Three New Monoterpene Alkaloids and a New Caffeic Acid Ester from *Incarvillea mairei* var. *multifoliolata*

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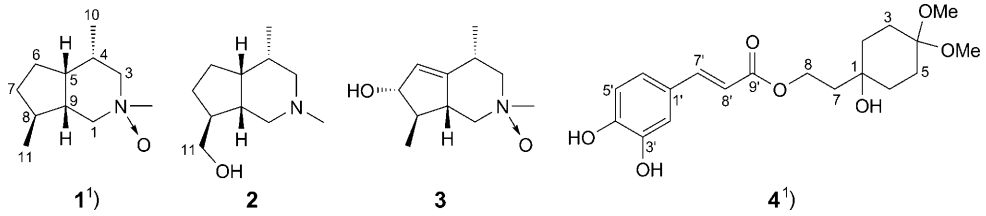
Three new monoterpene alkaloids, mairine A (**1**), mairine B (**2**), and mairine C (**3**), and a new caffeic acid ester, 2-(1-hydroxy-4,4-dimethoxycyclohexyl)ethyl caffeate (**4**), were isolated from the EtOH extract of the whole plants of *Incarvillea mairei* var. *multifoliolata*. The structures of these compounds were established on the basis of 1D- and 2D-NMR and HR-ESI-MS analysis.

Introduction. – *Incarvillea mairei* var. *multifoliolata* C. Y. WU et W. C. YIN (Bignoniaceae) is mainly distributed in Yunnan and Sichuan provinces of China [1][2]. Previous phytochemical studies on *Incarvillea* species have afforded alkaloids and caffeic acid esters [3][4]. This species was found to be rich of actinidine-type monoterpene alkaloids especially, which possess significant antinociceptive activity [5–8]. Further chemical investigation for more bioactive constituents from this plant led to the isolation of three new monoterpene alkaloids, mairine A[1]) (**1**), mairine B[1]) (**2**), and mairine C[1]) (**3**), and a new caffeic acid ester, 2-(1-hydroxy-4,4-dimethoxycyclohexyl)ethyl caffeate[1]) (**4**) (*Fig. 1*). Herein, we describe the isolation and the structural elucidation of compounds **1**–**4** from *Incarvillea mairei* var. *multifoliolata*.



1[1]) **2** **3** **4**[1])

Fig. 1. *Compounds* **1**–**4** *isolated from* Incarvillea mairei *var.* multifoliolata

Results and Discussion. – Compound **1** was isolated as a yellow oil. The molecular formula was determined as $C_{11}H_{21}NO$ from the positive-ion-mode HR-ESI-MS (*m*/*z* 184.1667 ($[M+H]^+$)). Comparison of its ^{1}H- and ^{13}C-NMR data (*Table 1*) with those of the known compounds *δ*-skytanthine and *α*-skytanthine (=(4*S*,4a*R*,7*S*,7a*R*)- and (4*R*,4a*R*,7*S*,7a*R*)-octahydro-2,4,7-trimethyl-1*H*-cyclopenta[*c*]pyridine, resp.) [9][10]

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

Table 1. *^1H-* (600 MHz) *and ^{13}C-NMR* (125 MHz) *Data of* **1**–**3**[1]). δ in ppm, J in Hz.

	1[a])		**2**[b])		**3**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	3.04 (*dd*, *J* = 12.0, 6.0), 2.91 (*t*, *J* = 12.0)	66.0	2.56 (*dd*, *J* = 11.4, 6.0), 1.63 (*t*, *J* = 11.4)	57.5	3.57 (*dd*, *J* = 11.4, 6.0), 3.07 (*t*, *J* = 11.4)	71.6
CH_2(3)	3.07 (*t*, *J* = 12.0), 2.94 (*dd*, *J* = 12.0, 4.2)	67.3	2.49 (*dd*, *J* = 11.4, 3.0), 1.68 (*t*, *J* = 11.4)	58.0	3.38 (*dd*, *J* = 11.4, 4.2), 3.05 (*t*, *J* = 11.4)	72.1
CH(4)	2.68–2.75 (*m*)	27.2	2.04–2.10 (*m*)	30.8	2.97–3.03 (*m*)	28.8
CH(5) or C(5)	2.19–2.24 (*m*)	40.1	1.90–1.95 (*m*)	40.9		145.5
CH_2(6) or H–C(6)	1.63–1.68 (*m*), 1.48–1.56 (*m*)	22.9	1.43–1.49 (*m*)	22.0	5.52–5.53 (br. *s*)	127.1
CH_2(7) or H–C(7)	2.01–2.07 (*m*), 1.21–1.27 (*m*)	32.3	1.82–1.87 (*m*), 1.14–1.21 (*m*)	25.9	4.38–4.40 (*m*)	84.4
CH(8)	1.73–1.76 (*m*)	36.9	1.69–1.72 (*m*)	45.1	1.64–1.68 (*m*)	48.3
CH(9)	2.32–2.36 (*m*)	43.7	2.04–2.10 (*m*)	41.3	2.83–2.87 (*m*)	47.0
Me(10)	0.95 (*d*, *J* = 6.6)	16.5	0.84 (*d*, *J* = 7.2)	17.5	1.15 (*d*, *J* = 6.6)	14.3
Me(11) or CH_2(11)	1.04 (*d*, *J* = 7.2)	22.8	3.36–3.42 (*m*)	66.5	1.20 (*d*, *J* = 7.2)	17.2
MeN	3.15 (*s*)	60.7	2.21 (*s*)	46.2	3.27 (*s*)	60.5

