Two New Alkaloids from *Incarvillea sinensis*

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Two new alkaloids, incarvines E (**1**) and F (**2**), were isolated from the EtOH extract of the whole plant of *Incarvillea sinensis*. Their structures were elucidated by spectroscopic methods, including 1D- and 2D-NMR.

Introduction. – *Incarvillea sinensis* LAM (Bignoniaceae), Chinese name 'Jiaohao (Kakko)' or 'Tougucao', is a wild plant which is prevalent in northern China, and the dried whole plant has been historically used for treating rheumatism and relieving pain in traditional Chinese medicine [1]. Until now, about 20 alkaloids have been isolated from this plant [2–4], some of which exhibit significant bioactivities, such as incarvillateine, a new dimeric monoterpene alkaloid containing a characteristic cyclobutane ring, has been found to produce more potent pain-relieving activity than morphine in a formalin-induced pain response [5]. So, it is worthwhile to study the constituents of *I. sinensis*. In this article, we report two new alkaloids from the EtOH extract of *I. sinensis*.

Results and Disscusion. – The $CHCl_3$-soluble fraction of the EtOH extract of *I. sinensis* was purified by repeated column chromatography to afford compounds **1** and **2**. Their structures were identified on the basis of NMR and mass spectrometric data.

Compound **1** was obtained as a yellow oil, and exhibited a positive reaction to *Dragendorff*'s reagent. The molecular formula of **1** was determined to be $C_{21}H_{35}NO_3$ by the *quasi*-molecular ion $[M+H]^+$ at m/z 350.2698 in the HR-Q-TOF-MS (positive-ion mode; calc. 350.2695). The ^{13}C-NMR spectrum (*Table*) showed 21 C-atom signals, including five Me, six CH_2, and seven CH groups, as well as three quaternary C-atoms. Among the three quaternary C-atoms, one is an ester CO group according to the chemical shift at δ(C) 167.5. The 1D-NMR and HSQC data indicate the presence of two olefinic H-atoms at δ(H) 6.67 (*t*, $J = 6.0$, 1 H; δ(C) 141.9) and δ(H) 5.38 (*t*, $J = 5.4$, 1 H; δ(C) 124.6), one MeN group at δ(H) 2.72 (*s*), δ(C) 43.9, four Me groups at δ(H) 1.78 (*s*), δ(C) 12.2, δ(H) 1.64 (*s*), δ(C) 16.5, δ(H) 0.96 (*d*, $J = 7.8$), δ(C) 14.3, and δ(H) 0.90 (*d*, $J = 7.2$), δ(C) 16.1, respectively, one OCH_2 group at δ(H) 4.39–4.42 (*m*), δ(C) 58.9, and an O-bearing CH group at δ(H) 5.24–5.26 (*m*), δ(C) 75.0. The key HMBCs are shown in *Fig. 1*. Based on the above spectral data, the skeleton of **1** is quite similar

Table. *[1]H- and [13]C-NMR Data* (600 and 150 MHz, resp.; $CDCl_3$) *of* **1** *and* **2**. δ in ppm, J in Hz.

	1[1])		**2**[1])	
	δ(C)	δ(H)	δ(C)	δ(H)
H_a–C(1)	54.7	3.23–3.25 (*m*)	54.5	3.27–3.28 (*m*)
H_b–C(1)		2.40–2.44 (*m*)		2.41–2.50 (*m*)
H_a–C(3)	55.4	3.06–3.08 (*m*)	55.2	2.50–2.60 (*m*)
H_b–C(3)		2.56–2.57 (*m*)		3.10–3.12 (*m*)
H–C(4)	28.4	1.70–1.71 (*m*)	28.1	1.69–1.73 (*m*)
H–C(5)	36.7	2.50–2.52 (*m*)	36.5	2.50–2.60 (*m*)
H_a–C(6)	29.8	2.40–2.44 (*m*)	29.7	2.35–2.36 (*m*)
H_b–C(6)		1.90–1.94 (*m*)		1.69–1.73 (*m*)
H–C(7)	75.0	5.24–5.26 (*m*)	74.1	5.26–5.28(*m*)
H–C(8)	40.9	2.05–2.08 (*m*)	40.9	2.07–2.09 (*m*)
H–C(9)	43.3	2.34–2.38 (*m*)	42.9	2.34–2.36 (*m*)
MeN	43.9	2.72 (*s*)	43.7	2.74 (*s*)
Me–C(4)	16.1	0.90 (*d*, J = 7.2)	16.5	0.93 (*d*, J = 6.6)
Me–C(8)	14.3	0.96 (*d*, J = 7.8)	14.3	0.97 (*d*, J = 7.2)
C(1′)=O	167.5	–	167.8	–
Me(2′)	12.2	1.78 (*s*)	12.4	1.82 (*s*)
C(3′)	127.7	–	128.7	–
H–C(4′)	141.9	6.67 (*t*, J = 6.0)	140.2	6.64 (*t*, J = 7.2)
CH_2(5′)	26.8	2.24–2.28 (*m*)	26.4	2.34–2.36 (*m*)
CH_2(6′)	37.9	2.10–2.12 (*m*)	39.3	2.26–2.28 (*m*)
Me(7′)	16.5	1.64 (*s*)	18.7	2.15 (*s*)
C(8′)	137.5		158.8	
H–C(9′)	124.6	5.38 (*t*, J = 5.4)	116.0	5.64 (*s*)
CH_2(10′) or C(10′)=O	58.9	4.39–4.42 (*m*)	166.7	–
C(1″)			70.5	
H_a–C(2″,6″)			33.2	1.77–1.81 (*m*)
H_b–C(2″,6″)				1.44–1.47 (*m*)
H_a–C(3″,5″)			29.8	1.87–1.90 (*m*)
H_b–C(3″,5″)				1.50–1.53 (*m*)
H–C(4″)			67.2	3.98–4.00 (*m*)
CH_2(7″)			39.3	1.83–1.91 (*m*)
CH_2(8″)			60.9	4.31–4.33 (*m*)

