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SAPONINS FROM *MUSSAENDA PUBESCENS*

WEIMIN ZHAO, JUNPING XU, GUOWEI QIN* and RENSHENG XU

Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China

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Key Word Index—*Mussaenda pubescens*; Rubiaceae; triterpenoid saponins; mussaendoside R and S.

Abstract—Two new triterpenoid saponins named mussaendoside R and S have been isolated from the whole plant of *Mussaenda pubescens* Ait. f. Their structures have been determined on the basis of spectral methods.

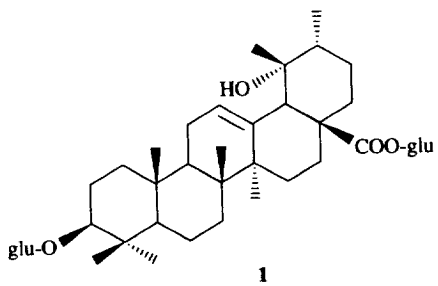
INTRODUCTION

Mussaenda pubescens Ait. f. (Rubiaceae) is a Chinese folk medicine, used for treatment of laryngopharyngitis, acute gastroenteritis and dysentery [1]. The hydrophilic fraction of the extract of this plant showed significant antifertility activity in *in vivo* tests [2]. To elucidate its active principles, we systematically studied the hydrophilic components of the whole plant collected in the Guangdong province. In previous papers, we reported the isolation and structure elucidation of several new saponins, namely mussaendosides A–C, M and N with cyclolanostene-type aglycone [3, 4]. As a part of our continuing chemical study on this plant, four compounds were isolated and identified on the basis of spectral methods. Among them, two were known compounds (**3**, **4**), and the other two were new triterpenoid saponins, namely mussaendoside R (**1**) and S (**2**).

RESULTS AND DISCUSSION

Compounds **3** and **4** were determined to be 6α-hydroxygeniposide and 3β-*O*-β-D-glucopyranosyl quinovic acid 28-*O*-β-D-glucopyranosyl ester by comparing their physical and spectral data with literature values [5–7]. Compound **4** was found in this plant for the first time.

Mussaendoside R (**1**), amorphous powder, showed a positive reaction to the Liebermann–Burchard and Molish tests, which indicated that it could be a triterpenoid saponin. The FAB-mass spectrum revealed a quasimolecular ion peak at *m/z* 794 $[M - H - H]^+$. In the ^1H NMR spectrum, six singlet and one doublet methyl signals were observed. On the basis of spectroscopic analyses, its aglycone was deduced to possess an α-amyrin type skeleton with a carboxyl at C-17 (δ48.5 and 176.9), a double bond at C-12, C-13 (δ128.3 and 139.1) and a β-OH at C-3 (δ88.9). In addition, the presence of a quaternary



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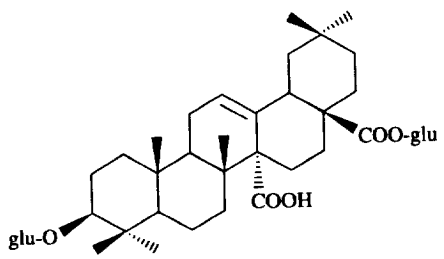

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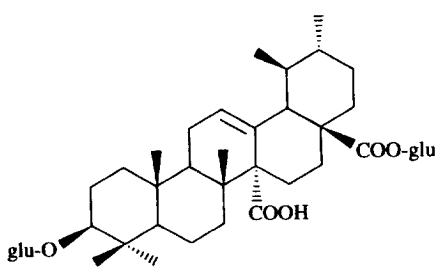

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*Author to whom correspondence should be addressed.

carbon signal at δ_C 72.5 and a characteristic allylic proton shown as a broad singlet at δ_H 2.93, and a singlet methyl signal at δ_H 1.70 led us to allocate an hydroxyl group to C-19. Therefore, the genin of **1** was considered to be pomolic acid. It was confirmed by comparison of its ^{13}C NMR data with published data of this triterpenic acid [8, 9].

Hydrolysis of **1** yielded D-glucose identified only by HPTLC detection. Its ^{13}C NMR spectrum revealed two D-glucose moieties besides its aglycone. The presence of two anomeric carbon signals at δ106.8 and 95.7, along with the C-3 signal at δ88.9 and C-28 signal at 176.9, indicated that **1** was a bisdesmoside, in which the C-3 hydroxy group was glycosylated and the C-28 carboxy group was esterified with glucose, respectively. In the ^{1}H NMR spectrum, the two anomeric proton signals at δ6.32 (*d*, J = 8.1 Hz) and 4.94 (*d*, J = 7.8 Hz) confirmed the existence of ester and ether glucose moieties, both of which were in β-glycosyl linkage. Therefore, the structure of mussaendoside R was established as 3β-*O*-β-D-glucopyranosyl pomolic acid 28-*O*-β-D-glucopyranosyl ester.

