Mussaendosides M and N, New Saponins from Mussaenda pubescens

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MUSSAENDOSIDES M AND N, NEW SAPONINS FROM *MUSSAENDA PUBESCENS*

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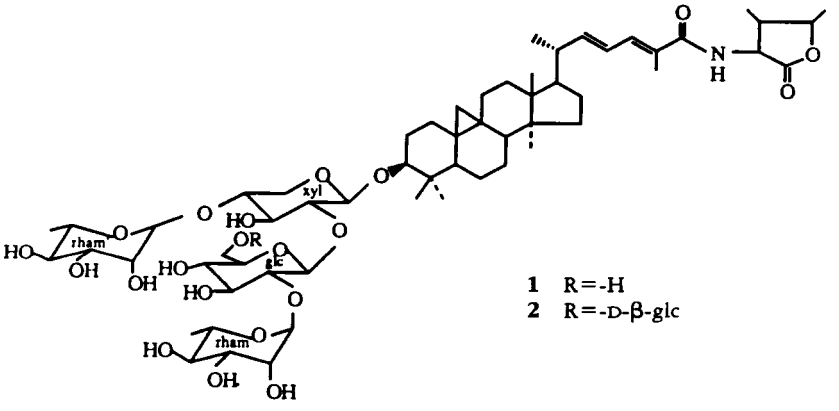
ABSTRACT.—Two new cycloartane-type triterpenoidal saponins, named mussaendosides M [**1**] and N [**2**], were isolated from whole plants of *Mussaenda pubescens* (Rubiaceae). Their structures were determined by 2D nmr techniques, such as TOCSY, HMQC, HMBC, and ROESY, and chemical evidence.

The whole plants of *Mussaenda pubescens* Ait. f. (Rubiaceae) have been used in Chinese folk medicine against laryngopharyngitis, acute gastroenteritis, and dysentery and have also been used as a contraceptive agent. Screening for pregnancy inhibition in rats indicated that both the 60% and 95% EtOH extracts of *M. pubesecus* gave a 28% fecundation rate, thus showing significant antifertility activity. In the preceding paper we described the isolation and structural elucidation of a cycloartane-type triterpene with an α-amino γ-lactone in an amide form in the side chain terminal and three new saponins, namely mussaendosides A, B, and C (1). We now report the isolation and structural elucidation of two new saponins, named mussaendosides M and N.

EtOH extracts of the whole plants were purified by passing through a column of DA-201 polymeric adsorbent (Nankai University, China), followed by cc on Si gel to afford two products, mussaendosides M [**1**] and N [**2**]. On acidic hydrolysis, with 2 N H_2SO_4-C_6H_6 (1:1), both **1** and **2** gave the same aglycone (MP-C), which was identified as *N*-[(2*S*,3*R*,4*R*)-3-methyl-4-pentanolid-2-yl]-3β-hydroxy-9,19-cyclolanosta-(22*E*,24*E*)-dien-27-amide. Both hydrolysates gave D-glucose, D-xylose, and L-rhamnose.

Mussaendoside M [**1**] showed fabms fragments at *m/z* 1174 $[M+Na]^+$, 1190 $[M+K]^+$, 1152 $[M+H]^+$, and 566 [aglycone + H]$^+$, corresponding to the molecular formula $C_{59}H_{93}NO_{21}$. The ^1H- and ^{13}C-nmr spectral data showed four anomeric signals (δ 4.88 d, 5.88 d, 5.50 s, 6.40 s, and 99.73, 102.17 × 2, 105.42). This indi-



1 R=-H
2 R=-D-β-glc

cated that **1** consisted of 1 mol of aglycone, D-glucose, and D-xylose and 2 mol of L-rhamnose. The β configurations at the anomeric centers of the D-glucopyranosyl and D-xylopyranosyl moieties were suggested by the chemical shifts, the large $J_{1,2}$ couplings (7.0 and 7.6 Hz, respectively) of the anomeric proton in the ^{1}H-nmr spectrum, and by ^{13}C-nmr data of the two sugars. The α configuration at the anomeric center of the L-rhamnopyranosyl moiety was derived from their ^{13}C-nmr spectral data.

