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Chemical Constituents from *Massaenda Pubescens*

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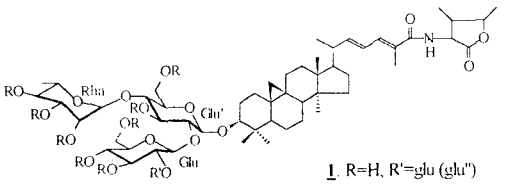
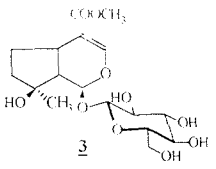
Abstract—A new triterpenoid saponin named Mussaendoside F(1), along with five known compounds (2-6) were isolated from aerial part of *Mussaenda pubescens*.

Keywords—*Mussaenda pubescens* · Rubiaceae · saponin · Mussaendoside F

Mussaenda pubescens Ait. f. (Rubiaceae) is a Chinese folk medicine commonly used as diuretic, antiphlogistic and antipyretic (Jiangsu New Medical College, 1986). It is also used to treat acute gastroenteritis and to detoxify mushroom poison in some part of southeast China (Fujian Institute of Medicine, 1970; Liu *et al.*, 1986). In previous papers, we have reported the isolation and structural determination of several saponins and iridoid glycosides from whole plant (Xu, *et al.*, 1992; Zhao, *et al.*, 1994, 1995). In our continuing investigation, the aerial plant of the materials collected from Yongtai county, Fujian Province were further studied. As a result, two saponins (1 and 2) and four iridoid compounds (3~6) were isolated and their structures were elucidated on the basis of chemical and spectral evidence. Among them, 1 was proved to be a new triterpenoid saponin named Mussaendoside F. The others (2-6) were first obtained from the aerial part of the plant. Compound 2 showed some significant activities in our preliminary vitro and vivo tests.



1. R=H, R'=glu (glu'')
1a. R=Ac, R'=glu(Ac)$_4$
2. R=H, R'=Rha.



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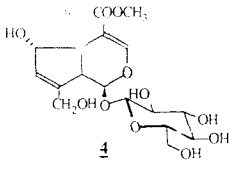

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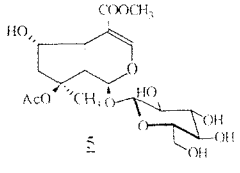

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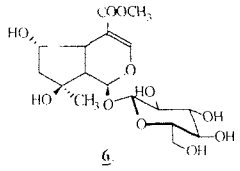

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Experimental

General - $[\alpha]_D$: JASCO, DIP-181 polarimeter. IR: Perkin-Elmer 599B spectrometer. FAB-MS: Finnigan-MAT-8430. ^1H and ^{13}C NMR spectra: Bruker AC-80, AM-300 and AM-400 instruments. ^1H-^1H DQF COSY, NOESY spectra were obtained on Bruker AM-400 instrument. Chemical shifts are reported in ppm, with solvents signals as int. standards.

Plant materials - The aerial parts of *Mussaenda pubescens* were collected from Yongtai County, Fujian Province in Dec. 1993. A voucher specimen was identified by

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Table 1. ^{13}C NMR (100 MHz, C_5D_5N) Data of Aglycone of 1.

C	δc	DEPT	C	δc	DEPT
1	31.5	t	19	29.2	t
2	29.1	t	20	40.8	d
3	89.1	d	21	19.2	q
4	40.7	s	22	147.3	d
5	47.0	d	23	123.0	d
6	20.6	t	24	134.2	d
7	25.6	t	25	128.4	s
8	47.3	d	26	12.9	q
9	19.4	s	27	170.1	s
10	25.8	s	28	14.7	q
11	26.1	t	29	25.4	q
12	32.5	t	30	18.9	q
13	45.1	s	1′	175.1	s
14	48.6	s	2′	54.9	d
15	35.2	t	3′	38.0	d
16	28.2	t	4′	76.4	d
17	51.4	d	3′-Me	7.4	d
18	17.8	q	4′-Me	14.9	q

Rentong Chen of Fujian Institute of Traditional Chinese Medicines and deposited in the Herbarium of Shanghai Institute of Materia Medica, China.

