Alkaloids from the Roots of *Stemona tuberosa*

by **Jun-Ping Hu**[a])[b]), **Dong-Hui Yang**[a]), **Wen-Han Lin**[a]), and **Shao-Qing Cai***[a])

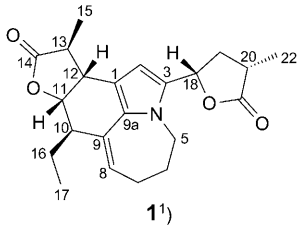
[a]) State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing 100191, P. R. China (phone/fax: +86-10-82801693; e-mail: sqcai@bjmu.edu.cn)

[b]) School of Pharmaceutical Sciences, Xinjiang Medical University, Urumqi 830054, P. R. China

Four new alkaloids, didehydrotuberostemonine A (**1**), stemoninone (**2**), tuberostemospiroline (**3**), and tuberostemonine L (**4**), together with seven known alkaloids, were isolated from the roots of *Stemona tuberosa*. Their structures were elucidated on the basis of spectroscopic analysis. The known alkaloids were identified as 2-oxostenine (**5**), tuberostemonine (**6**), sessilifoliamide H (**7**), tuberostemonone (**8**), didehydrotuberostemonine (**9**), bisdehydrostemoninine (**10**), and tuberostemoamide (**11**).

Introduction. – Stemonae Radix ('Baibu' in Chinese), the dried root of *Stemona tuberosa* LOUR. and related *Stemona* species (Stemonaceae family), has a long history in traditional Chinese medicine. It is often used as an antitussive drug to treat respiratory disorders, *e.g.* bronchitis, pertussis, and tuberculosis, and also as an anthelmintic agent for domestic animals [1]. The total alkaloid extract of *S. tuberosa* exhibited potent antitussive activity in guinea pigs [2][3]. Several stemona alkaloids have been found to exert anticough and insecticidal activities [3–6]. To date, it has been discovered more than 80 stemona alkaloids isolated from *Stemona* species, which can be structurally classified into six different groups, the stenine group, stemoamide group, tuberostemospironine group, stemonamine group, parvistemoline group, and miscellaneous group, respectively [7]. Chemical diversity and variability were significant in *S. tuberosa* [8]. In the research for biologically active alkaloids of *S. tuberosa*, four new stemona alkaloids were isolated and identified as didehydrotuberostemonine A (**1**), stemoninone (**2**), tuberostemospiroline (**3**), and tuberostemonine L (**4**), together with the seven known alkaloids 2-oxostenine (**5**), tuberostemonine (**6**), sessilifoliamide H (**7**), tuberostemonone (**8**), didehydrotuberostemonine (**9**), bisdehydrostemoninine (**10**), and tuberostemoamide (**11**), from the roots of this plant.

Results and Discussion. – A 95% EtOH extract of the plant material was acidified with dilute HCl (4%, *v*/*v*), and the acid soluble fraction was adjusted to pH 9 with 36% aqueous NH_3, and then extracted with $CHCl_3$. The $CHCl_3$ extract was used to isolate alkaloids by chromatographic methods. Altogether eleven alkaloids were obtained (**1**–**11**, resp.). The structures of the new compounds **1**–**4** were elucidated through 1D- and 2D-NMR analysis. The spectroscopic data for **5** was reported fully for the first time. Compounds **6**–**11** were identified by comparing their spectroscopic data with those reported in the literature [9–14].



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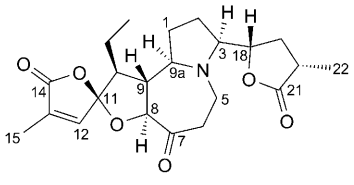

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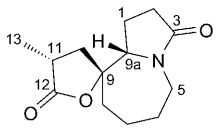

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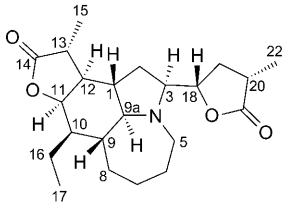

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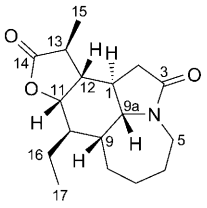

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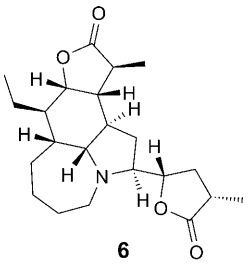

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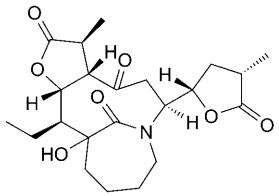

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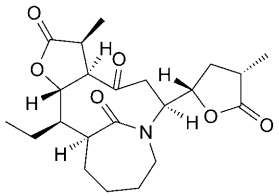