In order to reveal the sequence of the sugar unit, the acetate of **1** was examined by eims; three peaks were recorded due to *O*-acetylated sugars at *m/z* (%) 1007 [xyl(-rha)-glc-rha(OAc)$_{10}$]$^+$ (0.05), 561 [glc-rha(OAc)$_6$]$^+$ (17), and 273 [rha(OAc)$_3$)]$^+$ (100) (2,3). This indicated that both L-rhamnosyl moieties are in terminal locations, the glucosyl moiety is in the center of the tetrasaccharide chain, and the branching of the sugar chain is at the inner D-xylopyranosyl moiety. Because only one carbon (C-3, 89.23 ppm) in the aglycone was shifted downfield by 11.38 ppm, the tetrasaccharide chain was suggested to be linked to the C-3 hydroxyl group of the aglycone.

By comparison of the ^{13}C-nmr data of **1** with those of the corresponding sugars of mussaendosides B and C and methylrhamnopyranoside (4), it was found that the anomeric carbon signals of the xylosyl (105.42 ppm), glucosyl (102.17 ppm), and one rhamnosyl (102.17 ppm) moieties shifted upfield by 1.98, 3.99, and 2.67 ppm, respectively. Because of the presence of abnormal glycosylation effects created by 1,2-glycosidic bonds (5) in the tetrasaccharide chain, the C-2 signals due to xylosyl (78.64 ppm) and glucosyl (79.27 ppm) moieties were shifted downfield by 4.00 and 4.47 ppm, respectively, in the glycosylation. This analysis showed that the D-glucosyl moiety was connected to the C-2 hydroxyl groups of the D-xylosyl moiety, and one of the terminal L-rhamnosyl moiety was joined to the C-2 hydroxyl group of the D-glucosyl moiety. In addition, glycosylation shifts were observed at C-4 (+5.95 ppm) and C-5 (−3.60 ppm) of the xylopyranosyl moiety. Thus, another terminal L-rhamnosyl moiety was linked to the C-4 hydroxyl group of the inner D-xylopyranosyl moiety.

However, since signals due to the sugar units overlap, assignment of the ^{1}H- and ^{13}C-nmr signals was difficult and it was necessary to further confirm the structure of **1**.

Inspection of cross peaks in the ^{1}H-^{1}H COSY, TOCSY, and HMQC spectra of the acetate of **1** enabled ^{1}H and ^{13}C assignments to be made with certainty (6,7). Furthermore, long-range coupling (^3J) interactions between rha-C-1 and glc-H-2, glc-C-1 and xyl-H-2, rha′-H-1, and xyl-C-4, xyl-H-1, and aglycone-C-3 were evident from HMBC (Table 1). This result was further confirmed by using a 2D nOe in the rotating frame (ROESY) (8) where four nOe cross peaks were observed between aglycone-H-3 and xyl-H-1; xyl-H-2 and glc-H-1; xyl-H-4 and rha′-H-1; glc-H-2 and rha-H-1

TABLE 1. Cross Peaks in ^{1}H-detected Long-range ^{1}H-^{13}C Multiple Bond Shift Correlation (HMBC) Spectrum of Acetate of Mussaendoside M [**1**].

^{1}H	(δ ppm)	^{13}C (δ ppm)
xyl-1	(4.38)	C-3 (99.25)
xyl-2	(3.87)	glc-1 (100.10), xyl-3 (73.83), xyl-1 (103.45)
xyl-3	(5.16)	xyl-2 (75.05), xyl-4 (72.85)
glc-2	(3.65)	rha-1 (97.20), glc-1 (100.10), glc-3 (74.55)
glc-3	(5.19)	glc-2 (76.13), glc-4 (68.08)
rha-1	(4.92)	glc-2 (76.13), rha-5 (66.87), rha-3 (68.82)
rha-2	(5.02)	rha-1 (97.20), rha-3 (68.82)
rha′-1	(4.77)	xyl-4 (72.85), rha′-3 (69.17), rha′-5 (66.48), rha′-2 (70.08)
rha′-2	(5.14)	rha′-3 (69.13)
rha′-4	(5.04)	rha′-3 (69.13)

(Table 2). Consequently, the structure of mussaendoside M was established as MP-C 3-*O*-α-L-rhamnopyranosyl-(1↦2)-*O*-β-D-glucopyranosyl-(1↦2)-[*O*-α-L-rhamnopyranosyl-(1↦4)]-*O*-β-D-xylopyranoside [**1**].

TABLE 2. Cross Peaks in 2D ROESY Spectrum of Acetate of Mussaendoside M [**1**].

