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Virosaines A and B, Two New Birdcage-Shaped *Securinega* Alkaloids with an Unprecedented Skeleton from *Flueggea virosa*

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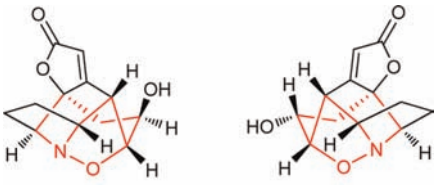
ABSTRACT



Two new *Securinega* alkaloids, virosaines A (1) and B (2), were isolated from the twigs and leaves of *Flueggea virosa*. The structures and absolute configurations were elucidated by means of NMR, X-ray diffraction, and CD analyses. Compounds 1 and 2 represent the first examples of *Securinega* alkaloids bearing a 7-oxa-1-azabicyclo[3.2.1]octane ring system, whose plausible biogenetic pathways were also proposed.

Indolizidine alkaloids were divided into simple indolizidine and *Securinega* alkaloids. The latter mostly occur in the plants of Euphorbiaceae,[1] which present a unique tetracyclic skeleton with an α,β-unsaturated γ-lactone ring. The complex ring system and diversity of stereostructure as well as significant biological effects on the central nervous system of *Securinega* alkaloids has attracted great interest as challenging targets for total synthesis and biogenetic studies.[2−4] The plant *Flueggea virosa* (Roxb. ex Willd.) Voigt is widely distributed in southern China, which is used for the treatment of eczema, allergic dermatitis, and scald in Chinese folk medicine.[5] A number of *Securinega* alkaloids have been isolated from this plant in previous phytochemical investigations, some of which showed significant biological activities on cytotoxicity and the central nervous system.[6−13]

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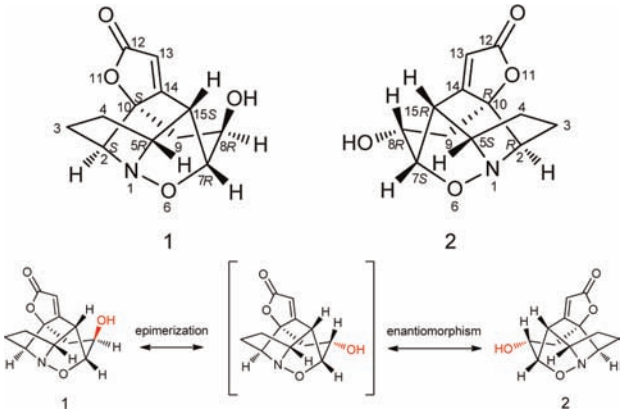
In a search for structurally unique and biologically active constituents from Chinese herbal medicines,[14,15] our group had reported the isolation of several new indolizidine alkaloids from the plants of genus *Flueggea*.[11−13] In our continuing investigation, two new *Securinega* alkaloids with an unprecedented skeleton, virosaines A (**1**) and B (**2**), were isolated from the twigs and leaves of *F. virosa*. Interestingly, **2** is an enantiomer of the epimer of **1**. We report herein the structure elucidation of **1** and **2**. In this letter, the plausible biogenetic pathways and cytotoxic activities of **1** and **2** are also discussed.



The air-dried and powdered twigs and leaves (50 kg) of *F. virosa* were percolated with EtOH/H_2O (95:5, v/v) three times at room temperature. The combined EtOH solution was concentrated under vacuum to yield a residue (7.5 kg). The crude extract was dissolved in H_2O and adjusted to pH 3 with 10% HCl. After removal of the neutral components by using $CHCl_3$ as a solution, the acidic suspension was then basified using NH_4OH to pH 9 and re-extracted with $CHCl_3$ to afford a total alkaloid fraction (450 g). Subsequently, the alkaloid fraction was subjected to column chromatographies over silica gel and preparative HPLC to yield **1** (6.5 mg) and **2** (7.3 mg).

