Natural Products

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Suffrutines A and B: A Pair of *Z*/*E* Isomeric Indolizidine Alkaloids from the Roots of *Flueggea suffruticosa***

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Abstract: *Suffrutines A (**1**) and B (**2**), a pair of novel photochemical Z/E isomeric indolizidine alkaloids, with a unique and highly conjugated C_{20} skeleton, were isolated from the roots of Flueggea suffruticosa. The structures were elucidated by extensive analysis of NMR spectra and single-crystal X-ray diffraction. The light-induced isomerization and hypothetical biogenetic pathway to **1** and **2**, as well as their activity for regulating the morphology of Neuro-2a cells are also discussed.*

The plants of genus *Flueggea*, belonging to the multitudinous and medicinally important family Euphorbiaceae,[1] are a rich source of a class of structurally unique indolizidine alkaloids known as *Securinega* alkaloids.[2] Previous phytochemistry investigations on several species of this genus had led to the isolation of a number of *Securinega* alkaloids, some of which had been reported to show significant biological activities on the central nervous system and cytotoxicity.[3]

Flueggea suffruticosa (Pall.) Rehd. is a shrub that grows widely in southeast Asia.[1] The twigs and leaves of this plant are used as a traditional Chinese medicine for the treatment of lumbago, numbness of the limbs, and indigestion. Our group had reported the isolation of a series of new *Securinega* alkaloids.[4] In our current research, suffrutines A (**1**) and B (**2**; Figure 1), a pair of novel indolizidine alkaloids with an unprecedented C_{20} framework constructed from an indolizidine heterocycle and a benzofuranone moiety tethered by a conjugated diene chain, were isolated from the roots of *F. suffruticosa*. As a pair of *Z*/*E* isomers, **1** and **2** appeared to be interconvertible by light. Herein, we describe the isolation, structural elucidation, and photoisomerization of **1** and **2**. In addition, a hypothetical biogenetic pathway for **1** and **2** and their ability to promote Neuro-2a cell differentiation are discussed.

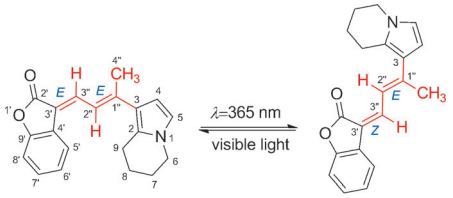

Figure 1. Chemical structures and photoisomerization process of **1** and **2**.

Suffrutine A (**1**) was obtained as orange-red needles. The molecular formula of **1** was established as $C_{20}H_{19}NO_2$ from its HR-ESI-MS (*m*/*z* 306.1488 $[M+H]^+$, calcd for $C_{20}H_{20}NO_2$: 306.1489). The UV/Vis spectrum of **1** showed the maximum absorption at $\lambda = 459$ nm, which revealed the presence of an extended conjugation system. The IR spectrum exhibited the characteristic absorptions for a lactone (1745 cm^{-1}) and benzene ring (1588, 1485, and 778 cm^{-1}). The ^1H NMR spectrum of **1** showed the presence of two conjugated olefinic protons [$\delta_H = 7.89$ and 7.01 ppm (each 1H, d, $J = 13.0$ Hz)], an *ortho*-disubstituted benzene ring [$\delta_H = 7.60$, 7.11 (each 1H, d, $J = 7.5$ Hz), 7.25 (1H, overlapped), and 7.15 ppm (1H, t, $J = 7.5$ Hz)], an α,β-disubstituted pyrrole ring [$\delta_H = 6.59$ and 6.42 ppm (each 1H, d, $J = 3.0$ Hz)], four methylenes [$\delta_H =$ 4.00, 3.07 (each 2H, t, $J = 7.5$ Hz) and 2.02, 1.94 ppm (each 2H, m)], and a methyl group [$\delta_H = 2.41$ ppm (3H, br s)]. The ^{13}C NMR and DEPT spectra of **1** revealed the presence of twenty carbon signals, including a carbonyl ($\delta_C = 170.2$ ppm), fourteen aromatic and olefinic carbon atoms, four methylenes ($\delta_C = 46.0$, 25.6, 22.8, and 22.1 ppm), as well as one methyl group ($\delta_C = 17.6$ ppm). Based on the analysis of the ^1H-^1H COSY, HSQC, and HMBC spectra, all the ^1H and ^{13}C NMR signals of **1** were assigned as shown in Table 1.

