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SAPONINS FROM *MUSSAENDA PUBESCENS*

WEIMIN ZHAO, PING WANG, RENSHENG XU, GUOWEI QIN,* SHAOKAI JIANG† and HOUMING WU*†

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China; †The State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China

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Key Word Index—*Mussaenda pubescens*; Rubiaceae; saponin; mussaendosides D, E and H.

Abstract—From the hydrophilic fractions of aerial parts of *Mussaenda pubescens*, three new saponins named mussaendosides D, E and H, along with a known saponin, mussaendoside S, were isolated. Their structures were elucidated on the basis of chemical and spectral evidence.

INTRODUCTION

Mussaenda pubescens Ait.f. is a Chinese folk medicine used as a diuretic, antiphlogistic and antipyretic [1]. It is also used to detoxify mushroom poison and terminate early pregnancy in some districts of Fujian Province, southeast China [2, 3]. In previous papers, we have reported the isolation and structural determination of several saponins and iridoid glycoside from whole plant [4–6]. In continuation of our studies, hydrophilic components of aerial plant materials collected from Yongtai county, Fujian Province, were further investigated. As a result, four saponins were isolated and their structures were elucidated on the basis of chemical and spectral evidence. Among them, three were new saponins named mussaendosides D (**1**), E (**2**) and H (**3**), and one was the known mussaendoside S (**4**).

RESULTS AND DISCUSSION

Saponins **1** and **2** both showed a positive reaction to the Liebermann–Burchard and Molish tests, which indicated them to be triterpenoid saponins. The UV spectrum (λ_{max} 265 nm) revealed the existence of a conjugated diene. The ^{1}HNMR spectra showed these protons corresponding to cyclopropane, conjugated diene, and an α-amino-3,4-dimethyl-γ-lactone moiety, which features are similar to those of heinsiagenin A, a common aglycone of saponins of this plant. Heinsiagenin A was further confirmed to be the aglycone of **1** and **2** by comparison of ^{13}C NMR data with the literature.

Saponin **1**, an amorphous powder, showed a quasimolecular ion at *m/z* 913 in the FAB-mass spectrum, corresponding to $[M(C_{48}H_{75}NO_{14}) + Na + H]^+$. On acidic hydrolysis, **1** provided glucose only (TLC) as the sugar moiety. From the above evidence, **1** should contain two glucose units and heinsiagenin A as the aglycone. In its ^{1}H NMR spectrum, two anomeric protons appeared at δ 5.41 (1H, *d*, J = 7.4 Hz) and 4.95 (1H, *d*, J = 7.5 Hz), which suggested both the glucose moieties had the β-glycosidic linkage. Among the ^{13}C NMR signals of two glucose units (Table 1), two anomeric carbon signals were seen at δ 106.0 and 104.9, two C-6 signals overlapped at δ 62.7, and two C-4 signals were exhibited at δ 71.6 and 71.5, respectively, which suggested that the two glucose moieties cannot be linked in the 1→6 or 1→4 manner. Furthermore, the signal at δ 83.3 was considered to be C-2 of a glucose which shifted about 7.4 ppm downfield upon glycosylation. Therefore, **1** was deduced to be heinsiagenin A 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside, which is a new triterpenoid saponin now named mussaendoside D.

Saponin **2**, an amorphous powder, showed a quasimolecular ion at *m/z* 1074 in the FAB-mass spectrum, corresponding to $[M(C_{54}H_{85}NO_{19}) + Na]^+$. On acid hydrolysis, **2** yielded glucose only (TLC) as the sugar moiety. From the above evidence, compound **2** should contain three glucose units and heinsiagenin A as the aglycone. In the ^{13}C NMR spectrum (Table 1), compound **2** exhibited three anomeric carbon signals at δ 105.7, 105.3 and 104.8, respectively. In addition, three oxygen-bearing methylene carbon signals appeared at δ 70.0, 62.8 and 62.8, corresponding to C-6 of three glucose units in **2**. The presence of a methylene signal at δ 70.0 and a methine signal at δ 82.9 suggested the 1→6 and 1→2 linkages among the three glucose units by considering the glycosylation. In the ^{1}H NMR spectrum, three anomeric proton signals were observed at δ 5.13 (1H, *d*, J = 7.8 Hz), 5.55 (1H, *d*, J = 7.6 Hz) and 5.32 (1H, *d*, J = 7.8 Hz), respectively, indicating all were in β-glycosidic linkages. On the basis of ^{1}H–^{1}H COSY and TOCSY spectra, all protons of the three glucose units were assigned. Subsequently,

*Authors to whom correspondence should be addressed.