Virosaine A (**1**) was obtained as colorless needles, mp 177−178 °C, $[\alpha]_{20}^{D}$ −51.6° (c = 0.50, CH_3OH). The molecular formula of **1** was established as $C_{12}H_{13}NO_4$ by its HR-ESI-MS (m/z 258.0736 $[M + Na]^+$, calcd for $C_{12}H_{13}NO_4Na$: 258.0737). The UV absorption maximum at 238 nm and IR bands at 3422, 1725, 1647 cm^{-1} implied the presence of an α,β-unsaturated γ-lactone ring and a hydroxyl group. The NMR spectra revealed that **1** possessed 12 carbons including an α,β-unsaturated γ-lactone ring [δ_H 5.84 (1H, br s); δ_C 175.5, 171.9, 110.8, and 85.4]. The above spectral data suggested that **1** could be a norsecurinine-type alkaloid. With the aid of $^1H-^1H$ COSY, HSQC, and HMBC experiments, the ^1H and ^{13}C NMR signals of **1** were assigned as shown in Table 1.

Table 1. NMR Data of **1** and **2** (in CD_3OD, J in Hz)[a]

	1		**2**	
no.	δ_H	δ_C	δ_H	δ_C
2	3.88	74.1	3.92	74.1
3	α 1.20 (m) β 1.70 (m)	22.1	α 1.24 (m) β 1.77 (m)	22.4
4	α 1.48 (m) β 1.91 (m)	20.8	α 1.44 (m) β 1.91 (m)	20.9
5	4.01 (m)	70.9	3.93	70.3
7	4.67 (ddd, 5.7, 4.8, 0.9)	88.0	4.58 (ddd, 5.7, 0.9, 0.9)	90.2
8	4.29 (dd, 5.7, 5.7)	65.4	3.78 (ddd, 8.4, 8.4, 0.9)	67.5
9	a 2.92 (dd, 14.1, 5.7) b 1.83 (dd, 14.1, 0.9)	44.7	a 2.43 (dd, 12.9, 8.4) b 2.16 (ddd, 12.9, 8.4, 0.9)	41.0
10	–	85.4	–	85.5
12	–	175.5	–	174.9
13	5.84 (br s)	110.8	5.89 (br s)	112.4
14	–	171.9	–	169.8
15	3.89	50.2	3.86 (dd, 5.7, 5.1)	50.3

[a] Overlapped signals were reported without designating multiplicity.

The $^1H-^1H$ COSY spectrum of **1** revealed the presence of a spin system (C-2 to C-9) (Figure 1). In the HMBC spectrum, the correlations between H-2 and C-4/C-5/C-9/C-14, between H-5 and C-7/C-14, between H-8 and C-10/C-15, and between H-15 and C-4/C-13 allowed the assignment of the planar structure of **1** (Figure 1). Furthermore, according to the molecular formula information and the obvious downfield shift at C-7, the remaining oxygen atom could be assigned to bridge C-7 and N-1 to form a 7-oxa-1-azabicyclo[3.2.1]octane ring system composed of a hexahydro-1,2-oxazepine ring (ring B) and an isoxazolidine ring (ring E) (Figure 1).

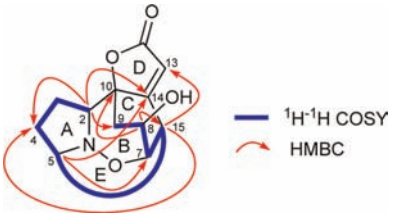


Figure 1. Key $^1H-^1H$ COSY and HMBC correlations of **1**.

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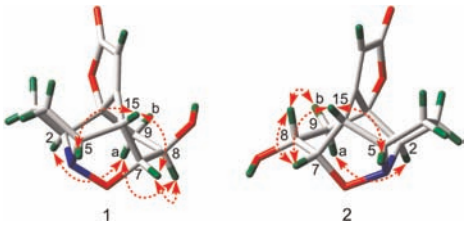


Figure 2. Key ROESY correlations of **1** and **2**.

The relative stereochemistry of **1** could be deduced by a ROESY experiment. Thus, the NOE correlations between H-9a and H-2/H-8, between H-7 and H-8/H-15, and between H-15 and H-5/H-7 established the relative configuration of **1** as shown in Figure 2. Finally, suitable crystals for an X-ray diffraction experiment were obtained, and the complete structure and stereochemistry were established (Figure 3). The final refinement on the Cu Kα data resulted in a small Flack parameter of 0.01 (16), allowing the unambiguous assignment of the absolute configuration of **1** as 2*S*, 5*R*, 7*R*, 8*R*, 10*S*, and 15*S*.[16] Compound **1** showed a birdcage-shaped structure through the X-ray stereoscopic view.

