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THREE MONOTERPENES FROM *MUSSAENDA PUBESCENS*

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Key Word Index—*Mussaenda pubescens*: Rubiaceae: monoterpene lactones; mussaenins A, B and C.

Abstract—Three new monoterpenes named mussaenins A, B and C, together with a known monoterpene, argyol, were isolated from the aerial parts of *Mussaenda pubescens* by repeated chromatography on silica gel. Their structures have been elucidated by various spectral methods.

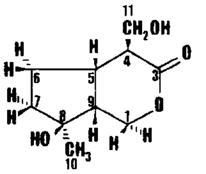
INTRODUCTION

Mussaenda pubescens Ait.f is a liana-like shrub, distributed in shady hillside, valley and shrub jungle of east, south and southwest China. It has been used in Chinese folk medicine as a diuretic, antiphlogistic and antipyretic [1]. It is also used to detoxify mushroom poisons and terminate early pregnancy [2, 3]. In previous papers, we have reported the isolation and structural determination of several saponins and an iridoid glycoside from the plant [4–6]. As a continuation of our studies, lipophilic components of aerial parts of the plant, collected from Guangzhou suburbs, Guangdong Province, were investigated and three new monoterpene lactones, named mussaenin A (**1**), B (**2**) and C (**3**), together with a known monoterpene, argyol (**4**), were isolated by repeated chromatography on a silica gel column. This paper deals with the isolation of structural elucidation of the four monoterpenes.

RESULTS AND DISCUSSION

Mussaenin A (**1**) a gum, $[\alpha]_D^{23}$ $-7.7°$ (acetone, *c* 0.53), showed a quasimolecular ion peak at *m/z* 201 in its EI mass spectrum, analysing for $[M(C_{10}H_{16}O_4) + H]^+$. The IR (KBr) spectrum indicated the presence of hydroxyl (3340 cm^{-1}) and lactone moieties (1735 cm^{-1}). Its ^1H NMR spectrum revealed the signals of one methyl at δ1.35 and two oxygen-bearing methylenes at δ4.15, 4.22 and 4.06, 4.34 (Table 1). In ^{13}C NMR spectrum (Table 2), 10 carbon signals were observed, including two quaternary carbon, three methine, four methylene and one methyl. From the above evidence, **1** is a monoterpenoid. Measurement of its ^1H NMR spectrum in pyridine-d_5 gave better isolated signals than that in $CDCl_3$. Analysing the ^1H–^1H DQF COSY spectrum of **1** resulted in the establishment of its coupled proton systems as $-O-CH_2-CH-CH(-CH-CH_2-O)-CH_2-CH_2-$. According to ^1H and ^{13}C NMR data, the quaternary carbon at δ80.1 ppm was assigned to C-8 in the iridolactone skeleton, with substitution of hydroxyl and methyl group on it, while the carboxyl carbon at δ175.3 ppm was assigned to C-3, which formed a lactone at C-1. Another oxygen-bearing methylene should be derived from C-11.

In the NOE difference spectrum, significant correlation peaks were observed between H-5 and H-9, H-11a, H-11b, H-6β; H-4 and H-1α, H-11α, H-11b; H-10 and H-7α, H-1α, respectively. Thus, the relative stereostructure of **1** was obtained, and the chemical shifts of two different oriented protons in all methylenes were differentiated from the above NOE results. On the basis of ^1H–^{13}C HETCOR experiments, all carbon signals were also assigned unequivocally. Regarding the NOE between H-1α and H-4, the lactone ring was supposed to possess a_1B_4 boat conformation. Similar situations were also found in conformations of isoiridoidmyrmecin and gibboside [7–9].



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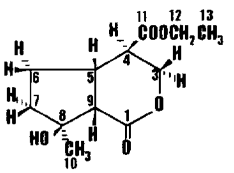

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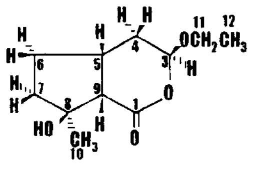

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Table 1. ^{1}H NMR data for monoterpenes **1** (400 MHz, pyridine-d_5), **2** and **3** (300 MHz, $CDCl_3$)

