CHEMICAL CONSTITUENTS OF *Aristolochia manshuriensis*

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UDC 547.972

Aristolochia manshuriensis Kom., a climbing plant belonging to the Aristolochiaceae family, grows mainly in northeast of China. It has been used as a folk medicine in China for many years. As a diuretic and anti-inflammatory reagent, it was reported as *Guanmutong* in China pharmacopoeia in 1963 to cure edema and rheumatic pain [1]. The use of *Guanmutong* was banned in the mainland of China from 2003, mainly because of its nephrotoxicity caused by the aristolochic acids contained [2]. Pharmacological studies on aristolochic acids also revealed that they possess antitumor activity as well as enhance cytoimmunity and macrophagus function [3]. In order to find more bioactive compounds, the chemical constituents of this plant were investigated.

Dried and powdered stems of *Aristolochia manshuriensis* Kom. (5 kg) were extracted three times with 95% ethanol under reflux. The solutions were combined and concentrated to yield a crude extract. The residue was suspended in water and extracted successively with petroleum ether, ethyl acetate, and *n*-butanol. The ethyl acetate extract (100 g) was subjected to silica gel column chromatography (CC) using gradient mixtures of chloroform–methanol (100:0–0:100) as eluants to afford 10 fractions (Fr. 1–10). Fraction 1 was separated on silica gel CC with chloroform–methanol (100:0–10:1) as eluants to afford **9** (50 mg). Fraction 2 was resubjected to silica gel CC with chloroform–methanol (100:0–10:1) as eluants to afford **7** (150 mg) and **8** (100 mg). Fraction 3 was purified by recrystallization to yield **1** (30 g), and the solution was subjected to silica gel CC using mixtures of chloroform–methanol (100:0–0:100) to afford **2** (15 mg), **3** (12 mg), and **6** (10 mg). Fraction 4 was subjected to silica gel CC eluting with chloroform–methanol gradient (100:0–5:1) to afford **3** (12 mg) and **6** (10 mg). Fraction 5 was purified by recrystallization to yield **10** (20 mg). Fraction 6 was subjected to silica gel CC using mixtures of chloroform–methanol (100:0–2:1) as eluants to afford **11** (15 mg).

Their structures were identified to be aristolochic acid I (**1**) [4], aristolochic acid II (**2**) [4], aristolic acid I (**3**) [5], aristolochic acid Ia (**4**) [4], aristolochic acid IIa (**5**) [6], aristolic acid II (**6**) [4], *p*-hydroxycinnamic acid (**7**) [7], ferulic acid (**8**) [8], lignoceric acid (**9**) [9], vanillic acid (**10**) [10], and syringic acid (**11**) [11] on the basis of spectral data as well as by comparison with literature. Compounds **1**, **2**, **4**–**7**, **10**, and **11** were found before in this plant, and compounds **3**, **8**, and **9** were isolated from this plant for the first time.

Aristolic Acid I (3). Yellow needles, mp 270–272°C. IR (KBr, ν_{max}, cm^{-1}): 3100–2510 (COOH), 1660 (C=O), 1600, 1500 (Ar), 1053 (C-O-C), 960 (OCH_2O). ESI-MS (*m/z*): 311 $[M]^+$. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.32 (1H, s, COOH), 8.79 (1H, d, J = 9.8, H-9), 8.65 (1H, d, J = 8.0, H-5), 8.06 (1H, d, J = 9.8, H-10), 7.88 (1H, s, H-2), 7.62 (1H, t, J = 8.0, H-6), 7.25 (1H, d, J = 8.0, H-7), 6.47 (2H, s, OCH_2O), 4.05 (3H, s, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 168.4 (COOH), 154.8 (C-8), 146.7 (C-4), 144.4 (C-3), 128.6 (C-10a), 128.3 (C-4b), 127.2 (C-6), 123.1 (C-10), 121.9 (C-1), 121.9 (C-8a), 120.1 (C-9), 119.0 (C-5), 115.8 (C-4a), 112.1 (C-2), 107.3 (C-7), 102.4 (OCH_2O), 55.8 (OCH_3).

Ferulic Acid (8). Colorless needles, mp 170–172°C. ^1H NMR (300 MHz, acetone-d_6, δ, ppm, J/Hz): 10.50 (1H, br.s, COOH), 8.15 (1H, s, OH), 7.47 (1H, d, J = 15.9, H-3), 7.32 (1H, d, J = 2.0, H-2′), 7.12 (1H, dd, J = 2.0, 8.4, H-6′), 6.86 (1H, d, J = 8.4, H-5′), 6.34 (1H, d, J = 15.9, H-2), 3.90 (3H, s, -OCH_3). ^{13}C NMR (100 MHz, acetone-d_6, δ): 168.0 (COOH), 149.0 (C-4′), 147.9 (C-3′), 144.4 (C-3), 125.7 (C-1′), 122.7 (C-6′), 115.6 (C-5′), 115.5 (C-2′), 111.2 (C-2), 55.7 (-OCH_3).

Lignoceric Acid (9). White amorphous powder, mp 86–88°C. IR (KBr, ν_{max}, cm^{-1}): 2910, 2834, 1716, 1458, 1398, 1361, 1260, 789, 730. EI-MS (*m/z*): 368 $[M]^+$, 354, 340, 325, 311. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.36 (2H, t, J = 7.5, H-2), 1.63 (2H, m, H-23), 1.21–1.30 (40H, br.s), 0.89 (3H, t, J = 6.4, H-24). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 179.6 (C-1), 22.7–33.9 (22C), 14.2 (C-24).

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Published in Khimiya Prirodnykh Soedinenii, No. 6, p. 815, November–December, 2010. Original article submitted June 6, 2009.

0009-3130/11/4606-0957

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