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Flueggenines A and B, Two Novel C,C-Linked Dimeric Indolizidine Alkaloids from *Flueggea virosa*

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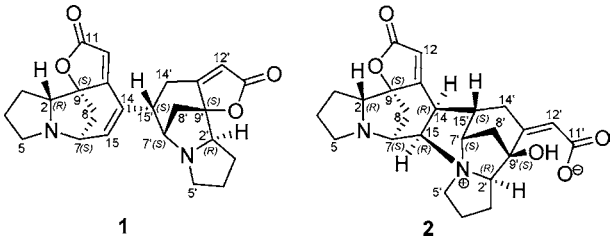
ABSTRACT



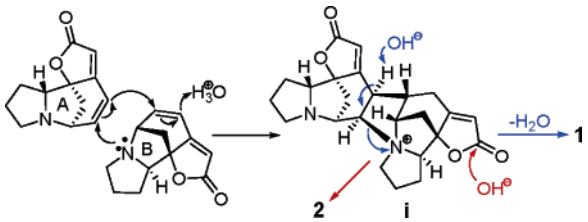
1 **2**

Two unprecedented C,C-linked dimeric indolizidine alkaloids, flueggenines A (1) and B (2), as well as their biosynthetic precursor (–)-norsecurinine, were isolated from the roots of *Flueggea virosa*. Their structures and absolute configurations were elucidated by spectroscopic methods, especially 2D NMR and CD spectral analyses, and supported by their unique biosynthetic pathway as proposed. Both 1 and 2 were tested against two tumor cell lines, and alkaloid 1 showed weak activity against the P-388 cell line.

The roots of *Flueggea virosa* Roxb. ex Willd (Euphorbiaceae), a traditional Chinese medicine, have been used for the treatment of rheumatism, pruritus, cephalic eczema, leucorrhoea, and injuries.[1] Chemical studies on this plant[2] afforded a number of indolizidine-type alkaloids known as Securinega alkaloids, and some of them showed strong cytotoxicity.[2f] A nonalkaloid, bergenin, isolated from its aerial part also exhibited antiarrhythmic activity.[3] The interesting stereochemistry and biological importance of Securinega alkaloids have been attracting the attention of synthetic chemists.[4]

In the current project, two unprecedented C,C-linked dimeric indolizidine alkaloids, flueggenines A (**1**) and B (**2**), along with their precursor (−)-norsecurinine,[2e] were isolated from the roots of *F. virosa*. Their structures and absolute configurations were elucidated by extensive spectroscopic analyses, especially by their 2D NMR and CD spectra. The biogenetic origin of **1** and **2** could be traced back to the coexisting major alkaloid (−)-norsecurinine (Scheme 1) via

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



a unique biosynthetic pathway involving a self-catalyzed Baylis−Hillman reaction as the key step. This biogenetic

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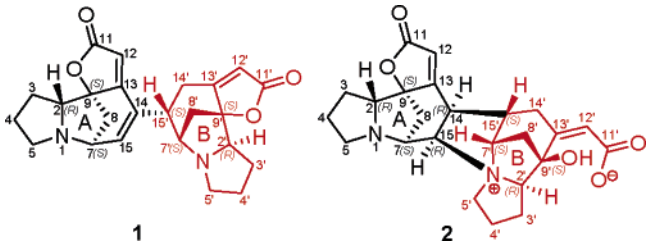
pathway fully supports the structures of flueggenines A (**1**) and B (**2**) as assigned.



1 **2**

The air-dried and ground root material (2.8 kg) of *F. virosa* was percolated with 95% EtOH at room temperature to give 190 g of crude extract, which was dissolved in 1 L of H_2O to form a suspension and adjusted to pH ≈ 3 with 2 M H_2SO_4. The acidic suspension was first partitioned with EtOAc (1 L × 3) to remove the neutral components. The aqueous phase was then basified with 5% Na_2CO_3 to pH ≈ 10 and extracted with *n*-BuOH (800 mL × 3) to obtain 10 g of crude alkaloids. The crude alkaloids were subjected to a MCI gel column (H_2O−MeOH, 1:0−0:1) to give three major fractions 1−3. Fraction 2 was rechromatographed on a silica gel column (CH_2Cl_2−MeOH, 50:1−3:1) to afford (−)-norsecurinine (2.1 g) and two major subfractions 2a and 2b. Fraction 2a was subsequently purified on an RP-18 silica gel column (50% MeOH in H_2O) to give flueggenine A (**1**) (15.6 mg). Fraction 2b was purified by preparative HPLC using a reversed-phase C-8 silica gel column (30% CH_3CN in H_2O) to obtain flueggenine B (**2**) (10.2 mg).