	1		**2**		**3**	
No.	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)
1α	4.06	*dd* (12.4, 11.0)				
1β	4.34	*dd* (11.1, 5.9)				
3α			4.45	*dd* (11.3, 3.7)	5.31	*dd* (2.4, 2.3)
3β			4.20	*m*		
4α	2.71	*ddd* (9.0, 5.1, 3.7)	2.53	*ddd* (10.5, 10.5, 3.7)	1.52	*ddd* (11.1, 10.0, 2.3)
4β					2.10	*m*
5	2.89	*m*	2.83	*m*	2.88	*m*
6α	1.71	*m*	2.15	*m*	1.30	*m*
6β	2.50	*m*	1.53	*m*	2.10	*m*
7α	1.81	*m*	1.89	*m*	1.78	*m*
7β	1.87	*m*	1.83	*m*	1.80	*m*
9	2.63	*ddd* (12.4, 9.4, 5.9)	3.00	*d* (11.5)	2.93	*d* (11.1)
10	1.35	*s*	1.30	*s*	1.26	*s*
11a	4.15	*dd* (11.2, 5.1)			3.60	*m*
11b	4.22	*dd* (11.2, 3.7)			3.88	*m*
12			4.20	*m*	1.18	*t* (7.1)
13			1.26	*t* (7.1)		

Table 2. ^{13}C NMR data for monoterpenes **1** (20 MHz, $CDCl_3$), **2** and **3** (75 MHz, $CDCl_3$)

No.	**1**	**2**	**3**
1	67.2 *t*	172.2 *s*	173.0 *s*
3	175.3 *s*	68.1 *t*	101.7 *d*
4	45.5 *d*	45.6 *d*	34.2 *t*
5	35.4 *d*	36.5 *d*	28.1 *d*
6	29.7 *t*	28.0 *t*	28.5 *t*
7	39.1 *t*	38.7 *t*	39.1 *t*
8	80.1 *s*	80.3 *s*	80.1 *s*
9	49.2 *d*	52.3 *d*	53.2 *d*
10	23.4 *q*	24.9 *q*	25.1 *q*
11	60.9 *t*	170.6 *s*	65.0 *t*
12		61.5 *t*	15.0 *q*
13		14.1 *q*	

In the circular dichroic (CD) spectrum of **1**, a negative Cotton effect was observed at 215 nm and a positive Cotton effect at 260 nm. On the basis of the sector rule of a δ-lactone in CD spectroscopy, the absolute configuration of **1** was deduced to be 4*S*, 5*S*, 8*R* and 9*R*, which accorded with those of natural iridoid compounds. Compound **1** was proved to be a new monoterpene and named mussaenin A.

Mussaenin B (**2**), an oil, $[\alpha]_D^{24}$ + 7.7° ($CHCl_3$, *c* 0.12), exhibited a quasimolecular ion peak at *m*/*z* 243 $[M + H]^+$ in its EI mass spectrum. With a combination of mass spectral and NMR data, its molecular formula was deduced to be $C_{12}H_{18}O_5$. In the ^{13}C NMR spectrum (Table 2), 12 carbon signals, including two methyls, four methylenes, three methines and three quaternary carbons, were observed. Among them, there was one oxygen-bearing quarternary carbon (δ80.3 *s*), two $-OCH_2-$ (δ68.1, *t*, δ61.5 *t*) and two ester carbonyl carbons (δ172.2 *s*, δ170.6 *s*). The ^{1}H NMR data (Table 1) showed the presence of ester ethyl (δ1.26, 3H, *t*; δ4.20, 2H, *m*), oxygen-bearing methyl (δ1.30, *s*) and esterified methylene (δ4.45, 1H, *dd*; δ4.20, 1H, *m*). Thus, **2** is an iridoid. From the ^{1}H–^{1}H DQF COSY spectrum, all coupled proton systems were obtained, and the fragment $-O-CH_2-CH-CH(CH)-CH_2CH_2-$ was deduced. On the basis of ^{1}H NMR evidence and biogenetic consideration, its structure was elucidated as **2**. The relative configuration of **2** was determined from NOESY experiments. The NOEs between H-5 and H-9, H-5 and H-6β, H-6α and H-6β, H-6α and H-7α, H-6β and H-7β, 10-Me and H-7α suggested H-5, H-9 and 8-hydroxyls were all in β configuration. Besides which, in the ^{1}H NMR spectrum, H-4 exhibited as a *ddd* peak with coupling constants of 10.5, 10.5 and 3.7 Hz; and H-3α exhibited as a *dd* peak at δ4.45 ppm with coupling constants of 11.3 and 3.7 Hz. Due to 11.3 Hz should be geminal coupling between H-3β and H-3α, the H-4 should couple with H-3β and H-5 with equal coupling constant (10.5 Hz). According to Drieding model investigation, the dihedral angle between H-4 and H-3β, and between H-4 and H-5 should both be near 180° in a twisted boat conformation to satisfy its *J* value. Thus, the relative configuration was established as in **2**. All proton signals in the ^{1}H NMR spectrum could be assigned according to the above observation. Furthermore, all carbon signals were also ascribed by using a ^{1}H–^{13}C HETCOR experiment. Compound **2** is thus a new monoterpene, named mussaenin B.