[a]) Measured in CD_3OD. [b]) Measured in $CDCl_3$.

suggested that **1** has the same constitution as skytanthine. The ^{1}H-NMR spectrum of **1** exhibited two Me *d* at δ(H) 0.95 ($J = 6.6$ Hz) and 1.04 ($J = 7.2$ Hz), indicating the presence of two Me–CH moieties, as well as a Me *s* at δ(H) 3.15. ^{13}C-NMR and DEPT Spectra showed eleven C-atom signals, including three Me, four CH_2 (sp^3), and four CH groups (sp^3). These data implied that **1** is an *N*-methylated monoterpenoid alkaloid. From the DQF-COSY experiment, two fragments (*a*: C(3)–C(4)(Me)–C(5)–C(6)–C(7)–C(8)–Me; *b*: C(1)–C(9)) could be established (*Fig. 2*). The key HMBCs H–C(5)/C(9), Me(11)/C(9), MeN/C(1), and MeN/C(3) suggested that **1** has the same skeleton as δ-skytanthine. In analogy to δ-skytanthine, Me (δ(H) 3.15 and δ(C) 60.7), CH_2(1) (δ(H) 3.04 and 2.91 and δ(C) 66.0), and CH_2(3) (δ(H) 3.07 and 2.94 and δ(C) 67.3) were ascribed to groups linked to an N-atom, although their signals were shifted downfield with respect to δ-skytanthine [9][10], due to the *N*-oxide moiety in **1**. The relative configuration of **1** was determined by NOESY correlations. The NOESY correlations H–C(5)/H–C(4), H–C(9), and Me(11), and H–C(9)/Me(11) indicated that H–C(5), H–C(9), and Me(11) are β-oriented, while Me(10) is on the opposite face of the molecule (*Fig. 3*). Thus, compound **1** was assigned the structure of δ-skytanthine *N*-oxide, and named mairine A.

