8	**9**	**10**		**8**	**9**	**10**
C_3-O-				C_{28}-O-			
Ara-1	105.1	104.7	105.3	Glc-1	95.6	95.8	95.7
2	75.5	75.4	75.9	2	73.9	74.9	73.9
3	74.2	74.3	74.3	3	78.2	79.3	78.8
4	69.7	69.6	69.5	4	71.1	71.3	71.1
5	65.4	66.2	66.3	5	78.0	78.9	78.4
Rha-1	101.5	101.4	101.6	6	69.1	62.5	69.2
2	71.6	71.8	71.7	Glc-1	105.3		105.3
3	83.5	83.6	83.4	2	75.2		75.2
4	73.0	73.0	73.0	3	78.4		78.5
5	69.5	69.7	69.8	4	71.5		71.6
6	18.4	18.4	18.5	5	78.5		78.0
Glc-1	106.5	106.7	106.8	6	62.7		62.7
2	75.6	75.5	75.7				
3	76.7	76.7	78.6				
4	81.2	81.2	71.6				
5	76.6	76.7	78.4				
6	62.0	62.0	62.6				
Glc-1	105.3	105.3					
2	74.7	74.7					
3	78.7	78.3					
4	71.6	71.6					
5	78.4	78.4					
6	62.4	62.4					

TABLE 3. Chemical Shifts for ^{1}H Atoms of Glycosides **8** and **9** (δ, ppm, J/Hz, 0 = TMS, C_5D_5N)

C atom	Compound		C atom	Compound	
	8	**9**		**8**	**9**
3	3.25 (dd, J = 4.0, 11.6)	4.27 (m)	C_3-O-		
12	5.39 (br.s)	5.39 (br.s)	Ara-1	4.79 (d, J = 6.0)	4.99 (d, J = 6.7)
18	3.17 (dd, J = 3.8, 13.5)	3.15 (dd, J = 3.7, 13.2)	2	4.48 (m)	4.48 (m)
23	1.26 (s)	3.85 (m), 4.23 (m)	3	4.16 (m)	4.15 (m)
24	1.11 (s)	1.09 (s)	4	4.17 (m)	4.07 (m)
25	0.86 (s)	0.94 (s)	5	3.75 (m), 4.25 (m)	3.69 (m), 4.19 (m)
26	1.06 (s)	1.09 (s)	Rha-1	6.15 (s)	6.23 (s)
27	1.23 (s)	1.17 (s)	2	4.88 (s)	4.89 (s)
29	0.86 (s)	0.85 (s)	3	4.72 (dd, J = 2.9, 9.4)	4.76 (dd, J = 3.0, 9.4)
30	0.86 (s)	0.85 (s)	4	4.44 (m)	4.43 (m)
C_{28}-O-			5	4.69 (m)	4.64 (m)
Glc-1	6.20 (d, J = 8.1)	6.30 (d, J = 8.1)	6	1.51 (d, J = 6.1)	1.52 (d, J = 6.2)
2	4.08 (m)	3.94 (m)	Glc-1	5.39 (d, J = 7.9)	5.40 (d, J = 7.7)
3	4.15 (m)	3.99 (m)	2	4.05 (m)	4.05 (m)
4	4.28 (m)	4.30 (m)	3	4.25 (m)	4.24 (m)
5	4.28 (m)	4.23 (m)	4	4.32 (m)	4.27 (m)
6	4.32 (m), 4.68 (m)	4.16 (m), 4.42 (m)	5	3.88 (m)	3.88 (m)
Glc-1	4.99 (d, J = 7.8)		6	4.37 (m), 4.50 (m)	4.33 (m), 4.45 (m)
2	3.95 (m)		Glc-1	5.15 (d, J = 7.7)	5.13 (d, J = 7.9)
3	4.16 (m)		2	4.00 (m)	4.00 (m)
4	4.15 (m)		3	4.15 (m)	4.14 (m)
5	3.85 (m)		4	4.10 (m)	4.13 (m)
6	4.31 (m), 4.45 (m)		5	3.95 (m)	3.94 (m)
			6	4.23 (m), 4.43 (m)	4.22 (m), 4.43 (m)

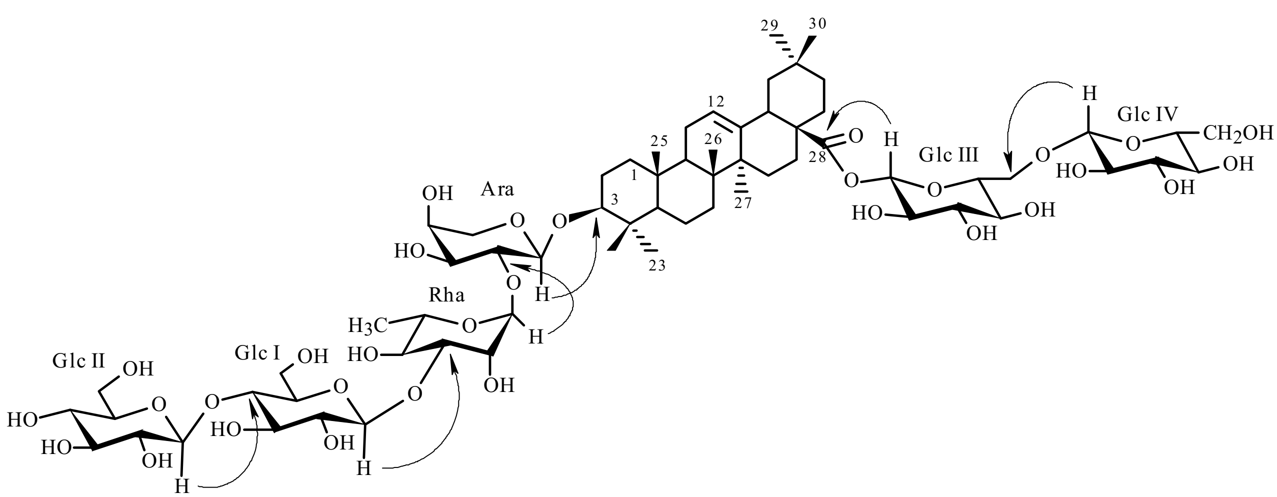

Fig. 1. Key HMBC correlations of **8** (from H to C).

