Alkaloids and Chemical Diversity of *Stemona tuberosa*

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Phytochemical investigation of the chemical components of *Stemona tuberosa* led to the isolation of two new alkaloids named tuberostemonine K (**1**) and tuberospironine (**2**), together with the known tuberostemonine (**3**). The new structures of **1** and **2** were elucidated through extensive spectroscopic analyses, while the molecular structure of **3** was confirmed by X-ray analysis. A gradient reversed-phase HPLC-ELSD method was established for the investigation of the chemical diversity of *S. tuberosa* from 13 localities, and four types of chemical variation featured by the major components **3**, neotuberostemonine (**4**), croomine (**5**), and stemoninine (**6**), respectively, were observed.

The root tuber of *Stemona tuberosa* Lour. ("Bai-Bu" in Chinese) has been applied in traditional Chinese medicine for centuries to manage respiratory diseases, e.g., bronchitis, pertussis, and tuberculosis, and to prevent human and cattle parasites, agricultural pests, and domestic insects.[1] The pronounced biological effects have motivated several phytochemical studies leading to the isolation of 27 *Stemona* alkaloids from species of different localities, whereas a total of 82 alkaloids were discovered from the family Stemonaceae.[2] These alkaloids were classified into three skeleton types (stichoneurine, protostemonine, and croomine types) based on biosynthetic considerations[2] and were also separated into eight groups (stenine, stemoamide, tuberostemospironine, stemoamine, parvistemoline, stemofoline, stemocurtisine, and miscellaneous groups) according to their structural features.[3] Tuberostemonine was reported to exhibit inhibitory activity on the excitatory transmission at the crayfish neuromuscular junction.[4] Neotuberostemonine was found to exhibit antitussive potency comparable to codeine but not involving the opioid receptors.[5]

During the course of our ongoing investigation of the chemical diversity of *S. tuberosa*, we have examined the alkaloidal contents of this herb collected from different localities. Seven stenine-type alkaloids were isolated from a sample purchased from a local herb shop. Neotuberostemonine (**4**, Figure 1) was identified as the major component in this sample, and two other compounds were found to be stereoisomers of **4**.[5,6] Subsequent phytochemical work on this herb from a collection made in Guangxi Province showed a different chemical profile with tuberostemonine (**3**) isolated as the major component. In addition, two minor but new compounds named tuberostemonine K (**1**) and tuberospironine (**2**) were also characterized from this sample (Figure 1). We report herein the isolation and structural elucidation of compounds **1**−**3**. In addition, reversed-phase HPLC coupled with evaporative light-scattering detection (ELSD) was applied for the investigation of the chemical diversity of *S. tuberosa* from different localities.

Results and Discussion

Compounds **1**−**3** were isolated from the total alkaloid fraction of *S. tuberosa* collected in Guangxi Province. The known compound **3** was characterized by X-ray analysis as tuberostemonine.[7] The asymmetric unit (Figure 2) consists of an independent molecule of **3** and four-tenths of a methanol molecule linked through a hydrogen bond (O-5-H···O-4, D = 2.912 Å). Compound **3** represents the first example among the stenine type of *Stemona* alkaloids that crystallizes in MeOH as its solvate. Crystals of this kind of compounds were normally obtained in a mixture of hexane and EtOAc,[5,6] though 1′,2′-didehydrostemofoline, an alkaloid belonging to the stemofoline group of *Stemona* alkaloids, was crystallized in a mixture of $CHCl_3$, hexane, and MeOH as a MeOH solvate.[8] Compound **3** had been isolated from *S. tuberosa* about four decades ago. The crystal structure of its methobromide dihydrate was reported, but the precision was low (R = 0.167 for 2017 visually estimated reflections), and no atomic coordinates or geometric parameters were published or deposited.[9] Total synthesis of this bioactive alkaloid has been done recently, but it was too unstable to be purified by silica gel chromatography or crystallization.[10,11] The present investigation not only revealed a rich natural source of compound **3** with a yield of 0.037% but also provided a detailed crystal profile, which is useful for comparison of the three-dimensional structure with its other isomers and for structure−activity relationship studies. Furthermore, we found that this compound was stable during the process of extraction, isolation, and crystallization.

