A Unique Indolo-[1,7]naphthyridine Alkaloid from *Incarvillea mairei* var. *grandiflora* (WEHRH.) GRIERSON

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A novel alkaloid with a unique indolo-[1,7]naphthyridine nucleus, incargranine B, was isolated from the whole plant of *Incarvillea mairei* var. *grandiflora* (WEHRH.) GRIERSON. Its structure was determined by means of spectroscopic methods.

Introduction. – *Incarvillea mairei* var. *grandiflora* (WEHRH.) GRIERSON (Bignoniaceae), mainly native to the mountains of Yunnan, Sichuan, and Qinghai provinces [1][2], is a member of the genus *Incarvillea*. Several structurally diverse actinidine-type monoterpenoid alkaloids with strong antinociceptive activity have been isolated from the genus *Incarvillea* [3–13], and attracted more attention due to their biological activities and structural complexities. We have previously reported the isolation and structural elucidation of two new alkaloids, isoincarvilline and incargranine A [14]. Further investigation of this plant led to another structurally unique decahydro-indolo-[1,7]naphthyridine alkaloid, incargranine B (**1**), isolated from the 80% EtOH extract of the whole plant of *I. mairei* var. *grandiflora*. Here, we report the isolation and structure elucidation of incargranine B (**1**; *Fig. 1*).

Results and Discussion. – The AcOEt extract (350 g) of the 80% EtOH extract of *I. mairei* var. *grandiflora* was chromatographed over silica-gel column with gradient

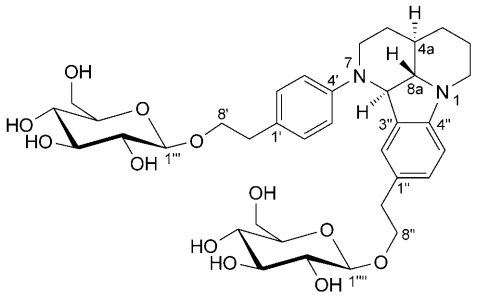

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Fig. 1. *Chemical structure of* **1**

$CHCl_3$/MeOH (100:0 → 0:100) to afford nine fractions 1–9, and *Fraction 9* was further purified by repeated column chromatography over silica gel and *Sephadex LH-20* (MeOH) to provide compound **1** (7 mg).

For incargranine B (**1**; *Fig. 1*), the molecular formula $C_{36}H_{50}N_2O_{12}$ was established by negative-ion-mode HR-ESI-MS (*m/z* 701.3282 ($[M-H]^-$, for $C_{36}H_{49}N_2O_{12}^-$; calc. 701.3286)), indicating 13 degrees of unsaturation. The ^{1}H-NMR spectrum (*Table*) of **1** exhibited the signals of one 1,4-substituted phenyl (δ(H) 6.59 (*d*, *J* = 8.4, 2 H), 7.09 (*d*, *J* = 8.4, 2 H)), one 1,3,4-substituted phenyl (δ(H) 7.03 (*d*, *J* = 7.9), 6.85 (*s*), 6.65 (*d*, *J* = 7.9)), and two sugar moieties (two anomeric H-atom signals at δ(H) 4.31 (*d*, *J* = 7.8) and 4.21 (*d*, *J* = 7.8), and a series of signals between δ(H) 3.13 and 3.90). The ^{13}C-NMR spectrum of **1** showed signals for 36 C-atoms, attributed to five sp^2 quaternary C-atoms, 20 CH groups (7 sp^2, 13 sp^3), and 11 sp^3 CH_2 groups. Analysis of the ^{1}H,^{1}H-COSY (*Fig. 2*) and HSQC data together with the 1D-NMR spectra of **1** led to identification of the partial structures **a** (N(1) to C(8)), **b** (C(1′) to C(6′)), **c** (C(7′) to C(8′)), **d** (C(1″) to C(6″)), **e** (C(7″) to C(8″)), **f** (C(1‴) to C(6‴)), and **g** (C(1⁗) to C(6⁗))[1]) (*Fig. 2*). Further analysis of the HMBC spectrum allowed attribution of the seven partial structures to five substructures: one decahydro-indole-[1,7]naphthyridine moiety, one 2-(4-substituted phenyl)ethanol moiety, one 2-(3,4-disubstituted phenyl)ethanol moiety, and two glucopyranoses (δ(C) 104.6, 75.3, 78.2, 71.8, 78.1, 63.0, and 104.6, 75.3, 78.2, 71.8, 78.1, 62.9, resp.). Of 13 degrees of unsaturation, two were assigned to the two glucoses, two to the decahydro-1,7-naphthyridine moiety, and eight to the two 2-phenylethanols, thus **1** was inferred to possess another ring. The result suggested the presence of a fused pentacyclic ring system for **1** between decahydro-1,7-naphthyridine moiety and 2-(3,4-disubstituted phenyl)ethanol moiety. The connections of C(8) to C(3″) and N(1) to C(4″) was further confirmed by the HMBC correlations H–C(8)/C(2″), C(3″), and C(4″). The HMBC correlation H–C(8)/C(4′) indicated that 2-(4-substituted phenyl)ethanol moiety was located at N(7). In the HMBC spectrum of **1**, the long correlations H–C(1‴)/C(8′) and H–C(1⁗)/C(8″) established the connections of two glucose moieties with two 2-phenylethanol moieties, respectively (*Fig. 2*). Thus, the gross planar structure of incargranine B was assigned as shown in *Figs. 1* and *2*.

