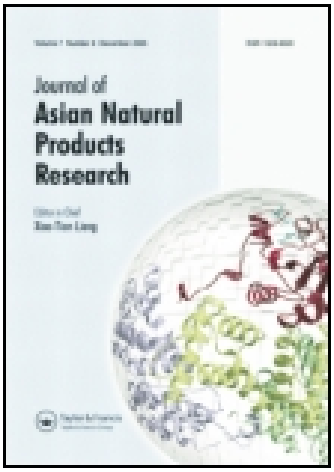

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A new aristolactam-type alkaloid from the roots of Aristolochia fangchi

Jun-Zhang Lu [a], Feng Wei [b] & Yu Cai [c]

[a] Department of Traditional Chinese Medicine, Chinese People's Liberation Army General Hospital, Beijing, 100853, China

[b] National Institutes for Food and Drug Control, State Food and Drug Administration, Beijing, 100050, China

[c] School of Pharmacy, Jinan University, Guangzhou, 510632, China

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NOTE

A new aristolactam-type alkaloid from the roots of *Aristolochia fangchi*

Jun-Zhang Lu[a]*, Feng Wei[b] and Yu Cai[c]

[a]*Department of Traditional Chinese Medicine, Chinese People's Liberation Army General Hospital, Beijing 100853, China;* [b]*National Institutes for Food and Drug Control, State Food and Drug Administration, Beijing 100050, China;* [c]*School of Pharmacy, Jinan University, Guangzhou 510632, China*

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Phytochemical investigation of 85% ethanol extracts from the roots of *Aristolochia fangchi* yielded a new aristolactam derivative named 6-methoxyl aristolactam I *N*-β-glucoside (**1**), together with four known compounds, aristolactam IVa (**2**), aristolactam I-β-D-glucoside (**3**), aristolactam I (**4**), and aristolactam-*N*-β-D-glucoside (**5**). Their structures were elucidated by spectral analysis. The cytotoxicity of the isolated compounds was also determined.

Keywords: Aristolochiaceae; *Aristolochia fangchi*; 6-methoxyl aristolactam I *N*-β-glucoside; cytotoxicity

1. Introduction

In recent years, much attention has been paid to the nephrotoxic and carcinogenic effects of aristolochic acid (AA) derivatives from medicinal plants. The toxic effects of AAs and aristololactams (ALs) have been reported during the past 20 years [1–5]. AAs have been found in the family Aristolochiaceae, whereas ALs have been isolated or detected from many other families, such as Annonaceae, Menispermaceae, Piperaceae, Monimiaceae, and Saururceae, except Aristolochiaceae [6–11]. The interest in phytochemical studies of the Aristolochiaceae family is due to the widespread use of its species in traditional Chinese medicine and homeopathy. Guang Fang Ji, the roots of *Aristolochia fangchi* Y. C. Wu ex L. D. Chou et S. M. Hwang (Aristolochiaceae), is distributed in many places of southeastern of China. For thousands of years it has been used in traditional Chinese medicine for the treatment of arthritis, rheumatism, and edema of the lower extremities. Several AAs and aristolactams have been isolated from this plant [12–14]. As part of an ongoing study on the chemical constituents and their toxicities [2,15], a new and four known aristolactam compounds were isolated from the roots of this plant. This paper deals with the structure determination of the new compound and the cytotoxicities of the obtained compounds.

2. Results and discussion

The air-dried and powdered roots of Guang Fang Ji were extracted with 85% ethanol, and the extracts were separated as described in the experimental section to yield compounds **1**–**5**. The four known compounds were identified as aristolactam IVa (**2**), aristolactam I-β-D-glucoside (**3**),

*Corresponding author. Email: lujunzhangpla@gmail.com

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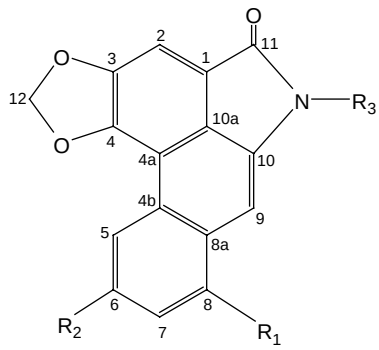

No.	R_1	R_2	R_3
1	OCH_3	OCH_3	Glc
2	OCH_3	OH	H
3	OCH_3	H	Glc
4	OCH_3	H	H
5	OCH_3	OH	Glc

Figure 1. Chemical structures of compounds **1**–**5**.

aristolactam I (**4**), and aristolactam *N*-β-D-glucoside (**5**) by comparing their physical and spectroscopic data with those of authentic samples and/or data reported in the literature [5,16,17] (Figure 1).