Compound **1** has the same molecular formula ($C_{22}H_{33}NO_4$) as that of **3**. The molecular ion $[M]^+$ at *m*/*z* 375 and a base peak at *m*/*z* 276 $[M - C_5H_7O_2]^+$ in the EIMS spectrum indicated the presence of a typical α-methyl-γ-lactone ring annexed to C-3 of the perhydroazaazulene ring,[12] commonly occurring in *Stemona* alkaloids. The ^{1}H NMR spectrum (Table 1) showed the signals of one primary methyl group at δ_H 0.87 (3H, t, J = 7.4 Hz, H-17), two secondary methyl groups at δ_H 1.23 (1H, d, J = 6.8 Hz, H-22) and 1.32 (1H, d, J = 7.4 Hz, H-15), two deshielded geminal protons at δ_H 2.81 (1H, dd, J = 9.3,15.1 Hz, H-5α) and 3.41 (1H, dd, J = 5.8, 9.3 Hz, H-5*β*), and two oxymethines at δ_H 4.26 (1H, m, H-18) and 4.20 (1H, d, J = 2.0 Hz, H-11). These data strongly resemble those of tuberostemonine-type alkaloids.[5] The skeleton was further confirmed by the ^{13}C NMR and DEPT spectra (Table 1), which indicate three methyl groups, seven methylenes, 10 methines, and two lactone carbonyls. With the two oxygenated protons H-11 and H-18 serving as starting points, the full assignments and connectivities of all the proton and carbon signals were determined by a combination of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. The ^{1}H−^{1}H COSY spectrum reveals spin systems involving H-11→H-12→H-13→H-15 and H-18→H-19→H-20→H-22. The HMQC

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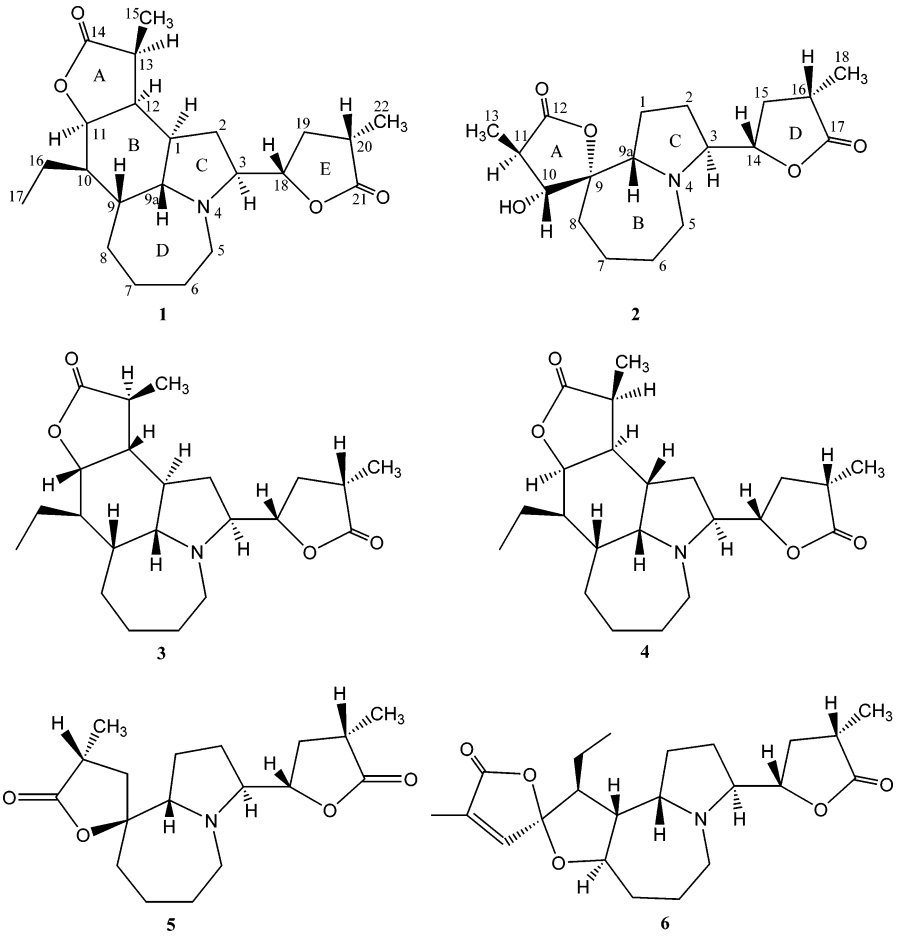

