Two New Alkaloids from *Stemona tuberosa*

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Two new alkaloids, named tuberostemoninol A (**1**) and tuberostemoninol B (**2**), along with the known compounds tuberostemoninol (**3**) and bisdehydroneotuberostemonine (**4**), were isolated from *Stemona tuberosa* LOUR. The structures were elucidated by means of spectroscopic analyses, including 1D- and 2D-NMR experiments. The four alkaloids **1**–**4** were analyzed by on-line high-performance liquid chromatography/electrospray-ionization mass spectrometry (HPLC/ESI-MS), and their fragmentation pathways were found to be similar. Compounds **1**, **2**, and **3** are isomers with the same molecular mass but which eluted at different retention times.

Introduction. – Stemonaceae is a small family with four genera, namely, *Croomia*, *Pentastemona*, *Stichoneuron*, and *Stemona*, the largest genus. The root tuber of *Stemona tuberosa* LOUR. (Chinese name 'Bai-Bu') has been used in traditional Chinese medicine for centuries as insecticide and as a cough remedy [1]. The crude extract of this plant was found to have antibacterial, antifungal, antiviral, and insecticidal activities [2]. The pronounced biological effects have attracted much interest in several phytochemical studies. So far, *ca.* 80 alkaloids have been isolated in this family [3]. In our investigation, two new alkaloids, named tuberostemoninol A (**1**) and tuberostemoniol B[1]) (**2**), along with the known compounds tuberostemoninol (**3**) [4] and bisdehydroneotuberostemonine (**4**) [5], were isolated from *Stemona tuberosa* LOUR. (*Fig. 1*). The structures were elucidated by means of spectral studies, including 1D- and 2D-NMR experiments. Four compounds were subjected to on-line HPLC/ESI-MS, and their fragmentation pathways were found to be similar. Compounds **1**, **2**, and **3** are stereoisomers which can be differentiated by their different retention times.

Results and Discussion. – Tuberostemoninol A (**1**) was obtained as colorless prismatic crystals. Its molecular formula was determined to be $C_{22}H_{31}NO_6$ from the M^+ peak at m/z 405.2160 in the HR-EI-MS. The ESI-MS spectrum showed a $[M+H]^+$ ion peak at m/z 406. Its IR (KBr) spectrum revealed the presence of OH (3480 cm^{-1}), two saturated γ-lactone (1765 and 1750 cm^{-1}), and lactam C=O groups (1681 cm^{-1}). The ^{1}H-NMR spectrum (*Table 1*) indicated the presence of a primary Me group at δ(H) 1.12 (*t*, $J = 7.4$ Hz), two secondary Me groups at δ(H) 1.14 (*d*, $J = 7.0$ Hz) and 1.71 (*d*, $J = 7.2$ Hz), two low-field H-atoms attached to C-atoms bearing an O-atom at δ(H) 4.43 (*dd*, $J = 2.7$, 11.5 Hz) and 4.72 (*dt*, $J = 4.8$, 10.0 Hz), and a CH and two geminal H-