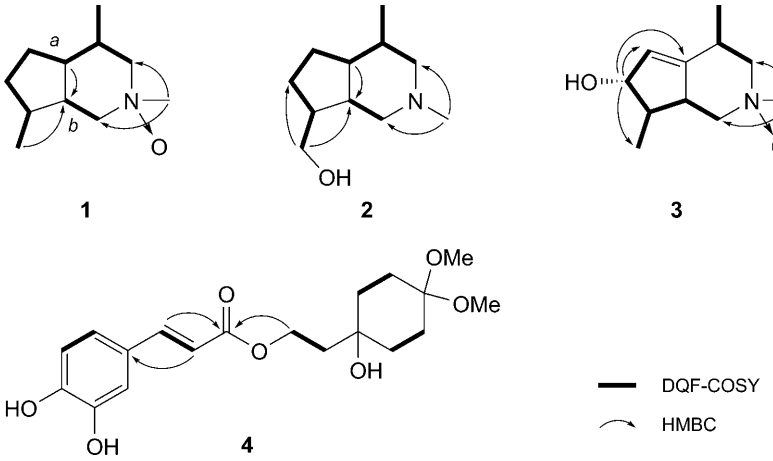


Fig. 2. *Selected 2D-NMR correlations of* **1**–**4**

Compound **2** was obtained as a yellow oil. The molecular formula was also determined as $C_{11}H_{21}NO$ from the positive-ion-mode HR-ESI-MS (*m*/*z* 184.1675 ($[M+H]^+$)). Comparison of the ^{13}C-NMR data of **2** (*Table 1*) with those of **1** revealed only a minor difference, namely a CH_2OH group in **2** instead of a Me group in **1**. The ^{1}H-NMR spectrum of **2** (*Table 1*) exhibited a Me *d* at δ(H) 0.84 ($J = 7.2$ Hz) and a Me *s* at δ(H) 2.21, indicating the presence of a Me–CH moiety and a MeN group. A CH_2 *m* at δ(H) 3.36–3.42 was ascribed to a CH_2OH group. ^{13}C-NMR and DEPT Spectra showed eleven C-atom signals, including two Me, five CH_2 (sp^3), and four CH groups (sp^3). These data implied that **2** is a derivative of δ-skytanthine. The HMBCs between CH_2(11) (δ(H) 3.36–3.42) and C(7) (δ(C) 25.9), C(8) (δ(C) 45.1), and C(9) (δ(C)

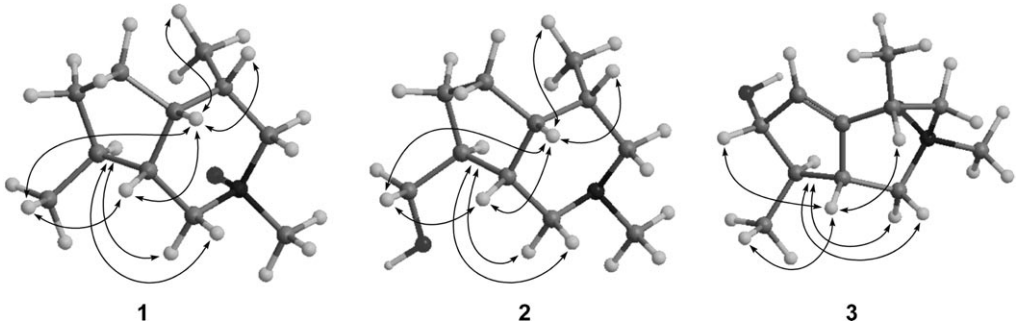


Fig. 3. *Key NOESY correlations of* **1**–**3**

41.3) implied that **2** had the constitution of a δ-skytanthin-11-ol (*Fig. 2*). This was confirmed by extensive 2D-NMR experiments, including DQF-COSY, HSQC, and HMBC (*Fig. 2*). The relative configuration of **2** was determined by the NOESY correlations H–C(5)/H–C(4), H–C(5)/H–C(9), H–C(5)/CH_2(11), and H–C(9)/CH_2(11) (*Fig. 3*). Thus, compound **2** was deduced as δ-skytanthin-11-ol, and named mairine B.

Compound **3** was obtained as a yellow oil. The molecular formula was determined as $C_{11}H_{19}NO_2$ from the positive-ion-mode HR-ESI-MS (*m/z* 198.1496 ($[M+H]^+$)). Comparison of the ^{1}H- and ^{13}C-NMR data of **3** (*Table 1*) with those of 5,6-didehydroskytanthine [11] suggested that **3** has a similar molecular skeleton as the latter. The ^{1}H-NMR spectrum of **3** exhibited an olefinic H-atom at δ(H) 5.52–5.53 (br. *s*) and two Me *d* at δ(H) 1.15 ($J=6.6$ Hz) and 1.20 ($J=7.2$ Hz), indicating the presence of two Me–CH moieties, as well as a Me *s* at δ(H) 3.27. ^{13}C-NMR and DEPT Spectra showed eleven C-atom signals, including three Me, two CH_2 (sp^3), one CH (sp^2), and three CH groups (sp^3), and a quaternary C-atom (sp^2). These data implied that **3** is the olefinic derivative of a known monoterpenoid alkaloid. Compared to **1**, the downfield chemical shifts of CH_2(1) (δ(H) 3.57 (*dd*, $J=11.4$, 6.0 Hz) and 3.07 (*t*, $J=11.4$ Hz) and δ(C) 71.6), CH_2(3) (δ(H) 3.38 (*dd*, $J=11.4$, 4.2 Hz) and 3.05 (*t*, $J=11.4$ Hz) and δ(C) 72.1), and a Me (δ(H) 3.27 (*s*)) implied the presence of an *N*-oxide. The CH at δ(H) 4.38–4.40 (*m*) and δ(C) 84.4 suggested that a OH group was attached to C(7). This inference was confirmed by the HMBCs MeN/C(1) and C(3), and H–C(7)/C(5), C(6), and C(11). The suggested constitution of **3** (5,6-didehydro-7-hydroxyskytanthine *N*-oxide) was confirmed by extensive 2D-NMR experiments, including DQF-COSY, HSQC, and HMBC (*Fig. 2*). The relative configuration of **3** was determined by the NOESY correlations H–C(4)/H–C(9), H–C(7)/H–C(9), and H–C(9)/Me(11) (*Fig. 3*). Thus, compound **3** was deduced as (7α)-5,6-didehydro-δ-skytanthin-7-ol *N*-oxide, and named mairine C.

