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Triterpenoid saponins from *Mussaenda glabra* Vahl

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Abstract

Five triterpenoid saponins, mussaendoside O (**1**), mussaendoside G (**2**), mussaendoside U (**3**), mussaendoside P (**4**), and mussaendoside Q (**5**) were isolated from the methanol extract of the aerial parts of *Mussaenda glabra*. Their chemical structures were determined using NMR spectra, ESI-MS, as well as in comparison with the reported data. These compounds have been reported from the *Mussaenda glabra* for the first time.

Keywords. *Mussaenda glabra*, Rhamnaceae, triterpenoid saponin.

1. INTRODUCTION

Mussaenda genus comprises about 200 species belonging to the Rhamnaceae family. In Vietnam, *Mussaenda* genus has been used in traditional medicine for the treatment of different ailments such as sore throat, and stomach troubles.[1] Phytochemical study of the *Mussaenda* genus revealed the presence of iridoids, triterpenoids, and flavonoids. These compounds have shown the potential significant biological effects as anti-inflammatory, antioxidant, and anticancer activities.[2] In previous papers, we have reported the isolation and structural determination of several saponins from this genus.[3] In our continuing investigation the chemical constituents from *Mussaenda glabra*, we report herein the isolation and structure elucidation of five known triterpenoid saponins.

2. MATERIALS AND METHODS

2.1. Plant materials

The aerial parts of *Mussaenda glabra* Vahl were collected at Me Linh, Vinh Phuc, Vietnam in February 2017 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (NCCT-P67) was deposited at the Institute of Marine Biochemistry, VAST.

2.2. General experimental procedures

All NMR spectra were recorded on a Varian MR400 or Bruker AM500 spectrometer. ESI mass spectra were recorded on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. Column chromatography was performed using a silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (150 μm, Fuji Silysia Chemical Ltd.), thin layer chromatography (TLC) using a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

2.3. Extraction and isolation

The dried powder of aerial parts of *M. glabra* (5.2 kg) was sonicated 3 times with hot methanol. The extract was filtered through filter paper, then solvent was removed under reduced pressure to yield 270 g of a dark solid extract. The extract was suspended in water and successively partitioned with *n*-hexane, dichloromethane, ethyl acetate giving *n*-hexane (MG1 45 g), dichloromethane (MG2 90 g), ethyl acetate extracts (MG3 23 g) and water layer (MG4). The water layer was chromatographed on a Diaion HP-20 column eluting with water to remove sugars, then increase the concentration of methanol in water (25, 50, 75, and 100 %) to obtain four fractions, MG4A-MG4D. The MG4D extract was chromatographed on a silica gel column eluting with dichloromethane: methanol (100:0 → 0:1, v/v) to

