Two New Alkaloids from *Incarvillea mairei* var. *grandiflora*

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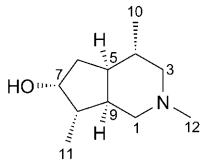
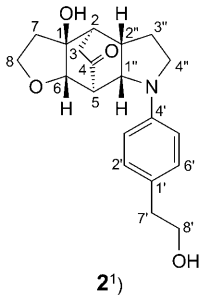
Two new alkaloids, isoincarvilline (**1**) and incargranine A (**2**), together with two known ones, were isolated from the 80% EtOH extract of the whole plant of *Incarvillea mairei* var. *grandiflora*. Their structures were identified on the basis of their spectroscopic analysis.

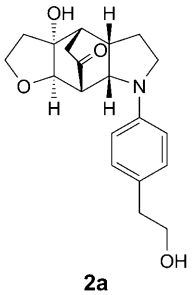
Introduction. – *Incarvillea mairei* var. *grandiflora* (WEHRHAHN) GRIERSON (Bignoniaceae), a beautiful mountain flower, is mainly distributed in the mountains of Yunnan, Sichuan, and Qinghai provinces [1][2]. The investigations on *Incarvillea* species have let to the isolation of many novel actinidine-type monoterpenoid alkaloids with strong antinociceptive activity [3–14]. Although no evidences were found for the use of the title plant in traditional Chinese medicine, the structural diverse and novel alkaloids from *Invarvillea* species still attracted our interest and decided us to perform the pharmacological and phytochemical investigations of *I. mairei* var. *grandiflora*. In our investigation of the components from this plant, two new alkaloids, isoincarvilline[1]) (**1**) and incargranine A[1]) (**2**), together with two known ones, were isolated from the 80% EtOH extract of the whole plant of *I. mairei* var. *grandiflora*. Their structures were elucidated on the basis of their spectroscopic analysis.



1[1])



2[1])



2a

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

Results and Discussion. – The $CHCl_3$ fraction of the 80% EtOH extract of *I. mairei* var. *grandiflora* was purified by repeated column chromatography to afford four compounds. On the basis of physical and spectroscopic analysis, including 2D-NMR techniques (HMQC, HMBC, and NOESY), the structures of two new compounds were determined and named isoincarvilline[1]) (**1**) and incargranine A[1]) (**2**), and two known alkaloids were deduced to be β-skytanthine (=(4*S*,4a*R*,7*S*,7a*S*)-octahydro-2,4,7-trimethyl-1*H*-cyclopenta[*c*]pyridine) [15] and incarvine C (=(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid (4*S*,4a*R*,6*S*,7*R*,7a*S*)-octahydro-2,4,7-trimethyl-1*H*-cyclopenta[*c*]pyridin-6-yl ester) [10] by comparing their spectroscopic data with those reported in the literature.

Compound **1** was isolated as colorless needles which showed a positive reaction to the *Dragendorff* reagent, indicating that **1** was an alkaloid. The molecular formula of **1** was determined to be $C_{11}H_{21}NO$ by the quasimolecular-ion peak $[M+H]^+$ at *m/z* 184.1700 in the HR-ESI-MS (positive mode). Comparison of its ^{1}H- and ^{13}C-NMR data (*Table*) with those of the known β-skytanthine [15] and incarvilline (=(4*R*,4a*S*,6*R*,7*S*,7a*R*)-octahydro-2,4,7-trimethyl-1*H*-cyclopenta[*c*]pyridin-6-ol) [9] suggested that **1** has the same planar structure as the latter, consistent with the HMBC and COSY (*Fig. 1*). An X-ray diffraction analysis of **1** (*Fig. 2*) was performed to establish the relative α-configurations of H–C(5), H–C(9), Me(10), Me(11), and OH–C(7). Consequently, the structure of isoincarvilline (**1**) was determined.

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1**[1]) *and* **2**[1]). δ in ppm, *J* in Hz.

