Two Rare Hydroazulene-type Sesquiterpenes from the Roots of *Aristolochia yunnanensis*

Zhuo Du[a], Feng-Ni Wen[b], Ji-Ping Zhang[c], Jian-Feng Wu[a], Fang Liu[a], Xue-Qin Wan[a], Zhong Li[d], and Bin Han[d]

[a] Pharmacy Department, Medical School, Foshan University, Foshan 528000, P. R. China
[b] The 5th People's Hospital of Foshan City, Foshan 528211, P. R. China
[c] The 2nd People's Hospital of Foshan, Foshan 528000, P. R. China
[d] The School of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou 510006, P. R. China

Reprint requests to Prof. Feng-Ni Wen. Fax: +86757-86882001. E-mail: wfniys@126.com or wfniys@163.com

Z. Naturforsch. **2014**, *69b*, 742 – 746 / DOI: 10.5560/ZNB.2014-4059
Received March 6, 2014

Two new sesquiterpenes, named aristoyunnolin I (**1**) and J (**2**), together with eight known compounds (**3** – **10**) were isolated from the roots of *Aristolochia yunnanensis*. Compounds **1** and **2** feature a rare hydroazulene-type sesquiterpene skeleton and represent the third and fourth examples of this kind found in nature. The structures were determined from spectroscopic data, and the absolute configurations of **1** – **3** were assigned by comparing experimental with simulated electronic circular dichroism (ECD) spectra. Compounds **1**, **2**, **6** – **10** were isolated from this plant for the first time. The cytotoxic activities of **1** – **10** were evaluated against P-388 and A-549 cell lines. Only compounds **4** and **5** showed moderate activity with IC_{50} values ranging from 12.0 to 18.2 μM.

Key words: *Aristolochia yunnanensis*, Aristoyunnolin, Hydroazulene-type Sesquiterpene, Cytotoxic Activities

Introduction

Aristolochia yunnanensis (*Aristolochia griffithii*) (Aristolochiaceae), endemic to Yunnan Province of China, is known as "Nan Mu Xiang" in Traditional Chinese medicine (TCM) for the treatment of trichomoniasis, gastrointestinal diseases and rheumatic pain [1]. Previous chemical investigation of this plant led to the isolation of sesquiterpenes which exhibited selective inhibition of the phosphorylation of extracellular signal-regulated kinases (ERK1/2) [2]. As part of our systematic investigations on chemical and bioactive constituents of TCM plants, we carried out extensive chemical studies on the roots of *Aristolochia yunnanensis*, and obtained two rare hydroazulene-type sesquiterpenes [3], together with eight known compounds (Fig. 1). The cytotoxic activities of all the isolated compounds were evaluated against P-388 and A-549 cell lines. Only compounds **4** and **5** showed moderate activity with IC_{50} values ranging from 12.0 to 18.2 μM. Herein, the details of the isolation, structure elucidation, and cytotoxic activities of these compounds are described.

Results and Discussion

Compound **1**, a colorless powder, had a molecular formula of $C_{15}H_{20}O_3$ as determined by HREIMS at $m/z = 248.1409$ $[M]^+$ (calcd. 248.1407). The IR spectrum exhibited the absorption bands for lactone (1746 cm^{-1}) functionalities. The ^1H NMR (Table 1) spectrum showed two methyl singlets [$\delta_H = 1.20$ (H_3-14) and 1.75 ppm (H_3-12)], a terminal double bond [$\delta_H = 4.86$ (1H, s, H-13a) and 4.91 ppm (1H, s, H-13b)], an oxymethine [$\delta_H = 4.78$ ppm (1H, d, $J =$ 8.7, H-6)] and a series of aliphatic methylene multiplets. The ^{13}C NMR spectrum, in combination with DEPT experiments, resolved 15 carbon resonances attributable to a ketone group ($\delta_C = 219.1$ ppm), a lactone group ($\delta_C = 178.7$ ppm), one sp^2 quaternary carbon, one sp^2 methylene, two methyls, two sp^3 methines (one bearing oxygen), five sp^3 methylenes, and