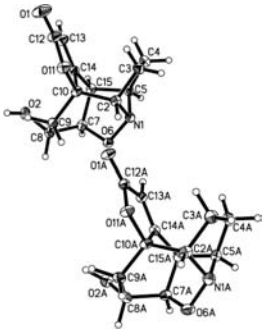


Figure 3. X-ray structure of **1**.

Virosaine B (**2**) was isolated as colorless oil. Compound **2** displayed the same molecular formula $C_{12}H_{13}NO_4$ as **1** by its HR-ESI-MS. The UV absorption maximum at 238 nm and IR bands at 1726, 1648 cm^{-1} implied the presence of an α,β-unsaturated γ-lactone ring. The NMR data of **2** (Table 1) were very similar to those of **1** except for the signals assigned to C-7, C-8, and C-9, indicating that **2** might be a C-8 epimer of **1**.

The relative stereochemistry of **2** could be elucidated by a ROESY experiment. The NOE correlations between H-2 and H-9a, between H-8 and H-7/H-9b, and between H-15 and H-5/H-7 were observed, which estabilshed the relative stereostructure of **2** (Figure 2). In order to furher confirm the relative configuration of **2**, a computational modeling study was performed by Sybyl 8.0 software. In an energy minimized conformation of **2**, the dihedral angles between H-8 and H-9a as well as between H-8 and H-9b were respectively assigned to 149.5° and 31.5°, which were consistent with the observed coupling constants ($J_{8,9a}$ = 8.4 Hz, $J_{8,9b}$ = 8.4 Hz). Furthermore, the theoretical computation of ^{13}C NMR data was performed using Gaussian 09 software. According to the result (Figure 4), the relative stereostructure of **2** was unambiguously determined due to the minor relative chemical shift errors (within 4 ppm).

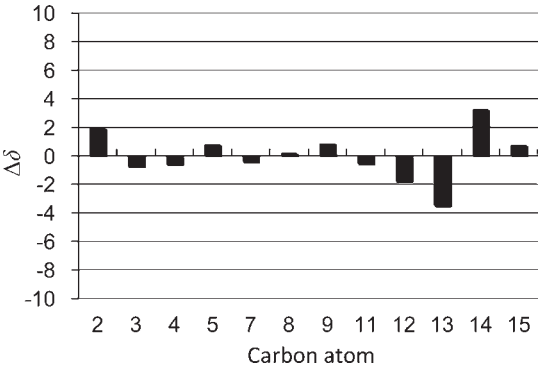


Figure 4. Relative errors between the calculated ^{13}C chemical shifts and recorded ones of **2**.

The quantum chemical CD calculation method was used to establish the absolute configuration of **2**. The overall predicted CD spectra of (2*R*, 5*S*, 7*S*, 8*R*, 10*R*, 15*R*)-**2** and (2*S*, 5*R*, 7*R*, 8*S*, 10*S*, 15*S*)-**2** were subsequently compared with the experimental one (Figure 5). The calculated CD curve of (2*R*, 5*S*, 7*S*, 8*R*, 10*R*, 15*R*)-**2** revealed a good agreement with the measured one. Thus, the absolute structure of **2** was determined as 2*R*, 5*S*, 7*S*, 8*R*, 10*R*, and 15*R*. In addition, the CD spectrum of **1** was also analyzed. Interestingly, the CD spectra of **1** and **2** displayed similar signal intensities but opposite Cotton

