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A novel alkaloid, aristopyridinone A and anti-inflammatory phenanthrenes isolated from *Aristolochia manshuriensis*

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ABSTRACT

A novel alkaloid, aristopyridinone A (**1**) and a new phenanthrene, aristolamide II (**2**), were isolated from *Aristolochia manshuriensis* (Guanmutong) together with eight known phenanthrenes (**3**–**10**). All structures were elucidated by spectroscopic methods. Compound **2** showed a selective inhibitory effect on elastase release by human neutrophils in response to fMLP with an IC_{50} value of 4.11 μg/mL. Compound **7** exhibited significant inhibitory effects on superoxide anion generation and elastase release with IC_{50} values of 0.12 and 0.20 μg/mL, respectively.

Aristolochia manshuriensis (Guanmutong), a perennial shrub belonging to the Aristolochiaceae family, is distributed throughout northeastern China and Korea.[1] *Aristolochia* species are traditional Chinese medicines (TCM) used as analgesic, antibacterial, anti-inflammatory, antitussive, and antiasthmatic agents as well as for the treatment of snake bites.[2] However, the major active constituents of *Aristolochia* species have been shown to be aristolochic acids, which induce nephrotoxic and carcinogenic effects due to the long period of human body metabolism.[3–7] Although the use of *Aristolochia* species leads to serious side effects, some TCM doctors still suggest that these natural medicines can be used in specific ways, including the ingestion of low doses over a short period of time and external use as anti-inflammation and antibacterial agents. Thus, their cytotoxicity must be explored.

During the screening of extracts of natural products to identify anti-inflammatory compounds, we found that the methanolic extract of *A. manshuriensis* was active against superoxide generation and elastase release by human neutrophils in response to fMLP. Further chemical investigation using bioassay-guided fractionation of *A. manshuriensis* extract led to the isolation of ten compounds, including a novel skeleton alkaloid, aristopyridinone A (**1**);[8] a new phenanthrene, aristolamide II (**2**);[9] and eight known compounds, aristolamide (**3**),[10] aristolochic acid I (**4**),[11] aristolochic acid-IVa (**5**),[12] aristolochic acid-IIIa (**6**),[11] aristolatams IIIa (**7**),[11] aristolochic acid-I methyl ester (**8**),[11] aristolic acid methyl ester (**9**),[13] and 6-methoxyaristolic acid methyl ester (**10**)[14] (Fig. 1).

The stems of *A. manshuriensis* (3.50 kg) were powdered and exhaustively extracted with MeOH (20 L × 5) at room temperature. The extract was filtered and concentrated to obtain the MeOH extract (297.80 g). The MeOH extract was partitioned with CH_2Cl_2 and *n*-BuOH, successively, yielding CH_2Cl_2- (104.60 g) and *n*-BuOH-soluble layers (43.80 g). The CH_2Cl_2-soluble layer displayed good anti-inflammatory effects. As indicated in prior studies,

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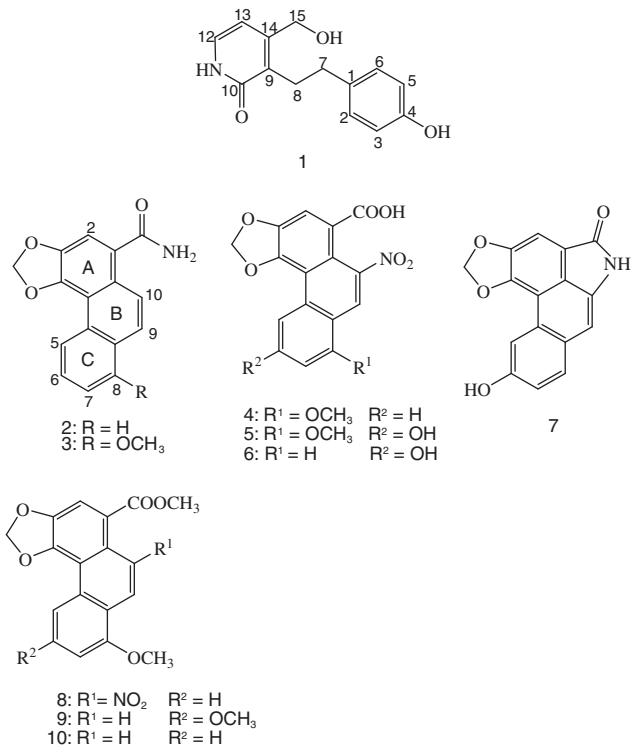

