Two New Cytotoxic Biphenyls from the Roots of *Incarvillea arguta*

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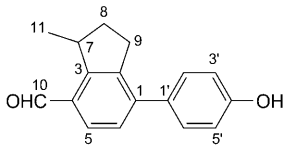
Two novel biphenyls, named incargutines A (**1**) and B (**2**), were isolated from the roots of *Incarvillea arguta*, and the structures were elucidated by detailed spectroscopic analysis, including HR-ESI-MS data and 2D-NMR spectroscopy. Compounds **1** and **2** contain an unprecedented C-atom skeleton, namely a 4-methylbiphenyl unit fused with a 7-methylcyclopentane unit at C(2) and C(3) (*i.e.*, a 1,7-dimethyl-4-phenylindane skeleton). The cytotoxicity of **1** and **2** was evaluated with four tumor cell lines, A549, LOVO, CEM, and MDA-MB-435 (MDA), by an MTT assay.

Introduction. – *Incarvillea arguta* (Bignoniaceae) mainly grows in the southwest area of China at an altitude 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]. A number of ceramides, triterpenes, monoterpene alkaloids, and flavones have been reported from *I. arguta* [2–6]. Further investigation of this plant led us to isolate two novel biphenyls **1** and **2** with an unprecedented C_{17} skeleton of the 4,7-dimethyl-1-phenylcyclopenta[*c*]benzene type (*i.e.*, of the 1,7-dimethyl-4-phenylindane type). To the best of our knowledge, this is the first example of a biphenyl natural product fused with a cyclopentane moiety at C(2) and C(3). We report on the isolation and structure elucidation of these novel biphenyls through extensive spectroscopic analysis and on their antitumor activities *in vitro*.



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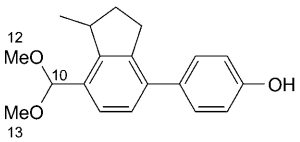

2 [1])

Results and Discussion. – The dried roots of *I. arguta* (24.9 kg) were chopped and percolated with 80% EtOH (4 × 50 l) at room temperature. The solvent was evaporated to give a crude extract (5.2 kg). The extract was taken up in 15 l of H_2O and the resulting suspension acidified to pH 2 with H_2SO_4 solution and filtered. The filtrate was basified to pH 10 with $NaHCO_3$ solution and then extracted with $CHCl_3$.

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

The $CHCl_3$ extract (93 g) was separated by column chromatography (silica gel) and prep. HPLC to yield **1** (3.2 mg) and **2** (16.0 mg).

Incargutine A[1]) (**1**) was obtained as a yellow oil. The molecular formula was determined as $C_{17}H_{16}O_2$ by HR-ESI-MS (*m/z* 251.1074 ($[M-H]^-$, $C_{17}H_{15}O_2^-$). The ^{13}C-NMR and DEPT spectra of **1** showed 19 C-atoms, including one Me, two CH_2, seven CH, and one CHO group, and six quaternary aromatic C-atoms (*Table 1*). There were two sets of mutually coupled H-atoms in the aromatic region of the ^{1}H-NMR spectrum of **1**: the one at δ(H) 7.69 (*d*, $J=7.8$ Hz) and 7.24 (*d*, $J=7.8$ Hz) indicated a 1,2,3,4-tetrasubstituted benzene moiety, and the other one at δ(H) 7.30 (*d*, $J=8.6$ Hz, H–C(2′), H–C(6′)) and 6.92 (*d*, $J=8.6$ Hz, H–C(3′), H–C(5′)) indicated a 1′,4′-disubstituted benzene ring. In the HSQC spectrum of **1**, the signals at δ(H) 10.18 (*s*) correlated with the signal at δ(C) 192.5 (C(10)). The CHO group of **1** was located at C(4) due to the HMBC cross-peaks H–C(10)/C(4) and C(5), and H–C(5)/C(4) and C(10) (*Fig. 1*), and the NOESY cross-peak H–C(10)/H–C(5). The analysis of the NOESY data is based on a 3D structure generated by molecular modeling (Chem-Office2006 Chem3D Ultra 10.0) with MM2 force-field calculations for energy minimization (*Fig. 2*).