The ^1H-^1H COSY spectrum of **1** revealed the presence of four spin systems (C4 to C5, C6 to C9, C5′ to C8′, and C2″ to C3″; Figure 2). In the HMBC spectrum, the correlations between H5 and C2/C3/C6, between $H_2$9 and C3, as well as between H4 and C2 indicated the presence of a reductive indolizidine moiety (**1a**). The HMBC correlations between H5′ and C3′/C9′, and between H8′ and C4′ allowed the establishment of a benzofuranone unit (**1b**). Moreover, the

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Table 1: NMR data of **1** and **2** (in $CDCl_3$, δ values in ppm, J values in Hz).[a,b]

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2	–	130.3	–	131.3
3	–	122.1	–	121.9
4	6.42 (d, 3.0)	108.3	6.40 (d, 3.0)	108.3
5	6.59 (d, 3.0)	120.4	6.55 (d, 3.0)	120.0
6	4.00 (2H, t, 7.5)	46.0	3.97 (2H, t, 6.0)	46.1
7	2.02 (2H, m)	22.8	1.97 (2H, m)	22.7
8	1.94 (2H, m)	22.1	1.92 (2H, m)	21.0
9	3.07 (2H, t, 7.5)	25.6	3.10 (2H, t, 6.0)	25.5
2′	–	170.2	–	167.8
3′	–	115.1	–	113.7
4′	–	124.4	–	125.9
5′	7.60 (d, 7.5)	122.0	7.46 (d, 7.5)	118.4
6′	7.15 (t, 7.5)	123.5	7.10 (t, 7.5)	123.2
7′	7.25	128.1	7.20 (t, 7.5)	127.6
8′	7.11 (d, 7.5)	110.6	7.06 (d, 7.5)	110.3
9′	–	153.0	–	152.1
1″	–	150.4	–	148.6
2″	7.01 (d, 13.0)	118.2	7.84 (d, 13.0)	118.8
3″	7.89 (d, 13.0)	137.3	7.79 (d, 13.0)	136.5
4″	2.41 (3H, br s)	17.6	2.38 (3H, br s)	16.9

[a] Data were recorded on Bruker AV-500 spectrometer. [b] Overlapped signals were reported without designating multiplicity.

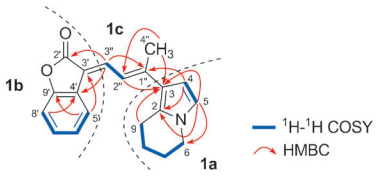


Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of **1**.

HMBC correlations between H3″ and C2′/C4′/C1″, between H2″ and C3, as well as between $H_3$4″ and C3/C2″ were observed, and suggested that the indolizidine moiety (**1a**) and benzofuranone unit (**1b**) were connected through a conjugated diene chain (**1c**; Figure 2).

The stereochemistry of the two C–C double bonds of **1** could be deduced by a ROESY experiment. The NOE correlations between H5′ and $H_2$9/H2″, between H2″ and $H_2$9, as well as between $H_3$4″ and H4/H3″ were observed, and indicated that the two double bonds were *trans* (Figure 3). Finally, suitable crystals for single-crystal X-ray diffraction experiment were obtained. The X-ray diffraction result

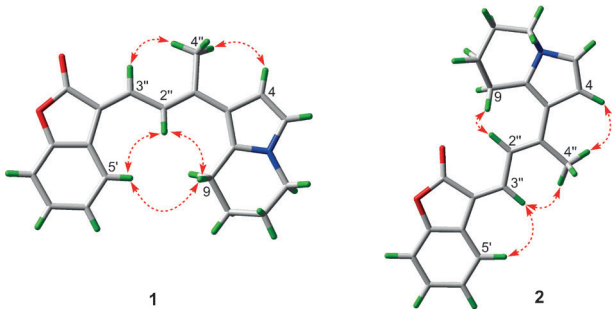


Figure 3. Key ROESY correlations of **1** and **2**.

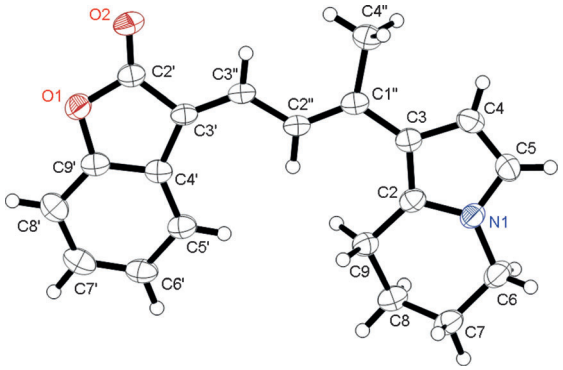


Figure 4. X-ray structure of **1**.

(Figure 4) further confirmed the structure and stereochemistry of **1**.