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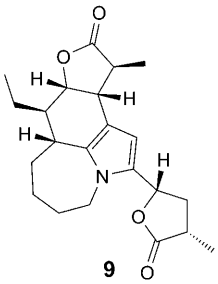

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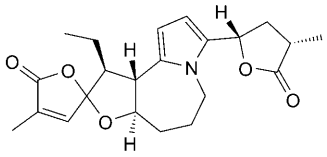

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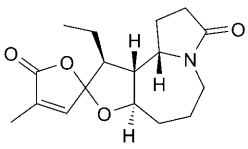

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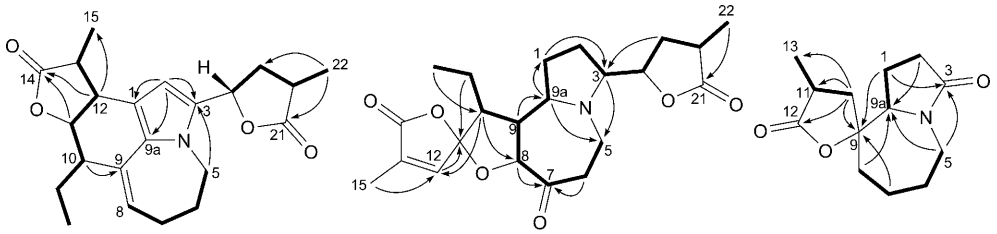
Compound **1** was obtained as pale yellow powder. HR-ESI-MS (*m/z* 370.2009, $[M + H]^+$; calc. 370.2018) suggested that **1** had the molecular formula $C_{22}H_{27}NO_4$, with ten degrees of unsaturation. The cleavage fragment *m/z* 271 $[M - C_5H_7O_2]^+$ in the EI-MS spectrum indicated the presence of an α-methyl-γ-lactone ring annexed to C(3) [11]. The IR absorption at 1768 cm^{-1}, in combination with ^{13}C-NMR data at δ(C) 178.73 (*s*) and 179.49 (*s*), indicated the presence of two saturated γ-lactones in the structure. The ^{1}H-NMR indicated the presence of three Me signals at δ(H) 0.93 (*t*, *J* = 7.5, Me(17)[1])), 1.40 (*d*, *J* = 7.0, Me(22)), and 1.43 (*d*, *J* = 7.5, Me(15)), two low-field H-atoms attached to C-atoms bearing an O-fragment at δ(H) 4.74 (*dd*, *J* = 4.0, 5.5, H–C(11)) and 5.35 (*dd*, *J* = 5.0, 10.0, H–C(18)). Additional two H-atom signals at

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

δ(H) 6.00 (*s*) and 5.46 (br. *t*, $J = 4.5$) indicated the presence of two olefinic H-atoms. The long-range correlations between δ(H) 6.00 (H–C(2)) and δ(C) 116.58 (C(1)), 128.61 (C(3)), and 129.70 (C(9a)), between δ(C) 128.61 (C(3)) and δ(H) 3.73 (*dd*, $J =$ 9.0, 11.0, H_α–C(5)) and 4.34 (*dd*, $J = 7.0, 13.0$, H_β–C(5)) in HMBC spectrum suggested the presence of a pyrrole ring in the structure. These data resembled those of **9** which belongs to the stenine-group alkaloid [7][12], except for the presence of two downfield C-atom signals at δ(C) 124.07, 125.33 and a downfield H-atom signal at δ(H) 5.46. According to the correlations of δ(H) 5.46 with 2.46–2.48 and 2.55–2.56 (each 1 H, *m*, CH_2(7)) in the ^{1}H,^{1}H-COSY spectrum, in combination with the long-range correlations between the C-atom signal at δ(C) 124.07 with the two H-atom signals at δ(H) 1.23–1.27 and 1.39–1.41 (each 1 H, *m*, CH_2(16)) in the HMBC spectrum and the correlation between δ(H) 5.46 with δ(C) 125.33 in the HMQC, the H-atom signal at δ(H) 5.46 and C-atom signal at δ(C) 125.33 were assigned to the C(8) position, and C-atom signal at δ(C) 124.07 was assigned to C(9), respectively (*Fig. 1*). All ^{1}H- and ^{13}C-NMR signals were assigned by ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra (*Tables 1* and *2*).