1			**2**					
	δ(H)[a])	δ(C)[b])		δ(H)[c])	δ(C)[d])		δ(H)[c])	δ(C)[d])
H_α–C(1)	2.77 (*d*, *J* = 12.0)	55.3 (*t*)	C(1)		81.7 (*s*)	H–C(5′)	6.62 (*d*, *J* = 8.4)	115.5 (*d*)
H_β–C(1)	2.11 (*dd*, *J* = 3.6, 12.0)		H–C(2)	2.37–2.39 (*m*)	42.7 (*d*)	H–C(6′)	7.05 (*d*, *J* = 8.4)	130.6 (*d*)
H_α–C(3)	2.68 (*d*, *J* = 10.2)	63.2 (*t*)	H_a–C(3)	2.49 (*dd*, *J* = 3.3, 18.9)	36.7 (*t*)	CH_2(7′)	2.70 (*t*, *J* = 7.2)	39.4 (*t*)
H_β–C(3)	1.41–1.47 (overlap)		H_b–C(3)	2.31 (*dd*, *J* = 3.1, 18.9)		CH_2(8′)	3.67 (*t*, *J* = 7.2)	64.6 (*t*)
H–C(4)	1.31–1.34 (*m*)	34.3 (*d*)	C(4)		214.1 (*s*)	H–C(1″)	4.06 (*dd*, *J* = 3.0, 8.6)	60.1 (*d*)
H–C(5)	1.41–1.47 (overlap)	42.0 (*d*)	H–C(5)	3.21 (*dd*, *J* = 3.6, 3.0)	53.9 (*d*)	H–C(2″)	3.34–3.36 (*m*)	36.1 (*d*)
H_α–C(6)	1.68 (*dd*, *J* = 8.4, 14.4)	39.3 (*t*)	H–C(6)	3.83 (*d*, *J* = 3.6)	86.7 (*d*)	H_a–C(3″)	2.15–2.21 (*m*)	27.4 (*t*)
H_β–C(6)	1.86 (*dd*, *J* = 6.0, 14.4)		CH_2(7)	1.97–2.00 (*m*)	39.6 (*t*)	H_b–C(3″)	1.92–1.95 (*m*)	
H–C(7)	4.12–4.14 (*m*)	74.6 (*d*)	H_a–C(8)	3.89–3.95 (*m*)	68.2 (*t*)	H_a–C(4″)	3.40–3.48 (*m*)	51.2 (*t*)
H–C(8)	1.99–2.05 (*m*)	38.7 (*d*)	H_b–C(8)	3.78–3.83 (*m*)		H_b–C(4″)	3.13–3.18 (*m*)	
H–C(9)	1.73 (*t*, *J* = 5.4)	43.5 (*d*)	C(1′)		129.1 (*s*)			
Me(10)	0.76 (*d*, *J* = 6.6)	17.7 (*q*)	H–C(2′)	7.05 (*d*, *J* = 8.4)	130.6 (*d*)			
Me(11)	0.93 (*d*, *J* = 6.0)	11.8 (*q*)	H–C(3′)	6.61 (*d*, *J* = 8.4)	115.5 (*d*)			
MeN	2.18 (*s*)	46.7 (*q*)	C(4′)		146.8 (*s*)			

[a]) At 600 MHz in $CDCl_3$. [b]) At 150 MHz in $CDCl_3$. [c]) At 600 MHz in CD_3OD. [d]) At 150 MHz in CD_3OD.

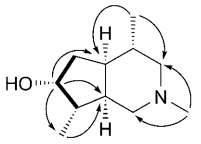


Fig. 1. *Key ^{1}H,^{1}H-COSY and HMBC of compound* **1**

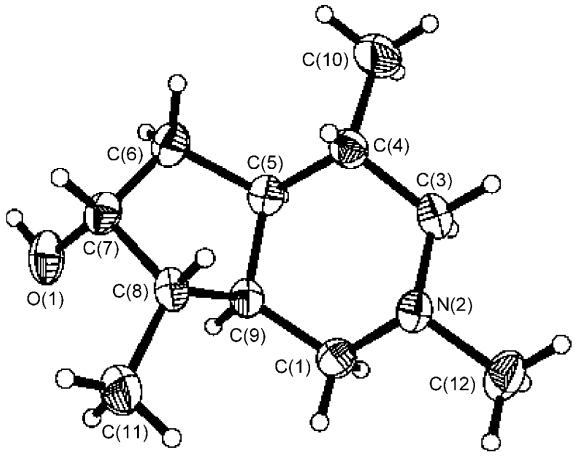


Fig. 2. *X-Ray crystallographic structure of* **1**[1]). The crystallographic data of **1** have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication number CCDC-691814. Copies of the data can be obtained, free of charge, *via* http://www.ccdc.cam.ac.uk/data_request/cif.

