Chemical constituents of *Arisaema franchetianum* tubers

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A novel pyrrolidine alkaloid, \((2R\ast*,3S\ast*,5S\ast*)-N,2-dimethyl-3-hydroxy-5-(10-phenyldecyl)pyrrolidine (1)\), and 17 known compounds were isolated from *Arisaema franchetianum* Engl. (Araceae) tubers. The 17 compounds were bergenin (2), emodin (3), caffeic acid (4), nobiletin (5), 3-O-β-D-galactopyranosyl-hederagenin 28-O-β-D-xylopyranosyl(1→6)-β-D-galactopyranosyl ester (6), coniferin (7), qingyangshenogenin (8), methylconiferin (9), syringaresinol 4-O-β-D-glucopyranoside (10), gagaminine (11), perlyrline (12), (S)-1-(1’-hydroxyethyl)-β-carbone (13), 1-(β-carbone-1-yl)-3,4,5-trihydroxy-1-pentane (14), 1-methoxy carbonyl-β-carbone (15), indolo[2,3-α]carbazo (16), 4-hydroxycinnamic acid methyl ester (17), and methyl 4-[2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-1-(hydroxymethyl)ethyl] ferulate (18). The inhibitory activities of compound 1 and its N-methyl derivative (1a) against porcine respiratory and reproductive syndrome virus (PRRSV), human leukemic K562 cells, and human breast cancer MCF-7 cells were evaluated. Compounds 1 \([\text{IC}_{50} = 12.5 \pm 0.6 \, \mu\text{M}]\) and 1a \([\text{IC}_{50} = 15.7 \pm 0.9 \, \mu\text{M}]\) were cytotoxic against K562 cells. Compound 1a also had a weak effect on PRRSV with an \(\text{IC}_{50}\) value of 31.9 \pm 6.0 \, \mu\text{M} [selectivity index (SI) = 18.7].

**Keywords:** Araceae; *Arisaema franchetianum*; alkaloids; C\(_{21}\) steroids; porcine respiratory and reproductive syndrome virus; cytotoxicity

1. Introduction

*Arisaema*, a large genus in the Araceae family, comprises about 150 species worldwide, with almost 100 species found in China [1]. The chemical constituents of *Arisaema* are mainly alkaloids and acylglycerylglycosides [2–6]. *Arisaema franchetianum* Engl., an herbaceous plant, has been used for a long time in Chinese folk medicine as an anti-inflammatory agent and for the treatment of snake bites. It is distributed in the Chinese provinces of Yunnan, Sichuan, Guizhou, and Guangxi [7]. The chemical constituents of the plant have not yet been reported. In a previous study [4], we isolated a cytotoxic piperidine alkaloid from *A. decipiens* Schott, and its N-methyl derivative showed potential inhibitory activity against the MCF-7 cell line and weak inhibitory activity against K562. In the present research, a new pyrrolidine alkaloid \((2R\ast*,3S\ast*,5S\ast*)-N,2-dimethyl-3-hydroxy-5-(10-phenyldecyl)pyrrolidine (1)\) was isolated from *A. franchetianum* tubers, along with 17 known compounds.

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The structural elucidation of 1, as well as the bioassay results of 1 and its N-methyl derivative (1a), is reported.

2. Results and discussion

Compound 1 was isolated as a colorless oil with the molecular formula of C_{22}H_{37}NO, which indicates five degrees of unsaturation, as determined by HR-ESI-MS at m/z 332.2945 [M + H]⁺. The IR spectrum of 1 revealed the absorption bands for its hydroxy (3406 cm⁻¹) and aromatic (1631, 1496, and 1454 cm⁻¹) groups. The ¹H NMR spectrum of 1 (Table 1) showed signals of a monosubstituted phenyl ring [δ_H 7.18 (3H, m) and 7.28 (2H, m)], one N-methyl group [δ_H 2.21 (3H, s)], and one methyl group [δ_H 1.14 (3H, d, J = 6.4 Hz)]. The NMR data of 1 are similar to those of the known pyrrolidine alkaloid irmine [8]. Based on ¹H-¹H COSY correlations (Figure 2), the fragment from 2-Me, C-2–C-5, to C-1 was confirmed. A polysubstituted pyrrolidine in 1 was supported by the HMBCs from 2-Me, C-2–C-5, to C-1. The length of the carbon chain was deduced as C_{10} based on the molecular formula of 1 (Figure 1). Accordingly, the planar structure of 1 was elucidated as N,2-dimethyl-3-hydroxy-5-(10-phenylethyl)pyrrolidine.

The relative configuration of 1 was deduced from its ROESY data (Figure 2). The key correlations of 2-Me/H-3 indicated that these hydrogen atoms are cofacial and arbitrarily assigned as β-oriented, whereas H-2 was α-oriented. H-5 was determined as α-oriented by the ROESY correlations of H-2/H-5. Finally, compound 1 was determined to be (2R*,3S*,5S*)-N,2-dimethyl-3-hydroxy-5-(10-phenylethyl)pyrrolidine.