Mussaenin C (**3**), an oil, $[\alpha]_D^{24}$ − 9.8° ($CHCl_3$, *c* 0.13), showed a quasimolecular ion peak at *m*/*z* 215 $[M + H]^+$ in its EI mass spectrum. With a combination of mass spectral and NMR data, its molecular formula was deduced to be $C_{11}H_{18}O_4$. Its ^{1}H NMR spectrum (Table 1) revealed the signals from CH_3-C- (δ1.26, *s*), O–CH–O (δ5.31, *dd*) and CH_3CH_2-O (δ1.18, *t*, 3.60, 3.88, *m*). The ^{13}C NMR spectrum of **3** (Table 2) exhibited 11 carbon signals, including two methyls, four methylenes, three methines and two quaternary carbons. Among them, there were –C=O (δ173.0), –C–O (80.1), O–CH–O (δ101.7) and –CH$_2$O (δ65.0) signals. From above evidence, **3** was considered to be a bicyclic monoterpene with an ethoxyl group on it. Analysis of the ^{1}H–^{1}H DQF COSY spectrum led to the establishment of a coupled proton system in structural fragment O–CH–CH$_2$–CH(CH)–CH$_2$–CH$_2$–. On further study of ^{1}H NMR data, along with biogenetic consideration, its structure was elucidated as shown in **3**.

In the NOESY spectrum, NOE correlation peaks were observed between H-5 and H-9; H-5 and H-4β; H-3 and two H-4 signals; 10-Me and H-7α; H-7α and H-6α; H-6β and H-7β. From NOE results in combination with ^{13}C NMR data, H-5 and H-9 should be both in β configuration, and 10-Me in α configuration. Thus, all proton signals in the five-membered ring were assigned. As to H-3 and two protons at C-4, the assignment was made on the following evidence (a) H-3 exhibited as a *dd* peak with *J* values of 2.4 and 2.3 Hz, thus H-3 should be *gauche* to the two H-4 protons. The above deduction was confirmed by the presence of cross peaks between H-3 and the two H-4 proton in the NOESY spectrum. (b) The H-4α proton exhibited as a *ddd* peak with coupling constants of 11.1, 10.0 and 2.3 Hz, in which the first two coupling constants should come from a geminal couple and its couple with H-5, Considering the presence of a large coupling constant, the dihedral angle between H-4α and H-5 should be near 180°. Although the signal of H-4β was basically overlapped with the signal of H-6β, H-4β could be deduced to be *gauche* to H-5 according to the shape of its cross peaks with other protons in the ^{1}H–^{1}H DQF COSY spectrum. Besides which, a cross peak was observed between H-4β and H-5 in the NOESY spectrum. From the above evidence, the H-4α proton should be near *anti* to H-5 and *gauche* to H-3; while the H-4β proton should be *gauche* to both H-3 and H-5. Further study on its Drieding model showed the lactone ring should be in twisted boat conformation, and the ethoxyl group at C-3 should be in β configuration. Since **2** and **3** have similar structures, it is reasonable to assume that they possess the same relative configuration and conformation. All proton and carbon signals of **3** were assigned unambiguously by using ^{1}H–^{1}H DQF COSY, NOESY and ^{1}H–^{13}C HETCOR experiments (Tables 1 and 2). Compound **3** is thus a new monoterpene, named mussaenin C.

Compound **4**, an oil, was identified as argyol on comparison of its ^{1}H and ^{13}C NMR data with literature data [7].

EXPERIMENTAL

Chemical shifts are reported in ppm, with solvents' signals as int. standards.

Plant materials. The aerial parts of *M. pubescens* were collected from Guangzhou suburbs in December 1993. A voucher specimen was identified by Prof. Bangyu Chen of the 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% EtOH (×3) at room temp. After removal of EtOH at 50° at *in vacuo*, H$_2$O was added to give a 1.5 l aq. suspension, which was extracted with EtOAc (4×500 ml). The EtOAc soln was evapd to dryness to give a residue (200 g), and subjected to CC repeatedly on silica gel, eluted with gradient solvents of petrol–Me$_2$CO or CHCl$_3$–Me$_2$CO. Compound **1** (300 mg) was obtained as a gum from the eluent of CHCl$_3$–Me$_2$CO (3:1); **2** (15 mg), **3** (10 mg) and **4** (50 mg) were obtained as oils from the frs of petrol–Me$_2$CO (4:1).

