Two Novel Monoterpene Alkaloid Dimers from *Incarvillea arguta*

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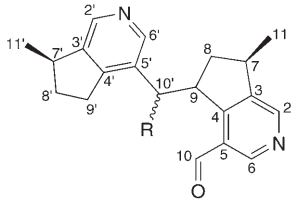
Two novel monoterpene alkaloid dimers, named argutanes A and B (**1** and **2**, resp.), together with the known alkaloid boschniakine (**3**), were isolated from the roots of *Incarvillea arguta*. The structures were elucidated on the basis of 1D- and 2D-NMR, as well as HR-ESI-MS data.

Introduction. – *Incarvillea arguta* (Bignoniaceae) meanly grows in the southwest area of China at altitudes of 1400–2700 m. It has been widely used as a herbal medicine of Yi nationality (known as '*Wabuyou*') to treat hepatitis and diarrhea in China [1]. Previous phytochemical studies on the genus *Incarvillea* have revealed that it is a rich source of monoterpene alkaloids [2–9]. A number of ceramides, triterpenes [10], monoterpene alkaloids [11], and flavones [12] have been reported from *I. arguta*. Further chemical studies of this plant led us to isolate two novel monoterpene alkaloid dimers, *i.e.*, **1** and **2**, of the boschniakine type (*Fig. 1*). Up to now, no boschniakine-type alkaloid dimers have been reported. Here, we describe the isolation and structural elucidation of these alkaloids.



1 R = ▬ OH
2 R = ┈ OH

Fig. 1. *Structures of compounds* **1** *and* **2**

Results and Discussion. – The dried roots of *I. arguta* were extracted with 80% EtOH four times at room temperature. After removal of solvent, the extract was suspended in H_2O and acidified to pH 2 with 20% H_2SO_4, and filtered. The filtrate was basified to pH 10 with $NaHCO_3$ and then extracted with $CHCl_3$. The aqueous phase was adjusted to pH 7 with 20% H_2SO_4 and partitioned successively with petroleum ether, AcOEt, and BuOH. After removal of the solvent, the petroleum ether extract was further purified by repeated column chromatography over silica gel and *Sephadex*

LH-20 to yield compounds **1**–**3**. The structures of **1** and **2** were elucidated mainly by NMR spectroscopy, including 1D- and 2D-NMR experiments (^{1}H,^{1}H-COSY, NOESY, HMQC, and HMBC), in combination with mass spectrometry (MS).

Compound **1** was obtained as yellow oil. The molecular formula was determined as $C_{20}H_{22}N_2O_2$ by HR-ESI-MS (*m/z* 345.1559 ($[M+Na]^+$ $C_{20}H_{22}N_2NaO_2^+$; calc. 345.1579)). The ^{13}C-NMR spectrum of **1** (*Table*) displayed 20 ^{13}C signals, and, combined with DEPT, revealed ^{13}C signals for two Me, three CH_2, nine CH groups, and six quaternary C-atoms. Comparison of the spectral data of **1** with those of the known compound **3** indicated that **1** is a dimer of a boschniakine-type alkaloid [13–15]. The ^{13}C-NMR signals of **1** disclosed the presence of two sets of boschniakine-type alkaloid moieties[1]) (δ(C) 149.9 (C(2)), 146.1 (C(3)), 154.1 (C(4)), 129.0 (C(5)), 150.8 (C(6)), 35.5 (C(7)), 39.1 (C(8)), 48.7 (C(9)), 193.1 (C(10)), and 19.0 (C(11)), and (δ(C) 142.9 (C(2′)), 146.1 (C(3′)), 155.1 (C(4′)), 127.1 (C(5′)), 144.9 (C(6′)), 37.7 (C(7′)), 34.4 (C(8′)), 30.3 (C(9′)), 73.0 (C(10′)), and 19.5 (C(11′))) (*Table*). The CHO group (δ(H) 10.21 (*s*); δ(C) 193.1 (CH)) was observed in the ^{1}H- and ^{13}C-NMR spectrum. A signal of an oxygenated methine group at δ(H) 4.75 (*d*, J = 8.6 Hz) correlated with the ^{13}C signal at δ(C) 73.0 (C(10′)) in the HMQC spectrum. The HMBC cross-peaks between H–C(10′), and C(4), C(8), C(9), C(4′), C(5′), and C(6′) suggested that these two boschniakine-type alkaloids are connected by a bond between C(9) and C(10′) (*Fig. 2*) leading to the constitutional formula **1**.

