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# Flueggines A and B, Two New Dimeric Indolizidine Alkaloids from *Flueggea virosa*

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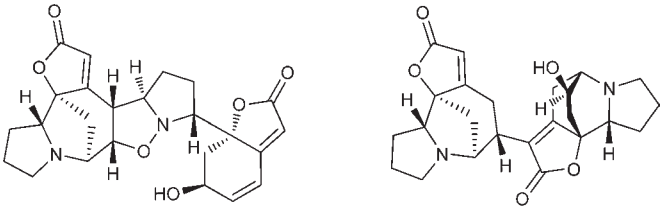
## ABSTRACT



1 2

**Two unprecedented C,C-linked dimeric indolizidine alkaloids, flueggines 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, single-crystal X-ray diffraction, and CD analyses. Compound 1 is the first example of *Securinega* alkaloids bearing an isoxazolidine ring, the plausible biogenetic pathway of which is also proposed. Compound 2 exhibited growth inhibitory activity against MCF-7 and MDA-MB-231 human breast cancer cells.**

The plant *Flueggea virosa* (Roxb. ex Willd.) Voigt is widely distributed in the southern part of China. The twigs and leaves of this plant are used in traditional Chinese medicine for the treatment of eczema, allergic dermatitis, and scald.[1] Phytochemical investigation on this plant has let to the isolation of a number of indolizidine alkaloids, known as *Securinega* alkaloids, some of which showed biological effects on the central nervous system and cytotoxicity.[2−8] Securinine, a typical *Securinega* alkaloid, which was isolated from plants of the *Securinega* genus, has been clinically used for the treatment of postpolio sequelae, prosopoplegia, and aplastic anemia as a selective antagonist of $GABA_A$ receptor.[9−12] The diverse bioactivities and unique skeletal structures of these alkaloids have presented challenges to chemists as novel targets for chemical synthesis.[13−15]

During the course of our search for structurally unique and biologically interesting constituents of natural

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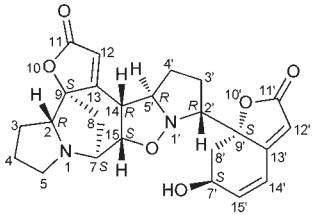
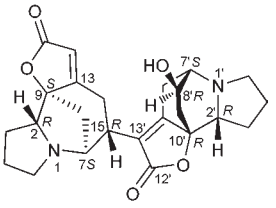
medicines,[16,17] our group has reported the isolation of new indolizidine alkaloids from the *Flueggea* genus.[7,8] Our further investigation on *F. virosa* now led to the isolation of two novel dimeric indolizidine alkaloids possessing unusual skeletons, named as flueggines A (**1**) and B (**2**). Herein, we describe the isolation and structural elucidation of **1** and **2**. In addition, a plausible biogenetic pathway of **1** is proposed.



1



2

The powder of the air-dried twigs and leaves (50 kg) of *F. virosa* was percolated with 95% EtOH at room temperature to afford 7.5 kg of crude extract, which was suspended in $H_2O$ and acidified with 10% HCl to pH 3. The acidic suspension was partitioned with $CHCl_3$ to remove the neutral components. The aqueous layer was then basified with $NH_4OH$ to pH 9 and re-extracted with $CHCl_3$ to obtain a total alkaloid fraction (450 g). The alkaloid fraction was purified repeatedly by column chromatography over silica gel, ODS, and preparative HPLC to yield compounds **1** (7.5 mg) and **2** (6.3 mg).

Flueggine A (**1**) was obtained as colorless needles: mp 221−222 °C, $[\alpha]^{20}_D$ −31.9 ($c$ = 0.25, $CH_3OH$). The molecular formula of **1** was established as $C_{24}H_{26}N_2O_6$ by its HR-ESI-MS ($m/z$ 439.1866 $[M + H]^+$, calcd for $C_{24}H_{27}N_2O_6$ 439.1864). The UV absorption maximum at 253 nm and IR band at 1756 $cm^{-1}$ implied the presence of an α,β-unsaturated γ-lactone ring. The analysis of NMR spectra revealed that **1** possessed 24 carbons, including two α,β-unsaturated γ-lactone rings [$\delta_H$ 5.78 (1H, br s) and 5.77 (1H, br s); $\delta_C$ 172.9, 172.0, 171.5, 166.8, 112.5, 112.0, 91.7 and 85.0] and a double bond [$\delta_H$ 6.50 (1H, d, $J$ = 9.6 Hz) and 6.21 (1H, dd, $J$ = 9.6, 4.8 Hz); $\delta_C$ 138.1 and 120.4]. The above spectral data were similar to those of norsecurinine,[2] suggesting that **1** could be a dimeric 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.