Extraction and Isolation - Dried plant materials (4.0 kg) were percolated with 95% ethanol at room temperature for four times. After evaporation of ethanol at 50°C *in vacuo*, the residual water solution was extracted with ethyl acetate and n-butanol successively.

The *n*-butanol fraction was concentrated to dryness and then subjected to polyporous resin DA-201, eluted with water, 40% and 90% ethanol successively, to give 70 g, 40 g and 40 g of respective residue.

The 40% ethanol fraction was chromatographed on silica gel column, eluted with gradient chloroform-methanol solvents. Compounds 3 (14.5 g), 4 (50 mg), 5 (70 mg), 6 (25 mg) were obtained.

The 90% ethanol fraction was subjected to silica gel chromatography with gradient chloroform-methanol-water as eluents. Fractions were further subjected to chromatography on RP-18 Lobar column, with gradient methanol-water or acetonitrile-water as eluents. Compounds 1 (100 mg) and 2 (7.5 g) were obtained.

1, *mussaendoside* F. Amorphous powder. $[\alpha]_D^{30}$-3.2° (C_5H_5N, c 0.12). FAB-MS *m/z*: 1221 $[M+Na+H]^+$ and 1236 $[M+K]^+$. UV (CH_3OH) λ_{max} 265 nm. ^1H NMR (400 MHz, C_5D_5N) δ ppm: 9.12 (1H, d, 7.7, NH), 7.31 (1H, br d, 10.9, H-24), 6.44 (1H, m, H-23), 5.75 (1H, m, H-22), 3.44 (1H, dd, 11.7, 4.0, H-3), 3.02 (1H, m, H-20), 2.12 (3H, br s, H-26), 1.38 (3H, s), 1.15 (2x3H, s+d), 0.96 (2x3H, s+d), 0.86 (2x3H, s+d), 0.41 (1H, d, 3.2, H-19a), 0.11 (1H, d, 3.2, H-19b); For sugar protons, see Table 2. ^{13}C NMR (100 MHz, C_5D_5N) data of sugar moieties, δ ppm: 105.5 (d), 105.4 (d), 102.7 (d), 102.2 (d), 82.1 (d), 80.2 (d), 79.0 (d), 79.0 (d), 78.9 (d), 78.9 (d), 78.1 (d), 77.9 (d), 77.7 (d), 76.7 (d), 75.6 (d), 75.1 (d), 73.6 (d), 73.4 (d), 72.0 (d), 70.2 (d), 64.2 (t), 62.7 (t), 62.7 (t), 19.1 (q); For data of aglycone, see Table 1.

Acidic hydrolysis of 1. 10 mg of 1 was dissolved in 2N HCl (3 ml) and heated at 90°C for 4hrs. After extraction with chloroform, the residue solution was neutralized with Ag_2CO_3 and filtrated. The filtrate was concentrated and then detected by silica gel TLC development in comparison with authentic sugar samples.

Peracetylation of 1. 10 mg of 1 was kept at room temperature in acetic anhydride-pyridine (1:1) for 48hrs, and then evaporated to dryness *in vacuo*. The residue was purified on silica gel column, eluted with pertroleum ether-acetone (1.5:1), and compound 1a (10 mg) was obtained.

1a. Amorphous powder. ^1H NMR (400 MHz, C_5D_5N) δ ppm: 9.09 (1H, d, 7.6, NH), 7.26 (1H, br d, 11.1, H-24), 6.41 (1H, dd, 14.8, 11.1, H-23), 3.38 (1H, dd, H-3), 2.89 (1H, m, H-3'), 2.35 (3H, s, H-26), 1.29 (3H, s), 1.18 (3H, d, 6.5), 1.11 (3H, s), 0.99 (3H, s), 0.97 (3H, d), 0.90 (3H, s), 0.84 (3H, d), 0.50 (1H, H-19a), 0.24 (1H, H-19b). For sugar protons,

Table 2. ^{1}H NMR Data (400 MHz, C_5D_5N) of Sugar Protons of 1 and 1a.