Figure 1. Structural formulas of alkaloids: tuberostemonine K (**1**), tuberospironine (**2**), tuberostemonine (**3**), neotuberostemonine (**4**), croomine (**5**), and stemoninine (**6**).

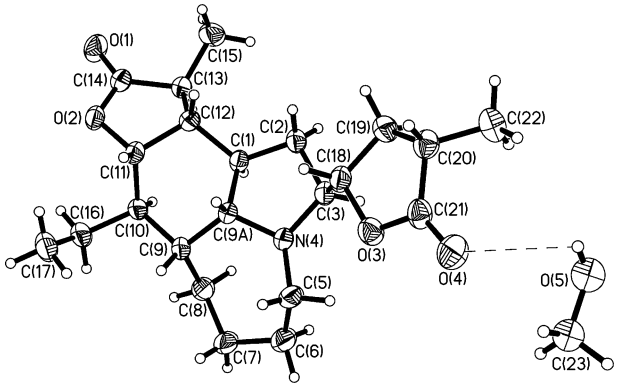

Figure 2. Molecular structure of tuberostemonine (**3**) with atom-labeling scheme. The dashed line represents a hydrogen bond between compound **3** and the methanol molecule in the asymmetric unit.

spectrum indicates that the signal at δ_H 4.20 (H-11) is attached to a carbon at δ 81.59 (C-11), and the signal at δ_H 2.17 (H-12) is attached to a carbon at δ 45.15 (C-12). The HMBC spectrum shows that C-11 correlates to H-10 and H-16, and C-12 correlates to H-1, H-2, and H-13, suggesting that an α-methyl-γ-lactone ring A is fused to ring B at C-11 and C-12. Similarly, the HMQC spectrum indicates that the signal at δ 4.26 (H-18) is attached to a carbon at δ_C 83.57 (C-18), which shows HMBC correlations to H-19 and H-3, further confirming the location of another α-methyl-γ-lactone ring at C-3. Thus the two-dimensional structure of **1** closely resembles that of **3**.

The relative configuration of compound **1** was established by means of a ROESY spectrum (Figure 3). The proton at δ 2.88 (H-13) is often α-oriented.[2,3,5,6] The cross-peaks of H-13/H-12, H-12/H-11, and H-11/H-10 and absence of correlation between H-10/H-9 reveal that H-10, H-11, and H-12 were all α-oriented and H-9 was β-oriented. The correlations between H-9/H-9a and H-3/H-1 and absence of correlation between H-1/H-9a indicate that H-9a, H-3, and H-1 were β-, α-, and α-oriented, respectively. The α-oriented H-3 was also confirmed by the cross-peak H-3/H-2α and absence of H-3/H-2β correlation. The proton at δ 4.26 (H-18) shows correlation with the proton at δ 2.64 (H-20), but not with the proton at δ 3.22 (H-3), which suggests that H-18 and H-20 are *cis*-related and both protons are *trans*-related to H-3. Indeed, the β-oriented H-18 and H-20 were observed in all *Stemona* alkaloids, and the *trans*-arrangement of H-3 and H-18 is common.[2,3,5,6] Accordingly, the relative configurations of the chiral centers C-1, C-3, C-9, C-9a, C-10, C-11, C-12, C-13, C-18, and C-20 were established to be *rel*-(*R*, *S*, *R*, *R*, *R*, *S*, *S*, *S*, *S*, and *S*), respectively. Compound **1** is an isomer of **3** at C-11 and C-12. It is here named tuberostemonine K. Compound **1** is also an isomer of neotuberostemonine[13] (**4**, also known as tuberostemonine L-G) at C-1. H-1 is α-oriented in compound **1**, but β-oriented in **4**.

