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Flueggether A and Virosinine A, Anti-HIV Alkaloids from *Flueggea virosa*

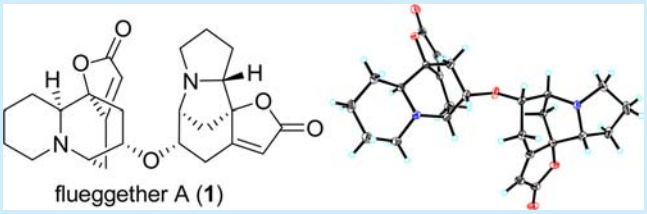
Hua Zhang,[†] Kong-Kai Zhu,[†] Ying-Shan Han,[‡] Cheng Luo,[†] Mark A. Wainberg,[‡] and Jian-Min Yue*[,†]

[†]State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, P. R. China

[‡]McGill University Aids Centre, The Lady Davis Institute for Medical Research, Jewish General Hospital, 3755 Cote Ste-Catherine Road, Montreal, Quebec H3T 1E2, Canada

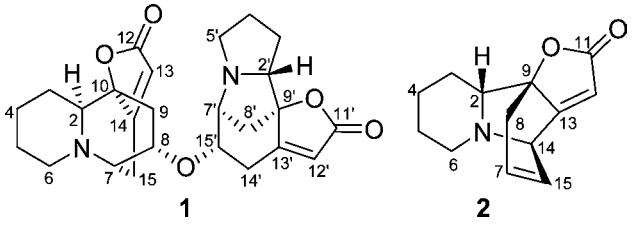
Supporting Information

ABSTRACT: Two new alkaloids, flueggether A (**1**) and virosinine A (**2**), were isolated from a Chinese medicinal plant, *Flueggea virosa*. Their structures were assigned via spectroscopic methods with the absolute configurations of **1** and **2** being established by X-ray diffraction analysis and calculated electronic circular dichroism data, respectively. Compound **1** represents the first example with an ether bridge of *Securinega* alkaloid oligomers, and **2** bears a new heterocyclic backbone. Both alkaloids showed mild in vitro anti-HIV activity.



Securinega alkaloids are an interesting class of natural products discovered only from plants of a limited number of genera, such as *Securinega* and *Flueggea* of the Euphorbiaceae family.[1] They have been known in the literature for over half a century, since securinine was isolated from *Securinega suffructicosa* by Russian researchers in 1956.[2] This structure family, with the rigid tetracyclic backbone incorporating a butenolide moiety, has attracted much attention from both natural products and synthetic chemists.[1] Of particular note, *Securinega* alkaloids reemerged as a hot research topic in the chemical community in this century, with 40+ new natural members and quite a few efficient total syntheses being reported.[1,3]

The *Flueggea* genus is a small tribe with about 12 species distributed all over the world.[4] *F. virosa* (Roxb. ex Willd.) Voigt is the star plant among the four native Chinese species owing to its diverse secondary metabolites, and it has been investigated intensively in the past decade.[3a,d,5] Previous chemical studies of the alkaloidal constituents from *F. virosa* have led to the discovery of all reported types of *Securinega* alkaloids,[1,5b] especially a series of norsecurinine-derived oligomers by our research group[3d,5a,f,g] Apart from the aforementioned efforts for alkaloid oligomers, we also observed other interesting signals from very minor constituents in the MS analysis. A further fractionation of the fractions showing different MS peaks returned two extra new alkaloids, flueggether A (**1**) and virosinine A (**2**). The structures of **1** and **2** were characterized on the basis of comprehensive spectroscopic data analysis, and their absolute configurations were established by single-crystal X-ray diffraction experiments and comparison of electronic circular dichroism (ECD) spectrum with the calculated one, respectively. Alkaloid **1** is the first example linked via an ether bond of *Securinega* alkaloid oligomers, while **2** incorporates a new architecture likely rearranged from its cometabolite viroallosecurinine.[5g,6] Both compounds displayed mild inhibitory effects in an in vitro anti-HIV bioassay.



A total of 28.2 g of crude alkaloid was generated from the stems and leaves (10 kg) of *F. virosa* via routine procedures.[5f] This alkaloid extract was first fractionated over silica gel using a petroleum ether−EtOAc system modified by $HNEt_2$ to yield six fractions. The fifth fraction was then processed by repeated silica gel column chromatography (CC) and finally via HPLC to afford alkaloid **1** (2.1 mg). Alkaloid **2** (2.0 mg) was obtained from the second fraction via silica gel CC, Sephadex LH-20 CC, and preparative TLC sequentially.