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**[1]). At 400/100 MHz, resp., in $CDCl_3$; δ in ppm, *J* in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		144.3		138.8
C(2)		131.0		132.2
C(3)		148.4		146.9
C(4)		146.4		141.9
H–C(5)	7.69 (*d*, $J=7.8$)	129.8	7.36 (*d*, $J=7.8$)	124.5
H–C(6)	7.24 (*d*, $J=7.8$)	128.4	7.07 (*d*, $J=7.8$)	127.6
H–C(7)	3.55–3.62 (*m*)	37.8	3.53–3.58 (*m*)	38.2
CH_2(8)	2.31–2.35 (*m*), 1.77–1.80 (*m*)	33.4	2.26–2.29 (*m*), 1.67–1.71 (*m*)	33.3
CH_2(9)	3.31–3.37 (*m*)	29.9	2.95–2.99 (*m*)	29.3
H–C(10)	10.18 (*s*)	192.5	5.41 (*s*)	102.7
Me(11)	0.80 (*d*, $J=7.0$)	19.8	0.80 (*d*, $J=6.9$)	19.9
Me(12)			3.37 (*s*)	53.2
Me(13)			3.37 (*s*)	53.2
C(1′)		132.9		133.5
H–C(2′)	7.30 (*d*, $J=8.6$)	129.7	7.24 (*d*, $J=8.5$)	129.7
H–C(3′)	6.92 (*d*, $J=8.6$)	115.4	6.87 (*d*, $J=8.5$)	115.2
C(4′)		155.6		155.4
H–C(5′)	6.92 (*d*, $J=8.6$)	115.4	6.87 (*d*, $J=8.5$)	115.2
H–C(6′)	7.30 (*d*, $J=8.6$)	129.7	7.24 (*d*, $J=8.5$)	129.7

The ^{1}H-NMR signals of **1** at δ(H) 3.55–3.62 (*m*), 2.31–2.35 (*m*), 1.77–1.80 (*m*), 3.31–3.37 (*m*, 2 H), and 0.80 (*d*, $J=7.0$ Hz) correlated with the ^{13}C-NMR signals at δ(C) 37.8 (C(7)), 33.4 (C(8)), 33.4 (C(8)), 29.9 (C(9)), and 19.8 (C(11)), respectively, in the HSQC spectrum. Furthermore, the HMBC cross-peaks H–C(7)/C(3), C(4), C(8), C(9), and C(11), H_a–C(8) and H_b–C(8)/C(7), C(9), and C(11), H_a–C(9) and H_b–C(9)/C(2), C(3), C(4), C(7), and C(8), and Me(11)/C(3), C(7), and C(8),

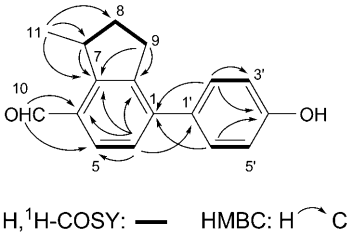

Fig. 1. *Selected 2D-NMR correlations for incargutine A* (**1**)[1])

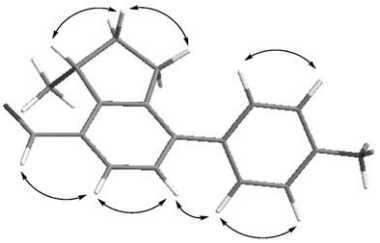

Fig. 2. *Selected NOE correlations for incargutine A* (**1**)

suggested the presence of a 7-methylcyclopentane unit fused with an arene ring at C(2) and C(3) in the structure of **1**. The IR spectrum revealed the presence of an OH group by the absorption at 3370 cm^{-1}, and the OH substituent at C(4′) (δ(C) 155.6) was deduced from its chemical shift and the molecular formula. The specific rotation of **1** was positive; thus the structure of **1** was elucidated as (+)-4′-hydroxy-7-methylcyclopenta[*c*]biphenyl-4-carboxaldehyde with as yet unknown absolute configuration.

Incargutine B[1]) (**2**) was obtained as a yellow oil. The molecular formula was determined as $C_{19}H_{22}O_3$ by HR-ESI-MS (*m*/*z* 321.1466 ($[M+Na]^+$, $C_{19}H_{22}NaO_3^+$). The NMR data (*Table 1*) were very similar to those of **1**, but two additional MeO signals at δ(H) 3.37 (*s*, Me(12), Me(13)) and δ(C) 53.2 (C(12), C(13)) were present instead of the CHO signals of **1**. These MeO groups were placed at C(10) (δ(C) 102.7) on the basis of their HMBC with C(10). The remaining 2D-NMR (^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY) data were identical to those of **1**. The specific rotation of **2** was positive; thus the structure of **2** was elucidated as (+)-12,13-dimethoxy-7-methylcyclopenta[*c*]biphenyl-4′-ol.