The $^1H-^1H$ COSY data of **1** revealed the presence of three spin systems (C-2 to C-5, C-8 to C-2′, and C-8′ to C-14′). In the HMBC spectrum, correlations between H-2 and C-4/C-5/C-7/C-8/C-13, between H-14 and C-7/C-9/C-12, as well as between H-15 and C-8/C-13 allowed the establishment of the planar structure of **1a**, which was similar to dihydronorsecurinine[18] except that the methylenes at C-14 and C-15 in dihydronorsecurinine were replaced by methines in **1a**. Similarly, the HMBC correlations between H-2′ and C-5′/C-8′/C-13′ and between H-14′ and C-7′/C-9′/C-12′ verified the planar structure of **1b**. Moreover, the $^1H$ and $^{13}C$ NMR signals of **1b** were similar to those of norsecurinine,[2] except that the methylene at C-5′ had become a methine, the N-1′−C-7′ bond was broken, and a hydroxyl group was attached to C-7′. The HMBC correlations between H-14 and C-4′ as well as between H-15 and C-5′ indicated that **1a** and **1b** were connected via C-14−C-5′ bonding. Furthermore, according to the molecular formula information and the obvious downfield shift at C-15, the remaining oxygen atom could be assigned as a

**Table 1.** NMR Data of **1** and **2** (in $CDCl_3$, $J$ in Hz)

| | **1** | | **2** | |
|---|---|---|---|---|
| no. | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | 3.15 (dd, 8.4, 6.6) | 65.5 | 3.21 (t, 7.2) | 66.4 |
| 3 | α 1.75 (m) | 29.1 | α 1.75 (m) | 29.0 |
| | β 1.94 (m) | | β 1.92 (m) | |
| 4 | α 1.98 (m) | 26.6 | α 1.99 (m) | 26.5 |
| | β 1.68 (m) | | β 1.66 (m) | |
| 5 | α 2.63 (m) | 57.2 | α 2.67 (m) | 57.4 |
| | β 3.35 (m) | | β 3.41 (m) | |
| 7 | 3.24 (m) | 60.7 | 3.47 (m) | 63.9 |
| 8 | a 2.32 | 29.5 | a 2.40 | 31.6 |
| | (dd, 11.2, 4.8) | | (dd, 11.4, 6.0) | |
| | b 1.80 (d, 11.2) | | b 1.20 (d, 11.4) | |
| 9 | | 91.7 | | 92.1 |
| 11 | | 171.5 | | 172.6 |
| 12 | 5.78 (br s) | 112.0 | 5.72 (d, 2.4) | 110.4 |
| 13 | | 172.9 | | 174.1 |
| 14 | 3.33 (dd, 8.8, 4.8) | 49.1 | α 3.10 | 25.5 |
| | | | (ddd, 17.4, 8.4, 2.4) | |
| | | | β 2.96 | |
| 15 | 4.30 (t, 4.8) | 76.3 | 3.34 (d, 8.4) | 40.2 |
| 2′ | 3.17 (dd, 10.0, 6.0) | 73.9 | 3.60 (dd, 9.0, 6.6) | 62.9 |
| 3′ | α 2.12 (m) | 25.9 | α 1.81 (m) | 27.3 |
| | β 1.77 (m) | | β 0.85 (m) | |
| 4′ | α 2.34 (m) | 30.1 | α 1.71 (m) | 24.6 |
| | β 1.72 (m) | | β 1.70 (m) | |
| 5′ | 4.01 (t, 8.0) | 68.9 | α 2.55 (m) | 50.6 |
| | | | β 3.03 (m) | |
| 7′ | 4.44 (m) | 63.7 | 3.15 (m) | 55.1 |
| 8′ | a 2.71 (d, 14.4) | 36.5 | 4.03 (m) | 67.2 |
| | b 2.06 | | | |
| | (dd, 14.4, 6.4) | | | |
| 9′ | | 85.0 | a 2.08 (d, 13.8) | 40.9 |
| | | | b 1.99 (d, 13.8) | |
| 10′ | | | | 82.9 |
| 11′ | | 172.0 | | |
| 12′ | 5.77 (br s) | 112.5 | | 172.3 |
| 13′ | | 166.8 | | 126.0 |
| 14′ | 6.50 (d, 9.6) | 120.4 | | 160.9 |
| 15′ | 6.21 (dd, 9.6, 4.8) | 138.1 | α 2.96 (d, 19.8) | 24.7 |
| | | | β 2.37 (d, 19.8) | |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bridge between C-15 and N-1′ to form an isoxazolidine ring (Figure 1).

