Two New Alkaloids from *Flueggea virosa*

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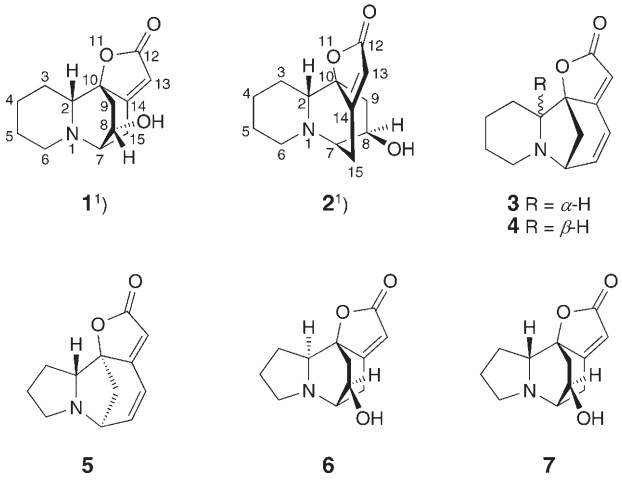
Two new securinega-type alkaloids and four known ones were isolated from the twigs and leaves of *Flueggea virosa*. The structures of the new compounds were elucidated by means of spectroscopic methods (UV, IR, HR-ESI-MS, and 1D- and 2D-NMR), and the absolute configurations were assigned by CD spectra. The structures of the known compounds were identified by comparison of their physical and spectroscopic data with those reported in the literature.

Introduction. –The plant *Flueggea virosa* ROXB. ex WILLD. (Euphorbiaceae) as a folk medicine was often used for the treatment of rheumatism, pruritus, cephalic eczema, leucorrhoea, and injuries. It also showed a curative effect in the clinical treatment of neonatal impetigo, allergic dermatitis and scald [1][2]. Previous phytochemical investigations of this plant led to the isolation of a number of securinega-type alkaloids [3–7]. Among them, several alkaloids showed strong cytotoxicities [6]. Recently, two C–C linked dimeric indolizidine alkaloids were isolated from the roots of this plant [8].

In our current project, two new securinega-type alkaloids named as virosines A (**1**) and B (**2**) were isolated from the twigs and leaves of *F. virosa*, as well as the four known ones virosecurinine (**3**), viroallosecurinine (**4**), norsecurinine (**5**), and bubbialidine (**6**). The structures of the new alkaloids were elucidated by extensive spectroscopic analyses, especially by their 2D-NMR and CD spectra. The known alkaloids were identified by comparison of their physical and spectroscopic data with those reported in the literature.

Results and Discussion. – The air-dried twigs and leaves of *F. virosa* were extracted with 95% EtOH. The residue of the extract was suspended in H_2O and acidified with HCl. The acidic suspension was partitioned with $CHCl_3$ to remove the neutral components. The aqueous layer was then basified with $NH_3 \cdot H_2O$ and re-extracted with $CHCl_3$ to obtain a residue which was subjected to silica gel columns to afford compounds **1**–**6**.

Compound **1** was obtained as colorless oil with $[\alpha]_D^{20} = +71$ ($c = 0.10$, MeOH). The HR-ESI-MS of **1** exhibited a quasimolecular ion at m/z 236.1284 (calc. 236.1287, for $[M+H]^+$), consistent with the molecular formula $C_{13}H_{17}NO_3$. The IR spectrum suggested the presence of a OH group (3370 cm^{-1}) and an α,β-unsaturated γ-lactone



ring (1763 and 1654 cm^{-1}). The structure of **1** was established on the basis of 1D- (^{1}H- and ^{13}C-NMR (*Table*), and DEPT) and 2D-NMR (COSY, HSQC, HMBC, and NOESY (*Fig. 1*)) data, as well as by comparison with the data of securinol A (**7**) [9]. The absolute configuration of **1** was determined through the CD spectrum (*Fig. 2*, *a*) analysis.

