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A New Phenolic Glycoside from *Mussaenda pubescens*

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Abstract – A new phenolic glycoside named mussaendoside L (**1**), along with four known iridoidal glycosides (**2~5**) were isolated from the aerial parts of *Mussaenda pubescens* Ait.f. Their structures were elucidated on the basis of chemical and spectroscopic evidence.

Key words – *Mussaenda pubescens;* Rubiaceae; phenolic glycoside; mussaendoside L.

Introduction

Mussaenda pubescens Ait.f.(Rubiaceae) is a liana-like shrub, distributed widely in shady hillside, valley and shrub jungle of east, south, and southwest China. It has been used as Chinese folk medicine for treatment of common cold, laryngopharyngitis, acute gastroenteritis, oedema and diarrhea.[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 iridoidal glycosides from the plant.[4-6] In continuation of our studies, hydrophilic fractions of the aerial part of the plant, collected from Guangzhou suburb, Guangdong Province, were further investigated. As a result, five compounds (**1~5**) were isolated. On the basis of chemical and spectral evidences, **1** was elucidated as a new phenolic glucoside named mussaendoside L, and **2~5** were identified as 6α-hydroxyl geniposide, 8-O-acetyl shanzhiside methyl ester, shanzhiside methyl ester and mussaenoside respectively. Compound **2** is first reported in this plant. This paper deals with the isolation and structure elucidation of **1~5**.

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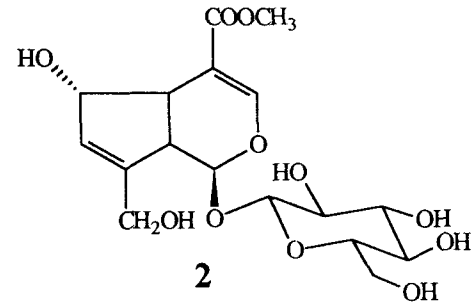
Experimental

$[\alpha]_D$: JASCO DIP-181 polarimeter. UV Shimatzu UV-250 spectrometer. IR: Perkin-Elmer 599B spectrometer. FAB-MS: Finnigan-MAT-8430. All NMR experiments were carried out using Bruker AC-80, AM-300, AM-400 and AMX-600 instruments. Chemical shifts are reported in ppm, with TMS as int. standards.

Plant materials – The aerial parts of *Mussaenda pubescens* were collected at Guangzhou suburb in Dec. 1993. A voucher specimen was identified by Prof. Bangyu Chen of South China Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation – Dried aerial parts of the plant (3.5 kg) were extracted with 95% ethanol at room temperature for three times. After removal of ethanol at

50°C *in vacuo*, water was added into the residue to give 1.5 liters of water suspension, which was extracted with ethyl acetate for four times (4×500 ml). Then the above water solution was evaporated *in vacuo* at 60°C to remove residual organic solvent. Water solution of 800 ml was remained, which was further subjected to polyporous resin (DA-201) column chromatography. The column was first eluted with water and followed with 40% and 90% EtOH solution, successively to give residue of 100 g (MP-0), 30 g (MP-40) and 20 g (MP-90), respectively. The 90% EtOH residue (10 g) was subjected to silica gel chromatography eluted by gradient chloroform-methanol-water(6:1:0.1~1:1:0.1) solvent. The eluents were divided into five fractions according to eluting sequence and TLC detection.



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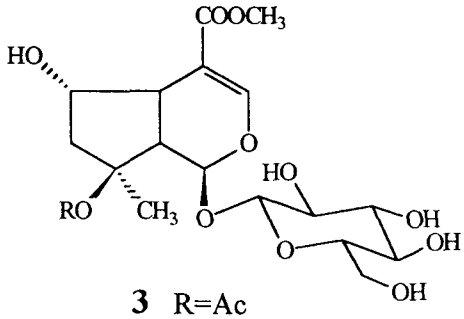
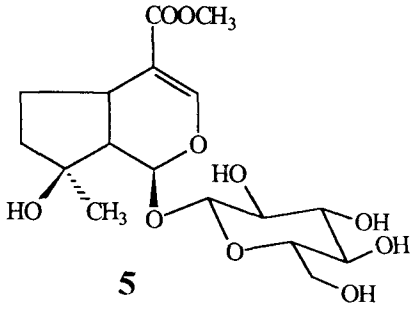


3 R=Ac

4 R=H



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Fraction 1 was chromatographed repeatedly on silica gel column, using chloroform-methanol-water (4:1:0.1) as a eluent. Compound **2** (120 mg), **3** (150 mg), **4** (20 mg), and **5** (1600 mg) were obtained.