1[1]) **2**[1]) **3**[1])

Fig. 1. *Key ^{1}H,^{1}H-COSY* (—) *and HMBC* (H → C) *correlations for* **1**–**3**

Considering the biogenetic relationships of the stenine-group alkaloids in *S. tuberosa*, the Me group attached to C(20) of the γ-butyrolactone ring attached at C(3) showed α-orientation, and the Et group at C(10) was represented with β-orientation, respectively [7], the relative configuration of **1** was assigned by NOESY spectrum. The β-orientation of H–C(18) was determined by its correlation with H–C(20) and no correlation with Me(22) in the NOESY spectrum. Correlations between H–C(12) and CH_2(16), which was further correlated with H–C(11), and between Me(15) and H–C(12) and H–C(11) suggested that H–C(11), H–C(12), and Me(15) were β-oriented (*Fig. 2*). As a consequence, H–C(10) and H–C(13) were α-oriented, which was further supported by missing NOESY cross-peaks between H–C(13) and H–C(11), and H–C(12) and H–C(10). Accordingly, the structure of **1** was named as didehydrotuberostemoninine A.

Compound **2**, white powder, was assigned the molecular formula of $C_{22}H_{29}NO_6$ by HR-ESI-MS (*m*/*z* 404.2064, $[M+H]^+$; calc. 404.2073), with nine degrees of unsaturation. The low-field CO C-atom signals at δ(C) 173.30 and 181.74, coupled with the strong IR absorptions at 1767 and 1711 cm^{-1}, suggested the presence of two γ-lactone moieties in **2**. Two olefinic C-atom signals at δ(C) 134.14 (*s*) and 147.10 (*d*) in association with one spiro C-atom signal at δ(C) 114.39 (*s*) indicated the presence of a ketal group and

Table 1. *^{1}H-NMR Spectroscopic Data for Alkaloids* **1**–**5**[1])[a])

Position	**1**	**2**	**3**	**4**	**5**
1α		2.41–2.46 (*m*)	1.66–1.69 (*m*)	1.96–1.98 (*m*)	2.08–2.15 (*m*)
1β		1.80–1.83 (*m*)	2.16–2.22 (*m*)		
2α	6.00 (*s*)	2.41–2.46 (*m*)	2.37–2.39 (*m*)	1.03–1.10 (*m*)	1.89–1.94 (*m*)
2β		1.81–1.84 (*m*)	2.37–2.39 (*m*)	2.10–2.15 (*m*)	2.02–2.05 (*m*)
3		3.28–3.30 (*m*)		2.77–2.81 (*m*)	
5α	3.73 (*dd*, *J* = 9.5, 11.0)	2.55 (*dd*, *J* = 4.0, 11.0)	3.17–3.22 (*m*)	2.27–2.32 (*m*)	2.54–2.57 (*m*)
5β	4.34 (*dd*, *J* = 7.0, 13.0)	2.98–3.00 (*m*)	3.64 (*dd*, *J* = 4.0, 12.5)	3.32–3.34 (*m*)	3.99 (*dd*, *J* = 1.5, 14.0)
6α	1.90–1.92 (*m*)	1.71–1.78 (*m*)	1.54–1.56 (*m*)	1.47–1.50 (*m*)	2.38–2.46 (*m*)
6β	2.12–2.14 (*m*)	2.10 (*dd*, *J* = 3.0, 10.5)	1.71–1.75 (*m*)	1.64–1.67 (*m*)	2.46–2.49 (*m*)
7α	2.46–2.48 (*m*)		1.54–1.56 (*m*)	1.17–1.19 (*m*)	1.42–1.45 (*m*)
7β	2.54–2.56 (*m*)		1.88–1.95 (*m*)	1.98–2.00 (*m*)	1.58–1.61 (*m*)
8α	5.46 (br. *t*, *J* = 4.5)	4.36 (*d*, *J* = 10)	1.88–1.95 (*m*)	1.42–1.46 (*m*)	1.67–1.70 (*m*)
8β			1.96–1.98 (*m*)	1.52–1.53 (*m*)	1.71–1.73 (*m*)
9		3.51–3.55 (*m*)		2.02–2.08 (*m*)	1.91–1.94 (*m*)
9a		3.48–3.50 (*m*)	4.02 (*dd*, *J* = 7.5, 8.5)	3.02 (*dd*, *J* = 9.0, 9.5)	3.55 (*dd*, *J* = 7.5, 11.0)
10α	2.69–2.71 (*m*)	2.79–2.81 (*m*)	1.76–1.78 (*m*)	1.56–1.61 (*m*)	1.47–1.53 (*m*)
10β			2.52 (*dd*, *J* = 3.5, 10.0)		
11	4.74 (*dd*, *J* = 4.0, 5.5)		2.82–2.88 (*m*)	4.40–4.44 (*m*)	4.56 (*dd*, *J* = 9.0, 12.0)
12	3.16–3.18 (*m*)	6.90 (*d*, *J* = 1.5)		2.13–2.15 (*m*)	2.34–2.38 (*m*)
13	2.68–2.70 (*m*)		1.29 (*d*, *J* = 7.0)	2.33–2.36 (*m*)	2.54–2.57 (*m*)
15	1.43 (*d*, *J* = 7.5)	1.90 (*s*)		1.32 (*d*, *J* = 7.5)	1.21 (*d*, *J* = 7.5)
16	1.23–1.27 (*m*)	1.33–1.35 (*m*)		1.50–1.52 (*m*)	1.56–1.60 (*m*)
	1.39–1.41 (*m*)	1.36–1.40 (*m*)		1.74–1.79 (*m*)	1.61–1.63 (*m*)
17	0.93 (*t*, *J* = 7.5)	0.77 (*t*, *J* = 7.5)		0.78 (*t*, *J* = 7.5)	0.95 (*t*, *J* = 7.5)
18	5.35 (*dd*, *J* = 5.0, 11.0)	4.49–4.51 (*m*)		4.36–4.39 (*m*)	
19α	2.15–2.20 (*m*)	1.49–1.54 (*m*)		1.53–1.56 (*m*)	
19β	2.69–2.71 (*m*)	2.46–2.50 (*m*)		2.45–2.50 (*m*)	
20	2.80–2.83 (*m*)	2.77–2.80 (*m*)		2.70–2.76 (*m*)	
22	1.40 (*d*, *J* = 7.0)	1.23 (*d*, *J* = 7.0)		1.20 (*d*, *J* = 7.0)	