^{1}H (δ ppm)		^{1}H (δ ppm)
xyl-1	(4.38)	C-3 (3.11), xyl-3 (5.16)
glc-1	(4.61)	xyl-2 (3.87), glc-3 (5.19), glc-5 (3.68)
rha-1	(4.91)	glc-2 (3.65)
rha′-1	(4.77)	xyl-4 (3.85)
rha-3	(5.24)	rha-5 (4.19)
rha′-1	(5.07)	rha′-5 (3.81)

Mussaendoside N [**2**] showed spectroscopic (uv, nmr) properties similar to those of mussaendoside M [**1**]. The molecular formula $C_{65}H_{103}NO_{26}$ was established by fabms. Comparison of the pseudo-molecular ion peaks observed at *m/z* 1336 $[M + Na]^+$ and 1352 $[M + K]^+$ with those of **1** indicated that **2** contained one more glucosyl moiety than **1**. The ^{1}H- and ^{13}C-nmr spectra showed five anomeric signals at δ 4.88 (d, $J = 7.2$ Hz, H-1 of xylose), 5.23 and 5.77 (each d, $J = 7.6$ Hz, H-1 of glucose), 5.44 and 6.40 (each s, H-1 of rhamnose); and 99.74, 101.88, 102.09, 105.27, and 105.58 (C-1 of each sugar). The ^{13}C-nmr signals due to the sugar units of **2** also showed abnormal glycosylation effects similar to those of **1**. Enzymatic hydrolysis of **2**, with emulsin at 37°, gave **1** and glucose. In the eims of the acetate of **2**, four fragments due to *O*-acetylated sugars at *m/z* 1295 $[xyl(-rha)-glc(-glc)-rha(OAc)_{13}]^+$, 849 $[glc(-glc)-rha(OAc)_9]^+$, 331 $[glc\ (OAc)_4]^+$, and 273 $[rha(OAc)_3]^+$ were observed (2,3). This indicated that another β-D-glucosyl moiety was present, in a terminal position, in addition to the sequence of sugar units of **1**. In the ^{13}C-nmr spectrum, C-6 of the inner glucosyl moiety was observed at δ 70.07, revealing deshielding of 7.21 ppm from the corresponding value of 62.86 ppm for **1**, showing it to be the point of glycosidation with the terminal glucosyl moiety. Therefore, the structure of mussaendoside N [**2**] was characterized as MP-C 3-*O*-α-L-rhamnopyranosyl-(1↦2)-[*O*-β-D-glucopyranosyl-(1↦6)]-*O*-β-D-glucopyranosyl-(1↦2)-[*O*-α-L-rhamnopyranosyl-(1↦4)]-*O*-β-D-xylopyranoside.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot plate and are uncorrected. Optical rotations were measured on a JASCO Dip-18 polarimeter. The uv spectra were obtained on a Shimazu UV-250 instrument. 1D and 2D ^{1}H- and ^{13}C-nmr spectra were recorded at ambient temperature on either Bruker AM-400 (1D, ^{1}H spectra); AM-300 (1D, ^{13}C spectra) or AMX-600 (2D, TOCSY, ROESY, HMQC, and HMBC) nmr spectrometers locked to the major deuterium of the solvent, C_5D_5N. The 2D TOCSY spectrum with a data matrix 512 × 1024 had 32 scans (with 2 dummy scans) per t_1 value and a delay time between scans of 0.9 sec. The 2D phase-sensitive ROESY spectrum with a data matrix 256 × 1024 had 48 scans (with 4 dummy scans) per t_1 value, a mixing time of 0.8 sec with a 2% random variation, and a delay time between scans of 2 sec. The 2D HMQC (and HMBC) spectrum with a 128 × 2048 (128 × 1024) data matrix had 296 scans and 2 dummy scans (400 + 4) per t_1 value and a delay between scans of 1 sec (1 sec). The value of the delay to optimize one-bond correlations in the HMQC spectrum and supress them in the HMBC spectrum was 3.45 msec, and the evolution delay for long range couplings in the latter was set to 70 msec. All 1D and 2D spectra were recorded using the standard Bruker software package, and data manipulation of the 2D spectra were performed on a Bruker Aspect ×32 data station. All chemical shifts were given in ppm relative to TMS, and couplings in Hz. Mass and fabms spectra were rcorded on a Varian MAT-212 mass spectrometer. Fabms were measured in the positive ion mode with an accelerating potential of 3.0 kV for an Xe beam source in 3-nitrobenzyl alcohol matrix.