Fraction 2 was separated on silica gel column with chloroform-methanol-water (10:3:0.3) as a eluent to yield compound **1** (30 mg).

1, mussaendoside L – Amorphous powder. $[\alpha]_D^{25}$+104.5° (CH_3OH, c 0.94). UV(CH_3OH) λ_{max} 304, 278, 227 nm. FAB-MS m/z 497[M+H]$^+$. ^{1}H NMR(400 MHz, CD_3OD) δ ppm: 7.51 (1H, *brs*, H-5), 7.50 (1H, *m* H-9), 6.62 (1H, *d*, 8.7, H-8), 6.20 (1H, *d*, 2.7), 5.93 (1H, *d*, 2.7), 5.31 (1H, *dd*, 11.3, 3.6, H-2), 4.62 (1H, *d*, 7.4, H_{G-1}), 3.72 (3H, *s*), 3.63 (3H, *s*). ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) data, δ ppm: see Table 1.

Recovery of compound 1 from CD_3OD – The CD_3OD solution of 1 for NMR experiment was evaporated to dryness *in vacuo* at 40°C to give a solid (15 mg). The solid was dissolved in 1 ml H_2O and followed by evaporation to dryness *in vacuo* at about 60°C. After repetition of the above dissolution and evaporation courses for three times, the residue was purified by silica gel column chromatography, with chloroform-methanol (4:1) as eluent. 10 mg of amorphous powder were obtained.

Hydrolysis of 1 – 10 mg of 1 was dissolved in 2N HCl (3 ml) and heated at 90°C for 4hrs. After extracted 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 ($CHCl_3$:MeOH:H_2O 7:3:0.3) in comparison with authentic sugar samples.

2, 6α-hydroxy geniposide – Amorphous powder. ^{1}H NMR (400 MHz, C_5D_5N) δ ppm: 7.93 (1H, *brs*, H-3), 6.56 (1H, *brs*, H-7), 5.85

Table 1. NMR data of **1** (DMSO-d_6).

Position	δ_C	δ_H	J(Hz)
2a	63.0t	4.13	m
2b		3.52	m
3	48.1 d	5.32	dd, 9.3, 3.2
4	196.9 s		
5	111.8 d	7.59	br s
6	147.5 s		
7	151.7 s		
8	115.2 d	6.83	d, 8.6
9	123.1 d	7.60	d, 8.6
10	128.6 s		
1'	132.7 s		
2'	135.9 s		
3'	153.9 s		
4'	99.9 d	6.31	d, 2.7
5'	154.4 s		
6'	104.9 d	6.03	d, 2.7
G-1	104.3 d	4.68	d, 7.7
G-2	74.6 d	3.34	m
G-3	76.5 d	3.32	m
G-4	69.3 d	3.30	m
G-5	77.0 d	3.12	m
G-6a	60.8 t	3.60	m
G-6b		3.65	m
3'MeO	56.0 q	3.71	s
6-MeO	55.8 q	3.81	s
2-OH		4.99	m
7-OH		9.98	s
5'-OH		9.25	s
G-2OH		4.91	d, 4.0
G-3OH		5.06	d, 4.6
G-4OH		5.00	d, 5.2
G-6OH		4.69	m

(1H, *d*, 8.8, H-1), 5.30 (2H, *m*, H-6 and H_{G-1}), 5.09 (1H, *brd* 15.7, H-10a). 4.61 (1H, *brd*, 15. 7, H-10b), 3.51 (3H, *s*, OMe), 3.27 (1H, *brdd*, H-5), 2.80 (1H, *brdd*, H-9). ^{13}CNMR (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 (2x*d*, C_{G-3} and C_{G-5}), 74.9 $(G_{G-2})^+$, 74.4 $(C-6)^+$, 71.4 (C_{G-4}), 62.4 (C-10)*, 61.2 (C_{G-6})*, 50.9 (*q*, MeO), 45.7 (*d*, C-9), 42.6(*d*, C-5). (data with + and * may be interchangeable. C-8 signal is under solvent signal at δ 149.8 ppm).[7]