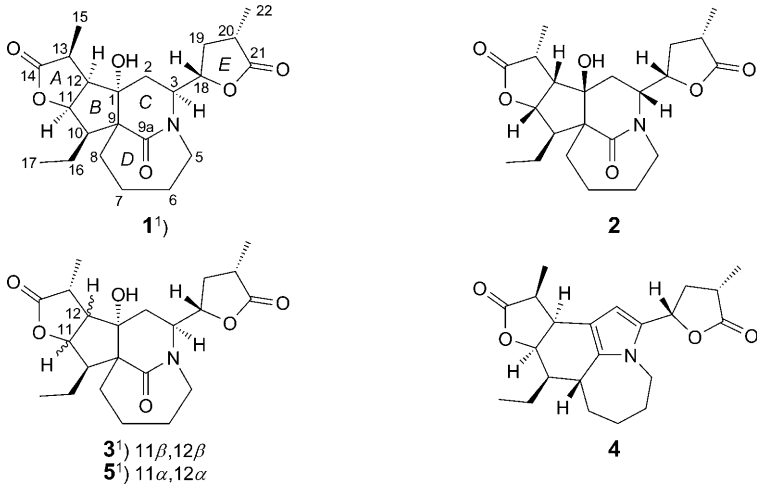


Fig. 1. *Tuberostemoninol A* (**1**), *tuberostemoninol B* (**2**), *tuberostemoninol* (**3**), *bisdehydroneotuberostemonine* (**4**), *and neotuberostemoninol* (**5**) *isolated from* Stemona tuberosa LOUR.

atoms attached to C-atoms bearing an N-atom at δ(H) 4.19–4.38 (*m*), 3.89–4.08 (*m*), and 3.57–3.61 (*m*). The ^{13}C-NMR spectrum of **1** (*Table 1*) showed 22 signals caused by three Me, seven CH_2, and seven CH groups, and five quaternary C-atoms of which three were C=O groups. These spectroscopic data were reminiscent of tetracyclic stenine-type alkaloids bearing an α-methyl-γ-lactone ring at C(3) [5]. The full assignments and connectivities were determined by ^{1}H,^{1}H-COSY, HMBC, and HMQC data. The ^{1}H,^{1}H-COSY plot established spin systems involving H–C(10)[1]), H–C(11), H–C(12), H–C(13), and Me(15), and CH_2(2), H–C(3), H–C(18), CH_2(19), CH_2(20), and Me(22). The HMQC spectrum revealed that the signal at δ(H) 4.43 arose from an H-atom attached to the C-atom at δ(C) 87.70 (C(11)), and the HMBCs from C(1), C(10), C(12), C(13), and C(14) to H–C(11) placed the γ-lactone between C(14) and C(11). Similarly, the HMQC spectrum revealed the proton of the signal at δ(H) 4.72 is attached to the C-atom at δ(C) 77.13 (C(18)), and the HMBCs from C(3), C(19), C(20), and C(21) to H–C(18) placed another γ-lactone between C(21) and C(18). The HMBCs from H–C(3), CH_2(5), CH_2(8), and H–C(10) to C(9a) placed the remaining C=O group (C(9a)) between the N-atom and C(9), and that from the OH H-atom to C(2) placed the OH group at C(1). From these observations, **1** was concluded to possess the same gross structure as tuberostemoninol (**3**) [4] and neotuberostemoninol (**5**) [6] isolated from *S. tuberosa*. Differences between the chemical shifts of the ^{1}H- and ^{13}C-NMR signals of those three compounds indicated that **1** is a stereoisomer of **3** and **5**. The relative configuration of **1** was deduced by NOESY analysis (*Fig. 2*). The NOESY data suggested that H–C(10), H–C(11), H–C(12), H–C(13), and H–C(3) were α-oriented due to the correlations H–C(11)/H–C(12), H–C(10), and H–C(13), H–C(10)/H$_\alpha$–C(8), and H–C(3)/H$_\alpha$–C(2) and H$_\alpha$–C(19). The α-orientation of OH–C(1) was proposed based on the NOESY cross-

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

Table 1. *^{1}H- and ^{13}C-NMR Data* (400 and 500 MHz, resp., C_5D_5N) *of* **1**[1]). δ in ppm, J in Hz.