Figure 1. The structures of compounds **1**–**10** isolated from *A. manshuriensis*.

aristolochic acids are major components of *A. manshuriensis*. The use of herbal medicines containing aristolochic acids have been associated with severe nephrotoxicity, which are characterized by chronic renal failure, tubulointerstitial fibrosis, and development of urothelial cancer.[15,16] Unexpectedly, all layers did not show obvious cytotoxicity toward liver (HepG2, Hep3B), gingival (Ca9-22), lung (A549), and breast (MCF7, MDA-MB-231) cancer cell lines.

Therefore, the CH_2Cl_2 layer was selected to be chromatographically separated using a bioassay-guided fractionation method. The remaining residue after vacuum drying (104.60 g) was subjected to fractionation using Celite545 eluted with *n*-hexane, *n*-hexane–EtOAc (1:1), EtOAc and MeOH, successively, to give four fractions (H, HE, E, and M, respectively). The four fractions were passed through silica gel, eluted with a mixture of CH_2Cl_2–MeOH (90:10 v/v) and further purified by reversed-phase preparative HPLC on a Thermo ODS Hypersil column (10 × 250 mm, 5 μm). Aristolochic acid I (**4**, 380.20 mg), aristolochic acid-I methyl ester (**8**, 10.08 mg), aristolic acid methyl ester (**9**, 7.22 mg), and 6-methoxyaristolic acid methyl ester (**10**, 2.71 mg) were isolated from the H fraction using reversed-phase HPLC with acetonitrile and water (20:80 v/v) as the eluent. Aristopyridinone A (**1**, 2.16 mg), aristolamide II (**2**, 5.89 mg), aristolamide (**3**, 9.18 mg) and aristolatam IIIa (**7**, 5.87 mg) were obtained from the HE fraction using reversed-phase HPLC with acetonitrile and water (25:75 v/v) as the eluent. The M fraction was chromatographically separated using reversed-phase HPLC with acetonitrile and water (10:90 v/v) as the eluent, yielding aristolochic acid-IVa (**5**, 0.70 mg) and aristolochic acid-IIIa (**6**, 17.6 mg). All known compounds were identified by comparison to the reported spectroscopic data.