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of Compounds* **1** *and* **2**[1]). δ in ppm, J in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
H–C(2)	65.1 (*d*)	2.64–2.70 (*m*)	63.2 (*d*)	2.24 (br. *d*, *J* = 10.4)
H_a–C(3)	25.5 (*t*)	1.46–1.54 (*m*)	25.7 (*t*)	1.56–1.62 (*m*)
H_b–C(3)		0.80–0.88 (*m*)		1.39–1.46 (*m*)
H_a–C(4)	23.8 (*t*)	1.78 (br. *d*, *J* = 12.6)	24.6 (*t*)	1.85 (*td*, *J* = 3.1, 9.7)
H_b–C(4)		1.24–1.32 (*m*)		1.28–1.36 (*m*)
H_a–C(5)	26.4 (*t*)	1.46–1.54 (*m*)	26.7 (*t*)	1.56–1.62 (*m*)
H_b–C(5)		1.46–1.54 (*m*)		1.56–1.62 (*m*)
H_a–C(6)	52.5 (*t*)	2.89–2.95 (*m*)	52.5 (*t*)	2.78–2.84 (*m*)
H_b–C(6)		2.66–2.72 (*m*)		2.64–2.72 (*m*)
H–C(7)	58.8 (*d*)	2.86–2.90 (*m*)	57.5 (*d*)	2.91–2.95 (*m*)
H–C(8)	64.6 (*d*)	4.29–4.35 (*m*)	66.7 (*d*)	4.23 (*dd*, *J* = 5.1, 8.5)
H_a–C(9)	40.6 (*t*)	2.65 (*dd*, *J* = 9.5, 12.2)	36.5 (*t*)	2.76 (*dd*, *J* = 8.6, 13.1)
H_b–C(9)		1.43 (*dd*, *J* = 4.9, 12.3)		1.20 (br. *d*, *J* = 13.1)
C(10)	84.6 (*s*)	–	84.8 (*s*)	–
C(12)	174.7 (*s*)	–	176.1 (*s*)	–
H–C(13)	111.2 (*d*)	5.65 (*s*)	108.8 (*d*)	5.63 (*s*)
C(14)	174.3 (*s*)	–	174.1 (*s*)	–
H_a–C(15)	29.3 (*t*)	2.96 (br. *d*, *J* = 18.5)	22.9 (*t*)	3.11 (*td*, *J* = 2.1, 19.3)
H_b–C(15)		2.72–2.78 (*m*)		2.78–2.84 (*m*)

[1]) Arbitrary numbering. For systematic names, see *Exper. Part.*

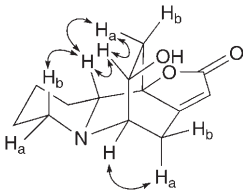


Fig. 1. *Key NOESY correlations of* **1**

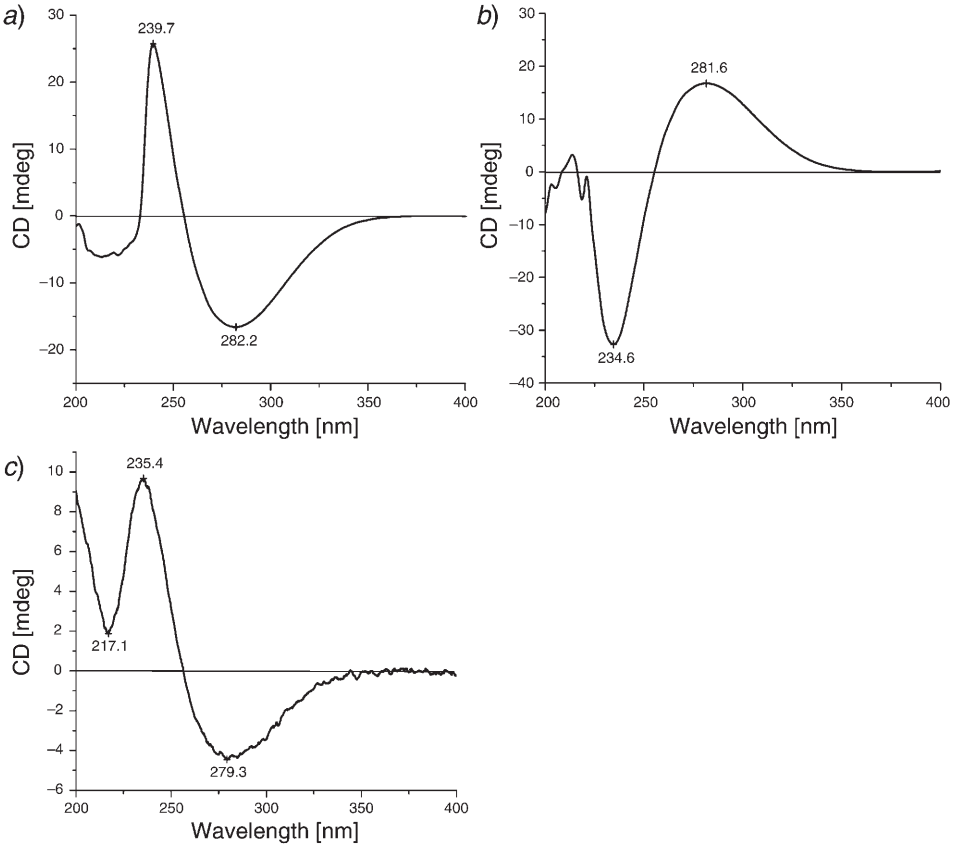


Fig. 2. *CD spectra of a*) **1**, *b*) **2**, *and c*) *securinol A* (**7**)