Flueggenine A (**1**),[5] a white amorphous powder, has a molecular formula of $C_{24}H_{26}N_2O_4$ as determined by HREIMS at *m*/*z* 406.1871 $[M]^+$ (calcd 406.1893). The IR spectrum exhibited typical absorptions for the norsecurinine type alkaloids at 1755 (α,β-unsaturated γ-lactone), 1649, 1622 cm^{-1} (olefinic bond).[6,2d] The ^1H, ^{13}C NMR, and DEPT spectra (Table 1) revealed the presence of two lactone carbonyls, three trisubstituted double bonds, and 16 sp^3 carbons including nine methylenes, five methines and two oxygenated quaternary carbons. The aforementioned data suggested that **1** was likely a dimer of two norsecurinine-

Table 1. ^1H and ^{13}C NMR Data of Flueggenines A (**1**) and B (**2**)[a]

	1		**2**	
no.	δ_H, *J* (Hz)	δ_C	δ_H, *J* (Hz)	δ_C
2	3.17 (1H, dd, 6.3, 8.2)	66.6 (CH)	3.41 (1H, dd, 8.5, 6.9)	69.2 (CH)
3	α 1.88 (1H, m)	30.4 (CH_2)	α 1.82 (1H, m)	30.9 (CH_2)
	β 1.97 (1H, m)		β 1.90 (1H, m)	
4	α 2.03 (1H, m)	27.9 (CH_2)	α 1.99 (1H, m)	28.0 (CH_2)
	β 1.73 (1H, m)		β 1.74 (1H, m)	
5	α 2.65 (1H, m)	56.3 (CH_2)	α 2.79 (1H, m)	58.2 (CH_2)
	β 3.24 (1H,dd, 8.6, 7.2)		β 3.36 (1H,m)	
7	3.81 (1H, dd, 1.8, 4.7)	61.0 (CH)	3.78 (1H, dd, 6.4, 2.8)	63.4 (CH)
8	a 1.75 (1H, d, 10.7)	37.2 (CH_2)	a 2.67 (1H, dd, 11.8, 6.7)	33.4 (CH_2)
	b 2.67 (1H, m)		b 1.52 (1H, d, 11.8)	
9		93.5 (qC)		92.7 (qC)
11		174.8 (qC)		174.4 (qC)
12	6.00 (1H, s)	108.4 (CH)	6.09 (1H, s)	114.2 (CH)
13		171.8 (qC)		173.1 (qC)
14		136.1 (qC)	3.55 (1H, m)	43.2 (CH)
15	6.93 (1H, d, 6.7)	140.6 (CH)	3.96 (1H, dd, 8.0, 3.0)	76.7 (CH)
2′	3.13 (1H, dd, 9.2, 7.1)	68.6 (CH)	3.84 (1H, t, 7.4)	90.8 (CH)
3′	α 1.84 (1H, m)	30.3 (CH_2)	α 2.24 (1H, m)	28.7 (CH_2)
	β 1.75 (1H, m)		β 2.51 (1H, m)	
4′	α 1.96 (1H, m)	27.9 (CH_2)	α 2.10 (1H, m)	27.7 (CH_2)
	β 1.68 (1H, m)		β 2.25 (1H, m)	
5′	α 2.63 (1H, m)	58.5 (CH_2)	α 4.12 (1H, m)	60.6 (CH_2)
	β 3.29 (1H, m)		β 3.57 (1H, m)	
7′	3.19 (1H, m)	66.3 (CH)	4.93 (1H, dd, 9.6, 4.3)	76.2 (CH)
8′	a 1.57 (1H, d, 11.0)	36.6 (CH_2)	a 2.28 (1H, m)	34.7 (CH_2)
	b 2.64 (1H, m)		b 2.21 (1H, m)	
9′		94.1 (qC)		77.1 (qC)
11′		175.6 (qC)		173.9 (qC)
12′	5.73 (1H, d, 1.9)	110.9 (CH)	5.94 (1H, d, 1.8)	130.3 (CH)
13′		176.4 (qC)		155.6 (qC)
14′	α 2.73 (1H, m)	29.3 (CH_2)	α 2.23 (1H, m)	36.3 (CH_2)
	β 3.00 (1H, m)		β 2.83 (1H, m)	
15′	2.71 (1H, m)	44.3 (CH)	2.94 (1H, m)	43.1 (CH)