Table 1. ^{13}C NMR data for compounds **1**, **2** and **3** (pyridine-d_5)

No.	1	2	3	No.	1	2	3
1	32.0	31.8	32.3	4′	77.0	77.0	77.2
2	29.5	29.6	29.8	3′-Me	8.1	8.1	8.1
3	88.7	88.9	89.7	4′-Me	15.4	15.5	15.4
4	41.2	41.3	41.3	G-1	104.9	104.8	104.7
5	47.3	47.7	47.9	G-2	83.3	82.9	79.9*
6	21.0	21.1	21.2	G-3	77.9	78.3	77.8
7	26.1	26.5	26.5	G-4	71.5*	71.7	79.4*
8	47.7	47.4	47.9	G-5	78.2	78.0	76.5
9	19.8	19.8	20.5	G-6	62.7	62.8	61.8
10	26.2	26.3	26.8	G′-1	106.0	105.7	102.3
11	26.5	26.5	27.0	G′-2	77.0	76.7	78.6
12	33.0	33.0	30.4	G′-3	78.2	78.2	77.5
13	45.5	45.6	49.9	G′-4	71.6*	71.5	73.1
14	49.1	49.2	52.4	G′-5	77.9	77.0	79.4*
15	35.6	35.7	36.6	G′-6	62.7	70.0	63.7
16	28.7	28.7	28.9	G″-1		105.3	
17	51.9	51.9	50.7	2		75.2	
18	18.3	18.2	64.7	3		78.3	
19	29.9	29.9	30.0	4		71.7	
20	41.2	41.3	41.5	5		78.3	
21	19.8	19.8	21.8	6		62.8	
22	147.9	147.9	149.2	R′-1			102.7
23	123.5	123.5	123.1	2			72.6
24	134.8	134.8	135.1	3			72.6
25	129.0	129.0	128.8	4			74.1
26	13.4	13.4	13.5	5			70.7
27	170.7	170.7	170.8	6			18.7
28	15.3	15.4	15.6	R-1			101.9
29	25.7	26.0	26.1	2			72.4
30	19.4	19.3	21.1	3			72.8
1′	175.7	175.7	175.7	4			74.3
2′	55.4	55.4	55.6	5			69.6
3′	38.6	38.6	38.8	6			19.1

*May be interchangeable in each column.

all glucose carbon signals were assigned by using a HMQC experiment (Table 2).

In the NOESY spectrum, the significant cross peaks among the glucose and aglycone were observed between $H_{G\text{-}1}$ (δ 5.13) and H-3 (δ 3.63), $H_{G'\text{-}1}$ (δ 5.55) and $H_{G\text{-}2}$ (δ 4.48), and $H_{G''\text{-}1}$ (δ 5.32) and $H_{G'\text{-}6}$ (δ 4.92, 4.66). Therefore, the structure of **2** was elucidated to be heinsiagenin A 3-*O*-β-D-glucopyranosyl(1→6)-*O*-β-D-glucopyranosyl(1→2)-*O*-β-D-glucopyranoside, which is a new triterpenoid saponin now named mussaendoside E.

Saponin **3**, an amorphous powder, showed quasimolecular ion peaks at *m/z* 1221 and 1237 in the FAB-mass spectrum, corresponding to $[M(C_{60}H_{95}NO_{23}) + NA + H]^+$ and $[M(C_{60}H_{95}NO_{23}) + K + H]^+$, respectively. Its ^1H NMR spectrum exhibited two cyclopropane proton signals at δ 0.11 (*d*, $J = 3.2$ Hz) and 0.41 (*d*, $J = 3.2$ Hz), and the signals corresponding to amide and olefinic protons at δ 9.12 (1H, *d*, $J = 7.7$ Hz, NH), 7.31 (1H, *br d*, $J = 10.9$ Hz, H-24), 6.44 (1H, *m*, H-23) and 5.75 (1H, *m*, H-22). The UV spectrum (λ_{max} 265 nm) suggested the presence of a conjugated diene. The above information revealed that the aglycone skeleton was similar to that of **1** and **2**. The obvious difference between them was the number of methyl signals in the ^1H NMR spectrum. Five singlet methyl signals belonging to the aglycone could be observed in the ^1H NMR spectrum of **1** and **2**, but only four singlet methyl signals were exhibited by compound **3**.