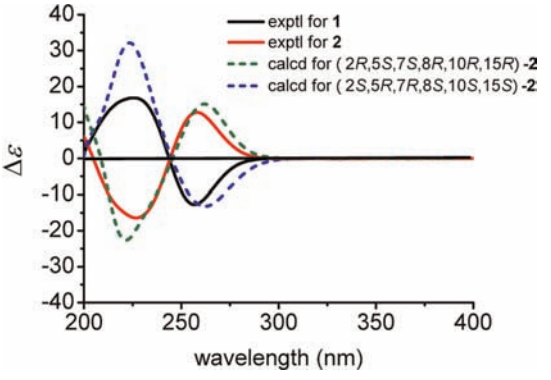
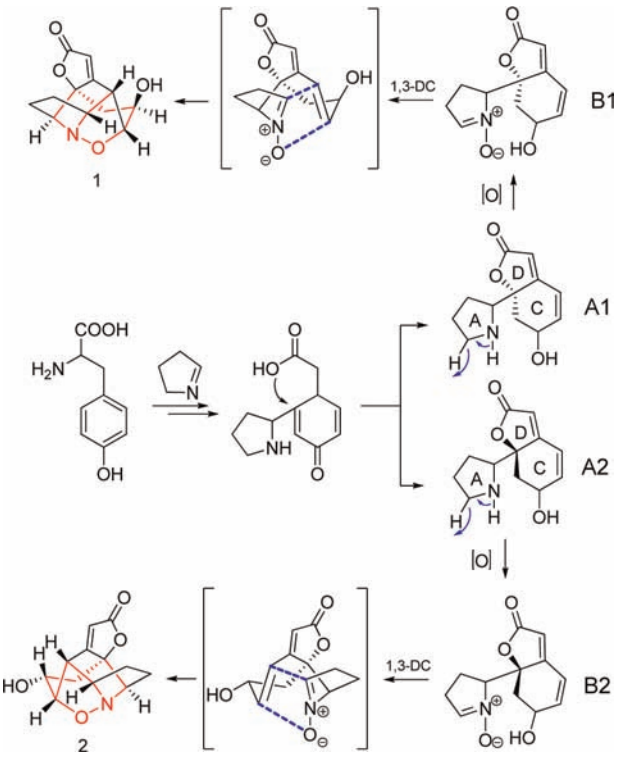


Figure 5. Calculated and experimental CD spectra of **2** as well as CD spectrum of **1**.

(16) Crystal data of compound **1** was deposited with the Cambridge Crystallographic Data Centre (CCDC 878240).

Scheme 1. Plausible Biogenetic Routes of **1** and **2**



effects, which further confirmed the stereochemical relationship between **1** and **2**.

Compounds **1** and **2** represent the first examples of *Securinega* alkaloids with a 7-oxa-1-azabicyclo[3.2.1] octane ring system. The biogenetic routes of **1** and **2** (Scheme 1) could be plausibly traced back to tyrosine and ornithine,[17,18] which would be initially transformed into a bicyclic intermediate. The route would be continued by an intramolecular 1,4-addition to form an α,β-unsaturated γ-lactone ring, furnishing the tricyclic precursors **A1** and **A2** with different configurations. Then, the *N*-lone-pair electrons in **A1** and **A2** were oxidated to form nitrones **B1** and **B2**, which trigger an intramolecular 1,3-dipolar cycloaddition (1,3-DC) reaction to form **1** and **2**, respectively.[19–21]

The inhibitory effects of **1** and **2** on the viability of several cancer cells were determined by MTT assay. Neither **1** nor **2** showed cytotoxic activity against MCF-7, MDA-MB-231, HepG2, HepG2/ADM, HL-60, K562, and Hep2 cells. In the previous paper,[13] we evaluated the growth inhibitory effect of two new dimeric indolizidine alkaloids, flueggines A and B, against three human breast cancer lines. As a result, flueggine B (with two indolizidine rings) showed potent cytotoxic activity, and flueggine A (only with one indolizidine ring) had weaker cytotoxic activity. In our present investigation, compounds **1** and **2** (with a rearranged *Securinega* alkaloid skeleton) had no cytotoxic activity. Based on these findings,[9,22–24] we supposed that the indolizidine ring of *Securinega* alkaloids is essential for their cytotoxicities. Nevertheless, the structure–activity relationship still needs to be further studied.

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Supporting Information Available. Detailed description of the experimental procedure, a listing of UV, IR, HR-ESI-MS, and NMR spectra, CIF files and bioassay data of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.