Compounds 2–18 were identified as bergenin (2) [9], emodin (3) [10], caffeic acid (4) [11], nobiletin (5) [12], 3-O-β-D-galactopyranosyl-hederagenin 28-O-β-D-xylopyranosyl(1→6)-β-D-galactopyranosyl ester (6) [13], coniferin (7) [14], qingyangshengenin (8) [15], methylconiferin (9) [14], syringaresinol 4’-O-β-D-glucopyranoside (10) [16], gagamine (11) [17], perlolyrine (12) [18], (S)-1-(1’-hydroxyethyl)-β-carboline (13) [19], 1-(β-carboline-1-yl)-3,4,5-trihydroxy-1-pentanone (14) [20], 1-methoxycarbonyl-β-carboline (15) [21], indolo[2,3-α]carbazole (16) [22], 4-hydroxycinnamic acid methyl ester (17) [23], and methyl 4-[2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-1-(hydroxymethyl)ethyl] ferulate (18) [24], respectively, by comparing their spectroscopic and physical data with those reported in literature.

We previously isolated a piperidine alkaloid from A. decipiens Schott and found that its N-methyl derivative is more cytotoxic to the MCF-7 and K562 cell lines than the original compound [4]. Therefore, the N-methyl derivative (1a) of 1 was prepared and their biological activities were evaluated (Figure 1). Table 2 shows that compounds 1 (IC₅₀ = 12.5 ± 0.6 μM) and 1a (IC₅₀ = 15.7 ± 0.9 μM) were cytotoxic against K562 cells. Compound 1a also showed weak effects on porcine respiratory and reproductive syndrome virus (PRRSV) with an IC₅₀ of 31.9 ± 6.0 μM (SI = 18.7).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter (JASCO Corporation, Tokyo, Japan). UV spectra were determined on a Shimadzu double-beam 210A spectrometer (Shimadzu Corporation, Shimadzu, Japan). IR spectra were measured on a Bio-Rad FTS-135 infrared spectrophotometer with KBr disks (Shimadzu Corporation, Shimadzu, Japan). 1D and 2D NMR spectra were obtained on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard.
Table 1. 

<table>
<thead>
<tr>
<th>Position</th>
<th>Compound 1</th>
<th>Compound 1a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta_C$</td>
<td>$\delta_H$ ($J$ in Hz)</td>
</tr>
<tr>
<td>2</td>
<td>66.7</td>
<td>2.11 (1H, qd, $J = 6.4, 4.3$)</td>
</tr>
<tr>
<td>3</td>
<td>72.7</td>
<td>3.93 (1H, ddd, $J = 7.8, 4.3, 1.7$)</td>
</tr>
<tr>
<td>4</td>
<td>40.2</td>
<td>2.34 (1H, ddd, $J = 14.2, 8.5, 7.8$); 1.39 (1H, ddd, $J = 14.2, 7.5, 1.7$)</td>
</tr>
<tr>
<td>5</td>
<td>65.7</td>
<td>2.04 (1H, m)</td>
</tr>
<tr>
<td>1$'$</td>
<td>34.5</td>
<td>1.71 (1H, m) 1.28 (1H, m)</td>
</tr>
<tr>
<td>2$'$</td>
<td>26.4</td>
<td>1.31 (2H, m)</td>
</tr>
<tr>
<td>3$'$–8$'$</td>
<td>29.5–29.9</td>
<td>1.27 (12H, m)</td>
</tr>
<tr>
<td>9$'$</td>
<td>31.5</td>
<td>1.61 (2H, m)</td>
</tr>
<tr>
<td>10$'$</td>
<td>36.0</td>
<td>2.60 (2H, t, $J = 7.7$)</td>
</tr>
<tr>
<td>1$''$</td>
<td>142.9</td>
<td>142.9 C</td>
</tr>
<tr>
<td>2$''$, 6$'$</td>
<td>128.4</td>
<td>7.18 (2H, m)</td>
</tr>
<tr>
<td>3$''$, 5$'$</td>
<td>128.2</td>
<td>7.28 (2H, m)</td>
</tr>
<tr>
<td>4$''$</td>
<td>125.5</td>
<td>7.18 (1H, m)</td>
</tr>
<tr>
<td>$N$-Me</td>
<td>38.1</td>
<td>2.21 (3H, s)</td>
</tr>
<tr>
<td>2-Me</td>
<td>12.8</td>
<td>1.14 (3H, d, $J = 6.4$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(Bruker Daltonics Inc., Billerica, MA, USA). MS analyses were performed on a VG Auto Spec-3000 mass spectrometer (Shanghai Inteli-test Instrument Co., Ltd, Shanghai, China). Silica gel G (80–100 and 300–400 mesh, Qingdao Makall Group Co., Ltd, Qingdao, China), RP18 silica gel (40–75 µm, Fuji Silysia Chemical Ltd, Kasugai, Japan), silica gel H (10–40 µm), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography (CC), and silica gel GF254 (Qingdao Makall Group Co., Ltd, Qingdao, China) for preparative thin layer chromatography (TLC) as precoated plates. The TLC spots were visualized under UV light and by dipping into 5% H2SO4 in alcohol, followed by heating.