[a]) Recorded at 500 MHz (**1** and **4** in $CDCl_3$, **2** and **3** in CD_3OD, **5** in CD_3COCD_3). Multiplicity and coupling constants (*J* values in Hz) are given in parentheses.

an α,β-unsaturated γ-lactone in the structure. Except for the signal of a ketone C-atom at δ(C) 213.22, the ^{1}H- and ^{13}C-NMR signals showed a close similarity to stemoninine [15], which belonged to the stemoamide-type of stemona alkaloids. The C-atom signal at δ(C) 213.22 was assigned to C(7)[1]), according to the long-range correlations between δ(C) 213.22 and the H-atoms δ(H) 4.36 (*d*, *J* = 12, H–C(8)) and 1.71–1.78 (*m*, H_α–C(6)) in the HMBC spectrum (*Fig. 1*). The assignments of all the H- and C-atom signals were done by a combination of ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra (*Tables 1* and *2*).

Table 2. *^{13}C-NMR Spectroscopic Data for Alkaloids* **1**–**5**[1])[a])

	1	**2**	**3**	**4**	**5**
C(1)	116.58 (*s*)	23.36 (*t*)	23.02 (*t*)	38.42 (*d*)	38.19 (*d*)
C(2)	105.99 (*d*)	22.54 (*t*)	30.88 (*t*)	30.39 (*t*)	30.45 (*t*)
C(3)	128.61 (*s*)	66.00 (*d*)	177.52 (*s*)	68.09 (*d*)	173.69 (*s*)
C(5)	46.42 (*t*)	56.41 (*t*)	43.32 (*t*)	53.72 (*t*)	41.91 (*t*)
C(6)	26.50 (*t*)	33.33 (*t*)	28.53 (*t*)	31.58 (*t*)	37.19 (*t*)
C(7)	31.35 (*t*)	213.22 (*s*)	23.48 (*t*)	32.59 (*t*)	29.14 (*t*)
C(8)	125.33 (*d*)	57.67 (*d*)	38.77 (*t*)	24.58 (*t*)	31.33 (*t*)
C(9)	124.07 (*s*)	87.94 (*d*)	89.22 (*s*)	40.95 (*d*)	42.05 (*d*)
C(9a)	129.70 (*s*)	65.84 (*d*)	67.67 (*d*)	71.09 (*d*)	61.60 (*d*)
C(10)	48.18 (*d*)	50.17 (*d*)	38.38 (*t*)	44.00 (*d*)	43.45 (*d*)
C(11)	80.63 (*d*)	114.39 (*s*)	36.53 (*d*)	85.06 (*d*)	79.46 (*d*)
C(12)	39.79 (*d*)	147.10 (*d*)	181.19 (*s*)	46.65 (*d*)	46.73 (*d*)
C(13)	45.02 (*t*)	134.14 (*s*)	17.41 (*q*)	41.86 (*d*)	39.89 (*d*)
C(14)	179.49 (*s*)	173.30 (*s*)		182.69 (*s*)	179.03 (*s*)
C(15)	15.61 (*q*)	10.27 (*q*)		17.97 (*q*)	15.05 (*q*)
C(16)	23.26 (*t*)	21.41 (*t*)		25.26 (*t*)	24.06 (*t*)
C(17)	12.09 (*q*)	13.02 (*q*)		12.25 (*q*)	10.49 (*q*)
C(18)	71.64 (*d*)	83.35 (*d*)		84.08 (*d*)	
C(19)	35.24 (*t*)	33.71 (*t*)		35.23 (*t*)	
C(20)	36.00 (*d*)	36.32 (*d*)		36.09 (*d*)	
C(21)	178.73 (*s*)	181.74 (*s*)		182.09 (*s*)	
C(22)	14.99 (*q*)	15.36 (*q*)		15.15 (*q*)	

[a]) Recorded at 125 MHz (**1** and **4** in $CDCl_3$, **2** and **3** in CD_3OD, **5** in CD_3COCD_3).