Compound **4** was isolated as a yellow oil. The molecular formula was determined as $C_{19}H_{26}O_7$ from the negative-ion-mode HR-ESI-MS (*m/z* 365.1576 ($[M-H]^-$)). Comparison of the ^{1}H- and ^{13}C-NMR data of **4** (*Table 2*) with those of (+)-2-(1-hydroxy-4-oxocyclohexyl)ethyl caffeate [4] suggested that **4** is a derivative of cyclohexylethyl caffeate (caffeic acid = (2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid). From the 1D-NMR spectra, two MeO and six CH_2 groups (sp^3), two sp^3 quaternary C-

atoms, five sp^2 CH groups, and four sp^2 quaternary C-atoms were deduced. Among them, five sp^2 CH (δ(H) 7.02 and δ(C) 115.1, δ(H) 6.77 and δ(C) 116.5, δ(H) 6.93 and δ(C) 122.9, δ(H) 7.52 and δ(C) 146.8, and δ(H) 6.23 and δ(C) 115.2), and four sp^2 quaternary C-atoms (δ(C) 127.7, 146.8, 149.6, and 169.3) were ascribed to those of a caffeoyl moiety. The six sp^3 CH_2 (δ(H) 1.54–1.63 and δ(C) 34.8, δ(H) 1.72–1.79 and δ(C) 29.0, δ(H) 1.72–1.79 and δ(C) 29.0, δ(H) 1.54–1.63 and δ(C) 34.8, δ(H) 1.85 (*t*, $J = 6.6$ Hz) and δ(C) 41.9, and δ(H) 4.32 (*t*, $J = 6.6$ Hz) and δ(C) 61.9), two sp^3 quaternary C-atoms (δ(C) 70.6 and 101.2), and two MeO (δ(H) 3.18 and 3.15 (2*s*) and δ(C) 47.9 and 48.0) suggested the presence of a 2-(1-hydroxy-4,4-dimethoxycyclohexyl)ethyl moiety. The above deductions were further confirmed by extensive 2D-NMR experiments, including DQF-COSY, HSQC, and HMBC (*Fig. 2*). The HMBC CH_2(8) (δ(H) 4.32)/C(9′) (δ(C) 169.3) implied that **4** was 2-(1-hydroxy-4,4-dimethoxycyclohexyl)ethyl caffeate.

Table 2. *^{1}H- and ^{13}C-NMR* (600 and 125 MHz, resp., CD_3OD) *Data of* **4**[1]). δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
C(1)		70.6	C(1′)		127.7
CH_2(2)	1.54–1.63 (*m*)	34.8	CH(2′)	7.02 (*d*, $J = 1.8$)	115.1
CH_2(3)	1.72–1.79 (*m*)	29.0	C(3′)		146.8
C(4)		101.2	C(4′)		149.6
CH_2(5)	1.72–1.79 (*m*)	29.0	CH(5′)	6.77 (*d*, $J = 7.8$)	116.5
CH_2(6)	1.54–1.63 (*m*)	34.8	CH(6′)	6.93 (*dd*, $J = 7.8$, 1.8)	122.9
CH_2(7)	1.85 (*t*, $J = 6.6$)	41.9	CH(7′)	7.52 (*d*, $J = 15.6$)	146.8
CH_2(8)	4.32 (*t*, $J = 6.6$)	61.9	CH(8′)	6.23 (*d*, $J = 15.6$)	115.2
*Me*O–C(4)	3.18 (*s*)	47.9	C(9′)		169.3
*Me*O–C(4)	3.15 (*s*)	48.0			

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Experimental Part

General. TLC: *$HSGF_{254}$* silica gel plates (SiO_2; 10–40 μm, *Yantai Chemical Industrial Institute*, P. R. China); detection by means of a UV (254, 365 nm) lamp and *Dragendroff* spray reagent. Column chromatography (CC): SiO_2 (200–300 mesh; *Yantai Chemical Industrial Institute*, P. R. China), SiO_2 *H* (10–40 μm, *Qingdao Haiyang Chemical Group Corporation*, P. R. China), and *Sephadex LH-20* (*Pharmacia Co., Ltd.*). Optical rotations: *Perkin-Elmer-341* polarimeter. IR Spectra: *Bruker Vector 22* instrument; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-Avance-II-600* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS: *Varian-MAT-212* mass spectrometer; in m/z (rel. %). HR-MS: *Q-Tof-micro-YA019* mass spectrometer; in m/z (rel. %).