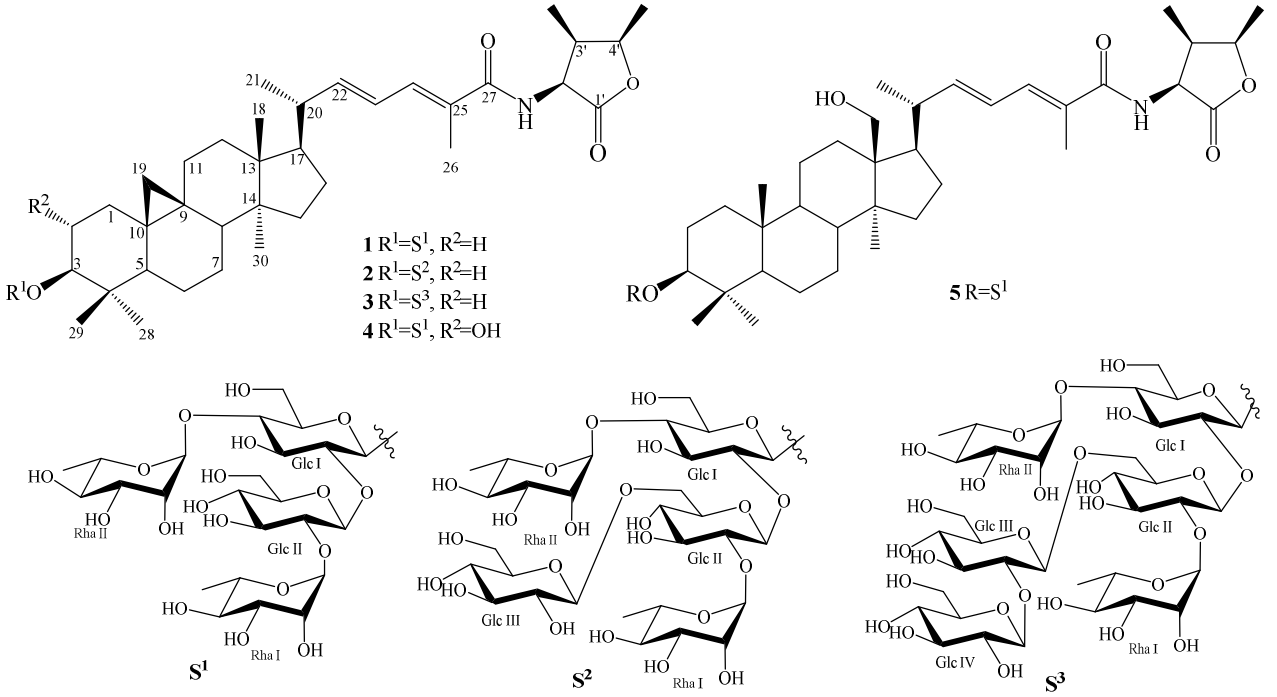

Figure 1: Chemical structures of compounds **1-5**

give 5 sub-fractions, MG4D1-MG4D5. MG4D5 was chromatographed on a RP-18 column eluting with acetone/water (1/1.2, v/v) to give six smaller fractions, MG4D5A-MG4D5F. Compound **1** (30.0 mg) was yielded from MG4D5A fraction using a silica gel column eluting with dichloromethane/acetone/water (1/4/0.3, v/v/v). MG4D5C fraction was chromatographed on a silica gel column eluting with dichloromethane /acetone/water (1/4/0.3, v/v/v) to give two fractions, MG4D5C1 and MG4D5C2. MG4D5C2 was chromatographed on a RP18 column eluting with methanol/water (1/1, v/v) to yield compounds **2** (14.0 mg) and **3** (20 mg). MG4D5E was continued to fractionate on a RP18 column eluting with methanol/water (1/1, v/v) to give two fractions, MG4D5E1 and MG4D5E2. MG4D5E1 was chromatographed on a silica gel column eluting with dichloromethane/methanol/water (3/1/0.15, v/v/v) to yield compounds **4** (12 mg) and **5** (6 mg).

Mussaendoside O (**1**): Amorphous powder; $[\alpha]_D^{25}$ + 2.0 (*c* 0.2, MeOH); ESI-MS *m/z* 1182 $[M+H]^+$, $C_{60}H_{95}O_{22}N$, M = 1181; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

Mussaendoside G (**2**): Amorphous powder; $[\alpha]_D^{25}$ +12.0 (*c* 0.2, MeOH); ESI-MS *m/z* 1344 $[M+H]^+$, $C_{66}H_{105}O_{27}N$, M = 1343; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

Mussaendoside U (**3**): Amorphous powder; $[\alpha]_D^{25}$ + 18.0 (*c* 0.2, MeOH); ESI-MS *m/z* 1528 $[M+Na]^+$, $C_{72}H_{115}O_{32}N$, M = 1505; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 1.

Mussaendoside P (**4**): Amorphous powder; $[\alpha]_D^{25}$ + 5.0 (*c* 0.2, MeOH); ESI-MS *m/z* 1198 $[M+H]^+$, $C_{60}H_{95}O_{23}N$, M = 1197; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 2.

Mussaendoside Q (**5**): Amorphous powder; $[\alpha]_D^{25}$ + 31 (*c* 0.2, MeOH); ESI-MS *m/z* 1198 $[M+H]^+$, $C_{60}H_{95}O_{23}N$, M = 1197; ^{1}H- and ^{13}C-NMR (CD_3OD), see table 2.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous powder. The ^{1}H-NMR spectrum of **1** showed the signals of three olefin protons at δ_H 5.96 (dd, *J* = 9.0, 15.0 Hz), 6.37 (dd, *J* = 11.0, 15.0 Hz), and 6.92 (d, *J* = 11.0 Hz), two cyclopropane protons at δ_H 0.40 (br s), and 0.61 (br s); five tertiary methyl groups at δ_H 0.92, 0.97, 1.09, 1.12, and 1.99; five secondary methyl groups at δ_H 0.85 (d, *J* = 7.5 Hz), 1.08 (d, *J* = 6.5 Hz), 1.28 (d, *J* = 6.5 Hz), 1.32 (d, *J* = 6.5 Hz), and 1.38 (d, *J* = 6.5 Hz) assigning to a triterpene aglycone and four anomeric protons at δ_H 5.23 (br s), 4.84 (br s), 4.90 (d, *J* = 7.6 Hz), and 4.41 (d, *J* = 8.0 Hz) suggesting the presence of four sugar units. The ^{13}C-NMR and DEPT spectra revealed the signals of 60 carbons, including 8 non-protonated carbons, 31 methines, 11 methylenes, and 10 methyl groups. The structure of **1** were determined by comparison with the reported data for mussaendoside O[4], and analysis of NMR spectra. The HMBC correlations from H-28 (δ_H 1.12)/H-29 (δ_H 0.92) to C-3 (δ_C 91.5)/C-4 (δ_C 42.2)/C-5 (δ_C 48.7) indicated the positions of oxygenated group