Flueggether A (**1**) was obtained as colorless crystals and displayed IR absorption bands at 1753 and 1651 cm^{-1} corresponding to conjugated γ-lactone(s).[7] The molecular formula of $C_{25}H_{30}N_2O_5$ was assigned to **1** on the basis of ^{13}C NMR data and (+)-HRESIMS analysis at m/z 439.2228 ($[M + H]^+$, Δmmu −0.5), consistent with 12 degrees of unsaturation (DOU). With the aid of DEPT and HSQC spectra (Figures S2 and S4, Supporting Information), the NMR data (Table 1) of **1** revealed the presence of two lactones (δ_C 174.2 and 172.8), two trisubstituted double bonds (δ_C 176.0, 109.1, 172.6, and

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Table 1. NMR Data for Alkaloids 1 and 2 in $CDCl_3$

	1			1			2	
no.	δ_C[a]	δ_H (mult, *J*, Hz)	no.	δ_C[a]	δ_H (mult, *J*, Hz)	no.	δ_C[a]	δ_H (mult, *J*, Hz)
2	63.7	2.23 (br d, 10.1)	2′	66.0	3.07 (dd, 8.8, 6.8)	2	65.4	2.91 (br d, 11.9)
3	26.0	1.66 (m), 1.35 (m)	3′	29.1	1.92 (m), 1.74 (m)	3	23.1[c]	1.74 (m), 1.65 (m)
4	24.8	1.87 (m), 1.28 (m)	4′	26.8[b]	1.96 (m), 1.73 (m)	4	20.4	1.84 (m), 1.47 (m)
5	26.9[b]	1.58 (m), 1.54 (m)	5′	57.4	3.31 (m), 2.64 (m)	5	23.2[c]	1.73 (2H, m)
6	52.7	2.81 (m), 2.67 (m)				6	45.1	3.08 (m), 2.67 (m)
7	56.2	2.94 (ddd, 4.9, 1.9, 1.9)	7′	64.6	3.01 (dd, 5.5, 4.4)	7	131.5	5.98 (ddd, 9.1, 3.2, 3.2)
8	72.3	3.87 (brdd, 8.2, 4.9)	8′	30.2	a 2.29 (dd, 11.3, 5.5)	8	38.2	a 3.11 (m)
					b 1.74 (d, 11.3)			b 2.46 (ddt, 18.0, 3.2, 2.2)
9	34.0	a 2.69 (dd, 13.2, 8.2)	9′	91.5		9	88.2	
		b 1.20 (d, 13.2)						
10	84.7		11′	172.8		11	174.1[d]	
12	174.2		12′	111.3	5.64 (br s)	12	104.0	5.67 (s)
13	109.1	5.62 (dd, 1.9, 1.4)	13′	172.6		13	174.0[d]	
14	176.0		14′	29.4	2.80 (2H, m)	14	54.7	4.09 (d, 5.3)
15	23.7	a 2.90 (ddd, 20.1, 1.9, 1.9)	15′	74.3	3.77 (m)	15	130.2	6.09 (ddt, 9.1, 5.3, 2.2)
		b 2.77 (ddd, 20.1, 1.9, 1.4)						

[a]Superscripts b–d indicate interchangeable assignments.

111.3; δ_H 5.62 and 5.64), and 19 sp^3 carbons ($CH_2 \times 11$, CH × 6, and C × 2 at δ_C 84.7 and 91.5). These observations accounted for four out of the 12 DOU and required **1** to be octacyclic. The above-mentioned analyses suggested that **1** was likely a *Securinega* alkaloid dimer incorporating one norsecurinine-type and one neosecurinane monomeric unit[1] as especially supported by the chemical shifts for the γ-carbon of their lactonyl rings at 91.5 and 84.7, respectively.