The relative configuration of **2** was assigned by the NOESY spectrum. According to the biogenetic relationships, an α-Me group attached to C(20) of the γ-butyrolactone ring attached to C(3) in the pyrrolidine ring indicated that H–C(20) was β-oriented. The correlation signal between H–C(20) and H–C(18) suggested that H–C(18) was β-oriented. Correlations of H–C(3) with H–C(9a) and H_α–C(5), but no correlation with H–C(18), revealed that H–C(3) and H–C(9a) were in α-orientation. In addition, the presence of NOESY cross-peak between H–C(9) and H_β–C(6), associated with the absence of a signal between H–C(9a) and H–C(9), showed that H–C(9) was in β-orientation. Based on the absolute (*R*)-configuration at C(11) [13][16] in analogous compounds, the bond C(11)–C(12) was in α-orientation. The NOESY correlations between H–C(12) and H–C(10), and H–C(10) and H–C(8) suggested that H–C(12), H–C(10), and H–C(8) were α-oriented (*Fig. 2*). Thus, the Et group at C(10) was β-oriented. Compound **2** was named stemoninone.

For compound **3**, a white powder, the molecular formula was established as $C_{13}H_{19}NO_3$ from the HR-ESI-MS (*m/z* 238.1437, $[M+H]^+$; calc. 238.1443). This formula required five degrees of unsaturation. The IR absorption at 1770 cm^{-1}, in association with the ^{1}H-NMR Me signal at δ(H) 1.29 (*d*, *J* = 7.0, Me(13)[1])) and the ^{13}C-NMR CO signal at δ(C) 181.19 (*s*), suggested the presence of an α-methyl-γ-lactone ring. Based on HMBCs of the quaternary C-atom at δ(C) 89.22 to δ(H) 4.02 (*dd*, *J* = 7.5, 8.5, H–C(9a)), 1.76–1.78 (*m*, H_α–C(10)), 2.52 (*dd*, *J* = 3.5, 10.0, H_β–C(10)), and 1.88–1.95 and 1.96–1.98 (each 1 H, *m*, CH_2(8)), the C-atom with

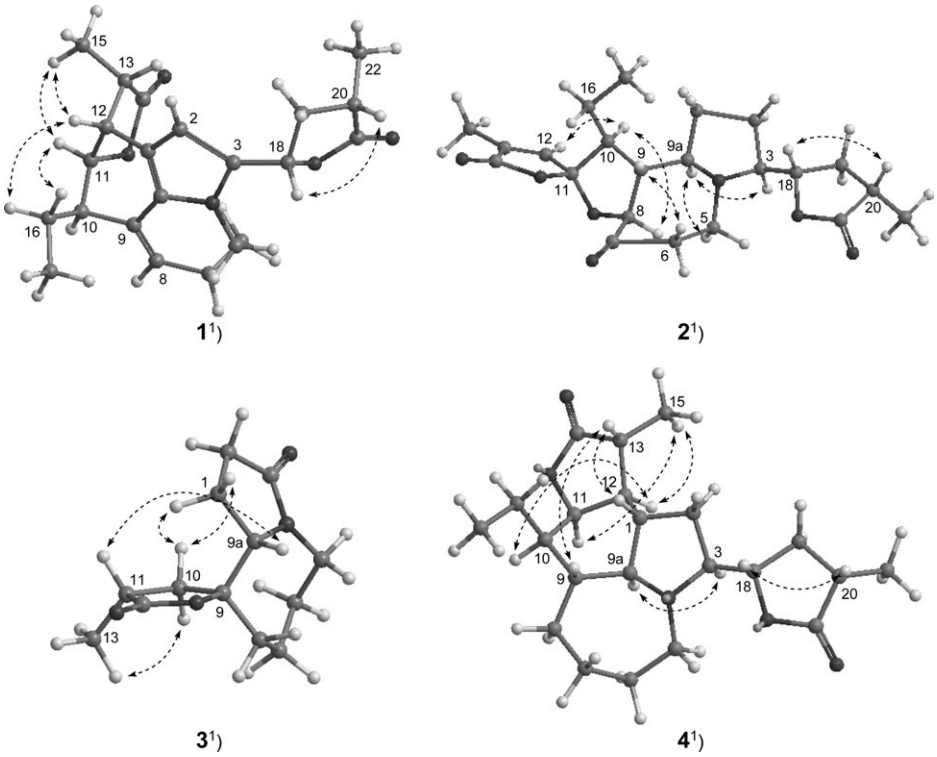


Fig. 2. *Key NOESY correlations* (broken arrows) *and relative configuration of* **1**–**4**