Table 1: NMR data for compounds **1**-**3** and reference compounds

C	1			2			3		
	δ_C*	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)	δ_C#	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)	δ_C@	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)
1	31.8	33.1	1.30 (m)/1.58 (m)	32.0	33.1	1.54 (m)/1.30 (m)	32.2	33.2	1.53 (m)/1.29 (m)
2	29.4	30.3	1.74 (m)/2.09 (m)	29.7	30.6	2.07 (m)/1.75 (m)	29.8	30.9	2.08 (m)/1.75 (m)
3	89.4	91.5	3.28 (m)	89.9	91.6	3.19 (m)	90.5	92.4	3.17 (m)
4	41.1	42.2	-	41.2	42.3	-	41.4	42.3	-
5	47.3	48.7	1.34 (m)	47.5	48.9	1.32 (m)	47.8	49.0	1.32 (m)
6	20.9	22.1	0.84 (m)/1.63 (m)	21.1	22.1	1.63 (m)/0.84 (m)	21.2	22.2	1.62 (m)/0.84 (m)
7	26.1	27.1	1.14 (m)/1.37 (m)	26.2	27.2	1.36 (m)/1.13 (m)	26.4	27.2	1.36 (m)/1.12 (m)
8	47.7	49.6	1.58 (m)	47.9	49.6	1.58 (m)	48.1	49.7	1.57 (m)
9	19.7	21.1	-	19.7	21.1	-	19.8	21.1	-
10	26.0	27.2	-	26.2	27.4	-	26.3	27.4	-
11	26.3	27.5	1.20 (m)/2.08 (m)	26.4	27.6	2.07 (m)/1.20 (m)	26.6	27.5	2.08 (m)/1.20 (m)
12	32.8	34.0	1.70 (m)	33.0	34.1	1.71 (m)	33.0	34.1	1.71 (m)
13	45.4	46.6	-	45.5	46.7	-	45.6	46.6	-
14	48.9	50.1	-	49.0	50.1	-	49.2	50.1	-
15	35.5	36.7	1.34 (m)/1.36 (m)	35.6	36.8	1.38 (m)/1.33 (m)	35.8	36.8	1.37 (m)/1.33 (m)
16	28.5	29.4	1.32 (m)/1.78 (m)	28.6	29.5	1.78 (m)/1.33 (m)	28.8	29.5	1.77 (m)/1.33 (m)
17	51.7	53.1	1.78 (m)	51.8	53.2	1.78 (m)	52.0	53.2	1.78 (m)
18	18.2	19.0	1.09 (s)	18.3	18.9	1.09 (s)	18.5	19.0	1.09 (s)
19	29.4	30.9	0.40 (br s)/0.61 (br s)	29.7	30.9	0.61 (br s)/0.39 (br s)	29.9	30.9	0.61 (br s)/0.40 (br s)
20	41.1	42.3	2.29 (m)	41.3	42.4	2.30 (m)	41.3	42.4	2.30 (m)
21	19.6	20.3	1.08 (d, 6.5)	19.7	20.2	1.08 (d, 6.5)	19.9	20.2	1.08 (d, 6.5)
22	147.7	149.6	5.96 (dd, 9.0, 15.0)	147.8	149.7	5.97 (dd, 9.0, 15.0)	147.9	149.7	5.97 (dd, 9.0, 15.0)
23	123.3	124.5	6.37 (dd, 11.0, 15.0)	123.5	124.5	6.38 (dd, 11.0, 15.0)	123.8	124.5	6.37 (dd, 11.0, 15.0)
24	134.6	136.4	6.92 (d, 11.0)	134.8	136.4	6.91 (d, 11.0)	134.8	136.4	6.91 (d, 11.0)
25	128.8	128.5	-	128.9	128.6	-	129.1	128.5	-
26	13.2	13.1	1.99 (s)	13.3	13.0	1.99 (s)	13.5	13.0	1.99 (s)
27	170.5	172.4	-	170.7	172.7	-	170.8	172.5	-
28	19.2	26.3	1.12 (s)	15.4	26.5	1.12 (s)	15.6	26.5	1.13 (s)