[a] Recorded in CD_3OD; 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR. ^{13}C multiplicities were determined by DEPT experiments.

type alkaloids. By comparison with norsecurinine,[2e] the ^{1}H and ^{13}C NMR data of **1** showed that the C-14 in the part A had become a quaternary olefinic carbon and the $\Delta^{14'}$ double bond in part B had been saturated to form the C-14′ methylene and C-15′ methine, indicating that the A and B parts of **1** were connected through the C-14 and C-15′ bond.

The structure of flueggenine A (**1**), especially the C-14 and C-15′ linkage between the A and B parts, was further confirmed by HMQC and HMBC spectra. The ^{13}C NMR and HMQC spectra first allowed the assignments of all the protons to their bonding carbons. The HMBC correlations (Figure 1, Supporting Information) then confirmed the

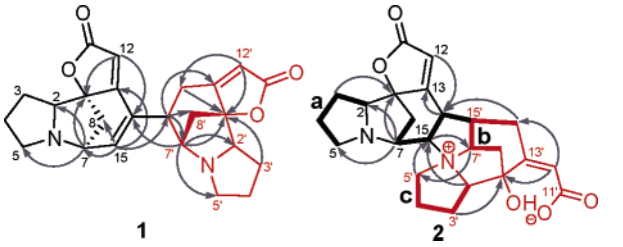


Figure 1. Key HMBC (H → C) and ^{1}H−^{1}H COSY (—) correlations of **1** and **2**.

backbones of the two monomers and their linkage. In the HMBC spectrum, the correlations of H-12 to C-14 and C-9, H-15 to C-13, C-7 and C-8, and H-7 to C-2, C-5 and C-9 verified the planar structure of part A; the correlations of H-12′ to C-14′ and C-9′, H-15′ to C-13′, C-7′ and C-8′, and H-7′ to C-9′, C-2′ and C-5′ proved the planar backbone of part B; the key correlation between H-7′ and C-14, and between H-15 and C-15′ established the C-14 and C-15′ linkage.

The relative stereochemistry of **1** was determined from the 500 MHz NOESY (in CD_3OD) spectrum (Figure 2). The

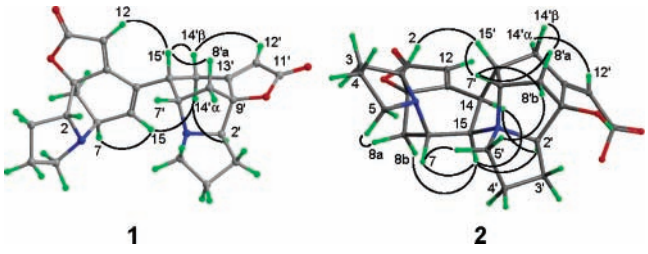


Figure 2. Key NOESY correlations of **1** and **2**.

key NOESY correlations between H-15′ and H-8′a revealed that H-15′ and CH_2-8′ were cofacial and arbitrarily defined as having a β-orientation. The correlations between H-2′ and H-14′α indicated that they were on the same side, and they were thus assigned the α-configuration. Although the C-14−C-15′ bond could rotate to some extent, the significant steric bulk of the two monomer parts A and B indicated it was fairly fixed. This was confirmed as shown in Figure 2 from their NOESY correlations between H-15 and H-14′α and between H-12 and H-15′.