Compound **1** was obtained as a amorphous brownish powder. The HR-FAB-MS of **1** showed a quasi-molecular ion peak at *m/z* 486.1403 $[M + H]^+$, indicating a molecular formula of $C_{24}H_{23}NO_{10}$. The UV absorption maxima at 223, 241, 262, 338, and 412 nm were characteristics of a phenanthrene chromophore [5]. The IR absorption bands at 3439 and 1672 cm^{-1} indicated the presence of hydroxy and lactam carboxyl groups [18]. In the ^1H NMR spectrum of **1**, a set of meta-coupled aromatic protons appeared at δ 8.05 (1H, d, $J = 2.0$ Hz) and 6.79 (1H, d, $J = 2.0$ Hz), which were assigned to H-5 and H-7, respectively. A singlet signal at δ 7.63 (1H, s) could be assigned to H-2 as it showed heteronuclear multiple-bond correlation (HMBC) with the carbonyl signal at δ 168.9. Similarly, a methylenedioxy group at δ 6.46 (2H, s) could be positioned to C-3 at δ 149.5 and C-4 at δ 147.3 according to HMBC analysis. Another singlet signal at δ 7.28 (1H, s) could be assigned to H-9, which showed the typical higher chemical shift of AL [19]. Signals of two methoxyl groups appearing at δ 3.90 (3H, s) and δ 3.98 (3H, s) could be positioned to C-6 at δ 150.4 and C-8 at δ 158.5, respectively, according to HMBC analysis. On acid hydrolysis of **1**, glucose was detected from the aqueous fraction by co-TLC analysis (*n*-BuOH–AcOH–H_2O, 4:1:1) with authentic samples. This was confirmed by ^{13}C NMR spectrum, which exhibited signals for a glucose moiety at δ 82.5, 71.0, 78.1, 70.5, 80.8, and 61.9. In the HMBC spectrum, significant correlations were observed between H-1′ at δ 4.88 and C-11 at δ 168.9, C-10 at δ 134.2, suggesting that the sugar moiety was located at N [16]. Consequently, the structure of compound **1** was determined as 6-methoxyl aristolactam I *N*-β-D-glucoside (Figure 2).

From the results of cytotoxic activity testing (Table 3) using aristolochic acid A as positive control, compounds **1**–**5** exhibited moderate cytotoxic activities against LLC-PK_1 cells with IC_{50} values of 76, 72, 79, 75, and 83 μmol/l, respectively. The results are consistent with the published data [2,15].

3. Experimental

3.1 General experimental procedures

For UV detection, an Agilent 8453 UV-vis spectrophotometer (Agilent, San Diego, CA, USA) was used. The IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer (Vernon Hills, IL, USA). For FAB-MS and HR-FAB-MS analyses, a JOEL JMS-SX 102A mass spectrometer (JEOL Ltd, Tokyo, Japan) was used. For NMR studies, a Varian

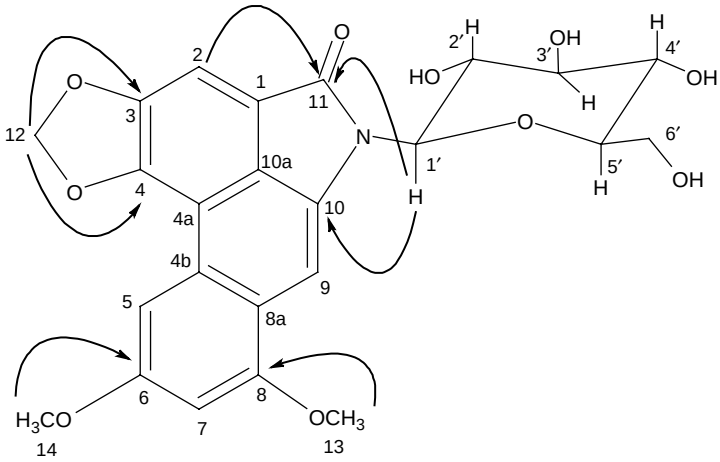

Figure 2. Key HMBC of compound **1**.

INOVA-500 Spectrometer (Varian NMR, Inc., Palo Alto, CA, USA) was used operating at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR including HMBC and nuclear overhauser enhancement spectroscopy (NOESY). Chemical shifts are given in δ relative to tetramethylsilane as internal standard. Silica gel (300–400 mesh, Qingdao Marine Chemical Co. Ltd, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Piscataway, NJ, USA) were used for column chromatography, and silica gel GF_{254} plates (Qingdao Marine Chemical Co., Ltd) were used for thin layer chromatography. For preparative HPLC, a Waters Prep Nova-Pak HR C-18 reverse-phase column (Milford, MA, USA) was used. For analytical HPLC (Waters Alliance 2690, 996 detector at wavelength 254 nm; Waters Separation Products) separation, an octadecylsilane column (Agilent C_{18} 150 mm × 4.6 mm I.D. 5μ, Agilent, Santa Clara, CA, USA) was used. LLC-PK_1 cells were obtained from Beijing NC, Inc. (Beijing, China), and dimethyl sulfoxide (DMSO) was obtained from Beijing Chemical Co. (Beijing, China).