29	25.7	15.3	0.92 (s)	26.0	15.9	0.94 (s)	26.2	16.0	0.96 (s)
30	15.1	19.9	0.97 (s)	19.3	19.8	0.97 (s)	19.5	19.9	0.97 (s)
Hil									
1′	175.5	176.8	-	175.7	176.9	-	175.8	176.9	-
2′	55.2	56.0	5.08 (d, 7.0)	55.3	56.1	5.07 (d, 7.0)	55.4	56.0	5.08 (d, 7.0)
3′	38.4	39.3	2.83 (m)	38.5	39.4	2.82 (m)	38.6	39.3	2.82 (m)
4′	76.8	78.7	4.77 (m)	76.9	78.7	4.76 (m)	77.0	78.7	4.77 (m)
3′-CH_3	7.9	8.1	0.85 (d, 7.5)	8.0	8.0	0.85 (d, 7.5)	8.1	8.0	0.85 (d, 7.5)
4′-CH_3	15.3	15.7	1.38 (d, 6.5)	15.4	15.7	1.38 (d, 6.5)	15.5	15.7	1.38 (d, 6.5)
Glc I									
1	104.4	105.3	4.45 (d, 8.0)	104.7	105.7	4.40 (d, 8.0)	105.1	106.3	4.38 (d, 8.0)
2	78.8	78.6	3.75 (m)	79.2	78.4	3.93 (m)	78.5	77.7	4.10 (m)
3	77.3	77.6	3.75 (m)	77.6	77.4	3.71 (m)	78.0	78.8	3.70 (m)
4	79.2	79.8	3.52 (m)	79.2	79.9	3.59 (m)	79.4	80.1	3.64 (m)
5	76.2	76.4	3.36 (m)	76.1	76.4	3.33 (m)	76.3	76.4	3.33 (m)
6	61.3	62.0	3.69/3.83 (br d, 11.0)	61.4	62.2	3.69/3.82 (brd, 11.0)	61.6	62.3	3.72*/3.81*
Glc II									
1	101.8	102.0	4.95 (d, 7.6)	101.9	102.0	4.87*	101.9	102.0	4.87*
2	78.1	78.9	3.42 (m)	78.1	79.1	3.43 (m)	79.3	79.2	3.45 (m)
3	79.1	79.2	3.50 (m)	78.1	79.4	3.49 (m)	78.3	79.3	3.50 (m)
4	72.5	72.7	3.11 (dd, 9.0, 9.0)	72.5	72.6	3.19 (m)	72.3	72.4	3.15 (m)
5	77.5	78.0	3.29 (m)	76.7	77.5	3.40 (m)	76.0	77.7	3.42 (m)
6	63.2	63.7	3.58*/3.88*	70.5	70.2	3.76*/4.15*	70.3	70.1	3.79*/4.11*
Rha I									
1	101.8	101.7	5.27 (br s)	101.9	101.9	5.25 (br s)	102.1	101.9	5.23 (br s)
2	72.1	72.2	3.95 (br s)	72.2	72.3	3.94 (br s)	72.4	72.3	3.95 (br s)
3	72.4	72.1	3.78 (m)	72.5	72.2	3.76 (m)	72.7	72.1	3.77 (m)
4	74.0	74.1	3.42 (m)	74.1	74.2	3.42 (m)	74.2	74.1	3.43 (m)
5	69.3	69.4	4.16 (m)	69.4	69.5	4.17 (m)	69.5	69.5	4.18 (m)
6	18.9	18.4	1.28 (d, 6.5)	19.0	18.4	1.28 (d, 6.5)	19.1	18.4	1.28 (d, 6.5)
Rha II									
1	102.4	102.7	4.89 (br s)	102.6	102.8	4.89 (br s)	102.8	102.9	4.95 (br s)
2	72.2	72.3	3.88 (br s)	72.4	72.5	3.86 (br s)	72.5	72.4	3.91 (br s)
3	72.3	72.1	3.68 (m)	72.4	72.1	3.68 (m)	72.7	72.1	3.68 (m)
4	73.7	73.7	3.42 (m)	73.8	73.8	3.42 (m)	73.9	73.8	3.43 (m)
5	70.3	70.6	4.00 (m)	70.5	70.7	4.00 (m)	70.7	70.8	3.96 (m)
6	18.4	18.1	1.32 (d, 6.5)	18.5	18.1	1.32 (d, 6.5)	18.7	18.3	1.36 (d, 6.5)