3, 8-O-acetyl shanzhiside methyl ester – Amorphous powder. UV(CH_3OH) λ_{max} 235 nm. ^{1}H NMR (300 MHz, D_2O) δ ppm: 7.49(1H, *s*, H-3), 6.00 (1H, *brs*, H-1), 4.77 (1H, *d*, 8.0, H_{G-1}), 4.36 (1H, *m*, H-6), 3.93(1H, *brd*, 11.0, H_{G-6a}), 3.75 (3H, *s*, MeO), 3.74 (1H, *m*, H_{G-6b}), 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, *brd*, 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}), 54.5 (*q*, MeO), 50.5 (*d*, C-9), 48.6 (*t*, C-7), 42.8 (*d*, C-5), 24.2 (*q*, $\underline{C}H_3$ CO), 23.5 (*q*, C-10).[8]

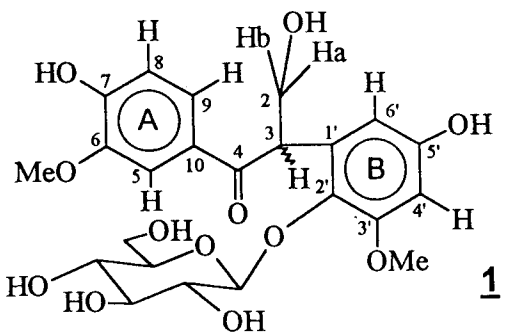
4, shanzhiside methyl ester – Amorphous powder. UV(CH_3OH) λ_{max} 235 nm. ^{1}H NMR (80 MHz, CD_3OD) δ ppm: 7.25 (1H, *brs*, H-3), 5.41 (1H, *d*, 2.4, H-1), 4.48(1H, *d*, 7.4, H_{G-1}), 3.58 (3H, *s*, MeO), 2.86 (1H, *dd*, 9.9, 3.1, H-5), 2.46 (1H, *dd*, 10.1, 2.4, H-9), 1.10 (3H, *s*, H-10). ^{13}C 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.[8,9]

5, mussaenoside – Amorphous powder, $[\alpha]_D^{14}$ -95.2° (CH_3OH, c 0.90). UV(CH_3OH) λ_{max} 235. ^{1}H NMR(400MHz, C_5D_5N) δ ppm: 7.69 (1H, *s*, H-3), 5.98 (1H, *d*, 4.0, H-1), 5.40 (2H, *d*, 7.8, H_{G-1}), 4.51 (1H, *brd*, 11.7, H_{G-6a}), 4.38 (1H, *dd*, 11.7, 5.1, H_{G-6b}), 4.28 (2H, *m*), 4.05 (1H, *dd*, 7.9, 7.9), 3.98 (1H, *m*, H_{G-5}), 3.57 (3H, *s*, MeO), 3.48 (1H, *brdd*, 15.2, 9.1, H-5), 2.77 (1H, *dd*, 9.1, 4.0, H-9), 2.50 (1H, *m*), 1.95 (1H, *m*), 1.81 (1H, *m*) 1.61 (3H, *s*, H-10), 1.58 (1H, *m*). ^{13}C NMR (75MHz, 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).[10]

Results and Discussion

1, amorphous powder, $[\alpha]_D^{25}$+104.5° (CH_3

OH, c 0.94), exhibited a quasimolecular ion peak at m/z 497[M+H]$^+$ in FAB-MS. Acidic hydrolysis of **1** yielded D-glucose as sugar component. Its ^{1}H NMR spectrum was first measured in CD_3OD, in which no hydroxyl signals could be observed. In order to utilize hydroxyl signals for spectral correlation studies, the CD_3OD solution of **1** was treated by repeated addition of water and evaporation to dryness to transform deuterium into hydrogen in its hydroxyl groups. Finally, the ^{1}H NMR spectrum was measured again in DMSO-d_6, in which all hydroxyl proton signals appeared clearly with about 30% integration compared with other proton signals. In its ^{1}H NMR spectrum, the signals of two methoxyls at δ3.71 and 3.81, one anomeric proton at δ4.68 (*d*, 7.7), one 1,2,3,5-tetra-substituted benzene ring (δ6.31, *d*, 2.7, H-4'; 6.03, *d*, 2.7, H-6'), one 1,3,4-tri-substituted benzene ring (δ7.59, *brs*, H-5; 6.83, *d*, 8.6, H-8; 7.60, *d*, 8.6, H-9) and seven hydroxyl protons were observed. In ^{13}C NMR spectrum, twenty-three carbon signals were exhibited, which covered one carbonyl, two methoxyls, twelve aromatic carbons, seven oxygen-bearing methine and methylene carbons and a methine carbon. From the above ^{1}H and ^{13}C NMR data(see Table 1), along with FAB-MS results, the molecular formula of **1** could be deduced as $C_{23}H_{28}O_{12}$. The UV(CH_3OH) λ_{max} 304, 278, 227 nm revealed the characteristic feature similar to flavanones or isoflavanones. Regarding 2 amu less than that of dimethoxyl-trihydroxyl flavanone monoglucoside in molecular weight, the dihydropyrone ring in **1** was considered to be cleaved in ether-oxygen linkage.