Analyses of 2D NMR data (Figure 1) enabled assembly of the planar structure for **1** as shown. In detail, $^1H-^1H$ COSY

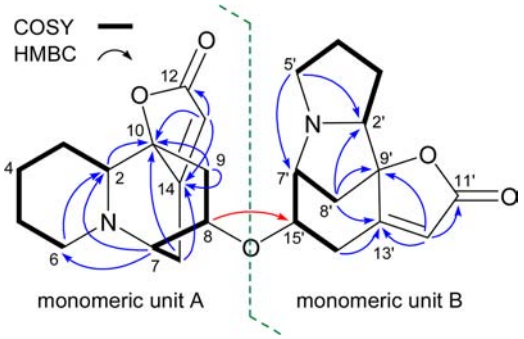

Figure 1. 2D NMR correlations for **1**.

correlations revealed structural fragments (H-2 to H_2-6, H_2-15 via H-7 and H-8 to H_2-9, H-2′ to H_2-5′, and H_2-8′ via H-7′ and H-15′ to H_2-14′) that were also found in virosine B[5c] and in the dihydronorsecurnine moiety of flueggenine A.[5a] Further examination of HMBC data corroborated the existence of the above-mentioned two monomeric units, and in particular, the connection of C-8 to C-15′ via an ether bond was supported by their deshielded chemical shifts (δ_C 74.3 and 72.3) and the HMBC correlation from H-8 (δ_H 3.87) to C-15′. Although ROESY data (Figure S6, Supporting Information) failed to provide enough information for determining the relative configuration of **1** due to severe signal overlapping, excellent NMR comparisons of its monomeric substructures with virosine B[5c] and 15α-methoxy-14,15-dihydronorsecurinine[8] were supportive of common configurations at all corresponding chiral centers. Finally, the relative structure of **1** assigned via NMR data was confirmed by a successful X-ray diffraction experiment which also established the absolute configuration of **1** (Figure 2) as 2*S*,7*S*,8*S*,10*R*,2′*R*,7′*S*,9′*S*,15′*S* [absolute

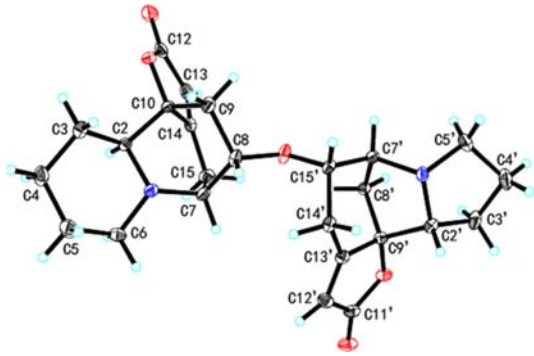

Figure 2. Single-crystal X-ray structure for **1**.

structure parameter: 0.01(8)].[9] On reviewing the literature, over 20 *Securinega* alkaloid oligomers have been discovered from *F. virosa* since 2006,[5a] but the connection between each of their two monomeric units was exclusively via the carbon–carbon bond. Although flueggeaine ether reported in 1985 by Chen and Hou[10] was claimed to be a flueggeainol ether dimer,[10] comparison of its chemical shifts for C-15 and C-15′ (δ_C 67.1 and 66.4) to those of an 15-epimer (δ_C 69.1) and two 15-*O*-methyl ethers (δ_C 79.8 and 79.1 for 15α- and 15β-epimers, respectively)[8] of flueggeainol apparently did not support this structural assignment. It is possible that flueggeaine ether reported in the literature was a mixture of the two 15-epimers of flueggeainol as judged by the upfield resonated chemical shifts of C-15 and C-15′. Therefore, alkaloid **1** was characterized to be the first the dimeric *Securinega* alkaloid formed via an ether bridge.

Alkaloid **2** showed a protonated molecular ion peak at *m*/*z* 218.1182 in the (+)-HRESIMS spectrum, consistent with a molecular formula of $C_{13}H_{15}NO_2$ (Δmmu 0.1) with seven DOU. As with **1**, the IR spectrum of **2** also revealed the presence of conjugated γ-lactone(s) (1772 and 1682 cm^{-1}). The NMR data (Table 1) of **2** showed signals for an α,β-

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conjugated lactone moiety (δ_C 104.0, 174.0, and 174.1; δ_H 5.67), an isolated double bond (δ_C 130.2 and 131.5; δ_H 5.98 and 6.09), four sp^3 methylenes (one nitrogenated), two sp^3 methines (*N*-bonded), and a sp^3 oxygenated quaternary carbon (δ_C 88.2). These functionalities occupied three DOU and the remaining four suggested a tetracyclic backbone for alkaloid **2**.