	δ(H)	δ(C)
C(1)	–	83.10 (*s*)
CH_2(2)	2.29–2.31 (*m*), 2.11 (*dd*, *J* = 4.2, 8.0)	32.03 (*t*)
CH(3)	4.19–4.38 (*m*)	59.56 (*d*)
CH_2(5)	3.89–4.08 (*m*), 3.57–3.61 (*m*)	48.22 (*t*)
CH_2(6)	2.23–2.27 (*m*), 1.34–1.39 (*m*)	22.99 (*t*)
CH_2(7)	1.64–1.73 (*m*), 1.28–1.36 (*m*)	29.67 (*t*)
CH_2(8)	1.82–1.86 (*m*), 1.39–1.49 (*m*)	26.80 (*t*)
C(9)	–	63.31 (*s*)
C(9a)	–	184.71 (*s*)
CH(10)	2.79–2.81 (*m*)	50.75 (*d*)
CH(11)	4.43 (*dd*, *J* = 2.7, 11.5)	87.70 (*d*)
CH(12)	3.14–3.18 (*m*)	53.49 (*d*)
CH(13)	2.99–3.04 (*m*)	35.59 (*d*)
C(14)	–	179.15 (*s*)
Me(15)	1.71 (*d*, *J* = 7.2)	14.76 (*q*)
CH_2(16)	1.58–1.63 (*m*), 1.39–1.46 (*m*)	33.78 (*t*)
Me(17)	1.12 (*t*, *J* = 7.4)	13.44 (*q*)
CH(18)	4.72 (*dt*, *J* = 4.8, 10.0)	77.13 (*d*)
CH_2(19)	2.52–2.60 (*m*), 1.51–1.54 (*m*)	35.92 (*t*)
CH(20)	2.78–2.86 (*m*)	35.24 (*d*)
C(21)	–	178.69 (*s*)
Me(22)	1.14 (*d*, *J* = 7.0)	10.82 (*q*)

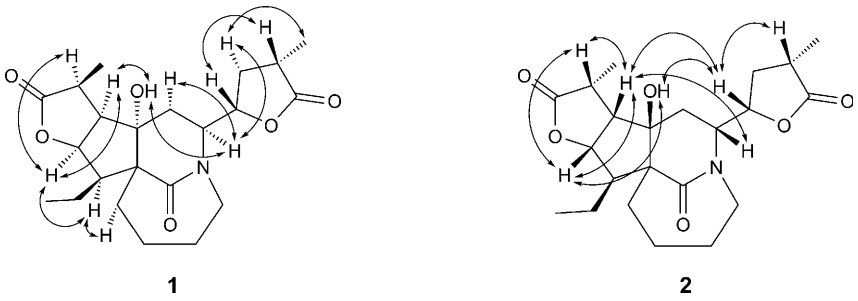


Fig. 2. *Selected NOE correlations of* **1** *and* **2**

peaks OH–C(1)/H–C(12), and OH–C(1)/H–C(3). Moreover, the NOESY correlations H–C(18)/H–C(20), H–C(20)/H_β–C(19), and H_α–C(19)/H–C(3) indicated that the α-methyl-γ-lactone ring was attached to C(3) in β-orientation together with β-configuration of H–C(20). The full assignments and connectivities were determined on the basis of ^{1}H,^{1}H-COSY, HMBC, HMQC, and NOESY data.

Tuberostemoninol B (**2**) was obtained as colorless prismatic crystals. Its molecular formula was determined to be $C_{22}H_{31}NO_6$ from the M^+ peak at m/z 405.2151 in the HR-EI-MS. The ESI-MS showed a $[M+H]^+$ ion peak at m/z 406. Its IR (KBr) spectrum revealed the presence of OH (3487 cm^{-1}), two saturated γ-lactone (1768 and 1760 cm^{-1}), and lactam C=O groups (1660 cm^{-1}). The ^{13}C-NMR and DEPT patterns

of **2** (*Table 2*) were indentical to those of **1** and the known tuberostemoninol (**3**) [4] and neotuberostemoninol (**5**) [6], having the same molecular formula. The relative configuration was established by the NOESY data (*Fig. 2*). The NOE correlations between H–C(11)[1]), H–C(12), and H–C(13) indicated that they were on the same side, tentatively assumed as β-oriented, and no response was observed at H–C(10) and H–C(12). As a consequence, H–C(10) and Me(15) were α-oriented. The NOE correlations H–C(12)/H–C(3), H–C(12)/H–C(18), and H–C(18)/H–C(20) indicated that H–C(3), H–C(18), H–C(20) were β-configured. Finally, the β-orientation of OH–C(1) was established by the NOESY cross-peak OH–C(1)/H–C(11). The full assignments and connectivities were determined on the basis of ^{1}H,^{1}H-COSY, HMBC, HMQC, and NOESY data.