The ^{1}H-NMR spectrum of **1** displayed signals for an olefinic H-atom at δ(H) 5.65 (*s*) and an oxygenated H-atom at δ(H) 4.29–4.35 (*m*). A total of 13 C-atom signals were observed in the ^{13}C-NMR and DEPT spectra of **1** (*Table*). All these data suggested the presence of a neosecurinan-12-one alkaloid skeleton [9]. Comparison of the ^{13}C-NMR data of **1** with those of securinol A (**7**) [9] revealed that the signals of the two compounds were very similar except for the signal of C(8)[1]) (δ(C) 70.1 in securinol A (**7**), and δ(C) 64.4 in **1**), which indicated that they had the same constitutional formula.

The *Cotton* effect of **1** was also similar to that of securinol A (**7**) (*Fig. 2*), which suggested that the absolute configuration of C(10) and C(7) in **1** was the same as that in securinol A (**7**). Therefore, the configurations of C(10) and C(7) were supposed to be (*R*) and (*S*), respectively. Furthermore, the NOESY spectrum of **1** showed the correlations H–C(2) (δ(H) 2.64–2.70)/H_a–C(9) (δ(H) 2.65) and H–C(8) (δ(H) 4.29–4.35), H–C(8)/H_a–C(9), and no correlations between H–C(2) and CH_2(15) (δ(H) 2.96 and 2.72–2.78) and between H–C(8) and CH_2(15) were observed, indicating that absolute configurations of C(2) and C(8) in **1** were (*R*) and (*S*), respectively. The above findings led to the conclusion that the configurations of C(2), C(7), C(8) and C(10) were (*R*), (*S*), (*S*), and (*R*), respectively.

Compound **2** was obtained as colorless oil with $[\alpha]_D^{20} = +92$ ($c = 0.10$, MeOH). The HR-ESI-MS of **2** exhibited a quasimolecular ion $[M+H]^+$ at *m*/*z* 236.1282, suggesting the presence of the same molecular formula as **1**. The IR spectrum displayed the presence of a OH group (3445 cm^{-1}) and an α,β-unsaturated γ-lactone ring (1740 and 1651 cm^{-1}). The structure of **2** was elucidated by ^{1}H- and ^{13}C-NMR (*Table*), COSY, HMBC and NOESY (*Fig. 3*) data. The absolute configuration of **2** was also determined by CD spectrum (*Fig. 2*, *b*) analysis.

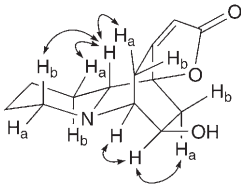


Fig. 3. *Key NOESY correlations of* **2**

The ^{1}H-NMR spectrum of **2** displayed signals for an olefinic H-atom at δ(H) 5.63 (*s*) and an oxygenated methine group at δ(H) 4.23 (*dd*, $J = 5.1, 8.5$). A total of 13 C-atom signals were observed in the ^{13}C-NMR and DEPT spectra of **2** (*Table*). These data suggested that **2** also possessed the skeleton of a neosecurinan-12-one alkaloid [9]. Comparison of the ^{13}C-NMR data of **2** with those of securinol A (**7**) [9] and **1** revealed that the signals of the three compounds were very similar, which indicated that **2** had the same constitutional formula as securinol A (**7**) and **1**.

The *Cotton* effect of **2** was opposite to those of securinol A (**7**) and **1** (*Fig. 2*), which suggested that the absolute configurations of C(10) and C(7)[1]) in **2** were opposite to those in securinol A (**7**) and **1**. Hence, the configurations of C(10) and C(7) were assigned as (*S*) and (*R*), respectively. Furthermore, the NOESY spectrum of **2** revealed the correlations H_a–C(9) (δ(H) 2.76)/H–C(8) (δ(H) 4.23), H–C(2) (δ(H) 2.24)/H_a–C(15) (δ(H) 3.11), and no correlations between H–C(8) and CH_2(15) and between H–C(2) and H_a–C(9) and H–C(8) were observed, suggesting that the absolute configurations of C(8) and C(2) in **2** were (*R*) and (*R*). The above findings led to the conclusion that the configurations of C(2), C(7), C(8) and C(10) were (*R*), (*R*), (*R*), and (*S*), respectively.