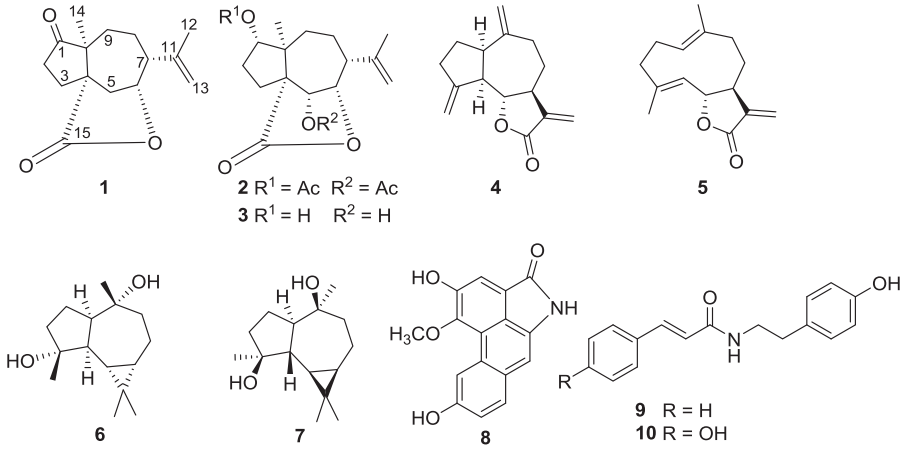

Fig. 1. The structures of **1** – **10** isolated from *Aristolochia yunnanensis*.

Table 1. NMR data for aristoyunnolin I (**1**) and aristoyunnolin J (**2**) (δ in ppm)[a].

No.	**1**[b]		**1**[c]		**2**[c]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		219.1		219.4	4.87 (dd, 9.7, 8.4)	81.7
2α	2.48 (ddd, 19.0, 9.3, 9.3)	34.9	2.56 (m)	34.6	1.70 (m)	26.3
2β	2.28 (m)		2.25 (m)		2.10 (m)	
3α	2.59 (ddd, 12.5, 12.5, 9.3)	29.6	2.59 (m)	29.3	2.23 (ddd, 14.8, 12.2, 4.2)	23.1
3β	1.75 (m)		1.84 (m)		1.83 (ddd, 14.7, 9.5, 6.7)	
4		51.6		51.4		54.9
5	α 2.40 (d, 13.2)	38.1	2.35 (m)	38.5	5.20 (s)	76.6
	β 2.20 (m)		2.26 (m)			
6	4.78 (d, 8.7)	79.3	4.77 (d, 8.3)	79.2	4.51 (s)	84.9
7	2.13 (dd, 11.2, 3.8)	52.3	2.14 (dd, 10.2, 4.5)	52.7	2.40 (dd, 9.6, 5.3)	49.9
8α	1.66 (m)	22.7	1.72 (m)	22.3	1.60 (m)	21.6
8β	1.60 (m)		1.68 (m)			
9α	1.83 (m)	33.0	1.86 (m)	32.8	1.73 (m)	35.8
9β	1.75 (m)		1.65 (m)		1.60 (m)	
10		54.7		54.7		48.8
11		147.0		146.1		145.3
12	1.75 (s)	22.0	1.79 (s)	21.8	1.77 (s)	21.8
13a	4.91 (s)	112.0	4.84 (s)	112.2	4.84 (s)	112.5
13b	4.86 (s)		4.84 (s)			
14		178.7		178.4		177.9
15	1.20 (s)	20.0	1.14 (s)	19.5	1.07 (s)	16.2
1-OAc					2.06 (s)	21.0, 170.5
6-OAc					2.11 (s)	20.8, 170.1

[a] ^{1}H and ^{13}C NMR were recorded at 400 and 100 MHz, respectively; [b] in [D$_5$]pyridine, signals of the ^{1}H NMR spectrum of **1** in $CDCl_3$ overlapped seriously; [c] in $CDCl_3$.

two sp^3 quaternary carbons. As three of the six degrees of unsaturation were consumed by a double bond and two carbonyls, the remaining three degrees of unsaturation required that **1** was tricyclic. The above-mentioned data were similar to those of the co-isolated analog versicolactone C (**3**) [2, 4], the first example of a hydroazulene-type sesquiterpene, except for the presence of a ketone group and one more methylene in **1** instead of the two oxymethines in **3**. HMBC correlations (Fig. 2) from CH_3-14, H_2-3, and H_2-9 to the carbonyl ($\delta_C = 219.5$ ppm) revealed that the ketone group was located at C-1. The ^{1}H-^{1}H COSY correlation (Fig. 2) from H-5 to H-6 and the HMBC correlation from H-5 to C-15 revealed that the oxymethine at C-5 in **3** was

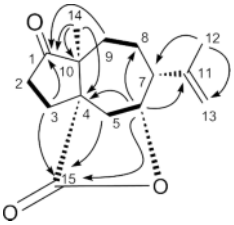

Fig. 2. Selected ^{1}H-^{1}H COSY (–) and HMBC (→) correlations of **1**.