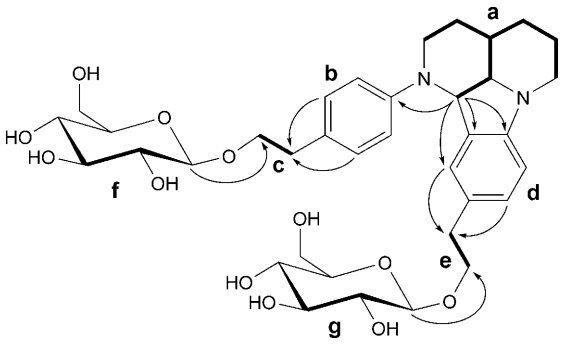

Fig. 2. *Selected HMBC* (H → C) *and* ^{1}*H,*^{1}*H-COSY* (—) *correlations of* **1**

[1]) Arbitrary atom numbering as indicated in *Fig. 1*. For systematic names, see *Exper. Part.*

Table. *^1H- (600 MHz) and ^{13}C-NMR (150 MHz) Data of* **1** (in CD_3OD, J in Hz, δ in ppm). Arbitrary C-atom numbering.

	δ(H)	δ(C)		δ(H)	δ(C)
CH_2(2)	3.64–3.65 (*m*, H_a),	49.3	C(1″)		130.3
	2.79–2.81 (*m*, H_b)		H–C(2″)	6.85 (*s*)	129.1
CH_2(3)	2.05–2.10 (*m*)	23.9	C(3″)		130.3
CH_2(4)	2.21–2.25 (overlap, H_a),	33.3	C(4″)		147.2
	1.70–1.75 (overlap, H_b)		H–C(5″)	6.65 (*d*, J = 7.9)	113.7
H–C(4a)	2.71–2.75 (overlap)	49.5	H–C(6″)	7.03 (*d*, J = 7.9)	129.0
CH_2(5)	2.21–2.25 (overlap, H_a),	31.7	CH_2(7″)	2.71–2.75 (overlap)	37.0
	1.70–1.75 (overlap, H_b)		CH_2(8″)	3.86–3.88 (*m*, H_a),	72.5
CH_2(6)	3.55–3.58 (overlap, H_a),	51.0		3.55–3.58 (overlap, H_b)	
	3.25–3.26 (*m*, H_b)		H–C(1‴)	4.31 (*d*, J = 7.8)	104.6
H–C(8)	4.27 (*d*, J = 9.0)	61.7	H–C(2‴)	3.13 (*t*, J = 7.8)	75.3
H–C(8a)	2.34–2.37 (*m*)	66.7	H–C(3‴)	3.19–3.22 (*m*)	78.2
C(1′)		128.5	H–C(4‴)	3.27–3.29 (overlap)	71.8
H–C(2′)	7.09 (*d*, J = 8.4)	130.8	H–C(5‴)	3.31–3.34 (overlap)	78.1
H–C(3′)	6.59 (*d*, J = 8.4)	114.8	CH_2(6‴)	3.90 (*dd*, J = 13.2, 9.6, H_a),	63.0
C(4′)		149.7		3.62 (*dd*, J = 13.2, 2.4, H_b)	
H–C(5′)	6.59 (*d*, J = 8.4)	114.8	H–C(1⁗)	4.21 (*d*, J = 7.8)	104.6
H–C(6′)	7.09 (*d*, J = 8.4)	130.8	H–C(2⁗)	3.23 (*t*, J = 7.8, 8.5)	75.3
CH_2(7′)	2.83–2.87 (*m*)	36.6	H–C(3⁗)	3.27–3.29 (overlap)	78.2
CH_2(8′)	4.04–4.07 (*m*, H_a),	72.5	H–C(4⁗)	3.27–3.29 (overlap)	71.8
	3.69–3.73 (*m*, H_b)		H–C(5⁗)	3.31–3.34 (overlap)	78.1
			CH_2(6⁗)	3.72 (*dd*, J = 13.2, 9.6, H_a),	62.9
				3.62 (*dd*, J = 13.2, 2.4, H_b)	