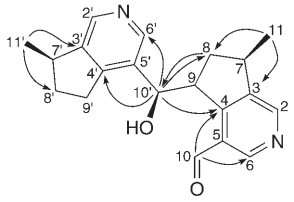


Fig. 2. *Key HMBC correlations of* **1**

The relative configuration of **1** was deduced from coupling constants, and the analysis of NOESY experiment as depicted on a 3D structure generated from the molecular modeling (ChemOffice2006, Chem3D Ultra 10.0) using MM2 force field calculations for energy minimization [16] (*Fig. 3*). In the NOESY, the cross-peaks observed between the H-atom pairs H_α–C(7)/H_α–C(8), H_α–C(8)/H–C(10′), H_α–C(7)/H–C(10′), H_α-(7′)/H_α–C(8′), H_α–C(8′)/H_α–C(9′), and H_α–C(9′)/H–C(10′) indicated that H–C(10′) is α-oriented. The large coupling constant (J(9β, 10′) = 8.6 Hz) observed between H–C(9) and H–C(10′) was indicative of a *trans*-coupling (τ 180°). Accordingly, the OH group at C(10′) was β-oriented. Thus, from the above data, the structure of **1** was elucidated as (7*R**)-5-{(*R**)-[(7*R**)-6,7-dihydro-7-methyl-5*H*-cyclopenta[*c*]pyridin-4-yl]hydroxymethyl}-6,7-dihydro-7-methyl-5*H*-cyclopenta[*c*]pyridine-4-carbaldehyde (absolute configuration unknown), and named argutane A.

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[1]) Arbitrary C-atom numbering as indicated in *Fig. 1*

Table. *[1]H- and [13]C-NMR Data of* **1** *and* **2**. At 400 and 100 MHz, respectively, in $CDCl_3$; δ in ppm, J in Hz. Arbitrary C-atom numbering according to *Fig. 1.*

Position	**1**		**2**	
	δ (H)	δ (C)	δ (H)	δ (C)
H–C(2)	8.64 (*s*)	149.9	8.65 (*s*)	150.5
C(3)		146.1		128.4
C(4)		154.1		152.4
C(5)		129.0		147.5
H–C(6)	8.89 (*s*)	150.8	8.86 (*s*)	153.6
H–C(7)	3.40–3.43 (*m*)	35.5	3.49–3.53 (*m*)	36.2
CH_2(8)	2.11–2.17 (*m*), 1.69–1.74 (*m*)	39.1	2.43–2.49 (*m*), 1.57–1.63 (*m*)	36.7
H–C(9)	4.18 (*t, J* = 8.6)	48.7	4.08 (*dd, J* = 3.4, 3.2)	48.2
H–C(10)	10.21 (*s*)	193.1	10.05 (*s*)	191.8
Me(11)	1.32 (*d, J*(11,7) = 6.9)	19.0	1.33 (*d, J* = 6.9)	19.7
H–C(2′)	8.34 (*s*)	142.9	8.33 (*s*)	140.0
C(3′)		146.1		146.3
C(4′)		155.1		146.7
C(5′)		127.1		124.6
H–C(6′)	8.45 (*s*)	144.9	8.63 (*s*)	142.9
H–C(7′)	3.41–3.45 (*m*)	37.7	3.29–3.33 (*m*)	37.7
CH_2(8′)	1.65–1.68 (*m*), 2.40–2.42 (*m*)	34.4	1.69–1.73 (*m*), 2.35–2.42 (*m*)	34.0
CH_2(9′)	3.05–3.11 (*m*), 2.73–2.77 (*m*)	30.3	3.11–3.17 (*m*), 3.01–3.06 (*m*)	30.5
H–C(10′)	4.75 (*d, J* = 8.6)	73.0	5.28 (*d, J* = 3.4)	71.6
Me(11′)	1.34 (*d, J* = 6.9)	19.5	1.35 (*d, J* = 6.9)	19.9

