

Two Triterpenoid Saponins from *Lonicera Japonica*

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Abstract: A new triterpenoid saponin, together with a known saponin, was isolated from the flowers of *Lonicera Japonica* Thunb. Using chemical and spectroscopic methods, mainly 2D NMR technique, their structures were deduced to be 3- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 3)- α -L-rhamnospyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl hederagenin 28-O- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside and 3- α -L-rhamnospyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl hederagenin 28-O- α -L-rhamnospyranosyl-(1 \rightarrow 2)-[β -D-xylcopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranoside, respectively.

Keywords: Triterpnoid saponin, *Lonicera Japonica*, 2D NMR.

The flowers of *Lonicera Japonica* Thunb are a Chinese traditional medicine and have the functions such as anti-bacteria, anti-virus and hepato-protective. A new triterpenoid saponin **2**, together with a known saponin **1**, was obtained from its flowers. This paper mainly discussed the structure elucidation of these two saponins.

Acid hydrolysis of saponin **1** and **2** yielded the same aglycon. By comparing ¹H and ¹³C NMR data, the aglycon was determined to be hederagenin¹. This result was also confirmed by 2D NMR spectra. Using HMQC and DQF-COSY, ¹³C and some of ¹H NMR signals of the aglycon were assigned (Table 1).

Saponin **1**, white amorphous powder, FDMS showed quasi-molecular ion peak at *m/z* 1213 [M+Na]⁺. In combination with ¹H and ¹³C NMR spectra, its molecular formula was deduced to be C₅₈H₉₄O₂₅. Acid hydrolysis of **1** on TLC gave glucose, xylose, arabinose, rhamnose by comparison with authentic samples.

HMQC spectra showed five anomeric proton signals at δ 5.10 (1H,d,J=5.97Hz), δ 6.22 (1H, brs), δ 6.13 (1H,d,J=7.8Hz), δ 6.51 (1H, brs), δ 4.88 (1H,d,J=7.53Hz) attached to five anomeric carbon signals at δ 104.5, δ 101.8, δ 94.9, δ 101.7, δ 105.6, respectively. It suggested that **1** contains five sugar moieties. As we know, HMQC-TOCSY is very useful to assign the ¹H and ¹³C NMR signals of sugar moieties especially when NMR signals are overlapped; From HMQC-TOCSY, ¹H and ¹³C NMR information of each sugar could be obtained and distinguished from each other²⁻³. For example, from cross-peak between anomeric proton δ 4.88 (1H,d,J=7.53Hz) and anomeric carbon δ 105.6, we find four

carbons at δ 78.1, δ 74.9, δ 71.2, δ 67.1 correlated to this anomeric proton. meanwhile, five protons δ 4.27 (1H,m), δ 4.25 (1H,m), δ 4.08 (1H,m), δ 3.91 (1H,t,J=8.8Hz) and δ 3.58 (1H,brd,J=10.12 Hz) correlated to that anomeric carbon. Compared with ^{13}C data of reference ⁴, it was deduced to be a β -D-xylcopyranose. Combined DQF-COSY and HMQC, those signals of carbons were assigned to C-3, C-2, C-4, C-5 and signals of protons were assigned to be H-4, H-5_a, H-3, H-2, H-5_b of the β -D-xylcopyranose respectively. The complete assignments of the other four sugar moieties were similar (Table 2). These assignments were also confirmed by HMBC.

The linkages of oligosaccharide chain and linkage sites to the aglycon were decided by HMBC which observed cross-peaks between the following carbons and protons in saponin 1: C-3 (δ 81.4) of aglycon and H-1 (δ 5.10) of ara., C-2 (δ 76.1) of ara. and H-1 (δ 6.22) of rha., C-28 (δ 176.8) of aglycon and H-1 (δ 6.13) of glc., C-6 (δ 69.3) of glc. and H-1 (δ 4.88) of xyl., and C-2 (δ 75.9) of glc. and H-1 (δ 6.51) of rha. So the structure of saponin 1 was deduced to be 3- α -L-rhamnospyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl hederagenin 28-O- α -L-rhamnospyranosyl-(1 \rightarrow 2)-[β -D-xylcopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranoside (Figure 1). It has been isolated by H. X. Lou⁵.

Saponin 2, white amorphous powder, FDMS showed quasi-molecular ion peak at m/z : 1421 [M+Na]⁺. In combination with ^1H and ^{13}C NMR spectra, its molecular formula was deduced to be C₆₅H₁₀₆O₃₂. Acid hydrolysis of 2 on TLC gave glucose, arabinose, rhamnose by comparison with authentic samples.