Table 1: NMR data for compounds **1-3** and reference compounds *(continue)*

Glc III						
1	105.1	104.5	4.68 (d, 8.0)	102.8	102.6	5.18 (d, 7.5)
2	75.2	75.4	3.20 (m)	84.5	83.5	3.42 (m)
3	78.7	77.6	3.52 (m)	77.8	78.4	3.33 (m)
4	71.5	71.6	3.35 (m)	71.2	71.2	3.41 (m)
5	78.0	77.9	3.49 (m)	77.8	76.9	3.56 (m)
6	62.7	62.7	3.72*/3.88*	62.5	62.1	3.72*/3.81*
Glc IV						
1				106.5	105.8	4.63 (d, 7.0)
2				76.5	76.1	3.42 (m)
3				78.1	77.1	3.89 (m)
4				71.1	71.2	3.41 (m)
5				78.8	77.7	3.68 (m)
6				62.3	62.6	3.74*/3.91*

[a]Measured in CD_3OD, [b]125 MHz, [c]500 MHz. *δ_C of mussaendoside O[4], #δ_C of mussaendoside G[5],@δ_C of mussaendoside U[6],*overlapped signals

at C-3, two 1.07). The HMBC correlations from H-20 (δ_H 2.29)/H-24 (δ_H 6.92) to C-22 (δ_C 149.6)/C-23 (δ_C 124.5); from H-21 (δ_H 1.08) to C-22 (δ_C 149.6); from H-23 (δ_H 6.37)/H-26 (δ_H 1.99) to C-24 (δ_C 136.4)/C-25 (δ_C 128.5); and from H-22 (δ_H 5.96) to C-24 (δ_C 136.4) confirmed the position of conjugated double bonds at C-22/C-23 and C-24/C-25. The position of cyclopropane at C-9/C-10 was confirmed by the HMBC correlation between H-19 (δ_H 0.61)/(0.40) and C-1 (δ_C 33.1)/C-9(δ_C 22.1)/C-10 (δ_C 27.2)/C-11 (δ_C 27.5). The ROESY correlations between H-19 (δ_H 0.61)/(δ_H 0.40) and H-8 (δ_H 1.58)/H-18 (δ_H 1.09) indicated that cyclopropane group was β-orientation. Comparison the ^{13}C-NMR data of α-amino-3,4-dimethyl-γ-lactone moiety (δ_C 176.8, 56.0, 39.3, 78.7, 8.1, and 15.7) to those reported in the literature,[4] and the ROESY correlations between H-2′ (δ_H 4.55) and H-3′ (δ_H 2.36)/H-4′ (δ_H 4.71), between H-3′ (δ_H 2.36) and H-4′ (δ_H 4.71), confirmed configurations of H-2′, H-3′, and H-4′ to be α orientation. The glucopyranosyl linkages (glc I and glc II) must be in the β-form as judged from the coupling constants (J = 8.0 Hz for glc I and J = 7.6 Hz for glc II) of the anomeric protons, and α-rhamnopyranosyl moieties (rha I and rha II) were based on coupling constants of anomeric protons of rha I H-1 and rha I H-2 (as both broad singlet signals). In addition, the HMBC cross peaks from rha II H-1 (δ_H 4.89) to glc I C-4 (δ_C 79.8); from rha I H-1 (δ_H 5.27) to glc II C-2 (δ_C 78.9), from glc II H-1 (δ_H 4.95) to glc I C-2 (δ_C 78.6), and from glc I H-1 (δ_H 4.45) to C-3 (δ_C 91.5) confirmed the sugar linkages as [α-L-rhamnopyranosyl(1→2)-*O*-β-D-glucopyranosyl (1→2)]-α-L-rhamnopyranosyl (1→4)-*O*-β-D-glucopyranoside and the location of sugar moiety was at C-3 of the aglycone. From the above evidence, and ESI-MS result (*m/z* 1182 $[M+H]^+$, corresponding to the molecular formula of $C_{60}H_{95}O_{22}N$), compound **1** was identified as mussaendoside O, a compound was previously isolated from *M. pubescens*.[4]