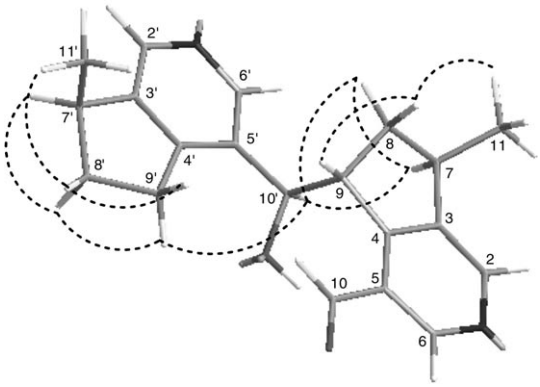

Fig. 3. *Key NOESY correlations of* **1**

Compound **2** was obtained as yellow oil. The molecular formula was determined as $C_{20}H_{22}N_2O_2$ by HR-ESI-MS (m/z 323.1769 ($[M+H]^+$; calc. 323.1760)), which showed the same molecular formula as **1**. The ^{1}H- and ^{13}C-NMR spectral data of **2** were similar to those of **1** (*Table*). The main difference between **2** and **1** was a small coupling constant observed between H–C(9) (δ(H) 4.08 (*dd* = 3.4, 3.2 Hz)) and H–C(10′) (δ(H) 5.28 (*d*, J = 3.4 Hz)). Furthermore, no interaction between H_α–C(7) and H–C(10′) was observed in the NOESY experiment. These results indicated that the

OH group at C(10′) was α-oriented. Thus, the structure of **2** was elucidated as (7*S**)-5-{(*S**)-[(7*S**)-6,7-dihydro-7-methyl-5*H*-cyclopenta[*c*]pyridin-4-yl]hydroxymethyl}-6,7-dihydro-7-methyl-5*H*-cyclopenta[*c*]pyridine-4-carbaldehyde.

Compound **3** (*Fig. 4*) was obtained as yellow oil. It was identified as boschniakine by comparing its NMR and specific-rotation data with those reported in the literature [13–15].

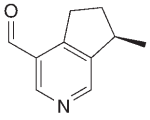

Fig. 4. *Structure of compound (+)-(*R*)-***3***

Experimental Part

General. TLC: *HSG* F_{254} Silica-gel plates (10–40 μm; *Yantai Huiyou*, China). Column chromatography (CC): Silica gel (200–300 mesh; *Yantai Jiangyou*, China), silica gel *H* (10–40 μm; *Qingdao Marine Chemical Ltd.*, China), and *Sephadex LH-20* (*Pharmacia Co. Ltd.*). *Dragendroff*'s reagent was used for alkaloid detection. Optical rotations: *Perkin-Elmer 341* polarimeter at r.t. NMR Spectra: in $CDCl_3$ on *Bruker AVANCE*^{II} *400* NMR, for ^{1}H-NMR at 400 MHz and ^{13}C-NMR at 100 MHz, resp. ESI- and HR-ESI-TOF-MS: *Varian MAT-212* and *Q-Tof micro YA019* spectrometers.

Plant Material. The roots of *I. arguta* were collected from Anning, Yunnan Province, China, in May 2006, and identified by Prof. *Bao-kang Huang* and *Han-chen Zheng*, Department of Pharmacognosy, Second Military Medical University. The voucher specimens (LTM20060514) have been deposited with the Herbarium of School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China.