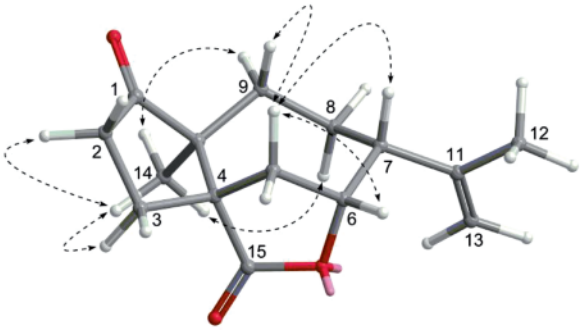

Fig. 3. Key NOE correlations (←- - -→) of **1**.

replaced by a methylene in **2**. Detailed 2D analyses (HSQC, ^{1}H-^{1}H COSY, and HMBC) further confirmed the planar structure of **1**. The relative configuration of **1** was established by analysis of the NOESY correlations (measured in [D_5]pyridine). The interactions of H-5 with H-6, H-7, and H-9β indicated that these protons were cofacial, and therefore they were tentatively assigned β-oriented. The interactions of CH_3-14 with H-2α, H-3α, H-8α, and H-9α indicated that H_3-14 was α-oriented (Fig. 3). The above correlations led to the conclusion that the skeletal rings were *cis*-fused. On the basis of the Beecham rule [5], the absolute configuration at C-4 was determined to be *S* by the negative Cotton effect at 225 nm in the electronic circular dichroism (ECD) spectrum, which was confirmed by the calculated ECD spectrum of **1** at the B3LYP/6-311++G(2d,2p)//B3LYP/6-31+G(d) level (Fig. 4). Thus, the absolute configurations of **1** at C-4, C-6, C-7, and C-10 were determined to be 4*S*, 6*R*, 7*R*, and 10*S*, respectively. Hydroazulene-type sesquiterpenes are very rare in nature, only two analogs [2, 4] have been reported before. Compound **1** is the third example of hydroazulene-type sesquiterpene found in nature and was named aristoyunnolin I.

Compound **2**, a colorless oil, had a molecular formula of $C_{19}H_{26}O_6$ as determined by HRESIMS at

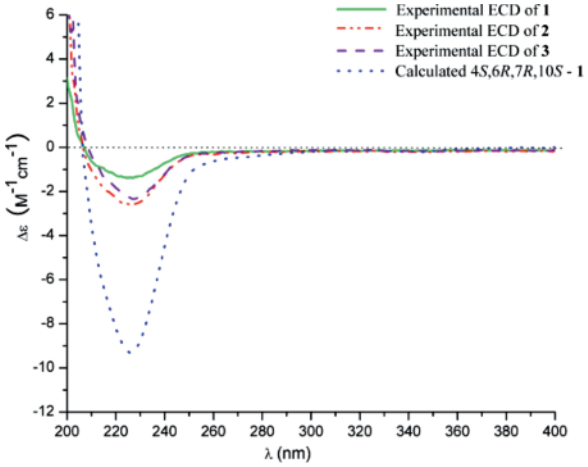

Fig. 4. ECD curves of **1**, **2** and **3** and the calculated ECD spectrum of the 4*S*,6*R*,7*R*,10*S* isomer of **1**.

$m/z = 351.1810$ $[M+H]^+$ (calcd. 351.1808). The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were very similar to those of **3** except for the presence of the signals of two acetyl groups [$\delta_H = 2.11$ (s), $\delta_C = 20.8$ and 170.1 ppm; $\delta_H = 2.06$ (s), $\delta_C = 21.0$ and 170.5 ppm], indicating that **2** was a fully acetylated derivative of **3**. The planar structure was confirmed by further analyses of its 2D NMR data. The relative configuration of **2** was assigned to be the same as that of **3** by comparing the NMR data and by the analysis of its NOESY spectrum. Finally, the chemical transformation of **3** to **2** by acetylation confirmed the structure of **2** as depicted in Fig. 1. The ECD curve of **2** matched well that of **1** and **3** (Fig. 4), indicating a 4*R* configuration (inverted by 5-OAc), and the absolute configurations at the other sites were determined to be 1*S*, 5*R*, 6*S*, 7*R*, and 10*S*, respectively. Thus, **2** was given the trivial name aristoyunnolin J and was the fourth hydroazulene-type sesquiterpene found in nature.