Compound **2** was isolated as an amorphous powder. The ^{1}H-NMR spectrum of **2** exhibited the signals of three olefin protons at δ_H 5.97 (dd, J = 9.0, 15.0 Hz), 6.38 (dd, J = 11.0, 15.0 Hz), and 6.91 (d, J = 11.0 Hz), two cyclopropane proton signals at δ_H 0.40 (br s), and 0.61 (br s); five tertiary methyl groups at δ_H 0.94, 0.97, 1.09, 1.12, and 1.99 (each 3H, s); five secondary methyl groups at δ_H 0.85 (3H, d, J = 7.5 Hz),1.08 (3H, d, J = 6.5 Hz), 1.28 (3H, d, J = 6.5 Hz), 1.32 (3H, d, J = 6.5 Hz), and 1.38 (3H, d, J = 6.5 Hz) assigning to a triterpene aglycone. Five anomeric protons at 5.25 (br s), 4.89 (br s), 4.87, 4.68 (d, J = 8.0 Hz), and 4.68 (d, J = 8.0 Hz) and two secondary methyl groups at 1.28 (3H, d, J = 6.5 Hz) and 1.32 (3H, d, J = 6.5 Hz) and suggesting the presence of five sugar units. The ^{13}C-NMR and DEPT spectra revealed the signals of 66 carbons, including 8 non-protonated carbons, 36 methines, 12 methylenes, and 10 methyl groups. The NMR spectra of **2** were almost similar to the corresponding spectra of **1**, excepted for the addition of a sugar unit at Glc II C-4 in the NMR spectra of **2**. The addition of a sugar unit at Glc II C-4 was further confirmed by HMBC correlations between glc III H-1 (δ_H 4.86) and glc II C-4 (δ_C 72.6). Furthermore, the ESI-MS of **2** exhibited an ion at *m/z* 1344 $[M+H]^+$, corresponding to the molecular formula of $C_{66}H_{105}O_{27}N$. Consequently, **2** was determined to be mussaendoside G, a compound previously reported from *M. pubescens*.[5]

The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of **2** except for an addition of sugar unit. The sugar moieties were proved as β-D-glucopyranosyl with multiplicity of glc I H-1 (δ_H 4.38, d, J=8.0 Hz), glc II H-1 (δ_H 4.87), glc III H-1 (δ_H 5.18, d, J=7.5 Hz), glc IV H-1 (δ_H 4.63, d, J=7.0 Hz); and α-L-rhamnopyranosyl with the multiplicity of rha I H-1 (δ_H 5.23 (br s)), and rha II H-1 (δ_H 4.95 (br s)). The difference in structure between **3** and **2** is the addition of β-D-glucopyranosyl at glc III C-2 of **3**, which further confirmed by HMBC correlations between glc IV H-1 (δ_H 4.63) and glc III C-2 (δ_C 83.5), and by the existence of an ion peak at *m/z* 1528 $[M+Na]^+$ in the ESI mass spectrum