Mussaendoside S (**2**), amorphous powder, showed a positive reaction to the Liebermann–Burchard and Molish tests, which indicated that it could be a triterpenoid saponin. The FAB-mass spectrum revealed a molecular ion peak at *m/z* 810, corresponding to $[C_{42}H_{66}O_{15}]^+$. In the ^{1}H NMR spectrum, six singlet methyl signals were observed. On the basis of the ^{13}C NMR data, the aglycone of **2** was supposed to possess a β-amyrin skeleton with a β-OH at C-3, a double bond at C-12/C-13 and two carboxyls derived from the remaining methyls in the skeleton. The chemical shifts of C-12 and C-13 at δ126.7 and 137.6 revealed a carboxyl substituted at C-14, and another carboxyl was at C-17 in the usual manner. Therefore, the genin of **2** was determined to be cincholic acid. It was confirmed by comparison of its ^{13}C NMR data with reported data of cincholic acid and its glycosides [7, 10].

Hydrolysis of **2** yielded D-glucose identified only by HPTLC detection. The ^{13}C NMR data revealed that **2** containing two D-glucose moieties besides its aglycone. By using the same methods described above, the two D-glucose units were deduced to be attached to C-3 and a carboxyl group, respectively, and both with a β-glucosyl linkage. In the ^{13}C NMR spectrum of **2**, the signal of C-28 shifted upfield about 3.4 ppm, but C-27 was almost coincident with those of cincholic acid and cincholic 3-*O*-monoglycoside [7]. Furthermore, the signal of C-17 in **2** shifted downfield about 0.2 ppm, while C-22 shifted upfield about 0.5 ppm compared to cincholic acid and its 3-*O*-monoglycoside [7]. Some other small differences were also observed for carbons neighbouring C-17. Similar changes of the ^{13}C NMR data could also be observed between some saponins bearing a C-28 ester glycoside and their alkaline hydrolysates [11]. From the above considerations, the two glucose units in **2** should be at C-3 and C-28, respectively. Therefore, mussaendoside S was determined to be 3β-*O*-β-D-glucopyranosyl cincholic acid 28-*O*-β-D-glucopyranoside.

EXPERIMENTAL

FAB-MS: Finnigan-MAT 8430. ^{1}H and ^{13}C NMR spectra: Bruker AM-400, Varian Gemini-300 or Burker AC-80 spectrometers, with TMS as int. standard. Chemical shifts are reported in ppm.

Plant material. The whole plants of *M. pubescens* were collected in Zhaoqing, Guangdong province, South China. The plant was identified by Dr Jian-yu Chen (Guangdong Medical College).

Isolation of compounds. The crude saponins (7 g) of *M. pubescens* were chromatographed on silica gel column, eluted with $CHCl_3$–MeOH–H_2O. From eluants of 14:6:1 of the solvent system, a mixture (500 mg) was obtained, which was rechromatographed on silica gel column, eluted with $CHCl_3$–MeOH (4:1) to give **3** (80 mg). The residue was separated on RP-18 Lobar column repeatedly, elution with MeOH–H_2O (7:3) gave **1** (56 mg), **2** (10 mg) and **4** (46 mg).

Acidic hydrolysis. A methanolic soln of **1** and **2**, together with standard sugar samples, were applied at points *ca* 1 cm from the bottom of a 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 (80:20:1) as eluant. The plate was then sprayed with 10% H_2SO_4 (in EtOH), and then heated at 110°.

Compound **1**. Amorphous powder. $[\alpha]_D^{15}$ + 4.6° (pyridine; *c* 0.13). FAB-MS *m/z* 794 $[M - H - H]^+$. ^{1}H NMR (400 MHz, pyridine-d_5): δ6.32 (1H, *d*, J = 8.1 Hz, g$'_1$-H), 5.55 (1H, *br s*, H-12), 4.94 (1H, *d*, J = 7.8 Hz, g$_1$-H), 3.37 (1H, *dd*, J = 11.5, 4.1 Hz, 3-H), 2.93 (1H, *br s*, H-18), 1.70 (3H, *s*, H-29), 1.40 (3H, *s*), 1.29 (3H, *s*), 1.18 (3H, *s*), 1.06 (3H, *d*, J = 6.5 Hz, H-30), 1.00 (3H, *s*), 0.87 (3H, *s*). For ^{13}C NMR data see Table 1.