Extraction and Isolation. The dried roots (24.9 kg) of *I. arguta* were chopped and percolated with 80% EtOH (4 × 50 l) at r.t. until the compounds of interest were exhaustively extracted. The solvent was removed by evaporation under reduced pressure to give a crude extract (5.2 kg). The extract was dissolved in 15 l of H_2O to form a suspension and acidified to pH 2 with 20% H_2SO_4, and filtered. The filtrate was basified to pH 10 with sat. $NaHCO_3$ soln. and then extracted repeatedly with $CHCl_3$. The org. fractions were combined and evaporated under vacuum to yield the $CHCl_3$ extract (93 g). Then, the aq. phase was adjusted to pH 7 with 20% H_2SO_4 and extracted successively with petroleum ether, AcOEt, and BuOH at r.t. Removal of the solvents from the extracts under reduced pressure gave three extracts, *i.e.*, the petroleum-ether extract (135 g), the AcOEt extract (272 g), and the BuOH extract (630 g). The petroleum-ether extract (107 g) was chromatographed over silica gel eluting with a gradient of increasing AcOEt (0–100%) in petroleum ether to give nine fractions. *Fr. 9* was purified by silica-gel CC with petroleum ether and AcOEt to yield compounds **1** (3 mg) and **2** (5 mg). *Fr. 3* was purified by *Sephadex LH-20* eluting with CH_2Cl_2/MeOH 1:1 to yield compound **3** (6.5 mg).

Argutane A (=*(7*R**)-5-{(*R**)-[(7*R**)-6,7-Dihydro-7-methyl-5*H*-cyclopenta[*c*]pyrdidin-4-yl]hydroxymethyl}-6,7-dihydro-7-methyl-5*H*-cyclopenta[*c*]pyridine-4-carbaldehyde*; **1**): Yellow oil. $[\alpha]_D^{25} = +25.4$ ($c = 0.055$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table.* ESI-MS: 323.3 ($[M+H]^+$). HR-ESI-TOF-MS: 345.1559 ($[M+Na]^+$, $C_{20}H_{22}N_2NaO_2^+$; calc. 345.1579).

Argutane B (=*(7*S**)-5-{(*S**)-[(7*S**)-6,7-Dihydro-7-methyl-5*H*-cyclopenta[*c*]pyridin-4-yl]hydroxymethyl}-6,7-dihydro-7-methyl-5*H*-cyclopenta[*c*]pyridine-4-carbaldehyde*; **2**): Yellow oil. $[\alpha]_D^{25} = +21.0$ ($c = 0.025$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table.* ESI-MS: 323.2 ($[M+H]^+$). HR-ESI-TOF-MS: 323.1769 ($[M+H]^+$, $C_{20}H_{23}N_2O_2^+$; calc. 323.1760).

Boschniakine (**3**). Yellow oil. $[\alpha]_D^{25} = +13.0$ ($c = 0.150$, MeOH). ^{1}H-NMR[1]): 10.22 (*s*, H–C(10)); 8.61 (*s*, H–C(2)); 8.83 (*s*, H–C(6)); 3.35–3.44 (*m*, H–C(9)); 3.30–3.34 (*m*, H–C(7)); 3.13–3.18 (*m*, H–C(9)); 2.40–2.46 (*m*, H–C(8)); 1.67–1.74 (*m*, H–C(8)); 1.36 (*d*, $J = 6.9$, H–C(11)) [13–15]. ^{13}C-NMR ($CDCl_3$)[1]): 191.64 (C(10)); 155.04 (C(4)); 150.96 (C(6)); 148.92 (C(2)); 146.12 (C(3)); 128.06 (C(5)); 38.40 (C(8)); 37.20 (C(7)); 30.77 (C(9)); 19.87 (C(11)). ESI-MS: 162.1 ($[M+H]^+$).

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