HMQC spectra showed that six anomeric proton signals at δ 4.99 (1H,d,J=7.20Hz), δ 6.25 (1H,brs.), δ 5.42 (1H,d,J=7.92Hz), δ 5.14 (1H,d,J=7.68Hz), δ 6.22 (1H,d,J=7.68Hz), δ 5.02 (1H,d,J=7.92Hz) attached to six anomeric carbon signals at δ 105.0, δ 101.4, δ 106.7, δ 104.8, δ 95.7, δ 105.3, respectively. It suggested that 2 contain six sugar moieties. Combining DQF-COSY and HMQC, we assigned the ^1H and ^{13}C NMR signals on the basis of the NMR information of all carbons and protons of each sugar moiety from HMQC-TOCSY spectra (Table 2). These assignments were confirmed by TOCSY, HMBC and ROESY.

The linkages of oligosaccharide chain and linkage sites to the aglycon were decided by HMBC and ROESY. ROESY spectra of 2 showed cross peaks between H-3 (δ 4.23) of aglycon and H-1 (δ 4.99) of ara., H-1 (δ 6.25) of rha. and H-2 (δ 4.51) of ara., H-1 (δ 5.42) of $^1\text{glc.}$ and H-3 (δ 4.77) of rha., H-1 (δ 5.14) of $^2\text{glc.}$ and H-4 (δ 4.31) of $^1\text{glc.}$, H-1 (δ 5.02) of $^2\text{glc.}$ and H-6 (δ 4.32) of $^1\text{glc.}$ Meanwhile, the HMBC spectra revealed cross peaks between C-3 (δ 81.2) of aglycon and H-1 (δ 4.99) of ara., C-2 (δ 76.1) of ara. and H-1 (δ 6.25) of rha., C-3 (δ 83.5) of rha. and H-1 (δ 5.42) of $^1\text{glc.}$, C-4 (δ 81.2) of $^1\text{glc.}$ and H-1 (δ 5.14) of $^2\text{glc.}$, C-28 (δ 176.6) of aglycon and H-1 (δ 6.22) of $^1\text{glc.}$, C-6 (δ 69.5) of $^1\text{glc.}$ and H-1 (δ 5.02) of $^2\text{glc.}$ So the structure of saponin 2 was deduced to be 3- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 3)- α -L-rhamnospyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl hederagenin 28-O- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (Figure.2).It is a new natural product.

Table 1. ¹H(500MHz) and ¹³C(125MHz) NMR data of aglycon of 1 and 2 (δ in pyrdine-d₅)

1				2					
	¹³ C	¹ H		¹³ C	¹ H		¹³ C	¹ H	
1	39.3		39.1		16	24.1	23.9		
2	26.4		26.4		17	47.4	47.1		
3	81.4	4.19(m)	81.2 ^a	4.23(m)	18	42.2	3.11(br.d,J=10.64Hz)	41.8	3.16(dd,J=14.16 Hz,4.08Hz)
4	43.7		43.7		19	46.6		46.3	
5	47.9		47.7		20	30.9		30.8	
6	18.5		18.2		21	34.2		34.1	
7	32.5		32.6		22	33.1		32.8	
8	40.2		40.0		23	64.2	4.06(m); 3.64(m)	64.1	4.23(m); 3.86(m)
9	48.4		48.3		24	14.2	0.98(s)	14.2	1.10(s)
10	37.1		37.0		25	16.5	0.99(s)	16.3	0.96(s)
11	23.6		23.5		26	17.7	1.08(s)	17.6	1.12(s)
12	123.0	5.40(br s.)	123.0	5.38(br s.)	27	26.2	1.17(s)	26.2	1.17(s)
13	144.3		144.2		28	176.8		176.6	
14	42.5		42.2		29	33.4	0.84(s)	33.2	0.85(s)
15	28.8		28.4		30	24.1	0.89(s)	23.8	0.85(s)