Flueggenine B (**2**)[7] was isolated as a white amorphous solid, and its molecular formula was determined as $C_{24}H_{28}N_2O_5$ on the basis of HRESIMS at *m/z* 447.1873 $[M + Na]^+$ (calcd 447.1896) and ^{13}C NMR data (Table 1). The molecular formula requires 12 degrees of unsaturation. The IR absorptions at 1765, 1635, and 1571 cm^{-1} suggested the presence of an α,β-unsaturated γ-lactone, an olefinic bond, and a $-COO^-$ group, respectively.[2d,6] The spectral data mentioned above also implied that **2** possessed the character of a dimeric norsecurinine-type alkaloid. Compared with flueggenine A (**1**), the ^{1}H and ^{13}C NMR data showed that both Δ^{14} and $\Delta^{14'}$ double bonds were saturated in **2** and that a linkage most likely existed between C-14 and C-15′. A quaternary carbon signal assignable to the C-9′ was strongly shifted upfield to δ 77.1 ppm, suggesting that the α,β-unsaturated γ-lactone ring in the B-part was possibly opened by saponification. Meanwhile, the C-15 signal at δ 76.7 in the A component and the carbon signals of C-2′ at δ 90.8, C-5′ at δ 60.6, and C-7′ at δ 76.2 in the B component were all significantly deshielded, indicating that the C-15 and the N-atom in part B were likely linked to form a quaternary ammonium, which actually caused the severe deshielding effects on its adjacent carbons. A dimeric indolizidine type alkaloid with an unprecedented octacyclic ring system was thus tentatively outlined, and was confirmed by 2D NMR spectra (HMQC, ^{1}H−^{1}H COSY, HMBC, and NOESY).

Three spin systems **a**, **b**, and **c** drawn with bold bonds in **2** were fixed by analysis of its ^{1}H−^{1}H COSY spectrum

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(5) Flueggenine A (**1**): white amorphous powder; $[\alpha]^{20}_D$ −24 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (4.34), 260 (4.10) nm; CD (MeOH) 213 ($\Delta\epsilon$ +7.35), 265 ($\Delta\epsilon$ −10.9) nm; IR (KBr) ν_{max} 3427, 2964, 1755, 1649, 1622, 1458, 914 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS 70 eV *m/z* (rel int) 406 $[M]^+$ (18), 337 (26), 269 (10), 221 (11), 191 (38), 177 (10), 149 (12), 96 (26), 70 (100); HREIMS *m/z* 406.1871 (calcd for $C_{24}H_{26}N_2O_4$, 406.1893).

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(7) Flueggenine B (**2**): white amorphous powder; $[\alpha]^{20}_D$ −70 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (4.22) nm; CD (MeOH) 202 ($\Delta\epsilon$ +8.45), 225 ($\Delta\epsilon$ −22.8) nm; IR (KBr) ν_{max} 3442, 2966, 2872, 1765, 1635, 1572, 1456, 1385, 1155, 1082, 914 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; ESIMS (positive) *m/z* 447 (52) $[M + Na]^+$, 425 (100) $[M + 1]^+$, 407 (9) $[M - H_2O + 1]^+$; (negative) 423 (100) $[M - 1]^-$, 375 (22); HRESIMS *m/z* 447.1873 (calcd for $C_{24}H_{28}N_2O_5Na$, 447.1896).

(Figure 1). The HMBC spectrum was then applied to assemble three subunits **a**, **b**, and **c** with the quaternary carbons and other functionalities. In the HMBC (Figure 1, Supporting Information), the basic skeleta of the two monomer parts A and B could be fulfilled by the relevant correlations as depicted; the key correlations of H-2′/C-15, H-5′/C-15, H-15/C-7′, and H-7′/C-5′ showed the linkage between C-15 and the *N*-atom in part B. The C-14 and C-15′ bond revealed by ^{1}H−^{1}H COSY was confirmed by the HMBC spectrum. No outer anion, such as Cl^- or SO_4^{2-}, was observed in both the positive and negative mode of the ESI-MS spectra, indicating that alkaloid **2** was an inner salt.

The relative configuration of **2** was established by its NOESY spectrum (Figure 2), in which, the correlations of H-2/H-15′, H-15′/H-7′, H-7′/H_2-8′, and H-8′a/H-14′β showed that they were all cofacial and arbitrarily assigned as β-oriented; the key correlations among H-2′, H-14, and H-15, as well as the correlations between H-15 and H-8b, and between H-8a and H-5α, indicated that they were on the same side and α-oriented.