Table 2: NMR data for compounds **4**, **5**, and reference compounds

C		4			5	
	δ_C*	δ_C[a,b]	δ_H[a,c](mult., *J* in Hz)	δ_C#	δ_C[a,d]	δ_H[a,c](mult., *J* in Hz)
Aglycon						
1	40.2	40.7	1.52 (m)/1.64 (m)	35.7	36.6	1.21 (m)/1.76 (m)
2	69.4	70.6	3.75 (ddd, 3.0, 7.0, 9.0)	26.8	27.6	1.73 (m)/2.04 (m)
3	96.3	96.7	3.08 (d, 9.0)	89.4	92.0	3.17 (dd, 4.0, 11.6)
4	42.2	43.0	-	39.5	40.6	-
5	47.4	48.8	1.40 (m)	50.8	52.1	1.05 (m)
6	21.1	22.1	0.85 (m)/1.65 (m)	18.2	19.2	1.51 (m)/1.68 (m)
7	26.0	27.0	1.17 (m)/1.39 (m)	25.3	27.5	1.74 (m)/1.93 (m)
8	47.7	49.5	1.60 (m)	133.6	134.8	-
9	19.4	20.5	-	136.0	137.2	-
10	25.0	25.9	-	36.8	38.0	-
11	26.7	27.7	1.25 (m)/2.10 (m)	21.4	22.1	1.59 (m)/2.09 (m)
12	32.9	34.0	1.74 (m)	26.5	26.1	1.63 (m)/2.23 (m)
13	45.6	46.7	-	49.3	50.9	-
14	49.2	50.1	-	50.0	50.3	-
15	35.7	36.7	1.34 (m)/1.36 (m)	31.0	31.8	1.18 (m)/1.44 (m)
16	28.7	29.5	1.35 (m)/1.78 (m)	28.8	29.5	1.28 (m)/1.41 (m)
17	51.9	53.2	1.77 (m)	50.4	51.5	1.67 (m)
18	18.3	18.9	1.09 (s)	62.2	62.9	3.44 (m)/3.56 (m)
19	29.5	30.8	0.49 (br s)/0.67 (br s)	19.2	19.8	1.03 (s)
20	41.2	42.4	2.30 (m)	41.6	42.7	2.54 (m)
21	19.8	20.2	1.09 (d, 6.5)	21.2	21.4	1.14 (d, 6.5)
22	147.9	149.7	5.98 (dd, 9.0, 15.0)	148.9	150.4	5.90 (dd, 9.2, 14.8)
23	123.8	124.7	6.39 (dd, 11.0, 15.0)	123.4	124.4	6.34 (dd, 11.2, 14.8)
24	134.8	136.4	6.90 (d, 11.0)	134.9	136.6	6.86 (d, 11.2)
25	129.0	128.6	-	128.4	128.4	-
26	13.4	13.0	1.99 (s)	13.2	13.0	1.95 (s)
27	170.7	172.7	-	170.6	172.7	-
28	26.0	26.5	1.16 (s)	25.8	28.7	1.10 (s)
29	16.2	16.4	0.97 (s)	28.0	16.7	0.88 (s)
30	19.2	20.0	0.97 (s)	16.4	26.0	0.93 (s)
1′	175.7	176.9	-	175.6	176.9	-
2′	55.4	56.1	5.07 (d, 7.0)	55.2	56.0	5.04 (d, 7.2)
3′	38.6	39.4	2.81 (m)	38.4	39.4	2.77 (m)
4′	77.0	78.7	4.76 (m)	76.9	78.7	4.72 (m)
3′-CH_3	8.0	8.0	0.85 (d, 7.5)	7.9	7.9	0.81 (d, 7.2)
4′-CH_3	15.4	15.7	1.37 (d, 6.5)	15.3	15.7	1.33 (d, 6.8)
Glc I						
1	104.5	104.8	4.47 (d, 8.0)	104.7	105.5	4.41 (d, 8.0)
2	79.0	78.7	3.85 (m)	79.1	78.7	3.70 (m)
3	77.6	77.7	3.80 (m)	77.4	77.7	3.71 (m)
4	79.3	79.3	3.60 (m)	79.1	79.9	3.46 (m)
5	76.4	76.8	3.47 (m)	76.2	76.6	3.33 (m)
6	61.1	61.6	3.70 (m)/3.84 (m)	61.4	62.0	3.64 (m)/3.80 (m)
Glc II						
1	102.2	101.9	4.99 (d, 7.6)	101.8	102.0	4.91 (d, 7.6)
2	78.4	79.0	3.44 (m)	78.2	78.9	3.40 (m)
3	77.8	79.3	3.50 (m)	77.4	79.3	3.46 (m)
4	72.8	72.8	3.11 (m)	72.6	72.7	3.04 (t, 9.0)
5	79.2	78.0	3.29 (m)	79.1	78.5	3.22 (m)
6	63.2	63.7	3.60 (m)/3.88 (m)	63.3	63.7	3.52 (m)/3.83*
Rha I						
1	102.1	101.9	5.27 (br s)	102.5	101.8	5.23 (br s)
2	72.2	72.1	3.94 (br s)	72.3	72.1	3.89 (br s)
3	72.6	72.4	3.77 (m)	72.4	72.2	3.72 (m)
4	74.2	74.2	3.42 (m)	73.7	74.2	3.38 (m)
5	69.5	69.5	4.13 (m)	70.4	69.5	4.11 (m)
6	19.0	18.4	1.27 (d, 6.5)	18.4	18.4	1.21 (d, 6.4)
Rha II						
1	102.7	102.8	4.89 (br s)	101.8	102.7	4.83 (br s)
2	72.3	72.3	3.86 (br s)	72.2	72.4	3.82 (br s)
3	72.4	72.1	3.67 (m)	72.6	72.1	3.63 (m)
4	73.8	73.8	3.42 (m)	73.9	73.8	3.38 (m)
5	70.7	70.7	4.00 (m)	69.2	70.7	3.96 (m)
6	18.6	18.1	1.31 (d, 6.5)	18.8	18.1	1.27 (d, 6.4)

[a)] Measured in CD_3OD, [b]125 MHz, [c]500 MHz, [d]100 MHz, [e]400 MHz. *δ_C of mussaendoside P[4], #δ_C of mussaendoside Q[4].