The known compounds versicolactone C [4] (**3**), dehydrocostuslactone [6] (**4**), costunolide [6] (**5**), aromadendrane-4β,10β-diol [7] (**6**), (–)-alloaromadendrane-4β,10β-diol [8] (**7**), 2,9-dihydroxy-1-methoxy-dibenz[*cd*,*f*]indol-4(5*H*)-one, [9] (**8**), cinnamoyltyramine [10] (**9**), and *trans*-*N*-*p*-coumaroyltyramine [11] (**10**), were identified by comparison of their NMR data with those in the literature.

The cytotoxic activities of all isolated compounds (**1**–**10**) against P-388 murine leukemia and A-549 human lung carcinoma cell lines were evaluated. Only compounds **4** and **5** showed moderate cytotoxic activi-

ties against the P-388 and A-549 cell line (IC_{50} values: 15.3 and 12.0 μM for **4**; 18.2 and 16.4 μM for **5**, respectively), while the other compounds were inactive against both the P-388 and A-549 cell lines.

Experimental Section

General methods

Optical rotations were measured on a Rudolph Autopol I automatic polarimeter. IR spectra were determined on a Bruker Tensor 37 infrared spectrophotometer. NMR spectra were measured on a Bruker AM-400 spectrometer at 25 °C. EIMS and HREIMS (70 eV) were recorded on a Finnigan MAT 95 mass spectrometer. ESIMS was measured on a Finnigan LCQ Deca instrument, and HRESIMS was performed on a Waters Micromass Q-TOF instrument. Silica gel (300 – 400 mesh, Qingdao Haiyang Chemical Co., Ltd.), C_{18} reversed-phase silica gel (12 nm, S-50 μm, YMC Co., Ltd.), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography. All solvents were of analytical grade (Guangzhou Chemical Reagents Company, Ltd.).

Plant material

Roots of *A. yunnanensis* were collected in October 2012 from Yunnan Province, P. R. China, and were identified by Prof. You-Kai Xu of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (accession number: Ari*st*yun201207) has been deposited at the Pharmacy Department of Foshan University.

Extraction and isolation

The air-dried powder of the roots of *A. yunnanensis* (1 kg) was extracted with 95% EtOH (3 × 3 L) at r. t. to give 60 g of crude extract. The extract was suspended in H_2O (1 L) and successively partitioned with petroleum ether (3 × 0.5 L) and EtOAc (3 × 0.5 L). The EtOAc extract (28 g) was subjected to MCI gel column chromatography eluted with a MeOH-H_2O gradient (2 : 8 → 10 : 0) to afford five fractions (Fr. I-V). Fr. I (4.3 g) was chromatographed over a C_{18} reversed-phase (RP-18) silica gel column eluting with MeOH-H_2O (4 : 6 → 10 : 0) to afford four fractions (Fr. Ia–Id). Fr. Id (0.8 g) was separated by silica gel column chromatography (petroleum ether-acetone, 3 : 1), followed by Sephadex LH-20 using ethanol as eluent to give **3** (16 mg). Fr. III (3 g) was subjected to silica gel column chromatography (petroleum ether-EtOAc, 5 : 1 → 1 : 1) to give three fractions (Fr. IIIa–IIIc). Fr. IIIb (220 mg) was subjected to RP-18 silica gel column chromatography (MeOH-H_2O, 6 : 4 → 10 : 0), followed by silica gel column chromatography (petroleum ether-acetone, 10 : 1 → 1 : 1) to afford **6** (23 mg), **7** (7 mg) and **10** (10 mg). Fr. IIIc (80 mg) was separated by silica gel column chromatography (petroleum ether-EtOAc, 50 : 1 → 30 : 1) to give **8** (8 mg). Fr. IV (1.8 g) was subjected to silica gel column chromatography (petroleum ether-EtOAc, 50 : 1 → 1 : 1) to give four fractions (fr. IVa-IVd). Fr. IVb (840 mg) was separated by silica gel column chromatography (petroleum ether-EtOAc, 50 : 1 → 20 : 1) to give **1** (4 mg), **2** (6 mg), **4** (20 mg), and **5** (52 mg). Fr. IVc (70 mg) was applied to a silica gel column (CH_2Cl_2-acetone, 100 : 1 → 30 : 1) to yield **9** (8 mg).