Plant Material. The whole plants of *I. mairei* var. *multifoliolata* were collected in Yunnan Province, P. R. China, in August 2007, and identified by Prof. *Han-Chen Zheng*. A voucher specimen (No. 20070801) was deposited with the Herbarium of the School of Pharmacy, Second Military Medical University.

Extraction and Isolation. The air-dried whole plants (21 kg) of *I. mairei* var. *multifoliolata* were powered and extracted with 80% EtOH three times under reflux. After evaporation of the EtOH, the extract was partitioned between $CHCl_3$ and 0.001N HCl. The aq. soln. was adjusted to pH 11 with 20% NaOH soln., followed by exhaustive extraction with petroleum ether, AcOEt, and BuOH. $CHCl_3$-Soluble materials were separated by CC (SiO_2, petroleum ether/AcOEt 10:1 → 1:1, then $CHCl_3$/MeOH 10:1 → 0:1): *Fractions T1 – T9*. *Fr. T5* was subjected to CC (*MCI* column, 90% → 100% aq. MeOH), in which a fraction that eluted with 90% MeOH was repeatedly purified by CC (SiO_2 and *Sephadex LH-20*): **1** (12 mg), **2** (150 mg), and **3** (27 mg). The AcOEt extract was subjected to CC (SiO_2 (200–300 mesh), petroleum ether/AcOEt 30:0, 30:1, 20:1, 10:1, 5:1, 2:1, and 0:1): *Frs. E1 – E7. Fr. E4* was purified repeatedly by CC (SiO_2 and *Sephadex LH-20*): **4** (5 mg).

Mairine A (= rel-*(4*R*,4a*S*,7*R*,7a*S*)-Octahydro-2,4,7-trimethyl-1*H*-cyclopenta[*c*]pyridine 2-Oxide*; **1**): Yellow oil. $[\alpha]_D^{23} = -12$ ($c = 0.71$, MeOH). IR (KBr): 3384, 2955, 2875, 1697, 1455, 1389. ^{1}H- and ^{13}C-NMR: *Table* 1. ESI-MS: 184.2 ($[M+H]^+$), 206.1 ($[M+Na]^+$). TOF-ESI-MS (pos.): 184.1667 ($[M+H]^+$, $C_{11}H_{22}NO^+$; calc. 184.1696).

Mairine B (= rel-*(4*R*,4a*S*,7*R*,7a*R*)-Octahydro-2,4-dimethyl-1*H*-cyclopenta[*c*]pyridine-7-methanol*; **2**): Yellow oil. $[\alpha]_D^{23} = -5$ ($c = 0.81$, MeOH). IR (KBr): 3332, 2950, 2872, 2793, 1506, 1463, 1388. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 184.3 ($[M+H]^+$), 206.2 ($[M+Na]^+$). TOF-ESI-MS (pos.): 184.1675 ($[M+H]^+$, $C_{11}H_{22}NO^+$; calc. 184.1696).

Mairine C (= rel-*(4*R*,6*R*,7*S*,7a*S*)-2,3,4,6,7,7a-Hexahydro-2,4,7-trimethyl-1*H*-cyclopenta[*c*]pyridin-6-ol 2-Oxide*; **3**): Yellow oil. $[\alpha]_D^{23} = +21$ ($c = 0.75$, MeOH). IR (KBr): 3420, 2959, 2929, 2873, 1457, 1418, 1048. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 198.1 ($[M+H]^+$), 220.1 ($[M+Na]^+$). TOF-ESI-MS (pos.): 198.1496 ($[M+H]^+$, $C_{11}H_{20}NO_2^+$; calc. 198.1489).

2-(1-Hydroxy-4,4-dimethoxycyclohexyl)ethyl Caffeate (=*2-(1-Hydroxy-4,4-dimethoxycyclohexyl)-ethyl (2*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoate*; **4**): Yellow oil. $[\alpha]_D^{23} = -16$ ($c = 0.57$, MeOH). IR (KBr): 3503, 3421, 2956, 2924, 2853, 1653, 1384, 1271, 1176, 1116. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 350.4 ($[M+H]^+$), 372.3 ($[M+Na]^+$). TOF-ESI-MS (neg.): 365.1576 ($[M-H]^-$, $C_{19}H_{25}O_7^-$; calc. 365.1606).

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