The ^{1}H-NMR spectrum of **1** showed two Me *d* at δ(H) 0.93 ($J = 6.0$ Hz) and 0.76 ($J = 6.6$ Hz), indicating the presences of two MeCH moieties. A *s* at δ(H) 2.18 was ascribed to an MeN group. The ^{13}C-NMR spectrum exhibited resonances for 11 C-atoms (one MeN, two Me, three CH_2 (sp^3), five CH (sp^3)). These data suggested that **1** was the derivative of a monoterpenoid alkaloid, and shared the same skeleton as β-skytanthine [15]. In the HMBC plot, the H-atoms of MeN were correlated to C(1), and C(3) (*Fig. 1*). The Me groups at δ(H) 0.76 (Me(10) and 0.93 (Me(11)) showed long-range correlations with C(3), C(4), and (5), and with C(7), C(8), and C(9), respectively. An oxygenated H-atom resonance at δ(H) 4.12–4.14 exhibited COSY cross-peaks with the signals of H–C(6) and H–C(8), implying that an OH group may be attached at C(7) (*Fig. 2*). This was further confirmed by the HMBC of H–C(7) at δ(H) 4.12–4.14 with C(5), C(9), and C(11). Compound **1** was then determined to have the same planar structure as the known compound incarvilline [9]. Although the NOESY correlations H–C(5)/H–C(9), Me(11)/H–C(9), and H–C(9)/Me(10) were observed, it was still difficult to determine the relative configuration. The X-ray diffraction analysis confirmed the suggested relative configuration (*Fig. 2*).

Compound **2**, a light yellow oil, showed a positive reaction to the *Dragendorff* reagent. The ESI-MS (positive mode) gave a quasimolecular-ion peak $[M + Na]^+$ at *m*/*z* 366.3. Combined with the ^{1}H- and ^{13}C-NMR data (*Table*), its molecular formula was deduced to be $C_{20}H_{25}NO_4$ with 9 degrees of unsaturation. The HR-ESI-MS (negative mode; *m*/*z* 378.1469 ($[M + Cl]^-$)) further confirmed the above deduction. The NMR data of **2** were quite close to those of the previously reported biotransformation

product **2a** [16], implying that their structures may be very similar. The ^{1}H,H-COSY (*Fig. 3*), HMBC (*Fig. 3*) and NOESY data (*Fig. 4*) of **2** confirmed that incargranine A (**2**) is a stereoisomer of **2a**.

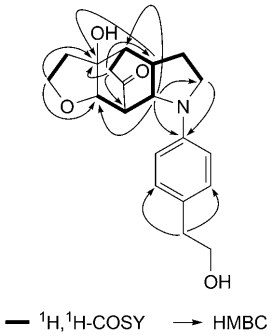


Fig. 3. *Key ^{1}H,^{1}H-COSY and HMBC of compound* **2**

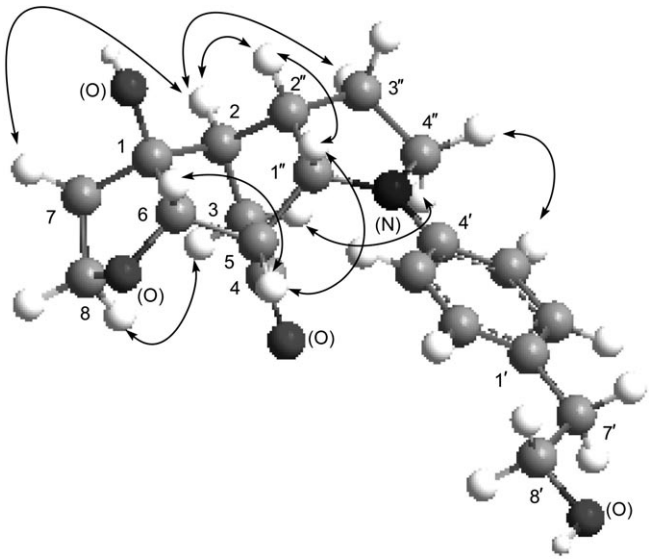


Fig. 4. *Key NOESY correlations of Compound* **2**

The ^{1}H-NMR of **2** exhibited two *d* at δ(H) 7.05 (J = 8.4 Hz, 2 H) and 6.61 (J = 8.4 Hz, 2 H), suggesting the presence of a 4-substituted phenyl group. Two mutually coupling CH_2 at δ(H) 2.70 (t, J = 7.2 Hz) and 3.67 (t, J = 7.2 Hz) indicated a hydroxyethyl moiety. The HMBC of the CH_2 H-atoms at δ(H) 2.70 with C(1′), C(2′), and C(6′) of the aryl group were consistent with a 4-(hydroxyethyl)phenyl group. The ^{13}C-NMR displayed resonances for twenty C-atoms, including a C=O at δ(C) 214.1. Beside the aryl and C=O group, the remaining 4 degrees of unsaturation indicated a four-ring structural moiety. The ^{1}H,^{1}H-COSY plot showed the following correlations: CH_2(8)/CH_2(7), CH_2(3)/H−C(2)/H−C(2″)/H−C(1″)/H−C(5)/H−C(6), and H−C(2″)/CH_2(3″)/CH_2(4″) (*Fig. 3*). Moreover, the HMBCs CH_2(8)/C(1), and C(6), CH_2(7)/C(2) and C(6), H−C(6)/C(2), C(4), C(7), C(8), and C(1″), H−C(1″)/C(2),