3.2 Plant material

The roots of *A. fangchi* were collected at Shantou City, Guangdong Province, China, in September 2008, and identified by Dr Wei Feng. A voucher specimen (No. 2569-30) is deposited at the Herbal Museum of National Institute for the Control of Pharmaceutical and Biological Products, State Food and Drug Administration (Beijing, China).

3.3 Extraction and isolation

The powdered and air-dried roots (30 kg) were extracted three times with 85% EtOH under reflux for 2 h. After removal of the solvent *in vacuo*, the extract (2.9 kg) was further partitioned between H_2O and $CHCl_3$ to give H_2O-soluble and $CHCl_3$-soluble fractions. The $CHCl_3$-soluble (0.6 kg) fraction was subjected to column chromatography on silica gel and eluted with $CHCl_3$–MeOH (20:0, 18:1, 10:1, 8:1) to yield four subfractions. The $CHCl_3$–MeOH (18:1) subfraction (4.1 g) was purified by Sephadex LH-20 column with $CHCl_3$–MeOH (1:2) as eluent, then followed by preparative HPLC (75% MeOH; flow rate, 25 ml/min; detector, 254 nm; retention time, 28 min) to yield compound **4** (86 mg). The $CHCl_3$–MeOH (10:1)

subfraction (3.2 g) was purified by preparative HPLC (70% MeOH; flow rate, 25 ml/min; detector, 254 nm; retention time, 23 min) to yield compound **2** (62 mg). The $CHCl_3$–MeOH (8:1) subfraction (4.6 g) was purified by preparative HPLC [MeOH − 0.5% HOAc aq. (68:32, v/v); flow rate, 25 ml/min; detector, 254 nm; retention time, 15, 21, 29 min] to yield **5** (17 mg), **1** (19 mg), and **3** (56 mg), respectively. The purity of the compounds was analyzed by HPLC [MeCN–0.5%HOAc aq. (69:31, v/v); flow rate, 1.0 ml/min; detector, 254 nm; column temperature, 25°C].

*3.3.1 6-Methoxyl aristolactam I N-β-D-glucoside (**1**)*

Brownish powder, UV λ_{max} (MeOH) nm: 223, 241, 262, 338, 412; IR (KBr) ν_{max} cm^{-1}: 3439, 1672, 1364, 1272, 1187, 1046, 969, 676; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) spectral data: see Tables 1 and 2; HR-FAB-MS (positive mode) *m/z*: 486.1403 $[M + H]^+$ (calcd for $C_{24}H_{24}NO_{10}$, 486.1400).

Table 1. ^{1}H NMR spectral data for compound **1** (500 MHz in DMSO-d_6).

No	**1** (*J* in Hz)	No	**1** (*J* in Hz)
2	7.63 (1H, s)	1′	4.88 (1H, d, *J* = 7.5)
5-H	8.05 (1H, d. *J* = 2.0)	2′	4.43 (1H, m)
7-H	6.79 (1H, d. *J* = 2.0)	3′	4.18 (1H, m)
9-H	7.28 (1H, s)	4′	4.60 (1H, dd, *J* = 5.4, 7.0)
12-H	6.46 (2H, s)	5′	4.10 (1H, m)
13-H	3.90 (3H, s)	6′	4.48 (2H, m)
14-H	3.98 (3H, s)		

Table 2. ^{13}C NMR spectral data for compound **1** (125 MHz in DMSO-d_6).

No.	**1**	No.	**1**
1	120.4	10	134.2
2	105.6	10a	125.5
3	149.5	11	168.9
4	147.3	12	103.3
4a	111.4	13	57.8
4b	126.5	14	59.5
5	115.6	1′	82.5
6	150.4	2′	71.0
7	106.3	3′	78.1
8	158.5	4′	70.5
8a	120.3	5′	80.8
9	104.4	6′	61.9

Table 3. Cytotoxic potential of compounds **1**–**5** against LLC-PK_1 cells.

Compounds	IC_{50} (μmol/l)[a]
1	75
2	72
3	79
4	76
5	83
Doxorubicin (positive control)	2.3

[a] IC_{50} (μmol/l) was the content of compound that inhibited cell growth to 50%.

3.4 Acid hydrolysis of compound 1

Compound **1** (5 mg) was refluxed with 5% HCl in MeOH (5 ml) for 6 h. The mixture was diluted with H_2O and neutralized with $NaHCO_3$. The neutral hydrolysate revealed the presence of glucose by TLC (*n*-BuOH–AcOH–H_2O, 4:1:1) when compared with authentic samples.

3.5 Assay of cytotoxic activities against LLC-PK_1 cells

The isolated compounds **1**–**5** were evaluated for their cytotoxic activities against LLC-PK_1 cells using a method described in the literature [2,15]. Doxorubicin was used as the positive control and hederasaponin C was used as the negative control (Table 3). An IC_{50} value over 300 μmol/l was considered to indicate no cytotoxic activity against LLC-PK_1 cells.

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