Analysis of 2D ^{1}H−^{1}H COSY data (Figure 3) revealed two structural fragments of H_2-2 to H_2-6 and H_2-8 via H-7 and H-15 to H-14. The HMBC correlations (Figure 3) from both H_2-8 and H-14 to C-9 and C-13 established the linkage between the 1,2-disubstituted vinyl group and the γ-lactone ring. Further observation of the HMBC correlations from H_2-6 to C-2 and C-14, and from H_2-8 and H-14 to C-2, confirmed the planar structure of **2** with a new heterocyclic ring system as shown. The relative configuration of **2** was assigned by analysis of ROESY data acquired in C_5D_5N (Figure S15, Supporting Information). The ROESY correlation of H-2/H-8a indicated that H-2 and the allyl group (C-8−C-7−C15) were located at the same side. The absolute configuration of **2** was established to be as drawn by comparing its experimental ECD spectrum with the calculated ones (Figure 4), where the curve trend of **2** matched well with that of the (2*R*,9*R*,14*R*) enantiomer.

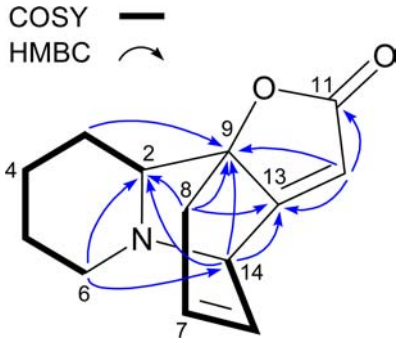


Figure 3. 2D NMR correlations for **2**.

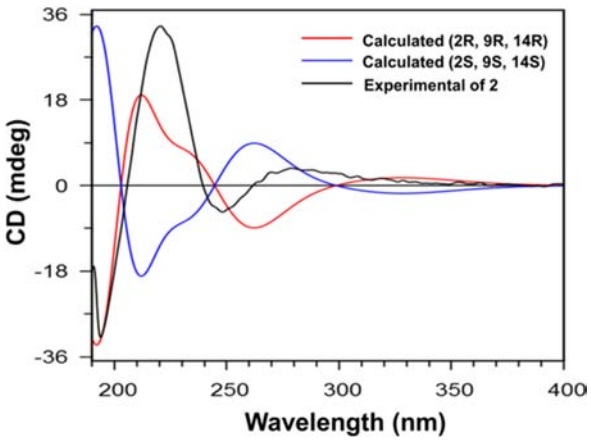
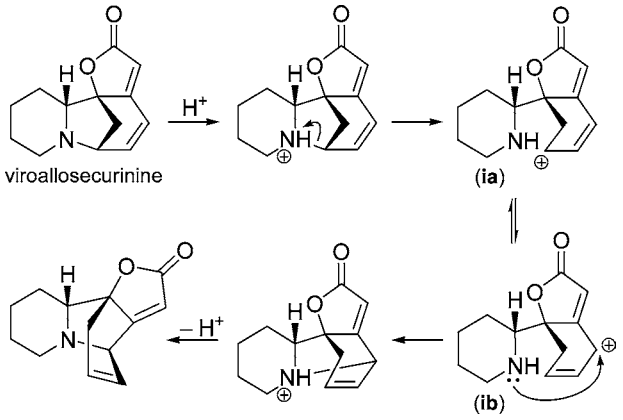


Figure 4. Experimental and calculated ECD spectra for **2**.

The biosynthetic origin of alkaloid **2** could be tracked back to its cometabolite viroallosecurinine[5g,6] as shown in Scheme 1. Protonation of the nitrogen atom and bond cleavage would return a key intermediate **ia** which could tautomerize to **ib**. Subsequent nucleophilic attack from the nitrogen lone pair electrons and loss of proton would afford **2** as the final product. It is also possible that alkaloid **2** and viroallosecurinine were derived from the same biosynthetic precursor with a skeleton represented by **ia**/**ib**.

Scheme 1. Proposed Biosynthetic Pathway for 2



The anti-HIV activity of **1** and **2** on HIV-1 NL 4-3 infected MT4 cells was evaluated in vitro with nevirapine as positive control.[3d,11] Both alkaloids exhibited mild inhibitory effect with EC_{50} values of 120 ± 12 (**1**) and 45.0 ± 4.5 (**2**) μM without showing cytotoxicity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03320.

Experimental section and raw spectroscopic data including IR, MS, and NMR spectra for alkaloids **1** and **2** (PDF)

X-ray crystallographic data for **1** (CIF)

■ AUTHOR INFORMATION

Corresponding Author

* E-mail: jmyue@simm.ac.cn. Tel: 86-21-5080-6718.

Notes

The authors declare no competing financial interest.

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