Acid hydrolysis of **3** yielded glucose and rhamnose (TLC). In the ^{13}C NMR spectrum, four anomeric carbons were observed at δ 104.7, 102.7, 102.3 and 101.9 (Table 1). In the ^1H NMR spectrum, two doublet methyl signals belonging to L-rhamnose were observed. Thus, compound **3** should contain two D-glucose units and two L-rhamnose units.

In the ^{13}C NMR spectrum, three oxygen-bearing methylene signals were observed at δ 64.7, 63.7 and 61.8. The latter two should be C-6 of D-glucose units, while the first one should be derived from the methyl group in the aglycone. In addition, the significant shifts of the carbon signals from those around C-13 were observed in comparison with those of **1** and **2** (Table 1). Therefore, a hydroxyl group was suggested to be located at C-18, and the aglycone of **3** was deduced to

Table 2. ^{1}H NMR data for sugar units in compounds **2** and **3** (pyridine-d_5)

	2		**3**	
No.	δ_H	J (Hz)	δ_H	J (Hz)
G-1	5.13	*d* 7.8	4.90	*d* 7.1
2	4.48	*m*	4.35	*m*
3	4.49	*m*	4.52	*m*
4	4.39	*m*	4.23	*m*
5	4.07	*m*	3.67	*m*
6a	4.68	*m*	4.09	*m*
b	4.53	*m*	4.26	*m*
G′-1	5.55	*d* 7.6	5.79	*m*
2	4.27	*m*	4.32	*m*
3	4.38	*m*	3.85	*m*
4	4.52	*m*	4.07	*m*
5	4.23	*m*	4.30	*m*
6a	4.96	*m*	4.32	*m*
b	4.66	*m*	4.48	*m*
G″-1	5.32	*d* 7.8		
2	4.24	*m*		
3	4.43	*m*		
4	4.42	*m*		
5	4.17	*m*		
6a	4.71	*m*		
b	4.57	*m*		
R′-1			5.80	*s*
2			4.68	*m*
3			4.58	*m*
4			4.32	*m*
5			4.91	*m*
6			1.66	*d* 6.0
R-1			6.45	*s*
2			4.80	*br s*
3			4.70	*m*
4			4.35	*m*
5			5.02	*m*
6			1.85	*d* 6.1

be 18-hydroxyheinsiagenin A, which is a new sapogenin.

In the ^{1}H NMR spectrum, four anomeric protons exhibited at δ 6.45 (1H, *s*), 5.80 (1H, *s*), 5.79 (1H, *m*) and 4.90 (1H, *d*, J = 7.1 Hz). All protons of the four sugar units were assigned unambiguously from the ^{1}H–^{1}H COSY spectrum (Table 2). In order to establish the linkage sites and sequence of four saccharides and the aglycone, a NOESY experiment was performed on **3**. The significant NOE correlation cross-peaks were observed from the following pairs: $H_{G'-1}$ (δ 4.90)/H-3 (δ 3.44), H_{G-1} (δ 5.79)/$H_{G'-2}$ (δ 4.35), H_{R-1} (δ 6.45)/H_{G-2} (δ 4.32) and $H_{R'-1}$ (δ 5.80)/$H_{G'-4}$(δ 4.23). Therefore, the structure of **3** was deduced to be 18-hydroxyheinsiagenin A 3-*O*-[α-L-rhamnopyranosyl-(1 → 2) - *O* - β - D - glucopyranosyl(1 → 2)] - α - L - rhamnopyranosyl(1→4)-*O*-β-D-glucopyranoside. This is a new triterpenoid saponin named mussaendoside H.

Saponin **4** was proved to be 3β-*O*-β-D-glucopyranosyl cincholic acid 28-*O*-β-D-glucopyranoside (mussaendoside S) by comparing its physical and spectral data with those of authentic sample isolated from the same species [5].

EXPERIMENTAL

$[\alpha]_D$: JASCO, DIP-181 polarimeter. IR: Perkin-Elmer 599B spectrometer. FAB-MS: Finnigan-MAT-8430. ^{1}H and ^{13}C NMR spectra of **1–4**: Bruker AM-300, AM-400 and AMX-600 MHz instruments. ^{1}H–^{1}H COSY, TOCSY, NOESY and HMQC of **2** and **3** spectra were obtained on Bruker AM-400 and AMX-600 MHz instruments. Chemical shifts are reported in ppm, with solvents signals as int. standards.