C(4), C(6), C(3″), C(4′), and C(4″), H–C(2″)/C(1), C(3), C(5), and C(4″), and CH_2(4″)/C(1″) and C(4′), indicated that **2** has the same planar structure as **2a**. In the NOESY plot of **2**, we observed the correlations H–C(2)/H–C(2″) and CH_2(3″), and H–C(5)/H–C(1″) (*Fig. 4*), indicating that the ethano bridge C(3)–C(4) was α-orientated and the bridgehead H–C(2) and H–C(5) β-orientated. Additionally, the relative β-configurations of H–C(6) and OH–C(1) were deduced from the NOESY correlations H–C(6)/H–C(5) and H–C(1″), and CH_2(7)/CH_2(3).

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh), *H60* (*Qingdao Marine Chemical Plant*, Qingdao, P. R. China); *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA). TLC: pre-coated SiO_2 GF_{254} plates (*Qingdao Marine Chemical Plant*, Qingdao, P. R. China). Optical rotation: *Perkin-Elmer-341* digital polarimeter (*Perkin-Elmer*, Norwalk, CT, USA); at 589 nm. IR: *Bruker-Vector-22* spectrophotometer; KBr pellets; in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Bruker-DRX-600* spectrometer; in $CDCl_3$; δ in ppm rel. to Me_4Si, J in Hz. MS: *Agilent-1100-LC/MSD-Trap* (ESI-MS) and *Agilent Micro-Q-Tof* (HR-ESI-MS) spectrometer; in m/z.

Plant Material. The whole plants of *I. mairei* var. *grandiflora* were collected in Zhongdian County, Yunnan Province, in late October 2006, and authenticated by Prof. *Li-Shan Xie* of the Kunming Institute of Botany, the Chinese Academy of Sciences. A voucher specimen (No. 2006101020) is deposited with the School of Pharmacy, Second Military Medical University.

Extraction and Isolation. The dried whole plants of *I. mairei* var. *grandiflora* (32.5 kg) were extracted 3 times with 80% EtOH under reflux. The EtOH extract was concentrated and the residue dissolved in 2% HCl soln. and filtered. The filtrate was adjusted to pH 9–10 by adding NH_4OH and then extracted with $CHCl_3$. The $CHCl_3$ fraction (120 g) was subjected to CC (SiO_2, petroleum ether/AcOEt (100:1 → 5:1): *Fractions 1–5*. *Fr. 3* was purified by repeated CC (SiO_2; *Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **1** (8 mg) and β-skytanthine (9 mg). *Fr. 5* ($CHCl_3$/MeOH 1:2) was purified by a similar procedure: **2** (7 mg) and incarvine C (8 mg).

Isoincarvilline (= rel-*(4*R*,4a*R*,6*S*,7*R*,7a*S*)-Octahydro-2,4,7-trimethyl-1*H*-cyclopenta[*c*]pyridin-6-ol*; **1**): Colorless needles. $[\alpha]_D^{22} = -77.3$ ($c = 0.2335$, $CHCl_3$). IR (KBr): 3215, 2962, 1460, 1376. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS: 184 ($[M+H]^+$). HR-ESI-MS: 184.1700 ($[M+H]^+$, $C_{11}H_{22}NO^+$; calc. 184.1701).

Incargranine A (= rel-*(3a*R*,4*S*,4a*R*,7a*R*,8*R*,8a*R*)-Decahydro-3a-hydroxy-7-[4-(2-hydroxyethyl)-phenyl]-4,8-ethano-2*H*-furo[3,2-*f*]indol-9-one*; **2**): Light yellow oil. $[\alpha]_D^{20} = +2$ ($c = 0.1750$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS: 366.3 ($[M+Na]^+$), 378.2 ($[M+Cl]^-$). HR-ESI-MS: 378.1469 ($[M+Cl]^-$, $C_{20}H_{25}ClNO_4^-$; calc. 378.1472).

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