Aristopyridinone A (**1**) was obtained as a reddish amorphous powder, and its molecular formula, $C_{14}H_{15}NO_3$, was determined by the HRESI-MS peak at *m/z* 246.1131 $[M+Na]^+$ ($C_{14}H_{15}NO_3Na$, calcd for 246.1130). The IR spectrum of **1** revealed an amide absorption band at 1662 cm^{-1} and a hydroxyl group at 3325 cm^{-1}. In the ^1H NMR spectrum, compound **1** exhibited peaks characteristic of the presence of a typical *para*-substituted benzyl moiety with two equivalent pairs of *ortho*-coupled protons at 6.93 (d, *J* = 8.5 Hz, H-2, H-6) and 6.65 (d, *J* = 8.5 Hz, H-3, H-5) (Table 1). Additionally, ^1H NMR signals at 6.98 and 6.15 with a coupling constant of 4.0 Hz assigned to intramolecular olefinic protons neighboring a nitrogen atom. They were eventually determined to be H-12 and H-13 of the pyridin-2-one moiety. Two D_2O-exchangeable signals at δ_H 9.21 (s, 4-OH) and 5.25 (d, *J* = 5.5 Hz, 15-OH) indicated the presence of two hydroxyl groups. Fourteen carbon signals, including five quaternary, six methane, and three methylene carbons were observed in the ^{13}C NMR and DEPT spectra. In the HSQC experiment, three additional methylene proton signals at δ_H 4.38 (t, *J* = 7.5 Hz, H-8), 4.27 (d, *J* = 5.5 Hz, H-15), and 2.80 (t, *J* = 7.5 Hz, H-7) were observed and correlated with the corresponding carbon signals at δ_C 46.6 (C-8), 54.3 (C-15), and 35.8 (C-7), respectively. The ^1H–^1H COSY and HMBC correlations of compound **1** are shown in Figure 2. The HMBC correlations of H-7/C-2 and C-6; H-8/C-1, C-9 and C-14; H-12/C-9, C10, C13 and C-14; and H-15/C-13 and C-14 established the presence of a *para*-substituted benzyl moiety, a dimethylene bridge, a pyridin-2-one moiety, and a hydroxymethyl function, respectively. Based on a detailed literature search, we concluded that the structure of compound **1** possesses a novel skeleton, which was named aristopyridinone A.

Aristolamide II (**2**) was isolated as a white amorphous powder. The HRESI-MS of **2** displayed a pseudomolecular ion peak at *m/z* 266.0817 $[M+H]^+$, corresponding to the formula of $C_{16}H_{11}NO_3$ (calcd for 266.0818). The UV absorption maxima at 220, 258, 320, and 367 nm together with the IR absorption bands at 3182 (NH_2) and 1744 (C=O) cm^{-1} (the lack of a typical NO_2 band around 1550 and 1350 cm^{-1}) suggested that compound **2** is a denitroaristolochic acid derivative.[10] In the ^1H NMR spectrum, the aromatic region exhibited the presence of *ortho*-substituted aromatic signals at δ_H 9.01 (1H, m, H-5), 7.96 (1H, m, H-8) and 7.66 (2H, m, H-6,

Table 1
^1H (600 MHz) and ^{13}C NMR (150 MHz) data of compounds **1** and **2** in DMSO-d_6

Position	**1**		Position	**2**	
	δ_C	δ_H		δ_C	δ_H
1	128.0		1	129.5	
2,6	129.3	6.93 (d, 8.5)	2	108.9	7.78 (s)
3,5	114.7	6.65 (d, 8.5)	3	143.9	
4	155.5		4	144.2	
7	35.8	2.80 (t, 7.5)	4a	115.6	
8	46.6	4.38 (t, 7.5)	4b	126.6	
9	131.0		5	126.7	9.01 (m)
10	178.6		6,7	127.3	7.66 (m)
12	123.7	6.98 (d, 4.0)	8	127.9	7.96 (m)
13	109.0	6.15 (d, 4.0)	8a	131.5	
14	143.0		9	125.5	7.70 (d, 9.0)
15	54.3	4.27 (d, 5.5)	10	124.3	8.17 (d, 9.0)
4-OH		9.21 (s)	10a	125.2	
11-NH		9.47 (s)	C=O	170.1	
15-OH		5.25 (d, 5.5)	O–CH_2–O	101.9	6.39 (s)

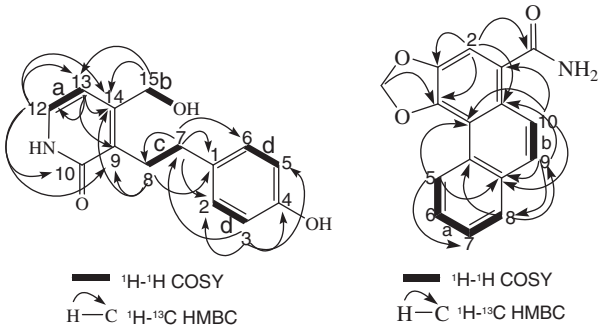

Figure 2. COSY and HMBC corrections of compounds **1** and **2**.