PLANT COLLECTION.—The whole plant of *M. pubescus* was collected at Gundong, China in 1987. The plant specimen, which was identified by Dr. Long-jing Hua from the Department of Pharmacy Sciences, Shanghai College of Traditional Chinese Medicine, has been deposited in the Shanghai Institute of Materia Medica, Academia Sinica and Shanghai College of Traditional Chinese Medicine.

ISOLATION.—Ground air-dried whole plants (6 kg) were extracted by cold percolation with EtOH. The concentrated extract was partitioned between H_2O and petroleum ether, then between H_2O and EtOAc, and finally between H_2O and *n*-BuOH saturated with H_2O three times. The *n*-BuOH extract was introduced into a DA-201 column (PSD-type polymeric adsorbent, made in Nankai University, China) and eluted with H_2O, 20% EtOH, 40% EtOH, and 60–90% EtOH, successively. Crude saponin (14 g) was obtained from the last fraction. The crude saponin (7 g) was then repeatedly separated by Si gel cc with $CHCl_3$-MeOH-H_2O (7:3:0.5) as eluent, to afford mussaendoside M [**1**] (271 mg) and mussaendoside N [**2**] (374 mg).

Mussaendoside M [**1**].—White powder, mp 178°, $[\alpha]_D$ +20.79° (c = 4.33, MeOH); uv λ max (MeOH) 270 nm; fabms *m/z* $[M+K]^+$ 1190, $[M+Na]^+$ 1174, $[M+H]^+$ 1152; ^{1}H nmr (C_5D_5N) δ 0.21 and 0.46 (each 1 H, d, J = 4.0 Hz, H-19), 0.85 (3H, d, J = 7.5, H-3′ , 0.87 (3H, s, Me-18), 1.03 (3H, s, Me-29), 1.04 (3H, d, J = 6.5, Me-21), 1.13 (3H, s, Me-28), 1.15 (3H, d, J = 6.6, Me-4′), 1.31 (3H, s, Me-30), 1.60 (3H, d, J = 6.0, rham-6), 1.85 (3H, d, J = 6.0, rham′-6), 2.20 (3H, s, Me-26), 3.46 (1H, m, H-3), 4.88 (1H, d, J = 7.2, xyl-1), 5.50 (1H, s, rha-1), 5.60 (1H, dd, J = 15.0, 9.3, H-22), 5.69 (1H, dd, J = 7.5, 5.0, H-2′), 5.88 (1H, d, J = 7.6, glc-1), 6.41 (1H, dd, J = 15.0, 11.0, H-23), 6.45 (1H, s, rha′-1), 7.27 (1H, d, J = 11.0, H-24), 9.16 (1H, d, J = 5.0, NH); ^{13}C nmr see Table 3.

Mussaendoside N [**2**].—White powder: mp 194°; $[\alpha]_D$ +19.63° (c = 3.50, MeOH); uv λ max (MeOH) 268 nm; fabms *m/z* $[M+K]^+$ 1352, $[M+Na]^+$ 1336; ^{1}H nmr (C_5D_5N) δ 0.26 and 0.48 (each 1H, d, J = 4.0, H-19), 0.85 (3H, d, J = 7.5, Me-3′), 0.87 (3H, s, Me-18), 0.96 (3H, s, Me-29), 0.98 (3H, d, J = 6.5, Me-21), 1.15 (3H, d, J = 6.6, Me-4′), 1.21 (3H, s, Me-28), 1.37 (3H, s, Me-30), 1.63 (3H, d, J = 6.2, rha-6), 1.84 (3H, d, J = 6.2, rha′-6), 2.20 (3H, s, Me-26), 3.44 (1H, m, H-3), 4.88 (1H, d, J = 7.2, xyl-1), 5.23 (1H, d, J = 7.6, glc-1), 5.44 (1H, s, rha-1), 5.60 (1H, dd, J = 15.0, 9.3, H-22), 5.69 (1H, dd, J = 7.5, 5.0, H-2′), 5.78 (1H, d, J = 7.6, glc′-1), 6.40 (1H, s, rha′-1), 6.42 (1H, dd, J = 15.0, 11.0, H-23), 7.26 (1H, d, J = 11.0, H-24), 9.16 (1H, d, J = 5.0, NH); ^{13}C nmr see Table 3.

TABLE 3. ^{13}C-nmr Data of Mussaendosides M [**1**] and N [**2**] (100 MHz, in C_5D_5N).