Suffrutine B (**2**) showed the same molecular formula, $C_{20}H_{19}NO_2$, as **1** given its HR-ESI-MS (m/z 306.1487 $[M+H]^+$, calcd for $C_{20}H_{20}NO_2$: 306.1489). The UV and IR spectra of **2** displayed characteristic absorptions similar to those of **1**. The NMR data of **2** (Table 1) were very close to those of **1** except for the obvious downfield chemical shift value of H2″ (δ_H = 7.84 ppm), thus indicating that **2** might be a geometric isomer of **1**. In the ROESY spectrum of **2**, the correlations between H2″ and $H_2$9, between H3″ and H5′, as well as between $H_3$4″ and H4/H3″ were observed (Figure 3), thus indicating the presence of a *trans* C1″–C2″ double bond and a *cis* C3′–C3″ double bond.

It is very interesting that **1** and **2**, a pair of *Z*/*E* isomeric indolizidine alkaloids, appeared to be interconvertible when stored in solution (see Figures S23 and 24 in the Supporting Information). Because the *Z*/*E* isomerization of the C–C double bond could occur under either thermal or photochemical conditions,[5] the influence of thermal treatment and light exposure on **1** and **2** were further studied. Firstly, a variable-temperature NMR experiment was conducted using a natural mixture of **1** and **2** (in a proportion about 5:3; Figure 5a) to investigate the influence of the thermal process. As the temperature increased, there was no obvious

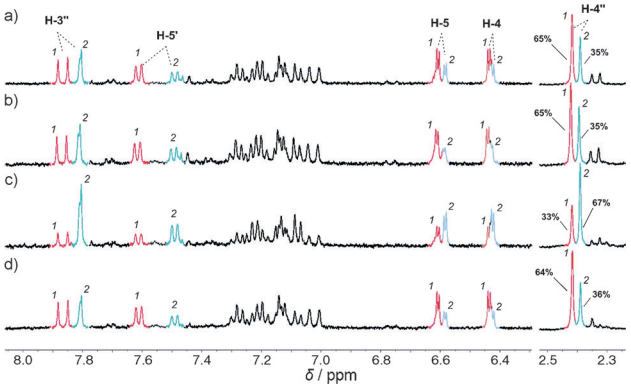
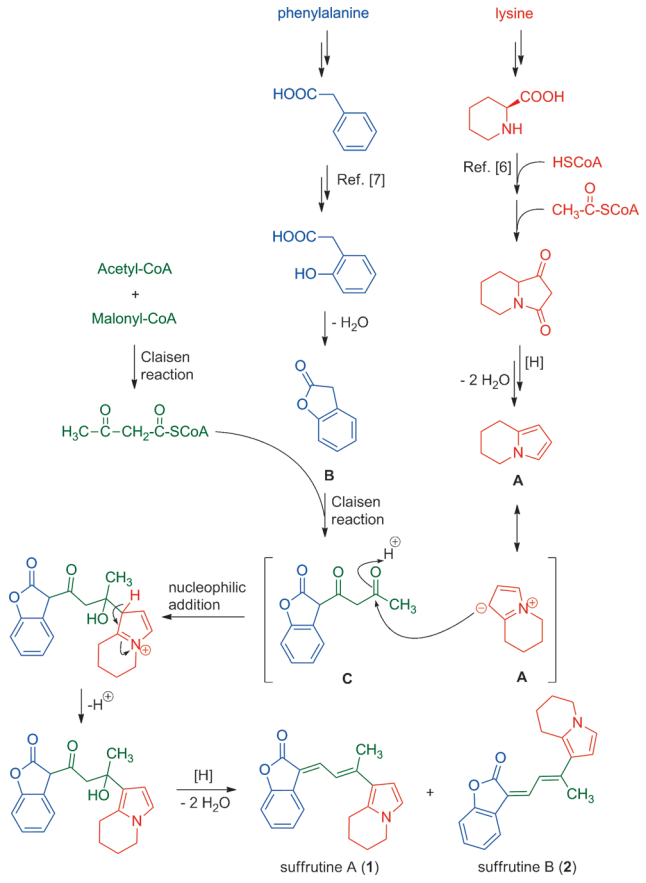


Figure 5. ^{1}H NMR spectra for the mixture of **1** and **2** during the isomerization process (400 MHz, $C_2D_2Cl_4$, 298 K). a) Natural mixture. b) After 4 h of UV irradiation in 254 nm. c) After 4 h of UV irradiation in 365 nm. d) Exposed to the incandescent lamp for 4 h after UV irradiation in 365 nm.

variation in the population of the two isomers (see Figure S25). Subsequently, the irradiation experiment was used to study the effect of the light. When the mixture was irradiated at $\lambda = 254$ nm, the proportion of the two isomers did not change (Figure 5b). However, after irradiation at $\lambda =$ 365 nm, the amount of the *cis*-isomer **2** was significantly increased (Figure 5c). Then, when the irradiated mixture was exposed to the incandescent lamp (used as a source of visible light) for 4 hours, the proportion of the two isomers (**1** and **2**) returned to the initial ratio (about 5:3, Figure 5d). Based on the above results, the interconversion of **1** and **2** can be induced by light. Namely, the isomerization from **1** to **2** can be triggered by UV light at $\lambda = 365$ nm, whereas the inverse process could be induced by visible light.