Table 2. *^{1}H- and ^{13}C-NMR Data* (400 and 500 MHz, resp., C_5D_5N) *of* **2**[1]). δ in ppm, J in Hz.

	δ(H)	δ(C)
C(1)	–	84.87 (*s*)
CH_2(2)	2.89–2.97 (*m*), 2.04 (*s*)	29.16 (*t*)
CH(3)	3.59 (*t*, *J* = 9.3)	61.53 (*d*)
CH_2(5)	4.32–4.40 (*m*), 3.05–3.15 (*m*)	54.02 (*t*)
CH_2(6)	1.65–1.74 (*m*), 1.58–1.64 (*m*)	23.79 (*t*)
CH_2(7)	2.06–2.08 (*m*), 1.54–1.61 (*m*)	28.97 (*t*)
CH_2(8)	3.24 (*dq*, *J* = 2.5, 7.6), 1.78–1.83 (*m*)	25.99 (*t*)
C(9)	–	61.93 (*s*)
C(9a)	–	180.72 (*s*)
CH(10)	2.35 (*dd*, *J* = 2.2, 11.6)	58.49 (*d*)
CH(11)	5.05 (*d*, *J* = 8.0)	88.25 (*d*)
CH(12)	3.03–3.09 (*m*)	59.39 (*d*)
CH(13)	3.10–3.17 (*m*)	37.57 (*d*)
C(14)	–	179.76 (*s*)
Me(15)	1.49 (*d*, *J* = 7.2)	18.82 (*q*)
CH_2(16)	2.15–2.22 (*m*), 1.51–1.55 (*m*)	34.73 (*t*)
Me(17)	1.04 (*t*, *J* = 7.4)	14.90 (*q*)
CH(18)	4.96 (*ddd*, *J* = 3.7, 10.0, 10.4)	83.73 (*d*)
CH_2(19)	1.89–1.95 (*m*), 1.75–1.81 (*m*)	38.10 (*t*)
CH(20)	2.50–2.57 (*m*)	35.86 (*d*)
C(21)	–	178.91 (*s*)
Me(22)	1.13 (*d*, *J* = 7.0)	13.96 (*q*)

Most *Stemona* alkaloids are not sensitive to direct UV detection because they have no chromophore in the near UV, but they are easily detectable by ESI-MS. Therefore, the four alkaloids isolated from the plant were subjected to on-line HPLC/ESI-MS. Their fragmentation patterns and products were useful for their characterization. *Fig. 3* shows the HPLC/ESI-MS total-ion-current (TIC) chromatogram of the isolated alkaloids mixture. Four peaks were included. These four peaks were indentified as tuberostemoninol B (**2**), tuberostemoninol A (**1**), tuberostemoninol (**3**), and bisdehydroneotuberostemonine (**4**). The retention times of them were 16.9, 18.9, 21.9, and 50.4 min, respectively. The isolates **1**–**3** are isomers with the same molecular mass but which eluted at different retention times (t_R 16.9, 18.9, and 21.9 min, resp.).

The variation of their retention times can thus be used to differentiate these three compounds.