No.	1	*J*(Hz)	No.	1a	*J*(Hz)
G′-1	4.92	d, 7.6	G′-1	4.86	d, 7.6
2	4.43	m	2	4.28	dd, 9.5, 9.5
3	4.56	m	3	5.72	m
4	4.18	m	4	4.08	m
5	3.80	m	5	3.84	m
6a	4.52	m	6a	4.45	m
6b	4.41	m	6b	4.73	m
G-1	5.19	d, 7.9	G-1	5.23	d, 7.5
2	3.95	m	2	4.11	m
3	4.22	m	3	5.82	dd, 9.5, 9.6
4	4.04	m	4	5.40	m
5	5.82	m	5	4.14	m
6a	4.48	m	6a	4.35	m
6b	4.27	m	6b	4.62	m
G″-1	5.76	d, 7.5	G″-1	5.03	d, 7.5
2	4.30	m	2	5.35	m
3	4.20	m	3	5.67	m
4	–		4	5.42	m
5	–		5	4.13	m
6a	–		6a	4.22	m
6b	–		6b	4.66	m
R′-1	6.45	br s	R′-1	5.66	s
2	4.81	dd, 3.4, 1.4	2	5.68	br s
3	4.74	dd, 9.3, 3.4	3	5.93	dd, 9.3, 3.4
4	4.38	m	4	5.67	m
5	5.11	dq, 9.4, 6.1	5	4.77	m
6	1.88	d, 6.1	6	1.58	d, 6.1

see Table 2.

2, *mussaendoside O*. Amorphous powder. UV (CH_3OH) λ_{max} 265 nm. ^{1}H NMR (400 MHz, C_5D_5N) and ^{13}C NMR (75 MHz, C_5D_5N) data were identical with those of authentic sample. (Xu *et al.*, 1992)

3, *mussaenoside*. Amorphous powder. $[\alpha]_D^{14}$ –95° (CH_3OH, c 0.90). UV (CH_3OH) λ_{max} 235 nm. ^{1}H NMR (400 MHz, C_5D_5N) δ ppm: 7.69 (1H, s, H-3), 5.98 (1H, d, 4.0, H-1), 5.40 (1H, d, 7.8, H_{G-1}), 4.51 (1H, br d, 11.7, H_{G-6a}), 4.38 (1H, dd, 11.7, 5.1, H_{G-6b}), 3.98 (1H, m, H_{G-5}), 3.57 (3H, s, MeO), 3.48 (1H, br dd, 15.2, 9.1, H-5), 2.77 (1H, dd, 9.1, 4.0, H-9), 1.61 (3H, s, H-10). ^{13}C NMR (75 MHz, D_2O) δ ppm: 170.6 (s, C-11), 151.7 (d, C-3), 113.2 (s, C-4), 99.1 (d, C_{G-1}), 95.2 (d, C-1), 80.4 (s, C-8), 77.0 (d, C_{G-5}), 76.4 (d, C_{G-3}), 73.4 (d, C_{G-2}), 70.3 (d, C_{G-4}), 61.5 (t, C_{G-6}), 52.6 (q, MeO), 51.4 (d, C-9), 40.3 (t, C-7), 30.3 (d, C-5), 29.6 (t, C-6), 23.7 (q, C-10). (Gardner, D.R. et al., 1987)