signal at δ(C) 89.22 was suggested a spiro-atom connecting to the α-methyl-γ-lactone ring. The signal pattern of the ^{1}H- and ^{13}C-NMR spectra of **3** are similar to those of croomine [17–19], which belongs to the tuberostemospironine-type of stemona alkaloids. The major difference between **3** and croomine involved the absence of an α-methyl-γ-lactone ring annexed to C(3) in **3**. The IR spectrum absorption at 1679 cm^{-1}, coupled with CO C-atom signal at δ(C) 177.52 (*s*) suggested the presence of a lactam group. The lactam CO group was assigned to C(3) because of the presence of the characteristic signals of a CH group and two geminal H-atoms each attached to a C-atom bearing an N-atom at δ(H) 4.02 (H–C(9a)), 3.17–3.22 (*m*, H$_\alpha$–C(5)), and 3.64 (*dd*, J = 4.0, 12.5, H$_\beta$–C(5)). The ^{1}H- and ^{13}C-NMR data were assigned by HMQC, ^{1}H,^{1}H-COSY, and HMBC spectra (*Fig. 1*, and *Tables 1* and *2*).

Since the absolute configuration of croomine has been established by X-ray-analysis [20], the relative configuration of **3** was assigned by NOESY spectra. The (*S*)-configuration of C(9) was further confirmed by the NOE correlation between H$_\beta$–C(10) with CH$_2$(1). Significant NOESY correlations of H–C(13) with H$_\alpha$–C(10) suggested that H–C(13) was in α-orientation, whereas the association of H–C(11) with H$_\beta$–C(10) and H–C(9a) revealed that H–C(9a) was in β-orientation (*Fig. 2*). Therefore, the structure of **3** was established as tuberostemospiroline.

Compound **4**, a light yellow powder, was assigned a molecular composition of $C_{22}H_{33}NO_4$ by HR-ESI-MS (*m/z* 376.2480, $[M+H]^+$; calc. for 376.2488), with seven degrees of unsaturation. The characteristic cleavage fragment *m/z* 276 $[M-C_5H_7O_2]^+$ showed the presence of an α-methyl-γ-lactone ring annexed to C(3) in the pyrrolidine ring [7]. Its ^{13}C-NMR data at δ(C) 182.69 and 182.09 in association with the strong IR absorptions at 1770 and 1600 cm^{-1} suggested the presence of two γ-lactones. The ^{1}H-NMR spectrum showed two geminal H-atoms at δ(H) 2.27–2.32 (*m*) and 3.32–3.34 (*m*) attached to the C-atom bearing an N-function. The ^{1}H-NMR spectrum also showed δ(H) 0.78 (*t*, *J* = 7.5, Me(17)[1])), 1.20 (*d*, *J* = 7.0, Me(22)), and 1.32 (*d*, *J* = 7.5, Me(15)) assigned to three Me groups. Two low-field H-atoms attached to C-atoms bearing an O-function at δ(H) 4.40–4.44 (*m*, H–C(11)) and 4.36–4.39 (*m*, H–C(18)) pointed to a stenine-group alkaloid [7]. The full assignments and connectivities of all the H- and C-atom signals were determined by a combination of HMQC, ^{1}H,^{1}H-COSY, and HMBC spectra (*Tables 1* and *2*).

The relative configuration of **4** was determined by the NOESY spectrum and in accordance with the relative configuration of known stemona alkaloids with an α-Me group at the γ-lactone ring attached to C(3). Correlation was observed between H–C(18) and H–C(20), thus H–C(18) and H–C(20) were β-oriented. In addition, H–C(9a) correlated to H–C(3), but not to H–C(18) and H–C(9), which suggested that H–C(9a) and H–C(3) were α-oriented, and H–C(9) was β-oriented, respectively. The correlation between H–C(9) with H–C(13), which was further correlated with H–C(1), suggested that H–C(1) and H–C(13) were also β-oriented. Me(15) showed a correlation with H–C(11) and H–C(12), which was further correlated to H–C(10), these results suggested that H–C(10), H–C(12), Me(15), and H–C(11) were on the same side of the molecule and in α-orientation (*Fig. 2*). Compound **4** was identified as tuberostemonine L, which is one of the stereoisomers of tuberostemonine.