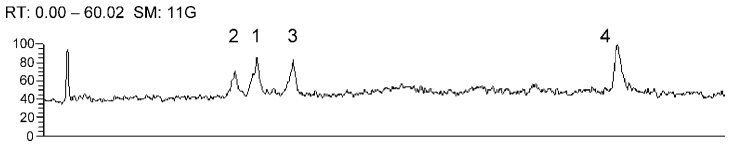
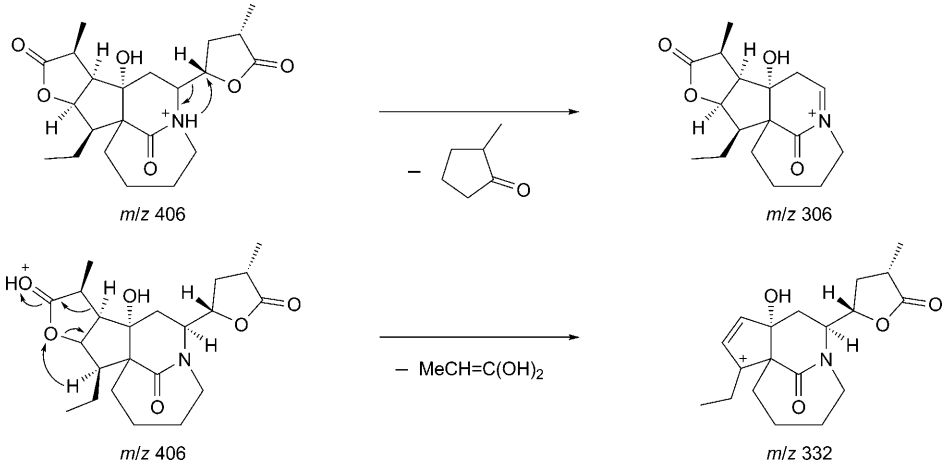


Fig. 3. *TIC Chromatogram of the four alkaloids* **1**–**4**

Compounds **1**–**4** belong to the stenine group. Their fragmentation pathways were found to be similar. All ESI-MS showed abundant $[M+H]^+$ ions with usually no fragmentation. The $[M+H-100]^+$ ion was prominent in all MS^2 spectra and was attributed to the loss of the α-methyl-γ-lactone group (see *Scheme*). This fragmentation was interpreted as being characteristic for *Stemona* alkaloids. Besides this, the $[M+H-74]^+$ ion was present, which was interpreted as arising *via* a H-atom transfer to the lactone ring and a four-membered transition state thus eliminating the neutral fragment $MeCH{=}C(OH)_2$ [7] (see *Scheme*). In addition, $[M+H-CO]^+$, $[M+H-H_2O]^+$, and $[M+H-CH_3]^+$ ions were sometimes observed.

Scheme. *Proposed Fragmentation Pathways of* $[M+H]^+$ *of Compound* **1** *in the ESI-MS*^{2}



Compounds **1**–**3** and **5** are alkaloids of unique structures, which are seldom seen in natural products. They have the same gross structure, but their relative configuration differs at several stereogenic centers. It is noteworthy that the five-membered ring *B* and six-membered ring *C* in **1** are changed into a six-membered and a five-membered ring in **4**, respectively. Stenine-type alkaloids are intriguing natural products with a wide structural diversity in the *A*, *B*, *C*, and *D* rings. However, ring *E* is always the same, and their fragmentation pathways were found to be similar. From this viewpoint, the absolute configurations of **1**, **2**, **3**, **5**, and **4** were inferred by considering the biogenetic relationships [6].

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; *Qingdao Haiyang Chemical Group Co., Ltd.*, China). M.p.: *Jeol-JNM-EX-400 Fisher-Scientific* instrument; uncorrected. IR Spectra: *Nicolet-Impact-410* IR spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra (^1H, ^{13}C, DEPT, NOESY, ^1H,^1H-COSY, HMQC, and HMBC): *Bruker-Avance-AV-400* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-EI-MS: *Thermo-MAT-95-XP* mass spectrometer; in m/z (rel. %).

Plant Material. The roots of *S. tuberosa* LOUR. were collected from Hubei Province, P. R. China. The material was identified at Guangzhou University of Chinese Medicine, where a voucher specimen (No. 061105) has been deposited.