4, *6α-hydroxyl geniposide*. Amorphous powder. ^{1}H NMR (400 MHz, C_5D_5N) δ ppm: 7.93 (1H, br s, H-3), 6.56 (1H, br s, H-7), 5.85 (1H, d, 8.8, H-1), 5.30 (2H, m, H-6 and H_{G-1}), 5.09 (1H, br d 15.7, H-10a), 4.61 (1H, br d, 15.7, H-10b), 3.51 (3H, s, OMe), 3.27 (1H, br dd, H-5), 2.80 (1H, br dd, H-9), ^{13}C NMR (20 MHz, C_5D_5N) δ ppm: 167.8 (s, C-11), 154.3 (d, C-3), 130.2 (d, C-7), 108.2 (s, C-4), 102.3 (d, C_{G-1}), 101.6 (d, C-1), 78.2 (2xd, C_{G-3} and C_{G-5}), 74.9 (C_{G-2})[a], 74.4 (C-6)[a], 71.4 (C_{G-4}), 62.4 (C-10)[b], 61.2 (C_{G-6})[b], 50.9 (q, MeO), 45.7 (d, C-9), 42.6

(d, C-5). (data with a and b may be interchangeable. C-8 signal is under solvent signal at δ 149.8 ppm). (Bailleul *et al.*, 1979)

5, 8-O-acetyl shanzhiside methyl ester. Amorphous powder. UV (CH_3OH) λ_{max} 235 nm. ^1H NMR (300 MHz, D_2O) δ ppm: 7.49 (1H, s, H-3), 6.00 (1H, br s, H-1), 4.77 (1H, d, 8.0, H_{G-1}), 4.36 (1H, m, H-6), 3.93 (1H, br d, 11.0, H_{G-6a}), 3.75 (3H, s, MeO), 3.74 (1H, m, H_{G6b}), 3.50 (2H, m, H_{G-3} and H_{G-5}), 3.38 (1H, dd, 9.4, 9.2, H_{G-4}), 3.26 (1H, dd, 9.2, 8.2, H_{G-2}), 3.08 (2H, m, H-5 and H-9), 2.20 (1H, br d, 15.3, H-7a), 2.05 (3H, s, $\underline{CH_3}CO$), 2.00 (1H, m, H-7b), 1.49 (3H, s, H-10). ^{13}C NMR (75 MHz, D_2O) δ ppm: 176.4 (s, $CH_3\underline{C}O$), 171.5 (s, C-11), 155.3 (d, C-3), 110.9 (s, C-4), 100.9 (d, C_{G-1}), 97.0 (d, C-1), 91.3 (s, C-8), 78.8 (d, C_{G-3}), 78.1 (d, C_{G-5}), 77.0 (d, C-6), 75.1 (d, C_{G-2}), 72.1 (d, C_{G-4}), 63.2 (t, C_{G-6}), 51.4 (q, MeO), 50.5 (d, C-9), 48.6 (t, C-7), 42.8 (d, C-5), 24.2 (q, $\underline{C}H_3CO$), 23.5 (q, C-10). (Damtoft *et al.*, 1982)

6, *shanzhiside methyl ester.* Amorphous powder. UV (CH_3OH) λ_{max} 235 nm. ^1H NMR(80 MHz, CD_3OD) δ ppm: 7.25 (1H, br s, H-3), 5.41 (1H, d, 2.4, H-1), 4.48 (1H, d, 7.4, H_{G-1}), 3.58 (3H, s, MeO), 2.86 (1H, dd, 9.9, 3.1, H-5), 2.46 (1H, dd, 10.1, 2.4, H-9), 1.10 (3H, s, H-10). ^{13}C NMR (20 MHz, CD_3OD) δ ppm: 170.6 (s, C-11), 153.7 (d, C-3), 112.2 (s, C-4), 100.7 (d, C_{G-1}), 95.8 (d, C-1), 80.0 (s, C-8), 79.2 (d, C_{G-3}), 78.9 (d, C-6), 78.4 (d, C_{G-5}), 75.5 (d, C_{G-2}), 72.5 (d, C_{G-4}), 63.7 (t, C_{G-6}), 49.9 (t, C-7), 42.4 (d, C-5), 25.6 (q, C-10). C-9 and MeO signals were overlapped by solvent peak. (Achenbach *et al.*, 1981; Damtoft *et al.*, 1982)