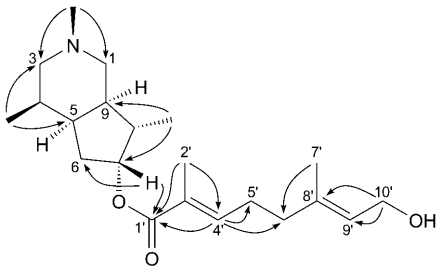

Fig. 1. *Structure and key HMBCs* (H → C) *of* **1**[1])

[1]) Arbitrary numbering. For systematic names, see *Exper. Part*.

to that of incarvine D, which containes an alkaloid moiety and a monoterpene moiety [6]. The only difference is the presence of an additional C=C bond at C(8′) (δ(C) 137.5) and H–C(9′)[1]) (δ(C) 141.9) in **1** instead of the C–C bond at H–C(8′) (δ(C) 29.4) and CH_2(9′) (δ(C) 39.7) in incarvine D. So the C-atom resonances at δ(C) 54.7, 55.4, 28.4, 36.7, 29.8, 75.0, 40.9, 43.3, 43.9, 16.1, and 14.3 could be assigned to C(1), C(3) to C(9), MeN, Me–C(4), and Me–C(8) of the monoterpene alkaloid incarvilline, the absolute configuration of which was determined by X-ray-analysis [7] and *Mosher*'s method [8]. The remaining NMR signals of the monoterpene moiety also were attributed (*Table*). Therefore, the structure of **1** was established, and named incarvine E.

Compound **2** was obtained as a yellow oil and showed a positive reaction to *Dragendorff*'s reagent. The ESI-MS showed signals at *m/z* 506.5 ($[M+H]^+$) and *m/z* 528.5 ($[M+Na]^+$) in the positive-ion mode, and *m/z* 504.4 ($[M-H]^-$) and *m/z* 540.4 ($[M+Cl]^-$ in the negative-ion mode, corresponding to a molecular formula $C_{29}H_{47}NO_6$. The C-atom resonances at δ(C) 54.5, 55.2, 28.1, 36.5, 29.7, 74.1, 40.9, and 42.9 suggested the presence of an incarvilline moiety. Furthermore, ten C-atom resonances at δ(C) 167.8, 166.7, 158.8, 140.2, 128.7, 116.0, 39.3, 26.4, 18.7, and 12.4 indicated the presence of two ester CO and two Me groups, two trisubstituted C=C bonds, and two CH_2 groups, which disclosed the presence of a monoterpene moiety identified as *Hildebrandt*'s acid as previously reported [9]. The remaining eight C-atoms, including one CH_2O group (δ(C) 60.9), five CH_2 groups (δ(C) 39.3, δ(C) 33.2 × 2, δ(C) 29.8 × 2), one O-bearing CH moiety (δ(C) 67.2), and one O-bearing sp^3 quaternary C-atom (δ(C) 70.5), indicated the presence of an isorengyol moiety determined previously [10]. The connection among incarvilline, *Hildebrandt*'s acid, and isorengyol moiety, were proved by HMBC correlations (*Fig. 2*). Comparison with the literature data of incarvine B [11] revealed that compound **2** is the isorengyol ester of incarvine B. Thus, the structure of compound **2** was determined, and named incarvine F.