Mussaenin A (**1**). Gum, $[\alpha]_D^{23}$ −7.7° (Me$_2$CO; *c* 0.53). EI-MS *m/z* 201 [M(C$_{10}$H$_{16}$O$_4$ + H]$^+$ IR λ_{max}^{KBr} cm^{-1}: 3340 (OH), 1735 (C=O). CD: $\Delta\varepsilon_{215\ nm}$: −1.35, $\Delta\varepsilon_{260\ nm}$: +0.097 (EtOH, *c* 0.018). ^{1}H NMR (400 MHz, pyridine-*d*$_5$) and ^{13}C NMR (20 MHz, CDCl$_3$) data, δ ppm: see Tables 1 and 2.

Mussaenin B (**2**). Oil, $[\alpha]_D^{24}$ +7.7° (CHCl$_3$, *c* 0.12). EI-MS *m/z* 243 [M(C$_{12}$H$_{18}$O$_5$) + H]$^+$. ^{1}H NMR (300 MHz, CDCl$_3$) and ^{13}C NMR (CDCl$_3$, 75 MHz) data, δ ppm: see Tables 1 and 2.

Mussaenin C (**3**). Oil, $[\alpha]_D^{24}$ −9.8° (CHCl$_3$; *c* 0.13). EI-MS *m/z* 215 [M(C$_{11}$H$_{18}$O$_4$) + H]$^+$. ^{1}H NMR (300 MHz, CDCl$_3$) and ^{13}C NMR (CDCl$_3$, 75 MHz) data, δ ppm: see Tables 1 and 2.

Argyol (**4**). Oil, ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm: 4.23 (1H, *dd*, $J = 11.8$, 5.5 Hz, H-1a), 4.07 (1H, *dd*, $J = 15.0$, 7.3 Hz, H-1b), 2.82 (1H, *m*, H-5), 2.59 (1H, *dd*, $J = 15.0$, 7.0 Hz, H-4a), 2.30 (1H, *dd*, $J = 15.0$, 7.3 Hz, H-4b), 2.24 (1H, *m*, H-9), 1.33 (*s*, H-10). ^{13}C NMR (20 MHz, CDCl$_3$) δ ppm: 173.4 (*s*, C-3), 81.0 (*s*, C-8), 67.3 (*t*, C-1), 48.2 (*d*, C-9), 39.9 (*t*, C-7), 34.8 (*t*, C-4), 33.9 (*d*, C-5), 30.9 (*t*, C-6), 24.2 (*q*, C-10).

REFERENCES

1. Jiangsu New Medical College (1986) *Dictionary of Chinese Traditional Medicine*, p. 176. Shanghai Science and Technology Press.
2. Liu, X. J., Liang, G. J., Cai, X., Chao, Q., Chu, Y. H., Bao, Y. M., Long, X. H. and Wang, G. Q. (1986) *Acta Acad. Med. Shanghai* **13**, 273.
3. Fujian Institute of Medicine (1979) *Encyclopedia of Fujian Plant Medicines*, Vol. 1, p. 447. Fujian People's Press.
4. Xu, J. P., Xu, R. S., Luo, Z. and Wu, H. M. (1992) *J. Nat. Prod.* **55**, 1124.
5. Zhao, W. M., Xu, J. P., Qin, G. W. and Xu, R. S. (1995) *Phytochemistry* **39**, 191.
6. Zhao, W. M., Xu, J. P:, Qin, G. W., Xu, R. S., Wu, H. M. and Weng, G. H. (1994) *J. Nat. Prod.* **57**, 1613.
7. Sisido, K., Inomata, K., Kageyema, T. and Utimoto, K. (1968) *J. Org. Chem.* **33**, 3149.
8. Uesato, S., Shan, X., Inouye, H., Shingu, T., Inoue, M. and Doi, M. (1987) *Phytochemistry* **26**, 561.
9. McConnell, J. F., Mathieson, A. M. and Schoenborn, B. P. (1987) *Tetrahedron Letters* **28**, 4699.
10. Bianco, A., Passacantilli, P., Garbarino, J. A., Gambaro, V., Serafini, M., Nicoletti, N., Rispolic, C. and Right, G. (1991) *Planta Medi.* **57**, 286.