3.2 Plant material

The tubers of A. franchetianum were collected from Songming County of Yunnan Province, China, in June 2010. The plant material was identified by Dr. Guang-Wan Hu, and a voucher specimen (No. SM0101) is deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The tubers of A. franchetianum (17.5 kg) were crushed and extracted with MeOH (4, 3, and 3 h, respectively) at 70°C. The extracts were dissolved in water and adjusted pH to about 1 with 1% HCl and then partitioned with AcOEt (310.0 g, A). The pH of water-soluble part was adjusted to about 10 with 5% NaOH and extracted by CHCl3 (5.2 g, B) and n-butanol (53.0 g, C), respectively.

Fr. B was partitioned by CC (C-18, MeOH/H2O, 10:90 → 95:5) to give 44 fractions (B1-B44). Fr. B15 (161.1 mg) was fractionated by CC (Sephadex LH-20, MeOH) and prep. TLC (CHCl3/MeOH, 5:1; CHCl3/MeOH, 20:1; CHCl3/MeOH, 10:1) to give 14 (9.7 mg), 17 (8.8 mg), and 18 (6.8 mg), respectively. Fr. B17 (265.2 mg) was fractionated by CC (Sephadex LH-20, MeOH) and prep. TLC (CHCl3/MeOH, 10:1) to give 13 (4.3 mg), 15 (6.2 mg), and 16 (2.7 mg). Fr. B22 (154.5 mg) was fractionated by CC (Sephadex LH-20, MeOH) to give 12 (15.8 mg). Fr. B30 (686.2 mg) was separated by CC (Sephadex LH-20, MeOH; silica gel, CHCl3/MeOH/Et2NH, 50:1:0.5) to give 1 (21.0 mg).

Figure 1. Structures of compounds 1 and 1a.

Figure 2. Key 2D NMR correlations of compound 1.
Fr. A was isolated by CC (silica gel, CHCl₃/MeOH, 1:0 → 0:1) to give six fractions (A₁–A₆). Fr. A₂ (117.0 g) was separated by CC (silica gel, petroleum ether/Me₂CO, 1:0 → 0:1) to give Fr. A₂₁–A₂₃. Fr. A₂₁ (4.6 g) was purified by CC (C-18, MeOH/H₂O, 75:25; Sephadex LH-20, MeOH) to give 3 (39.0 mg). Fr. A₂₂ (6.0 g) was purified to give 4 (C-18, MeOH/H₂O, 40:60; prep. TLC, CHCl₃/MeOH, 10:1; 6.2 mg) and 5 (C-18, MeOH/H₂O, 70:30; prep. TLC, CHCl₃/MeOH 15:1; 38.0 mg). Fr. A₂₃ (10.9 g) was purified to give 8 (C-18, MeOH/H₂O, 50:50; Sephadex LH-20, MeOH; CHCl₃/MeOH, 30:1; 17.1 mg) and 11 (C-18, MeOH/H₂O, 70:30; prep. TLC, CHCl₃/MeOH, 8:1; 7.8 mg). Fr. A₃ (40.0 g) was isolated by CC (C-18, MeOH/H₂O, 10:90 → 95:5) to give Fr. A₃₁ (C-18, MeOH/H₂O, 45:55) and Fr. A₃₂ (C-18, MeOH/H₂O, 70:30). Fr. A₃₁ (293.5 mg) was fractionated by CC (Sephadex LH-20, MeOH) and then recrystallized from MeOH to give 1 (9.1 mg). Fr. A₃₂ (1.7 g) was purified by prep. TLC (CHCl₃/MeOH, 4:1) to give 1 (53.9 mg).

Fr. C was isolated by CC (C-18, MeOH/H₂O, 10:90 → 95:5) to give Fr. C₁ (C-18, MeOH/H₂O, 20:80), C₂ (C-18, MeOH/H₂O, 50:50), and C₃ (C-18, MeOH/H₂O, 80:20). Fr. C₁ (727.2 mg) was fractionated by CC (Sephadex LH-20, MeOH; silica gel, CHCl₃/MeOH, 5:1) to give 7 (9.8 mg). Fr. C₂ (1.0 g) was fractionated by CC (Sephadex LH-20, MeOH; silica gel, AcOEt/MeOH, 25:1) to give 9 (17.1 mg) and 10 (6.0 mg). Fr. C₃ (2.3 g) was fractionated by CC (Sephadex LH-20, MeOH) and prep. TLC (n-butanol/AcOEt/H₂O, 4:1:5) to give 6 (41.9 mg).