Plant materials. The aerial parts of *M. pubescens* were collected from Yongtai County, Fujian Province, in December 1993. A voucher specimen was identified by Prof. Rentong Chen of the Fujian Institute of Traditional Chinese Medicines.

Extraction and isolation. Dried plant materials (4.0 kg) were percolated 4× with 95% EtOH at room temp. After evapn of EtOH at 50° *in vacuo*, the residual aq. soln was extracted with EtOAc and *n*-BuOH successively.

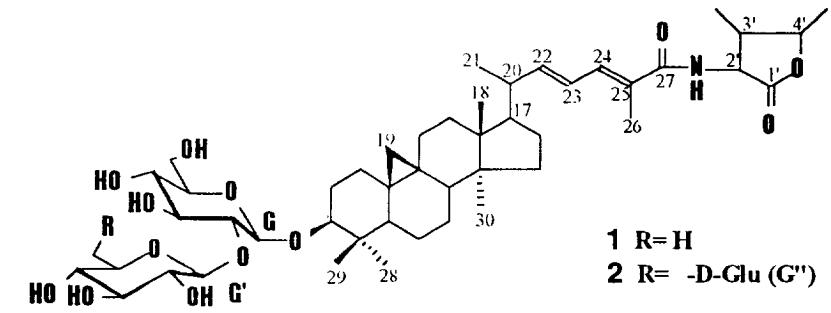
The *n*-BuOH fr. was concd to dryness and then applied to polyporous resin DA-201, eluting with H_2O and 40 and 90% EtOH successively, to give 70, 40 and 40 g of residue, respectively.

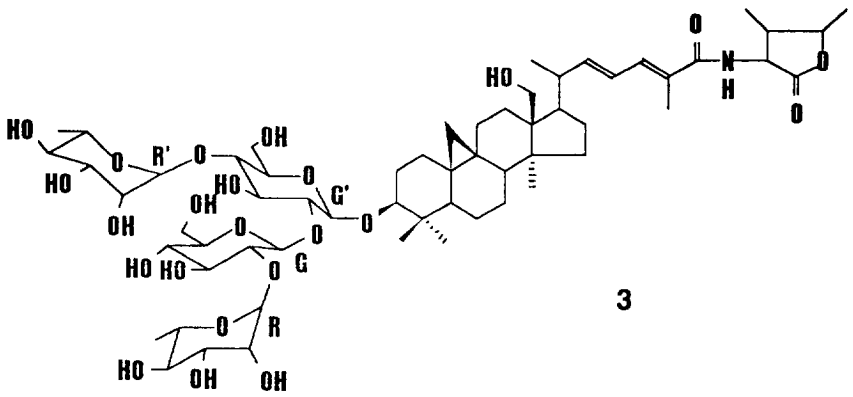
The 90% EtOH fr. was subjected to silica gel CC with a gradient of $CHCl_3$–MeOH–H_2O (6:1:0.1 → 1:1:0.1) as eluent. Frs were further subjected to chromatography on a RP-18 Lobar column, with a gradient of MeOH–H_2O (1:1 → 7:3) or MeCN–H_2O (1:1 → 7:3) as eluent. Frs were monitored by TLC and combined. Compounds **1** (15 mg), **2** (10 mg), **3** (20 mg) and **4** (30 mg) were obtained.

Compound **1**. Amorphous powder. $[\alpha]_D^{24}$ +30.2° (MeOH, *c* 0.54). UV λ_{max}^{MeOH} nm: 265. FAB-MS *m/z*: 913 $[M(C_{48}H_{75}NO_{14}) + Na]^+$. ^{1}H NMR (400 MHz, pyridine-d_5): δ 9.16 (1H, *d*, J = 7.5 Hz, NH), 7.27 (1H, *d*, J = 11.1 Hz, H-24), 6.41 (1H, *dd*, J = 14.8, 11.1 Hz, H-23), 5.68 (1H, *dd*, J = 7.3, 7.3 Hz, H-2′), 5.63 (1H, *dd*, J = 14.8, 8.7 Hz, H-22), 5.41 (1H, *d*, J = 7.4 Hz, $H_{G'-1}$), 4.95 (1H, *d*, J = 7.5 Hz, H_{G-1}), 4.66 (1H, *m*, H-4′), 3.45 (1H, *dd*, J = 11.0, 4.0 Hz, H-3), 2.90 (1H, *m*, H-20), 2.19 (3H, *br s*, H-26), 0.48 (1H, *d*, J = 3.7 Hz, H-19a), 0.19 (1H, *d*, J = 3.7 Hz, H-19b). ^{13}C NMR (75 MHz, pyridine-d_5): see Table 1.