The compounds **1** and **2** represent the first examples of a new class of indolizidine alkaloids with a unique and highly conjugated backbone. The biogenetic pathway for **1** and **2** could be proposed as shown in Scheme 1. The two key biogenetic intermediates, tetrahydroindolizidine (**A**) and benzofuranone (**B**), could be generated from the amino acid precursors lysine[6] and phenylalanine,[7] respectively, by a series of oxidative decarboxylation and acetylation processes. In the meantime, acetyl-CoA and malonyl-CoA could participate to form acetoacetyl-CoA through a Claisen condensation, and acetoacetyl-CoA reacts with **B** by another Claisen condensation to generate intermediate **C**. Finally, **1** and **2** could be formed through the nucleophilic addition between intermediates **A** and **C**, and the loss of hydride and H_2O.



Scheme 1. Hypothetical biogenetic pathway of **1** and **2**.

To screen the potential bioactivity of suffrutines A and B (**1** and **2**) on the nervous system, we took advantage of a mouse neuroblastoma cell model (Neuro-2a cells) to study the effect of these compounds on neuronal differentiation.[8] As a result, **1** could induce the differentiation of the cells into neuronlike morphology, such as elongation of cell bodies and extension of neurites (Figure 6a). Statistical analysis showed that a remarkable increase in the population of cells was differentiated upon treatment with **1** when compared to the control cells (Figure 6b). Moreover, the average length of total neurites and the longest neurites were substantially enhanced by **1** (Figure 6c,d). However, **2** did not show potent activity on regulating the morphology of Neuro-2a cells.

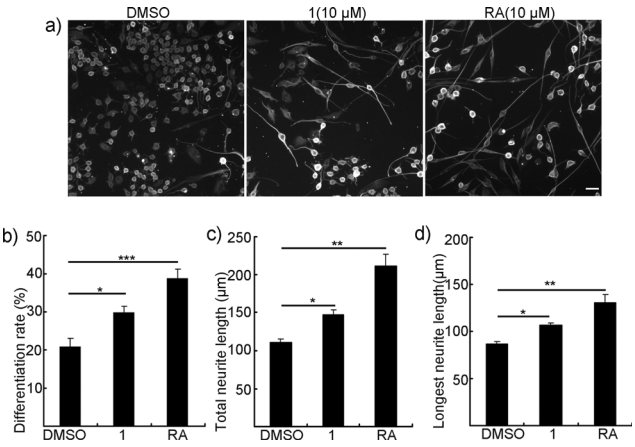


Figure 6. The compound **1** promotes differentiation of Neuro-2a cells. a) Neuro-2a cells were treated with **1** (10 μM) or retinoic acid (RA, 10 μM), a compound that promotes neural differentiation, for two days. DMSO was a solvent control. Cells were immunostained using β-tubulin III antibody for visualization of neurites. Scale bar, 50 μm. b) Cell differentiation rate (% of cells that bear neurites). c) Average length of total neurites, and d) average length of the longest neurites. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, student's *t*-test, at least 400 cells/group were analyzed in each experiment, $n = 4$. Error bars indicate ± s.e.m.

In summary, our current study describes in the discovery of a pair of novel *Z*/*E* isomeric indolizidine alkaloids (**1** and **2**) with an unprecedented skeleton from the roots of *F. suffruticosa*. The compounds **1** and **2** represent the first examples of simple indolizidine alkaloids with a unique and highly conjugated C_{20} skeleton. Because of the highly conjugated backbone, the energy barrier of *Z*–*E* interconversion of **1** and **2** is significantly low, and makes them easily interconvert under the irradiation of UV/Vis light. The compound **1** can absorb $\lambda = 365$ nm UV light and thus convert into **2**, which implies that **1** could be used as a sunscreen ingredient.[9] These compounds provide an interesting case wherein nature has the amazing ability to combine several different biosynthetic precursors (amino acids and polyketides) to generate structural diversity within molecules. Furthermore, the potential activity of **1** on regulating the

morphology of Neuro-2a cells might give some insight into the discovery of new lead compounds for the drug development on central nervous system.

Experimental Section

The isolation procedure, UV, IR, MS, and NMR spectra of **1** and **2**, detailed VT-NMR and irradiation NMR experiments data, as well as crystallographic data (CIF file) of **1** are given in the Supporting Information. CCDC 967597 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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