Compound **5** was obtained from the roots of *S. sessilifolia* for the first time in 1968 [9]. Its ^{1}H- (*Table 1*) and ^{13}C-NMR (*Table 2*) data are presented herein because it has not appeared in the literature previously. The known alkaloids **6** and **7** were also obtained from the roots of *S. sessilifolia* [9][10][21].

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; *Qingdao Marine Chemical Group Co. Ltd*, P. R. China). Prep. HPLC: *Alltech* system equipped with a *UV200* detector (210 nm) and an *Alltima C-18* column (10 μm, 10 mm × 250 mm), by using a mixed solvent of MeOH/H_2O or MeCN/H_2O at a flow rate of 3.0 ml/min. TLC: on precoated silica gel GF_{254} plates, and the TLC spots were viewed at 254 nm and visualized by spraying with *Dragendorff* reagent. M.p.: *XT-4A* melting point apparatus (uncorrected). Optical rotations: *AA-10R* automatic polarimeter (*Optical Activity Ltd.*, England). IR Spectra: *AVATAR 360* FT-IR spectrophotometer (*NICOLET*, USA). NMR (^{1}H- and ^{13}C-NMR, DEPT, ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY): *Bruker AVANCE DRX 500* spectrometer (Switzerland) with chemical shifts reported in δ using TMS as an internal standard. HR-ESI-MS and EI-MS: *APEX II FT-ICR-MS* spectrometer (*Bruker Daltonics*).

Plant Material. The roots of *Stemona tuberosa* LOUR. were purchased from Yulin County, Guangxi Province, P. R. China, in May, 2004. The samples were authenticated by Prof. *Shao-Qing Cai* (School of Pharmaceutical Sciences, Peking University Health Science Center) according to morphological and microscopic characteristics. A voucher specimen (4594) was deposited with the Herbarium of

Pharmacognosy, School of Pharmaceutical Sciences, Peking University Health Science Center (Beijing, P. R. China).

Extraction and Isolation. The roots (30 kg) were ground and percolated with 95% EtOH. After evaporation of the collected percolate, the crude extract was acidified with dilute HCl (4%, *v*/*v*) and filtered. The filtrate was basified with aq. NH_3 to pH 9 and extracted with $CHCl_3$ to give the total alkaloid extract (180 g, 0.6%). The dried residue (100 g) of crude alkaloid extract was further chromatographed on SiO_2 and eluted with the solvent system consisting of $CHCl_3$/MeOH 98:2, 97:3, 96:4, 95:5 in a discontinuous gradient elution. Elutes were monitored by TLC and the fractions containing same compounds were combined to afford four fractions, *Frs. A–D*. The dried residue of *Fr. A* (10 g) was further subjected to SiO_2 CC, eluted with petroleum ether (PE)/AcOEt 4:1, the collected fractions were further separated by reversed-phase HPLC (MeOH/H_2O 55:45) to give compounds **1** (17.3 mg) and **2** (8.6 mg). *Fr. B* (10.5 g) was collected from elutes with $CHCl_3$/MeOH 97:3 and purified by SiO_2 CC eluted with PE/acetone $8:1 \rightarrow 3:1$ to give two subfractions (*B1*, *B2*). *Fr. B1* (2.6 g) was further subjected to SiO_2 CC eluted with PE/AcOEt 2:1 to yield compound **10** (10 mg). *Fr. B2* (3.8 g) was purified by prep. HPLC separation (MeCN/H_2O 70:30) to yield compounds **4** (15 mg), **5** (16.4 mg), and **6** (8 mg). *Fr. C* (19.5 g) eluted with $CHCl_3$/MeOH 96:4 was dried to give 5.0 g of residue, which was further separated on by SiO_2 CC using PE/AcOEt/Et_2NH 3:1:0.1 and $CHCl_3$/MeOH/NH_4OH 95:5:0.05 resp., to yield compounds **3** (3 mg), **9** (9.6 mg), and **11** (150 mg). *Fr. D* (8.4 g) eluted with $CHCl_3$/MeOH 95:5 was also dried to give 6.0 g of residue, which was further separated by SiO_2 CC using gradient elution of PE/Me_2CO/Et_2NH 6:1:0.05, 4:1:0.05, 2:1:0.05 and hexane/AcOEt 3:1, 2:1, 1:1 successively, to yield compounds **7** (16.4 mg) and **8** (34 mg), resp.