The relative configuration of **1** was deduced from the ROESY spectrum as shown in the computer-generated three-dimensional drawing (*Fig. 3*). In the ROESY spectrum, no correlations H–C(8)/H–C(8a) and H–C(4a)/H–C(8a) were observed, while the presence of the strong correlation H–C(8)/H–C(4a) suggested that H–C(4a),

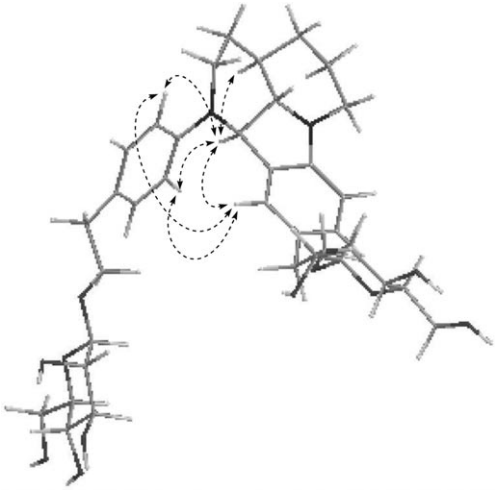

Fig. 3. *Key NOESY correlations of* **1**

H–C(8), and H–C(8a) were α-, α-, and β-oriented, respectively. Therefore, the structure of **1** was identified, and named incargranine B.

The structure of **1** represents a new type of alkaloid, namely decahydro-indolo-[1,7]naphthyridine alkaloid.

Experimental Part

General. Column chromatography (CC) was performed on silica gel (200–300 mesh), *H60* (*Qingdao Marine Chemical Plant*, Qingdao, P. R. China), and *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA). Optical rotation: *Perkin-Elmer 341* digital polarimeter; at 589 nm. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Bruker DRX-500* 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 Kunming Institute of Botany, 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 plant of *I. mairei* var. *grandiflora* (32.5 kg) was finely pulverized and extracted with 80% EtOH under reflux for three times. The combined extracts were concentrated to a small volume *in vacuo*, then dissolved in 2% HCl, and filtered. The filtrate was adjusted to pH 9–10 by adding NH_4OH and then extracted with $CHCl_3$. The remaining aq. layer was neutralized with by 2% HCl to pH 7 and extracted with AcOEt. The AcOEt extract (350 g) was subjected to CC (silica gel; gradient $CHCl_3$/MeOH 100:0 → 0:100) to afford *Fractions 1–9*. *Fr. 9* was purified by repeated CC (silica gel and *Sephadex LH-20* (MeOH)) to provide compound **1** (7 mg).

Incargranine B (=*2-(4-{*rel-*(3a*R,*11b*R,*11c*S*)-10-[2-(β*-D-*Glucopyranosyloxy)ethyl]-2,3,3a,4,5,6, 11b,11c-octahydro-1*H-*indolo[3,2,1-*ij*][1,7]naphthyridin-1-yl}phenyl)ethyl β*-D-*Glucopyranoside*; **1**). Brown powder. $[\alpha]_D^{20} = -12$ ($c = 0.275$, MeOH). ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (neg.): 701 ($[M-H]^-$), 737 ($[M+Cl]^-$). HR-ESI-MS (neg.): 701.3282 ($[M-H]^-$, $C_{36}H_{49}N_2O_{12}^-$; calc. 701.3286).

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