The antitumor activities of incargutines A (**1**) and B (**2**) against four tumor cell lines, A549, LOVO, CEM, and MDA-MB-435 (MDA), were determined by the MTT assay [7], with DOX (doxorubicin) as a positive control; the IC_{50} (μg/ml) values are listed in *Table 2*. Incargutine A (**1**) showed modest cytotoxicities against these four tumor cell lines with IC_{50} values in the range of 0.47–6.16 μg/ml.

Table 2. *Cytotoxicity* (IC_{50} [μg/ml]) *of Compounds* **1** *and* **2** *against A549, LOVO, CEM, and MDA-MB-435 Cell Lines*

	A549	LOVO	CEM	MDA
1	3.60	0.47	3.06	6.16
2	13.71	14.59	18.55	4.30
DOX	0.04	0.36	0.01	0.02

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Experimental Part

General. TLC: *HSG-F$_{254}$* silica gel plates (SiO_2, 10–40 μm; *Yantai Huiyou*, China). Column chromatography (CC): SiO_2 (200–300 mesh; *Yantai Jiangyou*, China); SiO_2 *H* (10–40 μm; *Qingdao Marine Chemical Ltd.*, China). Optical rotations: *Perkin-Elmer 341* polarimeter; at r.t. IR Spectra: *Bruker FTIR-Vector-22* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker Avance^{II}-400* spectrometer; in $CDCl_3$ at 400 (^{1}H) and 100 MHz (^{13}C); δ in ppm, *J* in Hz. ESI-MS: *Varian MAT-212* mass spectrometer; in *m*/*z*. TOF-ESI-MS: *Q-Tof-micro-YA019* mass spectrometer.

Plant Material. The roots of *I. arguta* were collected from Anning, Yunnan Province, P. R. China, in May 2006, and were identified by Prof. *Bao-Kang Huang*, Department of Pharmacognosy, Second Military Medical University. The voucher specimens (LTM20060514) were deposited with the Herbarium of the School of Pharmacy, Shanghai Jiao Tong University, Shanghai, P. R. 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 evaporated to give a crude extract (5.2 kg). The extract was suspended in H_2O (15 l) and the suspension acidified to pH 2 with 20% H_2SO_4 soln. and filtered. The filtrate was basified to pH 10 with aq. sat. $NaHCO_3$ soln. and then extracted repeatedly with $CHCl_3$. The org. fractions were concentrated to yield the $CHCl_3$ extract (93 g). The $CHCl_3$ extract was subjected to CC (SiO_2, CH_2Cl_2/MeOH 1:0 → 10:1): *Fractions 1–14*. *Fr. 4* (4.5 g) was separated by CC (SiO_2, CH_2Cl_2/MeOH 1:0 → 10:1) and further purified by prep. HPLC (MeOH/H_2O 65:35): **1** (3.2 mg) and **2** (16.0 mg).

Incargutine A (= rel-*(3*R*)-2,3-Dihydro-7-(4-hydroxyphenyl)-3-methyl-1*H*-inden-4-carboxaldehyde*; **1**): Yellow oil. $[\alpha]_D^{20} = +42.7$ ($c = 0.15$, MeOH). IR (KBr): 3370, 2958, 2867, 1689, 1612, 1591, 1517, 1454, 1382, 1227, 756. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 251.1074 ($[M-H]^-$, $C_{17}H_{15}O_2^-$; calc. 251.1072).

Incargutine B (= rel-*4-[(1*R*)-2,3-Dihydro-7-(dimethoxymethyl)-1-methyl-1*H*-inden-4-yl]phenol*; **2**): Yellow oil. $[\alpha]_D^{20} = +24.5$ ($c = 0.80$, MeOH). IR (KBr): 3375, 2956, 2867, 1612, 1593, 1519, 1454, 1363, 1269, 756. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 321.1466 ($[M+Na]^+$, $C_{19}H_{22}NaO_3^+$; calc. 321.1467).

Cytotoxicity Assay. A cytotoxicity assay was carried out according to *Denizot* and *Lang* [7]. Each cell (conc. $1 \cdot 10^4$) was seeded in each well containing 100 μl of DMEM (*Dulbecco*'s modified *Eagle*'s medium). Subsequently, various conc. of samples were added. The cells were incubated for 48 h at 37° in an atmosphere containing 5% of CO_2. Then 10 μl of FBS-free medium (FBS = fetal bovine serum) containing 5 mg/ml of MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium) soln. was added to the wells. After 4 h of incubation at 37°, the medium was discarded, and the formazan blue formed in the cells was dissolved by adding 100 μl of DMSO. The optical density was measured at 570 nm with a microplate reader (*Molecular Devices Co.*, Menlo Park, CA, USA).

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