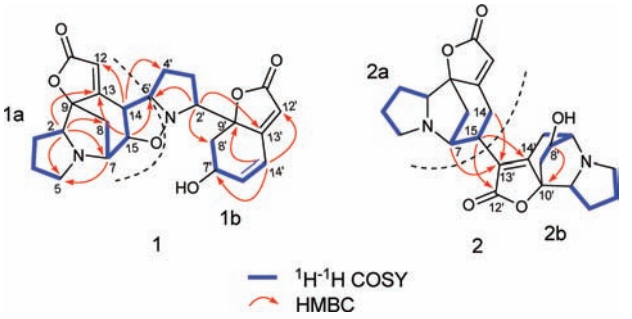


**Figure 1.** Key $^1$H−$^1$H COSY and HMBC correlations of **1** and **2**.

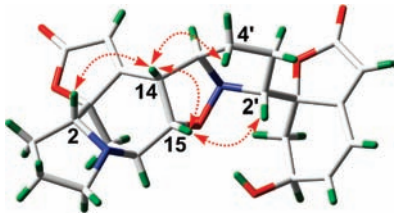


**Figure 2.** Key ROESY correlations of **1**.

The relative stereochemistry of **1** was deduced by a ROESY experiment, in which correlations between H-14 and H-2/H-15/H-4′a, as well as between H-15 and H-2′, suggested that these protons had the same orientation (Figure 2). Finally, suitable crystals for X-ray diffraction experiments were obtained, and the complete structure and stereochemistry were established. The final refinement on the CuKα data resulted in a small Flack parameter of 0.0 (1), allowing the unambiguous assignment of the absolute configuration of **1** (Figure 3).[19]

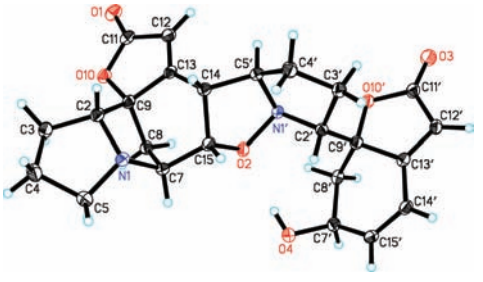

**Figure 3.** X-ray structure of **1**.

Flueggine B (**2**) was isolated as colorless needles, mp 235−236 °C, $[\alpha]^{20}_D$ +159.9 ($c$ = 0.25, $CH_3OH$). The molecular formula of **2** was assigned as $C_{24}H_{28}N_2O_5$ by HR-ESI-MS ($m/z$ 425.2072 $[M + H]^+$, calcd for $C_{24}H_{29}N_2O_5$ 425.2071). The NMR spectra of **2** also revealed the presence of 24 carbon signals including two α,β-unsaturated γ-lactone rings, suggesting that **2** was also a dimeric norsecurinine-type alkaloid. With the aid of $^1$H−$^1$H COSY, HSQC, and HMBC experiments, the $^1$H and $^{13}$C NMR signals of **2** were assigned as shown in Table 1.

The NMR data of **2a** and **2b** moieties were similar to those of dihydronorsecurinine[18] and bubbialine,[20] respectively, which were also isolated from the same plant (see the Supporting Information). Notably, the methylene at C-15 of dihydronorsecurinine was replaced by a methine in **2a**, and the sp$^2$ carbon at C-13′ has become a quaternary carbon. It was therefore suggested that the two monomeric fragments (**2a** and **2b**) were connected through a bonding between C-15 and C-13′. The HMBC correlations between H-15 and C-12′/C-14′ as well as between H-7/H-14 and C-13′ confirmed the above assignments (Figure 1). The complete structure and stereochemistry of **2** were finally determined by single-crystal X-ray diffraction analysis. Similar to **1**, the small Flack parameter 0.1 (1) allowed the unambiguous assignment of absolute configuration as shown in Figure 4.[19]