Acid hydrolysis afforded oleanolic acid and the component sugars, which were identified as arabinose, rhamnose, and glucose (1:1:4) by GC analysis with authentic monosaccharides. The chemical shifts of C-3 (δ 88.7) and C-28 (δ 176.5) revealed that **8** was a bisdesmosidic glycoside [2]. The sequence of the sugar linkages connected to C-3 of the aglycone was deduced from the following HMBC correlations: H-1 (δ 5.15) of Glc II with C-4 (δ 81.2) of Glc I, H-1 (δ 5.39) of Glc I with C-3 (δ 83.5) of Rha, H-1 (δ 6.15) of Rha with C-2 (δ 75.5) of Ara, H-1 (δ 4.79) of Ara with C-3 (δ 88.7) of aglycone (see Fig. 1). The second bisdesmosidic part at C-28 was established by the following HMBC information: the correlations between H-1 (δ 4.99) of Glc IV and C-6 (δ 69.1) of Glc III, and H-1 (δ 6.20) of Glc III and C-28 (δ 176.5) of the aglycone (see Fig. 1). The ^{1}H and

^{13}C NMR chemical shift assignments were accomplished by a combination of DEPT, HSQC HMQC-TOCSY, HMBC, and ROESY experiments. Thus, lonimacranthoide II (**8**) was elucidated as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Lonimacranthoide III (**9**) has the molecular formula $C_{59}H_{96}O_{27}$, determined from its negative ion HR-ESI-MS spectrum and from DEPT NMR data. Its ^{1}H and ^{13}C NMR spectra indicate that compound **9** possesses the same aglycone as that of macranthoidin A [1] but differs in the sugar moiety (see Tables 2 and 3). The ^{1}H and ^{13}C NMR spectra of **9** exhibited five anomeric protons and carbons (see Tables 2 and 3). Acid hydrolysis afforded hederagenin and the component sugars, which were identified as arabinose–rhamnose–glucose (1:1:3) by GC analysis. The chemical shifts of C-3 (δ 81.2) and C-28 (δ 176.4) revealed that **9** was a bisdesmosidic glycoside. From its 2D NMR data, it was evident that the sugar structure at C-3 was the same as that in **8**, and the sugar at C-28 was a D-glucose. On the basis of these results, lonimacranthoide III (**9**) was established as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester.

The known saponin **10** was identified as 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester [2] by analysis of its NMR spectroscopic data (see Tables 1 and 2). It was isolated from *Lonicera macranthoides* Hand.-Mazz. for the first time.

EXPERIMENTAL

General Comments. 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 operating 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 taxonomicly identified 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 for three times. After removal of ethanol, the residued 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 9 fractions (A-I). The fraction I (2.0 g) was repeatedly chromatographed on RP-C18 (YMC; 12 nm) and Sephadex LH-20 (Amersham Biosciences) columns using an MeOH–H_2O solvent system to give **8** (80 mg), **9** (36 mg), **10** (118 mg).

General Method for Acid Hydrolysis. Each saponin (5 mg) was heated in 1 mL of 1 M HCl (dioxane–H_2O 1:1) at 80° for 3 h in a water bath. Dioxane was removed and the solution was extracted with EtOAc (1 mL × 3). The EtOAc portion was washed with water. Then the aglycone was obtained after removal of EtOAc. The aglycone was tested by TLC with an authentic sample. The aqueous solution from acid hydrolysis of each 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 II (8), $C_{65}H_{106}O_{31}$, white amorphous powder; mp 225–226°C (MeOH); $[\alpha]_D^{21.9}$ –17.2° (*c* 0.065; MeOH); IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 2910 (CH), 1726 (C=O, ester), 1627 (unconjugated C=C), 1060 (C-O-C); ^{1}H and ^{13}C NMR see Tables 1, 2 and 3; HR-ESI-MS (positive mode): *m/z* 1405.6542 $[M+Na]^+$ (Calcd: 1405.6610).

Lonimacranthoide III (9), $C_{59}H_{96}O_{27}$, white amorphous powder; mp 240–242°C (MeOH); $[\alpha]_D^{21.9}$ –10.0° (*c* 0.050; MeOH); IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 2910 (CH), 1728 (C=O, ester), 1627 (unconjugated C=C), 1050 (C-O-C); ^{1}H and ^{13}C NMR see Tables 1, 2 and 3; HR-ESI-MS (negative mode): *m/z* 1235.6016 $[M-H]^-$ (Calcd: 1235.6066).

Glycoside 10, $C_{59}H_{96}O_{26}$, white amorphous powder, mp 220–222°C (MeOH), $[\alpha]_D^{21.9}$ –12.0° (*c* 0.105; MeOH), ESI-MS *m/z*: 1243 $[M+Na]^+$, ^{13}C NMR see Table 1 and 2.

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