The four known alkaloids virosecurinine (**3**) [10], viroallosecurinine (**4**) [11], norsecurinine (**5**) [5], and bubbialidine (**6**) [12] were also isolated from this plant and identified on the basis of their physical and spectroscopic data.

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Experimental Part

General. Column chromatography (CC): Silica gel (SiO_2; 200–300 mesh, *Qingdao Marine Chemical Factory*, Qingdao, P.R. China); *Sephadex LH-20* (*Pharmacia*). TLC: precoated SiO_2 GF_{254} plates (*Qingdao Marine Chemical Factory*, Qingdao, P. R. China). M.p.: *XT-4* micro-melting-point apparatus; uncorrected. Optical rotation: *Jasco P-1020* polarimeter. UV Spectra: *Perkin-Elmer Lambda 2* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. IR Spectra (KBr): *Perkin-Elmer 16 PC* FT-IR spectrometer; in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR spectra: *Bruker AV-400* spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. MS: *HP-1100 HPLC/EST* (ESI-MS) and *Biosystems Mariner™ 5140* (HR-ESI-MS) spectrometer; in *m/z*.

Plant Material. The twigs and leaves of *F. virosa* were collected in Conghua country, Guangdong province of China, in September of 2006, and authenticated by Mr. *Bo-Qing Wen* of Conghua Liuxihe Forestry Centre. A voucher specimen (No. 060915) was deposited in the herbarium of Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, P. R. China.

Extraction and Isolation. The air-dried twigs and leaves of *F. virosa* (3.2 kg) were extracted with 95% EtOH. The residue of the extract was dissolved in 5 l H_2O to form a suspension and then adjusted to pH 6 with 1M HCl. The acidic suspension was partitioned with $CHCl_3$ to remove the neutral components. The aq. phase was basified with 2% $NH_3 \cdot H_2O$ to pH 8 and extracted with $CHCl_3$ to obtain a residue (21 g). The residue was subjected to a SiO_2 column ($CHCl_3$/MeOH, 100:0 → 0:100) to give six major fractions 1–6. *Fr. 2* was re-chromatographed on a SiO_2 column ($CHCl_3$/MeOH, 99:1 to 70:30) to afford **3** (1.03 g), and **4** (130 mg). *Fr. 3* was re-chromatographed on a SiO_2 column ($CHCl_3$/MeOH, 98:2 to 70:30) to afford **5** (35 mg) and two major subfractions 3a and 3b. *Subfrs. 3a* and *3b* were purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1) to afford **1** (12 mg) and **2** (9 mg), resp. *Fr. 4* was separated by CC (SiO_2, $CHCl_3$/MeOH, 25:1) to afford **6** (30 mg).

Virosine A (=*(5S,10aR,10bR,12R)-4,5,8,9,10,10a-Hexahydro-12-hydroxy-7*H-*5,10b-ethano-2*H-*furo[2,3-*a*]quinolizin-2-one*; **1**): Colorless oil. $[\alpha]_D^{20} = +71$ ($c = 0.10$, MeOH). UV (MeOH): 228 (4.33). CD (MeOH): $\Delta\varepsilon_{240} = +25.6$, $\Delta\varepsilon_{282} = -16.3$. IR (KBr): 3370, 2948, 2848, 1799, 1763, 1654, 1141. ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 236.1284 ($[M+H]^+$, $C_{13}H_{18}NO_3^+$; calc. 236.1287).

Virosine B (=*(5R,10aR,10bS,12R)-4,5,8,9,10,10a-Hexahydro-12-hydroxy-7*H-*5,10b-ethano-2*H-*furo[2,3-*a*]quinolizin-2-one*; **2**): Colorless oil. $[\alpha]_D^{20} = +92$ ($c = 0.10$, MeOH). CD (MeOH): $\Delta\varepsilon_{235} = -33.3$, $\Delta\varepsilon_{282} = +17.4$. IR (KBr): 3445, 2926, 1740, 1651, 1150. ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 236.1282 ($[M+H]^+$, $C_{13}H_{18}NO_3^+$; calc. 236.1287).

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