*Aristoyunnolin I (**1**)*

Colorless powder. – $[\alpha]_D^{20} = -13.6$ ($c = 0.10$, $CHCl_3$). – IR (KBr): $\nu_{max} = 2922, 1746, 1464, 1363, 1207, 1183, 957, 894\ cm^{-1}$. – ^1H and ^{13}C NMR data: see Table 1. – MS (EI, 70 eV): $m/z(\%) = 248\ [M]^+$ (100). – HRMS (EI, 70 eV): $m/z = 248.1409$ (calcd. 248.1407 for $C_{15}H_{20}O_3$, $[M]^+$).

*Aristoyunnolin J (**2**)*

Colorless oil. – $[\alpha]_D^{20} = -28.6$ ($c = 0.10$, $CHCl_3$). – IR (KBr): $\nu_{max} = 2928, 1753, 1466, 1372, 1210, 1180, 953, 899\ cm^{-1}$. – ^1H and ^{13}C NMR data: see Table 1. – MS ((+)-ESI): $m/z = 351.2\ [M+H]^+$, $373.2\ [M+Na]^+$. – HRMS ((+)-ESI): $m/z = 351.1810$ (calcd. 351.1808 for $C_{19}H_{27}O_6$, $[M+H]^+$).

*Chemical transformation of **3** to **2***

100 μL acetic anhydride was added to a stirred solution of compound **3** (2 mg) in pyridine (1 mL). The mixture was stirred at r. t. for 2 h and then evaporated under vacuum. The residue was purified by Sephadex LH-20 chromatography eluted with ethanol to afford **2** (1.8 mg), which was identified by ^1H NMR spectroscopy and $[\alpha]_D^{20}$.

Cytotoxicity assays

Cytotoxicity against P-388 and A-549 cell lines was evaluated by using the MTT [12] and SRB [13] methods, respectively, according to the protocols described in the literature and with pseudolaric acid B [14] as a positive control ($IC_{50} = 0.85$ μM against P-388 and 0.42 μM against A-549).

Supporting information

1D-NMR and 2D-NMR data of aristoyunnolin I (**1**) and aristoyunnolin J (**2**) are given as Supporting Information available online (DOI: 10.5560/ZNB.2014-4059).

Acknowledgement

This work was supported by the Guangdong Natural Science Foundation (S2012040007298), the Foundation for Distinguished Young Talents in Higher Education of Guangdong (2012LYM_0130) and the Medical Scientific Research Foundation of Guangdong Province (A2013688).

[1] L. R. Song, *Chinese Materia Medica* (*Zhonghua Ben-Cao*), Vol. 8, Shanghai Science and Technology Press, Shanghai **1999**, pp. 486 – 487.
[2] Z.-B. Cheng, W.-W. Shao, Y.-N. Liu, Q. Liao, T.-T. Lin, X.-Y. Shen, S. Yin, *J. Nat. Prod.* **2013**, *76*, 664 – 671.
[3] T.-S. Wu, A. G. Damu, C.-R. Su, P.-C. Kuo, *Nat. Prod. Rep.* **2004**, *21*, 594 – 624.
[4] J. Zhang, L. He, *Acta Pharm. Sin.* **1986**, *21*, 273 – 278.
[5] A. F. Beecham, *Tetrahedron Lett.* **1968**, *32*, 3591 – 3594.
[6] C. Wei Ming, R. Mayer, H. Zimmermann, G. Rücker, *Phytochemistry* **1989**, *28*, 3233 – 3234.
[7] T.-S. Wu, Y.-Y. Chan, Y.-L. Leu, *Chem. Pharm. Bull.* **2000**, *48*, 357 – 361.
[8] Z.-H. Sun, C.-Q. Hu, J.-Y. Wang, *Chin. J. Chem.* **2008**, *26*, 831 – 834.
[9] V. R. Hegde, S. Borges, M. Patel, P. R. Das, B. Wu, V. P. Gullo, T.-M. Chan, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1344 – 1346.
[10] Y. Yang, Z.-G. Song, Z.-Q. Liu, *Free Radical Res.* **2011**, *45*, 445 – 453.
[11] L. Zhang, B. Bai, X. Liu, Y. Wang, M. Li, D. Zhao, *Food Chem.* **2011**, *126*, 203 – 206.
[12] M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, M. R. Boyd, *Cancer Res.* **1988**, *48*, 589 – 601.
[13] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107 – 1112.
[14] D. J. Pan, Z. L. Li, C. Q. Hu, K. Chen, J. J. Chang, K. H. Lee, *Planta Med.* **1990**, *56*, 383 – 385.