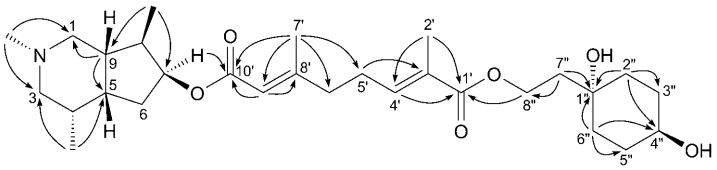

Fig. 2. *Structure and key HMBCs* (H → C) *of* **2**[1])

Experimental Part

General. TLC: *HSGF254* silica gel plates (10–40 μm, *Yantai*, P. R. China). The alkaloids were detected under UV (254, 365 nm) light and with *Dragendroff* spray reagent. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, *Yantai*, P. R. China), silica gel *H* (10–40 μm, *Qingdao*, P. R. China), and *Sephadex LH-20* (*Pharmacia Co. Ltd.*). Optical rotations: *Perkin-Elmer 341* polarimeter. CD Spectra: *JASCO J-810* instrument. NMR Spectra: in $CDCl_3$, on *Bruker AVANCE II 600* NMR, for ^{1}H-NMR at 600 MHz, and ^{13}C-NMR at 150 MHz; chemical shifts in ppm with TMS as internal standard. ESI-MS Spectra: *Varian MAT-212* mass spectrometer. TOF-MS: *Q-Tof micro YA019* mass spectrometer.

Plant Material. The whole plants of *Incarvillea sinensis* were collected in Shanxi Province, P. R. China, in August 2007, and identified by Prof. *Han-Chen Zheng*, Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai. A voucher specimen (No. 20070801) was

deposited with the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai, P. R. China.

Extraction and Isolation. The air-dried whole plants (15 kg) of *I. sinensis* was powered and extracted with 80% and 95% EtOH (30 l) under reflux. After removal of EtOH under reduced pressure, the aq. brownish syrup (6 l) was successively partitioned with petroleum ether (PE), AcOEt, $CHCl_3$, and BuOH. The $CHCl_3$ extract (68 g) was subjected to SiO_2 (200–300 mesh) CC, eluting with gradient $CHCl_3$/MeOH (20:0, 20:1, 10:1, 5:1, 2:1, 0:1) to yield six fractions. *Fr. 2* was repeatedly chromatographed on a SiO_2 column eluting with cyclohexane/$CHCl_3$/Et_2NH (6:4:1 → 5:3:1) and *Sephadex LH-20* to afford compounds **1** (21 mg) and **2** (6 mg).

Incarvine E (=*(4*R**,4a*S**,6*R**,7*S**,7a*R**)-Octahydro-2,4,7-trimethyl-1*H*-cyclopenta[*c*]pyridin-6-yl (2*E*,6*E*)-8-Hydroxy-2,6-dimethylocta-2,6-dienoate*; **1**). Yellow oil. $[\alpha]_D^{20} = -6.3$ ($c = 0.66$, $CHCl_3$). IR (KBr): 3398, 2964, 2935, 1702, 1652, 1540. ^{1}H- and ^{13}C-NMR: *Table.* HMBC: *Fig. 1.* ESI-MS (pos.): 350.4 ($[M+H]^+$), 372.3 ($[M+Na]^+$). ESI-MS (neg.): 348.3 ($[M-H]^-$). ESI-Q-TOF-MS (pos.): 350.2698 ($[M+H]^+$, $C_{21}H_{36}NO_3^+$; calc. 350.2695).

Incarvine F (=*1-[2-(*trans*-1,4-Dihydroxycyclohexyl)ethyl] 8-[(4*R**,4a*S**,6*R**,7*S**,7a*R**)-2,4,7-Trimethyloctahydro-1*H*-cyclopenta[*c*]pyridin-6-yl] (2*E*,6*E*)-2,6-Dimethylocta-2,6-dienedioate*; **2**). Yellow oil. $[\alpha]_D^{20} = -6.6$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3392, 2969, 2932, 1737, 1716, 1558. ^{1}H- and ^{13}C-NMR: *Table.* HMBC: *Fig. 2.* ESI-MS (pos.): 506.5 ($[M+H]^+$), 528.5 ($[M+Na]^+$). ESI-MS (neg.): 504.4 ($[M-H]^-$), 540.4 ($[M+Cl]^-$). ESI-Q-TOF-MS (pos.): 506.3510 ($[M+H]^+$, $C_{29}H_{48}NO_6^+$; calc. 506.3482).

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