Hydrolysis of **1**. A MeOH soln of **1**, together with standard sugar samples, were applied at points about 1 cm from the bottom of precoated HPTLC silica gel plate and hydrolysed with HCl vapour for 2 hr at 50°. The plate was then heated at 60° for 2 hr to remove residual HCl, and developed using $CHCl_3$–MeOH–H_2O (7:3:0.1) solvent. The plate was sprayed with 10% H_2SO_4 (EtOH) and then heating to locate spots.

Compound **2**. Amorphous powder. $[\alpha]_D^{24}$ −1.4° (MeOH, *c* 0.03). UV λ_{max}^{MeOH} nm: 265 FAB-MS *m/z*: 1074 $[M(C_{54}H_{85}NO_{19}) + Na]^+$. ^{13}C NMR (150 MHz, pyridine-d_5) data: see Table 1. ^{1}H NMR (600 MHz, pyridine-d_5): δ 9.25 (1H, *d*, J = 7.6 Hz, NH), 7.28 (1H,





d, J = 10.9 Hz, H-24), 6.43 (1H, *dd*, J = 14.9, 10.9 Hz, H-23), 5.66 (2H, *m*, H-22, H-2′), 3.50 (1H, *dd*, J = 11.7, 4.0 Hz, H-3), 2.92 (1H, *m*, H-3′), 2.20 (3H, *br s*, H-26), 1.36 (3H, *s*, H-29), 1.21 (3H, *s*, H-28), 1.18 (3H, *d*, J = 6.6 Hz, 4′-Me), 1.00 (3H, *m*, H-21), 0.99 (3H, *s*, H-18), 0.90 (3H, *s*, H-30), 0.87 (3H, *d*, J = 7.4 Hz, 3′-Me); for proton signals of sugar units: see Table 2.

Acidic hydrolysis of **2**. Similar to **1** in procedure.

Compound **3**. Amorphous powder. $[\alpha]_D^{24}$ +6.3° (pyridine-d_5, *c* 0.20). FAB-MS *m/z*: 1221 $[M+Na]^+$, 1237 $[M+K]^+$. UV λ_{max}^{MeOH} nm: 265. ^{13}C NMR (100 MHz, pyridine-d_5): see Table 1. ^1H NMR (400 MHz, pyridine-d_5): δ 9.12 (1H, *d*, J = 7.7 Hz, NH), 7.31 (1H, *br d*, J = 10.9 Hz, H-24), 6.44 (1H, *m*, H-23), 5.75 (1H, *m*, H-22), 5.67 (1H, *dd*, J = 7.5, 7.3 Hz, H-2′), 4.70 (1H, *m*, H-4′), 4.29 (1H, *m*, H-18a), 4.05 (1H, *m*, H-18b), 3.44 (1H, *dd*, J = 11.7, 4.0 Hz, H-3), 3.02 (1H, *m*, H-20), 2.90 (1H, *m*, H-3′), 2.11 (3H, *br s*, H-26), 1.18 (3H, *m*, H-21), 0.86 (3H, *d*, J = 7.3 Hz, 3′-Me), 0.41 (1H, *d*, J = 3.2 Hz, H-19a), 0.11 (1H, *d*, J = 3.2 Hz, H-19b); for ^1H signals of sugar units: see Table 2.

Acidic hydrolysis of **3**. Similar to **1** in proceduure.

Compound **4**. Amorphous powder. It was identical to co-TLC with an authentic sample. ^1H NMR (400 MHz, pyridine-d_5): δ 6.38 (1H, *d*, J = 7.8 Hz, $H_{G\text{-}1}$), 5.99 (1H, *br s*, H-12), 4.75 (1H, *d*, J = 6.3 Hz, $H_{G'\text{-}1}$), 3.34 (1H, *dd*, J = 12.5, 4.3 Hz, H-3), 1.20 (3H, *s*), 1.11 (3H, *s*), 0.93 (3H, *s*), 0.85 (3H, *s*), 0.83 (3H, *s*), 0.71 (3H, *s*).

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