Extraction and Isolation. A dry ground herbal sample (7.0 kg) was refluxed with 95% EtOH. After evaporation of the solvent, the residue was acidified with dilute (4%) HCl soln. and centrifuged. The supernatant was adjusted to pH 10 with aq. NH_3 soln. and extracted with CH_2Cl_2. The extract was concentrated and the residue subjected to CC (SiO_2, $CHCl_3$/MeOH 9:1, 8:2, and 7:3). The fraction eluted with $CHCl_3$/MeOH 9:1 was further subjected to CC (SiO_2, hexane/acetone 7:3): **1** (20 mg) and **2** (9 mg). Colorless prisms of **3** (30 mg) were obtained by slow evaporation of the fraction eluted with hexane/acetone 6:4 of this latter CC. The fraction eluted with $CHCl_3$/MeOH 8:2 of the former CC was further subjected to CC (SiO_2, hexane/acetone 7:3): **4** (50 mg).

Tuberostemoninol A (=*(3*S*,3a*R*,3b*S*,5*S*,6*R*,10a*R*,11*R*,11a*R*)-11-Ethyldecahydro-3b-hydroxy-3-methyl-5-[(2*S*,4*S*)-tetrahydro-4-methyl-5-oxofuran-2-yl]-6,10a-methanofuro[2′,3′:4,5]cyclopent[1,2-d]azonine-2,12(3*H*)-dione*; **1**): M.p. 277–280°. $[\alpha]_D^{20} = +129$ ($c = 0.1$, MeCN). IR (KBr): 3480 (OH), 1765 (sat. γ-lactone), 1750 (sat. γ-lactone), 1681 (lactam). ^1H- and ^{13}C-NMR: *Table 1*. EI-MS: 405 (M^+), 361 (25), 306 (7), 288 (10), 262 (100), 142 (24), 70 (42). HR-EI-MS: 405.2160 ($[M+H]^+$, $C_{22}H_{32}NO_6^+$; calc. 405.2146).

Tuberostemoninol B (=*(3*R*,3a*S*,3b*R*,5*R*,6*R*,10a*R*,11*R*,11a*S*-11-Ethyldecahydro-3b-hydroxy-3-methyl-5-[(2*S*,4*S*)-tetrahydro-4-methyl-5-oxofuran-2-yl]-6,10a-methanofuro[2′,3′:4,5]cyclopent[1,2-d]azonine-2,12(3*H*)-dione*; **2**). M.p. 185–188°. $[\alpha]_D^{20} = -41.5$ ($c = 0.2$, MeCN). IR (KBr): 3487 (OH), 1768 (sat. γ-lactone), 1760 (sat. γ-lactone), 1660 (lactam). ^1H- and ^{13}C-NMR: *Table 2*. EI-MS: 405 (M^+), 361 (12), 306 (2), 288 (10), 262 (54), 142 (53), 70 (100). HR-EI-MS: 405.2151 ($[M+H]^+$, $C_{22}H_{32}NO_6^+$; calc. 405.2146).

HPLC/ESI-MS Analysis. Anal. HPLC: *Thermo* instrument equipped with a *Surveyor* degasser, *Surveyor* autosampler, *Surveyor LC* pump, and a *Surveyor PDA detector*; *Hypersil GOLD* C_{18} column (150 × 2.1 mm, 5 μm, *Thermo*): solvent gradient of MeCN (*A*) and H_2O (*B*): 0–25 min, linear 80 to 65% of *B*; 26–50 min, linear 65 to 55% of *B*; 51–52 min, linear 55 to 80% of *B*; the HPLC effluent was splitted so that *ca.* 200 μl/min entered the ESI-MS source. ESI-MS: *TSQ-7000* quadrupole mass spectrometer (*Thermo*, USA), ESI source operating in pos.-ion mode; capillary temp. 350°, spray voltage 3.5 kV, capillary voltage fixed at 15.0 V; full scan (m/z 200–800) with 200 ms collection time in pos. mode; sheath gas flow 60 arbitrary units of N_2; auxiliary gas flow 10 arbitray units of N_2.

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