Results and Discussion

Compound 1 showed positive reaction to Liebermann-Burchard and Molisch tests, which indicated it to be triterpenoid saponin. UV (CH_3OH): λ_{max} 265 nm revealed the existence of an conjugated diene. Its ^1H NMR spectrum showed those protons corresponding to cyclopropane, conjugated diene and α-amino-3,4-dimethyl-γ-lactone moiety, which features are similar to heinsiagenin A, a common aglycone in reported saponins of this plant. ^{13}C NMR data further confirmed heinsiagenin A sa its aglycone.

Hydrolysis of 1 yielded D-glucose and L-rhamnose only. Four anomeric carbon signals were observed at δ 104.1, 104.0, 101.3 and 101.0 ppm in ^{13}C NMR spectrum (Table 1). Therefore, it should be a triterpenoid saponin with four sugar units in its saccharide moiety. In its ^1H NMR spectrum, four anomeric protons appeared at δ 6.45 (br s), 5.76 (d, 7.5), 5.19 (d, 7.9) and 4.92 (d, 7.6), corresponding to one L-rhamnose and three D-glucose units. The three glucose units were all in β glycosidic linkage according to their coupling constants. While the L-rhamnose unit was determined to be in α glycosidic linkage on the basis of ^{13}C NMR data.

Due to serious overlapment, the proton signals of sugar units could only be partially assigned from ^1H-^1H COSY spectrum of 1. In order to clarify the proton assignments of suger units, peracetylation of 1 was performed to give 1a. In ^1H NMR spectrum of 1a, the signals of those protons attached to acetoxyl groups exhibited at relative lower field, while H-5 of sugars and the protons in glycosylated position appeared at relative higher field. In ^1H-^1H COSY spectrum of 1a (C_5D_5N), all signals of sugar protons could be clearly ascribed, and no complete overlapment was observed among sugar protons (Table 2). For further determination of linkage sites and sequences among sugar units and aglycone, NOESY experiment was carried out. In NOESY spectrum, cross peaks were observed between H_{G-1} (δ 4.86) and H-3(δ 3.37); H_{G-1} (δ 5.23) and $H_{G'-2}$ (δ 4.28); $H_{G''-1}$ (δ 5.03) and H_{G-2} (δ 4.11); $H_{R'-1}$ (δ 5.66) and $H_{G'-4}$ (δ 4.08), which indicated the linkage site of each sugar unit. Therefore, the structure of 1 was identified to be heinsiagenin A 3-O-[β-D-glucopyranosyl(1$\rightarrow$2)-O-β-D-glucopyranosyl(1$\rightarrow$2)]-α-L-

rhamnopyranosyl-(1→4)-O-β-D-glucopyranoside. It's a new triterpenoid saponin named mussaendoside F.

In the course of our study, some isolated compounds were subjected to pharmacological tests. The results showed 2 can inhibit significantly the secretion of lacrimal gland and salivary gland induced by galanthamine at a dosage of 100 mg/kg in mice. Further test on 2 indicated its inhibitive effect on the contraction of the isolated longitudinal muscle strip from guinea-pig ileum evoked by an M-Ach receptor's agonist (carbachol, 10^{-6} M) at concentration of 10^{-4} and 10^{-5} M. From above results, compound 2 were considered to be an antagonist of M-Ach receptor. Regarding the abundant existence of compound 2 in the plant, it was supposed to be responsible for the antitoxicity of some poisonous mushroom containing muscarine and may also play a spasmolytic role in treatment of acute gastroenteritis. Besides, compound 2 promoted the proliferation of T cells of mice *in vitro* significantly at concentrations of 10^{-7} M, 10^{-6} M and 10^{-5} M, respectively.

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