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For elucidation of substitution pattern, a series of 2D NMR experiments including ^{1}H-^{1}H DQF COSY, HMQC, HMBC and NOESY were performed (see Table 2). Therefore, two methylene protons at δ4.13 and 3.52 were proved to be oxygen-beared due to these protons coupled with hydroxyl proton at δ4.99 in ^{1}H-^{1}H DQF COSY. The structural part of -CO-CH-CH_2OH was deduced from the correlation between CH proton at δ5.32 and CH_2 protons at δ4.13 and 3.52 in ^{1}H-^{1}H DQF COSY, as well as the correlation between CH_2 protons and carbonyl carbon at δ196.9 in HMBC. Thus, **1** was suggested to be a seco-isoflavanone.

The signal of isolated aromatic proton at δ7.59 was assigned to H-5 in ring A, which correlated with C-4 in HMBC and C_6-methoxyl protons in NOESY. The signals of adjacent two aromatic protons at δ6.83 and 7.60 were assigned as H-8 and H-9 in ring A,

Table 2. Summary of 2D NMR data of **1**.

Proton	DQF COSY (H)	HMBC (C)	NOESY (H)
2a	2b, 3, 2-OH	3, 4	2b, 3, 6'
2b	2a, 3, 2-OH	3, 4	2a, 3
3	2a, 2b	2, 1', 2', 6'	2a, 2b, 5
5	9	4, 7, 9	3, 6-MeO
8	9	6, 10	9
9	5, 8	4, 5, 7	8
4'	6'	2', 3', 5', 6'	3'-MeO
6'	4'	3, 2', 4', 5'	2a
G-1	G-2	2'	G-3, G-5
G-2	G-2, G-2OH		
G-3	G-2, G-4, G-3OH		G-1, G-5
G-4	G-3, G-5, G-4OH		
G-5	G-4, G-6a,b		G-1, G-3
G-6a	G-5, G-6b, G-6OH		G-6b
G-6b	G-5, G-6a, G-6OH		G-6a
3'-MeO		3'	4'
6-MeO		6	
2-OH	2a, 2b		
5'-OH		4', 5', 6'	
G-2OH	G-2		
G-3OH	G-3		
G-4OH	g-4		
G-6OH	G-6a,b		

among which the former correlated with C-6 and C-10, and the latter with C-4, C-5 and C-7 in HMBC. The arrangement of ring B was made in the same 2D NMR experiments. The H-6' was assigned from the correlation with C-3 in HMBC, H-4' from correlation with H-6' in ^{1}H-^{1}H DQF COSY, and 3'-MeO from correlation with H-4' in NOESY. The signal at δ_c 135.0 was suggested as C-2', which coupled with H-3 in HMBC. The glucose unit was deduced to be connected to C-2' from correlation bet- ween anomeric proton and C-2' in HMBC, with β-configuration from its large $J_{1,2}$ value (7.7Hz). The unambiguous assignment of ^{1}H and ^{13}C resonance was conformed by HMQC experiment. From the above evidence, the structure was established as new phenolic compound, named mussaendoside L.

On the basis of spectral evidence, compounds **2**~**5** were identified as 6α-hydroxyl geniposide, 8-O-acetyl shanzhiside methyl ester, shanzhiside methyl ester and mussaenoside respectively. As a result of our investigation, **2** was isolated from this plant for the first time.

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