The absolute configurations of flueggenines A (**1**) and B (**2**) were determined on the basis of the CD spectral analyses (Figure 3). The negative Cotton effect at 265 nm for **1**, relating to the conjugated transoid diene and the γ-lactone chromophore, was very similar to that of (−)-norsecurinine, whose absolute configuration was assigned as 9*S* by CD and chemical means,[2d,2e] suggesting that the A-part of alkaloid **1** also had the 9*S*-configuration. A 9′*S*-configuration in the B component of **1** was also assumed on the basis of biogenetic reasoning and the fact that it coexisted with (−)-norsecurinine in this plant. The absolute configuration of **1** was thus assigned as depicted. The CD spectrum of flueggenine B (**2**) showed a split Cotton effect at λ 225 nm ($\Delta\epsilon$ −22.8) and 202 nm ($\Delta\epsilon$ +8.45) centered at 213 nm; this corresponded to the UV maximum of α,β-unsaturated carbonyl groups, which could be well interpreted by the exciton chirality method.[8] The negative chirality of **2** resulted from the dipole−dipole interaction between the electric transition moments of the two chromophores of the α,β-unsaturated γ-lactone and α,β-unsaturated carboxylate, indicating that the two chromophores were oriented in a counterclockwise manner (Figure 3b). Accordingly, both the C-9 and C-9′ of **2** were assigned the *S*-configuration, which is also identical to that in (−)-norsecurinine.[2d] The absolute structure of **2** was therefore established as depicted.

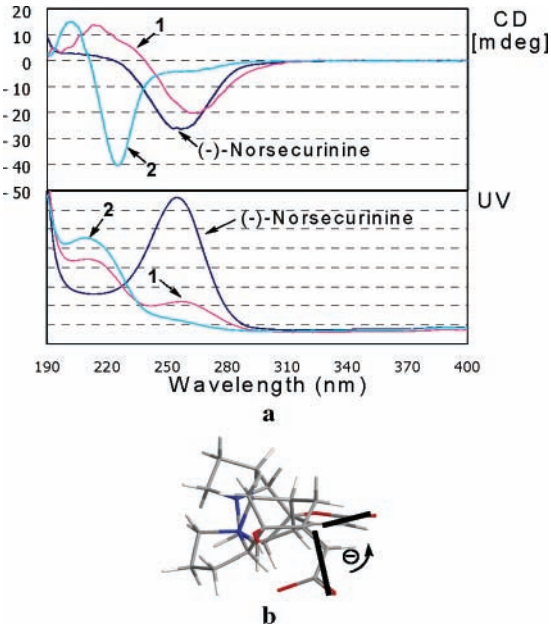

Figure 3. CD and UV spectra of **1**, **2**, and (−)-norsecurinine (a); chiral analysis of **2** (b). Bold lines denote to the electric transition dipole of the chromophores.

The unique biogenetic origin of flueggenines A and B can plausibly be traced back to (−)-norsecurinine,[2d,e] a coexisting major alkaloid in this plant (Scheme 1). Nucleophilic attack of the *N*-lone-pair of electrons in the B-component of (−)-norsecurinine on the C-15 position of the A-component of (−)-norsecurinine would trigger a self-catalyzed Baylis−Hillman reaction[9] to form a key intermediate (**i**), which would subsequently undergo a quaternary ammonium elimination[10] via an E2 mechanism to yield **1** or a simple saponification to produce **2**.

The in vitro activity of the two alkaloids against the P-388 murine leukemia and A-549 human lung carcinoma cell lines were tested by using the MTT[11] and SRB[12] methods, respectively, and with pseudolaric acid B[13] as a positive control (IC_{50} = 0.74 μM against P-388). Only alkaloid **1** showed weak activity (IC_{50} = 51.5 μM) against the P-388 tumor cell line.

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Supporting Information Available: Detailed HMBC correlations (table and figures); 1D and 2D NMR and IR spectra of flueggenines A (**1**) and B (**2**); EIMS of **1** and ESIMS of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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