Carbon	Compound		Carbon	Compound		Carbon	Compound	
	1	**2**		**1**	**2**		**1**	**2**
C-1 t	32.15	32.20	C-25 s	129.12	129.15	5 d	77.68	77.00
C-2 t	29.83	29.77	C-26 q	13.49	13.44	6 t	63.30	70.07
C-3 d	89.23	89.27	C-27 s	170.74	170.73	rha		
C-4 s	41.23	41.46	C-28 q	26.03	26.10	1 d	99.73	99.81
C-5 d	47.61	47.66	C-29 q	15.30	15.49	2 d	72.37	72.34
C-6 t	21.16	21.17	C-30 q	19.48	19.49	3 d	72.60	72.60
C-7 t	26.40	26.39	C-1′ s	175.73	175.71	4 d	73.93	73.90
C-8 d	47.95	48.01	C-2′ d	55.47	55.44	5 d	69.72	69.62
C-9 s	19.98	19.84	C-3′ d	38.63	38.61	6 q	18.59	18.59
C-10 s	26.05	26.10	C-4′ d	78.64	78.44	rha′		
C-11 t	26.63	26.56	3′-Me	8.07	8.07	1 d	102.17	102.05
C-12 t	33.05	33.03	4′-Me	15.51	15.49	2 d	72.37	72.34
C-13 s	45.65	45.63	xyl			3 d	72.72	72.79
C-14 s	49.21	49.35	1 d	105.42	105.26	4 d	74.27	74.24
C-15 t	35.77	35.76	2 d	78.64	78.70	5 d	69.99	70.03
C-16 t	28.75	28.74	3 d	75.49	76.03	6 q	19.07	18.99
C-17 d	52.00	51.98	4 d	76.85	76.88	glc′		
C-18 q	18.42	18.47	5 t	63.30	63.18	1 d		105.56
C-19 t	29.83	29.98	glc			2 d		75.43
C-20 d	41.35	41.58	1 d	102.17	102.08	3 d		78.22
C-21 d	19.85	19.85	2 d	79.27	79.31	4 d		71.72
C-22 d	147.91	147.92	3 d	77.02	77.00	5 d		78.22
C-23 d	123.68	123.84	4 d	72.37	72.34	6 t		62.86
C-24 d	134.82	134.82						

ACID HYDROLYSIS OF **1** AND **2**.—Each sample (30 mg) was refluxed with 2 N H_2SO_4-C_6H_6 (1:1) for 35 h on an H_2O bath. The reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ extract was purified by Si gel cc, eluting with *n*-hexane–$CHCl_3$ (4:1) to give aglycone MP-C, which was identified as *N*-[(2*S*,3*R*,4*R*)-3-methyl-4-pentanolid-2-yl]-3β-hydroxy-9,19-cyclolanosta-(22*E*,24*E*)-dien-27-amide (1). The aqueous layer was neutralized with 1 N NaOH and evaporated to dryness in vacuo. Both **1** and **2** showed the presence of D-glucose, D-xylose, and L-rhamnose on Si gel tlc [solvents *n*-BuOH–HOAc–H_2O (4:1:2) and $CHCl_3$-MeOH-H_2O (25:17:3)].

ENZYMATIC HYDROLYSIS OF **2**.—An H_2O solution containing **2** (80 mg) and emulsin (85 mg) was incubated at 38° for 4 days, then filtered and purified by RP-18 hplc with MeOH-H_2O (7:3) as eluent to give **1** (7.5 mg).

ACETYLATION OF **1** AND **2**.—Each solution of **1** and **2** (10 mg) in pyridine (1 ml) and Ac_2O (1 ml) was kept at room temperature overnight. Excess reagents were removed in vacuo, and the residue was partitioned between H_2O and Et_2O. The acetate was obtained from the Et_2O extract. Acetate of **1**: eims *m/z* 1007 [xyl(-rha)-glc-rha-OAc)$_{13}$]$^+$, 849 [glc(-glc)-rha(OAc)$_9$]$^+$, 331 [glc(OAc)$_4$]$^+$, and 273 [rha(OAc)$_3$]$^+$. Acetate of **2**: eims *m/z* 1295 [xyl(-rha)-glc(-glc)-rha(OAc)$_{13}$]$^+$, 849 [glc(-glc)-rha(OAc)$_9$]$^+$, 331 [glc(OAc)$_4$]$^+$, and 273 [rha(OAc)$_3$]$^+$ (2,3).

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