Two New *Securinega* Alkaloids from *Securinega suffruticosa*

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Two new *Securinega* alkaloids, 2-episecurinol A (**1**) and 8-(diethylamino)-2-episecurinol A (**2**), together with the five known related analogues **3**–**7**, were isolated from *Securinega suffruticosa* (PALL.) REHD. Their structures were determined by detailed analysis of 1D- and 2D-NMR spectra and by comparison with the related model compounds.

Introduction. – Plants within the Euphorbiaceae family elaborate a diverse number of alkaloids with diverse structures and biological properties. The *Securinega* alkaloids comprise a total of 42 compounds isolated mainly from two or three species of the S*ecurinega* and the *Phyllanthus* genera. The antitumor, antimalarial, antibacterial, and central nervous system (CNS) activity of these compounds has initiated great interest in the synthesis of this structurally unique class of alkaloids [1–5] and attracted considerable attention of pharmacologists [6–11]. *Securinega suffruticosa*, a kind of semi-shrub plant widely distributed in the subtropical zone, is one of Chinese folk medicines used to treat rheumatic disease, quadriplegia, impotence, and children's malnutrition, *etc.* As part of our ongoing research on the bioactive constituents from traditional Chinese medicinal herbs [12–14], we made a collection of the title plant in Anhui Province, P. R. China. Separation of the CH_3Cl-soluble fraction of the MeOH extract of this plant resulted in the isolation of two new *Securinega* alkaloids, namely 2-episecurinol A (**1**) and 8-(diethylamino)-2-episecurinol A (**2**), together with the five known related analogues **3**–**7**) (see *Fig. 1*). This paper describes the isolation and structure elucidation of the new compounds **1** and **2**.

Results and Discussion. – Air-dried, powdered stems of *S. suffruticosa* were extracted with MeOH, and this extract was partitioned between AcOEt and an acidic aqueous phase (pH 4–5). The aqueous layer, adjusted to pH 9–10 by addition of Na_2CO_3, was then extracted with $CHCl_3$. The $CHCl_3$-soluble material was subjected to repeated column chromatography on silica gel to give seven compounds, of which two, **1** and **2**, are new and the others, **3**–**7**, are known ones.

The known compounds were readily identified as allosecurinine (**3**) [15], virosecurinine (**4**) [16], securitinine (**5**) [17], 15α-methoxy-14,15-dihydrophyllochrysine (**6**) [16], and phyllanthidine (**7**) [18], respectively, by analysis of their NMR spectra and by comparison with the data reported in the literature.