3.3.1 (2R*,3S*,5S*)-N,2-dimethyl-3-hydroxy-5-(10-phenyldecyl)pyrrolidine (1)

A colorless oil; [α]D²²⁺ + 3.1 (c 0.25, MeOH); UV (MeOH) λmax (log ε) 267 (2.67) nm. IR (KBr) νmax 3406, 3026, 2924, 2853, 1631, 1496, and 1454 cm⁻¹. ¹H and ¹³C NMR spectral data (see Table 1). ESI-MS (positive): m/z 332 [M + H]⁺; HR-ESI-MS: m/z 332.2945 [M + H]⁺ (calcld for C₂₂H₃₈NO, 332.2953).

3.3.2 N-methylation of 1

A solution of 1 (10.0 mg, 0.302 mmol), MeI (0.1 ml), and K₂CO₃ (10.0 mg) in dry acetone (5 ml) was refluxed for 4 h and then concentrated under reduced pressure. The residue was dissolved in MeOH and purified by LH-20 CC (MeOH) to yield 1a (9.0 mg, 0.190 mmol, 62.9%) as a pale yellow solid. 1a: ¹H and ¹³C NMR spectral data (see Table 1). ESI-MS (positive): m/z 346 [M⁺].

3.4 Inhibitory activity against PRRSV

The following antiviral method has been described in our recently published paper [25].

<table>
<thead>
<tr>
<th>Compound</th>
<th>PRRSVᵃ</th>
<th>K562ᵇ</th>
<th>MCF-7ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC₅₀ (µM)</td>
<td>IC₅₀ (µM)</td>
<td>SI</td>
</tr>
<tr>
<td>1</td>
<td>NT</td>
<td>&gt;200</td>
<td>NT</td>
</tr>
<tr>
<td>1a</td>
<td>595.0 ± 7.7</td>
<td>31.9 ± 6.0</td>
<td>18.7</td>
</tr>
<tr>
<td>Positive control</td>
<td>855.4 ± 74.9</td>
<td>225.1 ± 27.4</td>
<td>3.8</td>
</tr>
</tbody>
</table>

ᵃ Positive control: tilmicosin phosphate.
ᵇ Positive control: adriamycin.
ᶜ NT, not tested.

Table 2. The inhibitory effects of 1 and 1a on PRRSV, K562 cells, and MCF-7 cells (means ± SD, n = 3).
3.4.1 Cell culture and cytotoxicity assays
Marc-145 cells (Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were grown in monolayer cultures in 5% carbon dioxide and 95% atmosphere at 37°C, with Dulbecco’s modified Eagle medium (DMEM; Hy-Clone) containing 10% fetal bovine serum (HyClone), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cytotoxicity assays were performed by WST-8 method using Cell Counting Kit-8 (CCK-8; Beyotime Co., Jiangsu, China) according to the supplier’s recommendations. Cells were incubated in a 96-well microculture plate (Corning, New York, USA) in the absence or presence of twofold serial dilutions of compound 1 and tilmicosin phosphate (Hubei Hengshuo Chemical Co. Ltd., China). After 3 days of culture, 10 μl of CCK-8 solution was added, and the cells were incubated for 1.5 h. The number of surviving cells was measured with a Bio-Tek ELx 800 enzyme-linked immunosorbent assay microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) at a detection wavelength of 450 nm (L1) and a reference wavelength of 650 nm (L2). The 50% cytotoxic concentration (CC50) was obtained by nonlinear regression analysis of logistic curves (the value of L1−L2 vs. different concentrations of the test compound).

3.4.2 Cytopathic effect inhibition assay
The YN-1 strain of PRRSV was isolated from local pigs in Yunnan Province, China. The antiviral activity of the test compounds against viruses was measured by the cytopathologic effect (CPE) inhibition assay. The tissue culture medium infective dose (TCID50) of 500 viral particles with twofold serial dilutions of the compounds was added to each test well, and the plates were reincubated for 4 days to allow the development of any CPE. A non-infected control that contained no natural products was prepared, and tilmicosin phosphate was used for drug control. The concentration reducing CPE by 50% with respect to the virus control was estimated from graphic plots and defined as IC50. SI was calculated from the ratio CC50/IC50.

3.5 Cytotoxic activity against K562 and MCF-7 cells
The cytotoxic activity against K562 and MCF-7 cells was measured by the methyl thiazolyl tetrazolium [26] and sulforhodamine B methods [27], respectively, according to previously described procedures.

Acknowledgments
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References