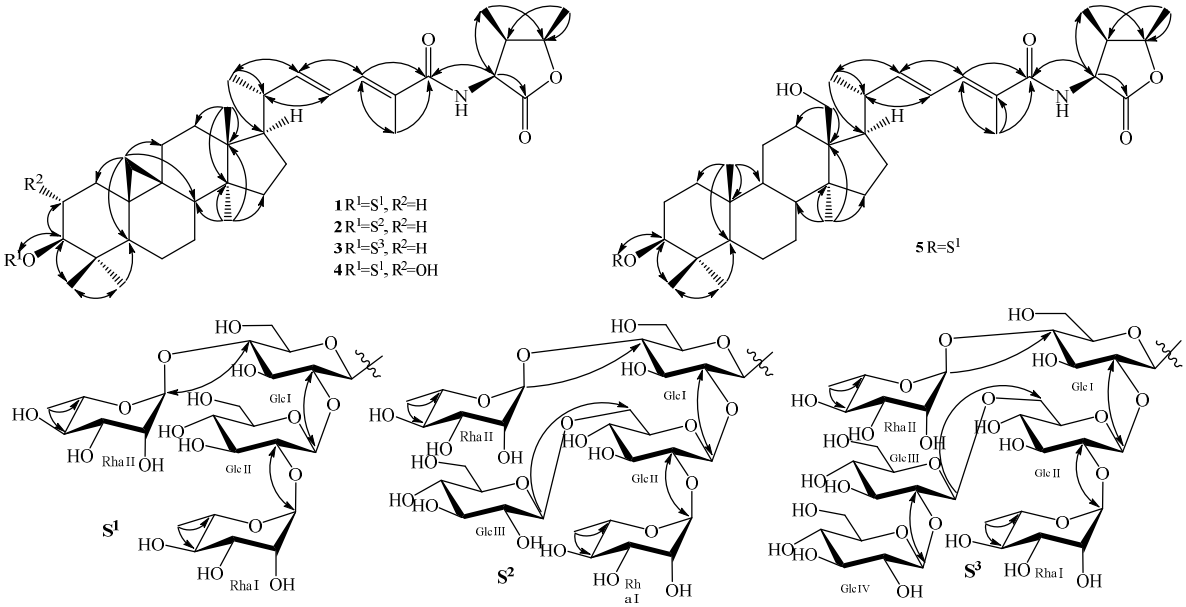

Figure 2: The key HMBC correlations of compounds **1-5**

(corresponding to the molecular formula of $C_{72}H_{115}O_{32}N$). Thus, the structure of **3** was elucidated to be mussaendoside U, a compound reported from *M. pubescens*.[6]

The NMR spectra of **4** showed similar with those of **1** except for addition of a hydroxyl group at C-2. The position of hydroxyl group at C-2 was proved by HMBC correlations between H-3 (δ_H 3.08) and C-2 (δ_C 70.6). The large coupling constant of H-2 and H-3, J = 9.0 Hz suggested the configuration of hydroxyl group at C-2 as α. Therefore, this aglycone was recognized to be 2α-hydroxylheinsiagenin A. The NMR data of **4** were compared to the corresponding data of mussaendoside P[4] and found to match. The sugar linkages were proved by HSQC and HMBC spectra. The ESI-MS of **4** exhibited an ion at *m/z* 1198 $[M+H]^+$, corresponding to the molecular formula of $C_{60}H_{95}O_{23}N$. Thus, compound **4** was identified as mussaendoside P, a compound was isolated from *M. pubescens*.[4]

The analysis of NMR spectra indicated the structure of **5** was similar to those of mussaendoside Q in the literature.[4] In addition, the position of hydroxyl group at C-18 was confirmed by HMBC correlations between H-18 (δ_H 3.44)/(δ_H 3.56) and C-12 (δ_C 26.1)/C-13(δ_C 50.9)/C-14 (δ_C 50.3)/C-17 (δ_C 51.5). The HMBC correlations between H-19 (δ_H 1.03) and C-1 (δ_C 36.6)/C-5(δ_C 52.1)/C-9 (δ_C 137.2)/C-10 (δ_C 38.0); between H-30 (δ_H 0.93) and C-8 (δ_C 134.8) suggested a double bond at C-8/C-9 and a methyl group at C-10. The ESI-MS of **4** exhibited an ion at *m/z* 1198 $[M+H]^+$, corresponding to the molecular formula of $C_{60}H_{95}O_{23}N$. Finally, the structure **5** was elucidated as mussaendoside Q, a compound was isolated from *M. pubescens*.[4]

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