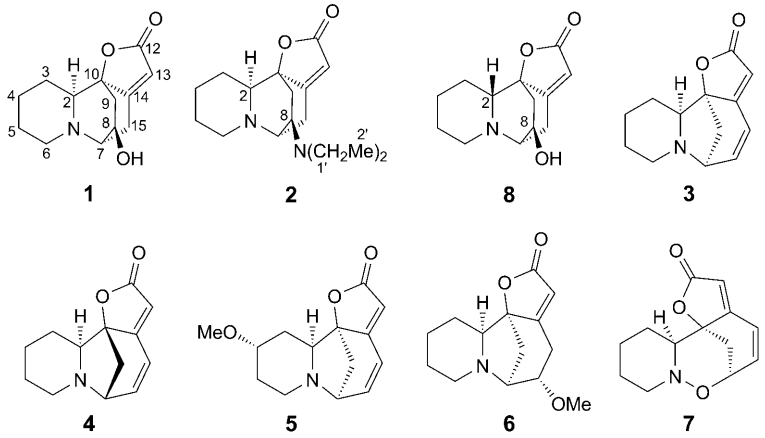

Fig. 1. *Structures of compounds* **1**–**8**

2-Episecurinol A (**1**) was obtained as a colorless oil. The molecular formula, $C_{13}H_{17}NO_3$, consistent with six degrees of unsaturation, was determined by HR-ESI-MS (*m*/*z* 236.1290 ($[M+H]^+$); calc. 236.1287). The IR spectrum of **1** showed absorption bands implying the presence of an α,β-unsaturated γ-lactone (1736 and 1637 cm^{-1}) moiety that was supported by the UV absorption at λ_{max} 254 nm (log ε 4.06, MeOH). The ^{13}C-NMR spectrum (*Table*) exhibited 13 signals (six CH_2, four CH, and three C) whose chemical shift values and multiplicities (DEPT) not only confirmed the presence of a butenolide moiety (δ(C) 85.0 (*s*), 108.5 (*d*), 174.5 (*s*), and 176.9 (*s*)), but also showed the presence of a OH-substituted methine group (δ(C) 69.4 (*d*)), and three N-bearing C-atoms (δ(C) 52.4 (*t*), 59.4 (*d*), and 63.0 (*d*)) in the molecule. From these spectral data, compound **1** was deduced to be tetracyclic, containing a C=C bond and a C=O group.

Detailed analysis of 1D- and 2D-NMR spectra revealed that the constitution of **1** was identical with the one of securinol A (**8**) [3], which was isolated from the leaves of the same plant for the first time in 1965. In fact, as shown in the *Table*, the ^{13}C-NMR data from C(2) to C(6) and from C(7) to C(12), and C(15) of **1** and **8** are almost identical, whereas the notable differences between them are mainly manifest at C(13) and C(14). Although the δ(C) value of C(2) in both **1** and **8** is almost the same, the ^{1}H-NMR chemical shifts of the bridgehead H–C(2) of **1** and **8** are distinctly different (δ(H) 2.20 in **1** and δ(H) 3.23 in **8**) indicating that compound **1** differs from **8** only in the relative configuration at C(2). Finally, the α-configuration of H–C(2) of **1** was unambiguously determined by the observation of the diagnostic ROESY correlation between H–C(2) and H_α–C(6), and no correlation between H–C(2) and CH_2(9) (see *Fig. 2*). Thus, compound **1** was determined to be the C(2) epimer of **8**.

Since compound **1** contains a secondary OH group at C(8), we tried to determine its absolute configuration by applying the modified *Mosher*'s method [19]. Unfortunately, we failed to obtain the corresponding MTPA esters when treating **1** with (*R*)- and (*S*)-MTPA chlorides in dry pyridine at room temperature. However, considering the close

Table. *^{1}H- and ^{13}C-NMR Data*[a]) *of Compounds* **1** *and* **2**, *and ^{13}C-NMR data*[a]) *of* **8**. At 400 MHz in $CDCl_3$; δ in ppm, J in Hz.

	1		**2**		**8**
	δ(H)	δ(C)	δ(H)	δ(C)	δ(C)
H_α–C(2)	2.20 (*dd*, *J* = 10.2, 1.6)	63.0 (*d*)	2.17–2.19 (*m*)	62.2 (*d*)	62.8 (*d*)
H_α–C(3)	1.60–1.62 (*m*)	25.6 (*t*)	1.53–1.55 (*m*)	24.7 (*t*)	24.2 (*t*)
H_β–C(3)	0.87–0.89 (*m*)		0.86–0.88 (*m*)		
H_α–C(4)	1.25–1.26 (*m*)	22.5 (*t*)	1.17–1.19 (*m*)	24.0 (*s*)	22.4 (*t*)
H_β–C(4)	1.86 (*dd*, *J* = 2.6, 12.5)		1.80 (*d*, *J* = 12.5)		
H_α–C(5)	1.52–1.53 (*m*)	26.5 (*t*)	1.58–1.60 (*m*)	27.0 (*d*)	26.6 (*t*)
H_β–C(5)	1.51–1.53 (*m*)		1.40–1.42 (*m*)		
H_α–C(6)	2.62–2.68 (*m*)	52.4 (*t*)	2.71 (*d*, 11.2)	51.4 (*t*)	52.5 (*t*)
H_β–C(6)	2.75–2.77 (*m*)		2.98–3.00 (*m*)		
H_α–C(7)	2.90 (*t*, *J* = 2.5)	59.4 (*d*)	3.30–3.32 (*m*)	59.6 (*t*)	59.2 (*d*)
H–C(8)	4.20 (*dd*, *J* = 4.9, 8.2)	69.4 (*d*)	3.00–3.02 (*m*)	67.0 (*d*)	69.8 (*d*)
H_α–C(9)	1.20 (*d*, *J* = 13.3)	41.1 (*t*)	2.03 (*d*, *J* = 10.0)	35.2 (*t*)	41.1 (*t*)
H_β–C(9)	2.78–2.79 (*m*)		2.28 (*dd*, *J* = 9.6,6.0)		
C(10)		85.0 (*s*)		89.8 (*s*)	84.5 (*s*)
C(12)		174.5 (*s*)		172.3 (*s*)	173.6 (*s*)
H–C(13)	5.60 (*s*)	108.5 (*d*)	5.70 (*s*)	112.5 (*d*)	112.7 (*d*)
C(14)		176.9 (*s*)		174.3 (*d*)	171.8 (*d*)
H_α–C(15)	3.10 (*d*, *J* = 19.1)	28.0 (*t*)	2.96–2.98 (*m*)	28.8 (*d*)	30.2 (*d*)
H_β–C(15)	2.74–2.77 (*m*)		2.85–2.87 (*m*)		
			2.63 (*m*, 2 CH_2(1′))	43.6 (*t*)	
			1.00 (*t*, *J* = 7.1, 2 Me(2′))	11.3 (*q*)	