Table 2
Inhibitory effects of compounds from *A. manshuriensis* on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB

Compounds	IC_{50} (μg/mL)[a]	
	Superoxide anion	Elastase
1	N.D.[c]	N.D.[c]
2	>10	4.11 ± 0.06
3	>10	>10
4	>10	>10
5	5.78 ± 0.24	8.49 ± 1.20
6	>10	>10
7	0.12 ± 0.03	0.20 ± 0.44
8	>10	>10
9	>10	>10
10	>10	>10
Genistein[b]	0.54 ± 0.11	6.99 ± 1.62

[a] Concentration necessary for 50% inhibition (IC_{50}) at 10 μg/mL concentration. Results are presented as mean ± SEM ($n = 3$).
[b] Genistein as positive control.
[c] Not determined

H-7) on the C ring of phenanthrene (Table 1). In addition, an AB set of doublets at δ_H 8.17 (d, J = 9.0 Hz) and 7.70 (d, J = 9.0 Hz) was assigned to H-10 and H-9, respectively. Moreover, the signal of the aromatic proton at δ_H 7.78 (s) was assigned to H-2, and the methylene dioxy function at δ_H 6.39 (s) should fuse the C-3 and C-4 positions of A ring. Sixteen carbon signals consisting of eight quaternary carbons, seven aromatic methines, and one methylene were observed in the ^{13}C NMR and DEPT spectra. Additionally, the planar structure of compound **2** was determined by analyzing 2D NMR spectra. HMBC correlations of δ_H 7.78 (H-2) to C-3, C-4, C-10a and a carbonyl group at 170.1 further confirmed the positions of the substitutions of the A ring. On the basis of the aforementioned analysis, **2** is a new compound, which was named aristolamide II.

This is the first report of the inhibitory effect of this plant on superoxide anion generation and elastase release by human neutrophils in response to fMLP. The inhibitory effects of aristolamide II (**2**) and all known compounds (**3**–**10**) on superoxide anion generation and elastase release by human neutrophils in response to fMLP were measured (Table 2).[17] Unfortunately, the insufficient amount of compound **1** led the absence of bioactive data. Aristolamide II (**2**) exhibited a selective inhibitory effect on elastase release, with an IC_{50} value of 4.11 μg/mL. Compounds **5** and **7** showed good anti-inflammatory activity against superoxide anion generation and elastase release, with IC_{50} values of 5.78/8.49 and 0.12/0.20 μg/mL, respectively. Compound **7** was 4.5- and 35-fold more potent than genistein (the positive control) for superoxide anion generation and elastase release, respectively. Furthermore, the aristolochic acid alkyl esters of compounds **8**, **9**, and **10** displayed no effect on both inflammatory mediators.

Compounds **4**, **5**, **6**, and **8** are the major components of *A. manshuriensis* and belong to the class of aristolochic acid derivatives. They are regarded as toxic agents; however, they did not have primary contributions to anti-inflammatory activity observed in this study. We wish to propose that the NO_2 functional group be biologically or chemically degraded or removed from the total aristolochic acid containing extracts of plants such as *A. manshuriensis* (Guanmutong) or *Aristolochia fangchi* (Guangfangji), which may allow the use of these plants in TCM without toxicity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.067.

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9. Aristolamide II (**2**): white amorphous powder; mp: 282–284 °C; IR (Neat) ν_{max} 3349, 3182, 1744, 1643, 1512, 1477, 1256, 1030 cm^{-1}; UV (MeOH) λ_{max} nm (log ε): 220 (4.12), 258 (4.55), 282 (4.17), 320 (3.91), 349 (3.35), 367 (3.31); ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) are given in Table 1; ESI-MS m/z 266 $[M+H]^+$; HRESI-MS m/z 266.0817 $[M+H]^+$ (calcd for $C_{16}H_{11}NO_3$, 266.0818).
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