Didehydrotuberostemonine A (=*(8*R*,8a*S*,11*S*,11a*R*)-8-Ethyl-5,6,8,8a,11,11a-hexahydro-11-methyl-2-[(2*S*,4*S*)-tetrahydro-4-methyl-5-oxofuran-2-yl]azepino[3,2,1-*hi*]furo[3,2-*e*]indol-10(4*H*)-one*; **1**). Pale yellow powder (MeOH). $[\alpha]_D^{25} = +150$ ($c = 0.32$, $CHCl_3$). IR (KBr): 3421, 2961, 2929, 1768, 1653, 1455, 1341, 1260, 1171, 994, 924, 898, 794, 730. ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 2*. EI-MS: 369 (M^+), 325, 310, 280, 271 ($[M - C_5H_7O_2]^+$), 225. HR-ESI-MS: 370.2009 ($[M+H]^+$, $C_{22}H_{28}NO_4^+$; calc. 370.2018).

Stemoninone (=*(1′*S*,2*R*,3a′*S*,8′*S*,10a′*R*,10b′*R*)-1′-Ethyl-3a′,5′,6′,8′,9′,10′,10a′,10b′-octahydro-4-methyl-8′-[(2*S*,4*S*)-tetrahydro-4-methyl-5-oxofuran-2-yl]-5*H*-spiro[furan-2,2′-furo[3,2-*c*]pyrrolo[1,2-*a*]azepine]-4′,5(1′*H*)-dione*; **2**). White powder (MeOH). $[\alpha]_D^{25} = +30$ ($c = 0.40$, MeOH). IR (KBr): 3400, 3087, 2928, 2863, 1767, 1711, 1668, 1453, 1379, 1317, 1290, 1186, 1158, 1114, 1062, 1036, 1014, 974, 921, 884, 855, 805, 762, 687, 598, 555. ^{1}H-NMR (CD_3OD, 500 MHz): *Table 1*. ^{13}C-NMR (CD_3OD, 125 MHz): *Table 2*. EI-MS: 404 ($[M+H]^+$), 375, 276 (100), 164. HR-ESI-MS: 404.2064 ($[M+H]^+$, $C_{22}H_{30}NO_6^+$; calc. 404.2073).

Tuberostemospiroline (=*(2*R*,4*R*,9a′*S*)-Hexahydro-4-methyl-3*H*-spiro[furan-2,9′-pyrrolo[1,2-*a*]azepine]-3′,5(2′*H*,4*H*)-dione*; **3**). White powder (MeOH). $[\alpha]_D^{25} = -33.90$ ($c = 0.59$, MeOH). IR (KBr): 3345, 3020, 2985, 2939, 2876, 2851, 1770, 1679, 1448, 1420, 1365, 1331, 1274, 1201, 1182, 1154, 1129, 1049, 1026, 977, 942, 925, 894. ^{1}H-NMR (CD_3OD, 500 MHz): *Table 1*. ^{13}C-NMR (CD_3OD, 125 MHz): *Table 2*. EI-MS: 237 (M^+), 138 (100), 99. HR-ESI-MS: 238.1437 ($[M+H]^+$, $C_{13}H_{20}NO_3^+$; calc. 238.1443).

Tuberostemonine L (=*(2*S*,7a*R*,8*R*,8a*R*,11*R*,11a*R*,11b*S*,11c*S*)-8-Ethyldodecahydro-11-methyl-2-[(2*S*,4*S*)-tetrahydro-4-methyl-5-oxofuran-2-yl]azepino[3,2,1-*hi*]furo[3,2-*e*]indol-10(2*H*)-one*; **4**). Light yellow powder. $[\alpha]_D^{25} = -25.53$ ($c = 0.47$, MeOH). IR (KBr): 3430, 2962, 2930, 2876, 1770, 1600, 1457, 1380, 1337, 1300, 1188, 1015, 960, 926. ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 2*. EI-MS: 376 ($[M+H]^+$), 277, 276 (100, $[M - C_5H_7O_2]^+$). HR-ESI-MS: 376.2480 ($[M+H]^+$, $C_{22}H_{34}NO_4^+$; calc. 376.2488).

2-Oxostenine (=*(7a*R*,8*R*,8a*S*,11*S*,11a*S*,11b*R*,11c*R*)-8-Ethyldodecahydro-11-methylazepino[3,2,1-*hi*]furo[3,2-*e*]indole-2,10-dione*; **5**). Light yellow crystals ($CHCl_3$/MeOH). IR (KBr): 3560, 3437, 2964, 2933, 2877, 1748, 1667, 1452, 1427, 1381, 1311, 1171, 1008, 931, 592. ^{1}H-NMR (CD_3COCD_3, 500 MHz): *Table 1*. ^{13}C-NMR (CD_3COCD_3, 125 MHz): *Table 2*. EI-MS: 291 (M^+), 290 (100, M^+), 276, 262, 234, 216, 188, 150, 137, 96, 67, 55, 41.

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