[a]) *Bruker-DRX-400* NMR spectrometer; assignments made with the aid of DEPT, ^{1}H,^{1}H-COSY, HMQC, HMBC, and ROESY experiments.

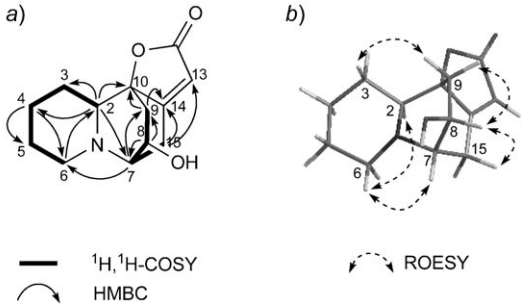

Fig. 2. *Selected ^{1}H,^{1}H-COSY and HMBC* (*a*), *and ROESY data* (*b*) *of compound* **1**

biogenetic relationship of **1** and **8** and for chemotaxonomic reasons, the absolute configuration of **1**, except for C(2), was tentatively assigned to be the same as that of **8**.

8-(Diethylamino)-2-episecurinol A (**2**) was obtained as a colorless oil. The molecular formula $C_{17}H_{26}N_2O_2$ was determined by HR-ESI-MS (*m*/*z* 313.1905 ([*M* + Na]$^+$); calc. 313.1892), consistent with six degrees of unsaturation. It was immediately apparent from the ^{1}H- and ^{13}C-NMR data, that **2** differs from **1** only in the substituent at

C(8), where the OH group in **1** was replaced by a diethylamino moiety (δ(H) 2.63 (*m*, 4 H), 1.00 (*t*, J = 7.1, 6 H); δ(C) 43.6 (*t*), 11.3 (*q*)) in **2**. The significant HMBC cross-peak from $CH_2(1')$ (δ(H) 2.63) to C(8) (δ(C) 67.0) clearly showed that a diethylamino moiety was linked to C(8) (*Fig. 3*). Finally, by analogy to compound **1**, the configuration of **2** was deduced to be the same as in **1**.

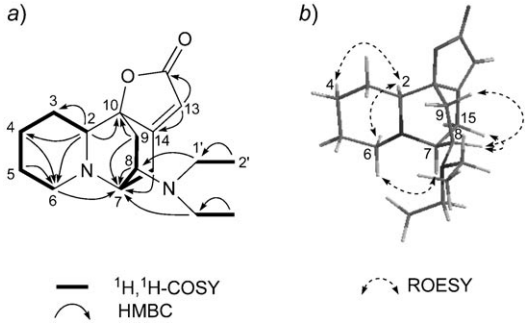

Fig. 3. *Selected ^{1}H,^{1}H-COSY and HMBC* (*a*)*, and ROESY data* (*b*) *of compound* **2**

The carbon skeleton of compounds **1**–**8** is quite rare in nature. To our knowledge, this is the second report about securinol A-like alkaloids from a natural source. The discovery of compounds **1** and **2** widened the knowledge of this intriguing group of compounds. It may be worth to point out that we have doubted about the origin of compound **2** because it differs from **1** only by the different substituent at C(8). In order to rule out the possibility that **2** might be produced during the isolation process, the MeOH extract of the plant was re-examined on the TLC with pure compound **2** as reference. The unequivocal detection of the same compound in the original extract with an identical TLC R_f value as that of the reference compound proved that **2** is a natural product and not an artifact of isolation.