Compound **2**. Amorphous powder. $[\alpha]_D^{16}$ + 53.3° (pyridine; *c* 0.12). FAB-MS *m/z* 810 $[M]^+$. ^{1}H NMR (400 MHz, pyridine-d_5): δ6.38 (1H, *d*, J = 7.8 Hz, g$'_1$-H), 5.99 (1H, *br s*, H-12), 4.75 (1H, *d*, J = 6.3 Hz, g$_1$-H), 3.34 (1H, *dd*, J = 12.5, 4.3 Hz, 3-H), 1.20 (3H, *s*), 1.11 (3H, *s*), 0.93 (3H, *s*), 0.85 (3H, *s*), 0.83 (3H, *s*) 0.71 (3H, *s*). For ^{13}C NMR data see Table 1.

Compound **3**. Amorphous powder. ^{1}H NMR (400 MHz, pyridine-d_5): δ7.93 (1H, *br s*, H-3), 6.56 (1H, *br s*. H-7), 5.85 (1H, *d*, J = 8.8 Hz, H-1), 5.30 (2H, *m*, H-6 and g$_1$-H), 5.09 (1H, *br d*, J = 15.7 Hz, Ha-10), 4.61 (1H, *br d*, J = 15.7 Hz, Hb-10), 3.51 (3H, *s*, OMe), 3.27 (1H, *br dd*, H-5), 2.80 (1H, *br dd*, H-9). ^{13}C NMR (20 MHz, pyridine-d_5): δ167.8 (C-11), 154.3 (C-3), 130.2 (C-7), 108.2 (C-4), 102.3 (g$_1$-C), 101.6 (C-1), 78.2 (g$_3$-C and g$_5$-C), 74.9 (g$_2$-C),[a] 74.4 (C-6),[a] 71.4 (g$_4$-C), 62.4 (C-10), 61.2 (g$_6$-C), 50.9 (MeO), 45.7 (C-9), 42.6 (C-5) (data with [a] may be interchanged. C-8 is under the pyridine-d_5 signal).

Compound **4**. Amorphous powder. FAB-MS *m/z* 810 $[M]^+$. ^{1}H NMR (400 MHz, pyridine-d_5): δ6.33 (1H, *d*, J = 7.9 Hz, g$'_1$-H), 5.95 (1H, *br s*, H-12), 4.74 (1H, *d*, J = 7.6 Hz, g$_1$-H), 3.18 (1H, *dd*, J = 11.5, 3.5 Hz, 3-H), 1.18 (3H, *s*), 1.14 (3H, *d*, J = 5.9 Hz), 1.09 (3H, *s*), 0.91 (3H, *s*), 0.84 (3H, *s*), 1.44 (3H, *d*, J = 6.1 Hz). For ^{13}C NMR data see Table 1.

Table 1. ^{13}C NMR spectral data of compounds **1**, **2** and **4** (75 MHz, pyridine-d_5)

C	1	2	4	C	1	2	4
1	38.7	39.2	38.7	22	37.6	32.4	36.0
2	26.5	25.5	26.3	23	28.1	28.3	27.6
3	88.9	89.0	88.4	24	15.5	16.7[a]	16.2[a]
4	39.4	39.6	39.0	25	17.3	17.3[a]	16.7[a]
5	55.8	56.1	55.4	26	16.6	19.1	18.8
6	18.5	18.8	18.2	27	24.5	178.7	177.8
7	33.4	37.5	37.1	28	176.9	176.8	176.2
8	40.4	40.3	39.8	29	26.9	33.3	17.7
9	47.6	47.8	46.8	30	16.9	24.0	20.8
10	36.8	37.3	36.6	g-1	106.8	106.9	106.4
11	23.9	23.7	23.0	2	75.6	75.9	75.3
12	128.3	126.7	129.2	3	79.1	79.4	78.8
13	139.1	137.6	132.9	4	71.7	72.1	71.4
14	42.0	56.9	56.4	5	78.6	78.9	78.2
15	29.1	26.9	25.8	6	62.9	63.2	62.6
16	26.0	25.0	25.1	g'-1	95.7	95.9	95.3
17	48.5	48.2	48.6	2	73.9	74.4	73.7
18	54.3	44.3	54.3	3	78.7	79.1	78.4
19	72.5	44.1	38.7	4	71.1	71.5	70.8
20	42.0	31.0	37.1	5	78.1	78.2	77.7
21	26.5	34.1	29.9	6	62.2	62.6	61.9

[a]Assignments may be interchanged in each vertical column.

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