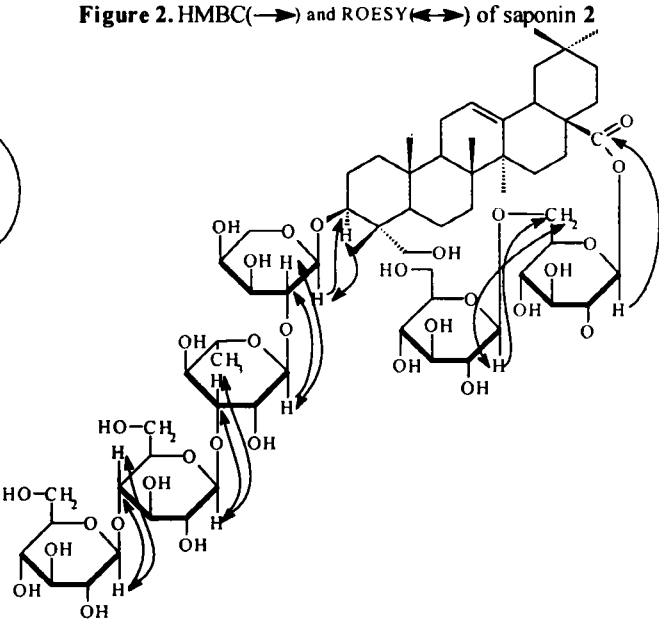
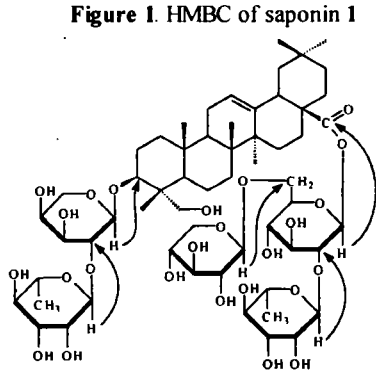


Table 2. ¹H(500MHz) and ¹³C(125MHz) NMR data of sugar moietis of **1** and **2** (δ in pyrdine-d₅)

1			2				
	¹³ C	¹ H		¹³ C	¹ H		
3-O-ara	1	104.5	5.10(d,J=5.97Hz)	1	105.0	4.99(d,J=7.20Hz)	
	2	76.1	4.50(m)	2	75.4	4.51(t,J=7.44Hz)	
	3	74.7	4.08(m)	3	75.0	3.94(m)	
	4	69.4	4.16(m)	4	69.7	4.08(m)	
	5	65.7	4.23(m); 3.68(dd,J=15.22, 8.35Hz)	5	66.3	4.20(m); 3.62(brd,J=13.68Hz)	
rha	1	101.8	6.22(brs.)	1	101.4	6.25(brs.)	
	2	72.4	4.59(m)	2	71.8	4.90(brs.)	
	3	72.7 ^f	4.68(brs.)	3	83.5	4.77(dd,J=8.40Hz, 3.60Hz)	
	4	73.9	4.23(m)	4	73.0	4.46(m)	
	5	69.9	4.62(m)	5	69.7	4.65(m)	
28-O-glc	6	18.7	1.64(d,J=5.79Hz)	6	18.5	1.52(d,J=6.00Hz)	
	1	94.9	6.13(d,J=7.80Hz)	¹ glc	1	106.7	5.42(d,J=7.92Hz)
	2	75.9	4.30(m)	2	75.5	4.08(m)	
	3	79.6	4.22(m)	3	76.8 ^c	4.25(m)	
	4	71.2 ^f	4.10(m)	4	81.2 ^a	4.31(m)	
rha	5	77.7	4.01(m)	5	76.8 ^c	3.90(m)	
	6	69.1	4.58(m); 4.23(m)	6	61.9	4.46(m); 4.36(m)	
	1	101.7	6.51(brs.)	² glc	1	104.8	5.14(d,J=7.68Hz)
	2	72.3	4.50(m)	2	74.8	4.02(m)	
	3	72.7 ^f	4.74(brs.)	3	78.5 ^b	4.12(m)	
xyl	4	74.2	4.30(m)	4	71.6 ^d	4.14(m)	
	5	70.1	4.46(m)	5	78.3	3.96(m)	
	6	19.0	1.76(d,J=5.79Hz)	6	62.5	4.50(m); 4.24(m)	
	1	105.6	4.88(d,J=7.53Hz)	28-O-			
	2	74.9	3.91(t,J=8.80Hz)	¹ glc	1	95.7	6.22(d,J=7.68Hz)
3	78.1	4.08(m)	2	74.0	4.09(m)		
4	71.2 ^c	4.27(m)	3	78.5 ^b	4.22(m)		
5	67.1	4.25(m); 3.58(brd,J=10.12Hz)	4	71.0	4.26(m)		
			5	78.0	4.19(m)		
			6	69.5	4.68(brd,J=9.36Hz); 4.32(m)		
			² glc	1	105.3	5.02(d,J=7.92Hz)	
			2	75.2	3.97(m)		
			3	78.8	4.16(m)		
			4	71.6 ^d	4.18(m)		
			5	78.5 ^b	3.84(m)		
			6	62.7	4.44(m); 4.32(m)		

^{a-f} signals may be interchangeable in the same column.

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