The new compounds **1** and **2** were evaluated for their inhibitory activity against hPTP1B (human protein tyrosine phosphatase 1B), a key target for the treatment of type II diabetes and obesity [20]. Unfortunately, the results indicated that both compounds were inactive. Other bioassay studies for antibacterial and anti-inflammatory activities are currently underway.

Experimental Part

General. Column chromatography (CC): commercial silica gel (SiO_2; *Qing Dao Hai Yang Chemical Group Co.*; 200–300 mesh), amino silica gel (*Merck*; *LiChroprep NH$_2$*, 40–63 μm), and *Sephadex LH-20* (*Amersham Biosciences*). TLC: precoated SiO_2 plates (*Yan Tai Zi Fu Chemical Group Co.*; *G60*, *F-254*). Optical rotation: *Perkin-Elmer 341* polarimeter. UV Spectra: *756 CRT* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Magna FT-IR 750* spectrophotometer; ν_{max} in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Varian Mercury 400* (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer; chemical shifts δ in ppm, with residual $CHCl_3$ (δ(H) 7.26, δ(C) 77.0) or CD_3OD (δ(H) 3.30, δ(C) 49.5) as internal standard, coupling constant J in Hz. ESI-MS and HR-ESI-MS: *Q-TOF Micro* LC/MS-MS spectrometer in *m*/*z*.

Plant Material. *S. suffruticosa* (PALL.) REHD. was collected in Anhui Province, P. R. China, in June, 2007, and identified by Assoc. Prof. *X.-H. Song* of China Pharmaceutical University. A voucher specimen (P06-39) is available for inspection at the Herbarium of Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The air-dried, powdered stems (2.1 kg) of *S. suffruticosa* were exhaustively extracted with MeOH (3×1 l, each 7 d) at r.t. Evaporation of the solvent gave a residue, which was suspended in H_2O (1 l) and adjusted to pH 4–5 with 2N H_2SO_4. The acidic mixture was defatted with AcOEt (3×1 l), and the aq. layer was basified to pH 9–10 with sat. Na_2CO_3, and then extracted with $CHCl_3$ (3×1 l) and BuOH (3×1 l). The $CHCl_3$-soluble material was subjected to SiO_2 chromatography eluted with a $CHCl_3$/MeOH/Et_2NH (50:1:0.1 to 1:1:0.1) gradient, and the fractions eluted with $CHCl_3$/MeOH/Et_2NH (25:1:0.1) were further purified by amino silica gel (eluted with petroleum ether/AcOEt (4:1) and $CHCl_3$/MeOH (95:5)) to afford pure compounds **1** (6.7 mg), **2** (12.7 mg), **3** (16.1 mg), **4** (232.1 mg), **6** (7.2 mg), and **7** (8.3 mg).

2-Episecurinol A (=*(5*S*,10a*S*,10b*R*,12*R*)-4,5,8,9,10,10a-Hexahydro-12-hydroxy-2*H*,7*H*-5,10b-ethanofuro[2,3-*a*]quinolizin-2-one*; **1**). Colorless oil. $[\alpha]_D^{23} = -51.1$ ($c = 0.15$, $CHCl_3$). UV (MeOH): 254 (4.06). IR (KBr): 1736, 1637. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 236 ($[M+H]^+$). HR-ESI-MS: 236.1290 ($[M+H]^+$; calc. 236.1287).

8-(Diethylamino)-2-episecurinol A (=*(5*S*,10a*S*,10b*R*,12*R*)-12-(Diethylamino)-4,5,8,9,10,10a-hexahydro-2*H*,7*H*-5,10b-ethanofuro[2,3-*a*]quinolizin-2-one*; **2**). Colorless oil. $[\alpha]_D^{23} = +77.4$ ($c = 0.05$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 291 ($[M+H]^+$). HR-ESI-MS: 313.1905 ($[M+Na]^+$; calc. 313.1892).

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