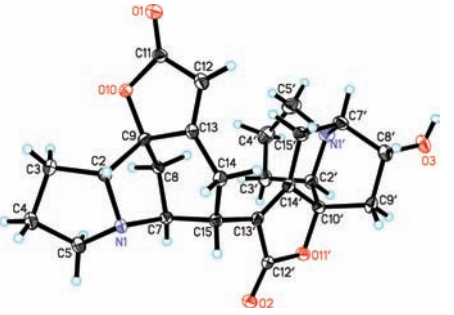

**Figure 4.** X-ray structure of **2**.

The absolute configurations of **1** and **2** were further confirmed by CD analysis. The negative Cotton effect at 268 nm for **1**, relating to the conjugated transoid diene and the γ-lactone chromophore, was similar to that of (−)-norsecurinine, whose absolute configuration had been previously assigned to be 9*S* based on CD and chemical means.[5] It followed that **1** also has a 9′*S*-configuration. On the other hand, the positive Cotton effects at 228 and 268 nm for **2** indicated that the absolute configurations of C-9 and C-10′ were the same as those in dihydronorsecurinine and bubbialine,[20] respectively. Thus, compound **2** has a 9*S*,10′*R*-configuration (Figure 5).

(19) Crystal data of compounds **1** and **2** were deposited with the Cambridge Crystallographic Data Centre (CCDC 825921 and 825922, respectively).

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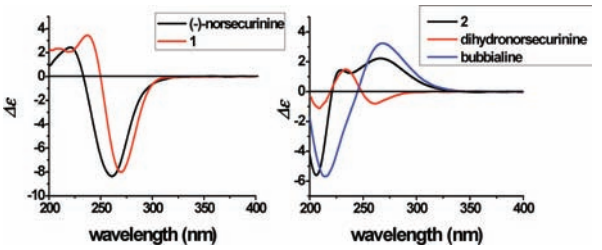
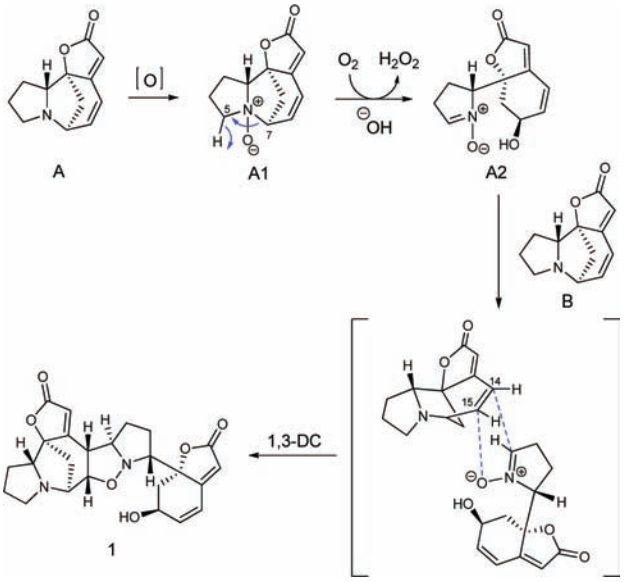



**Figure 5.** CD spectra of **1** and **2** and their references.

Compound **1** is the first example of *Securinega* alkaloids bearing an isoxazolidine ring. The unique biogenetic route of **1** (Scheme 1) could be plausibly traced back to norsecurinine. First, the *N*-lone-pair electrons in molecule **A** of norsecurinine is oxidated to form *N*-oxide **A1**. Then, the cleavage of N-1−C-7 bond, the formation of N-1−C-5 double bond[21] and nucleophilic attack of the hydroxyl group on C-7 will result in a nitrone **A2**, which triggers a 1,3-dipolar cycloaddition (1,3-DC) reaction with the molecule **B** to form compound **1**.[22−24] In the present biogenetic pathway (Scheme 1), the involvement of nitrone 1,3-DC reaction is proposed, which will provide chemists with a new insight in biomimetic synthesis.

Three different human breast cancer lines, MCF-7 (estrogen-dependent phenotype), MDA-MB-231 (estrogen-independent phenotype) and MCF-7/ADR (doxorubicin-induced multidrug resistant phenotype) were used to evaluate the growth inhibitory effect of **1** and **2**. Flueggine B (**2**) exhibited a significant inhibitory activity on the growth of MCF-7 and MDA-MB-231 cells, with $IC_{50}$ values of 135 ± 5 and 147 ± 3 nM, respectively, thereby suggesting that **2** inhibits the proliferation of breast cancer cells regardless of their estrogen receptor status. On the other hand, **2** only showed a weak activity against MCF-7/ADR cells, with an $IC_{50}$ value of 19 ± 3 $\mu$M, implying that **2** might be a substrate of *p*-glycoprotein.[25] Flueggine A (**1**) exhibited modest activities in all three cell lines, with $IC_{50}$ values of 60 ± 4. (MCF-7), 86 ± 9 (MDA-MB-231), and 68 ± 7 $\mu$M (MCF-7/ADR).

**Scheme 1.** Plausible Biogenetic Route of **1**

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

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