Although compound **1** is an isomer of **3** and **4**, it eluted much earlier from the HPLC column than the latter two compounds (compound **1**: 13.2 min; compound **3**: 19.7 min; compound **4**: 19.0 min) in the same injection. It is interesting to note that the dipole−dipole parameter of compound **1** (8.60) calculated by Chem3D MM2 software[14] is smaller than those of **3** (8.63) and **4** (8.62). The dipole−dipole parameters were inferred to be partly responsible for the different retention times of these isomers.

Compound **2** has the molecular formula $C_{18}H_{27}NO_5$ with six degrees of unsaturation, in contrast to the seven degrees in **1**. The molecular ion $[M]^+$ *m/z* 337 and the characteristic cleavage fragment

Table 1. NMR Data for Compounds **1** and **2**

	1		**2**	
position	δ_H mult., *J* in Hz	δ_C mult.	δ_H mult., *J* in Hz	δ_C mult.
1	1.79, 1H, m	35.60 d	2.07−2.10, m 2.12−2.15, m	27.02, t
2	1.10, 1H, m, H-2β 2.25, 1H, m, H-2α	33.64 t	1.75−1.81, m 1.40−1.42, m	26.69, t
3	3.22, 1H, dd, J = 9.0, 12.1	65.51 d	3.45,dd, J = 6.8, 6.4	63.51, d
5	2.81, 1H, dd, J = 9.3, 15.1, H-5α 3.41, 1H, dd, J = 5.8, 9.3, H-5β	50.41 t	3.50, dd, J = 1.0, 15.1, H-5α 3.27,dd, J = 15.1, 11.7, H-5β	46.07, t
6	1.28, 2H, m	27.57 t	1.32−1.35, m, H-6α 1.65−1.72, m, H-6β	22.39, t
7	1.57, 2H, m	30.08 t	1.40−1.45, m, H-7α 1.85, dt, J = 15.0, 4.5, H-7β	26.63, t
8	1.06, 1H, m, H-8α 1.88, 1H, m, H-8β	32.99 t	2.03−2.08, m, H-8α 2.20−2.25, m, H-8β	27.16, t
9	1.89, 1H, m	47.27 d		90.53, s
9a	3.11, dd, J = 3.9, 11.2	64.13 d	3.64, t, J = 8.4	71.13, d
10	1.89, 1H, m	40.99 d	4.09, d, J = 10.3	78.79, d
11	4.20, 1H, d, J = 2.0	81.59 d	2.87, dq, J = 10.3, 6.8	41.21, d
12	2.17, 1H, m	45.15 d		176.13, s
13	2.88, 1H, dq, J = 6.8, 7.4	41.58 d	1.36, d, J = 6.8	12.69, q
14		178.88 s	4.18, ddd, J = 5.3, 6.8, 1.5	82.83, d
15	1.32, 3H, d, J = 7.4	12.15 q	1.44−1.50, m, H-15α 2.14−2.18, m, H-15β	34.22, t
16	1.2−1.4, 2H, m	26.10 t	2.59, dq, J = 12.4, 6.8	35.06, d
17	0.87, 3H, t, J = 7.4	13.05 q		179.59, s
18	4.26, 1H, m	83.57 d	1.20, d, J = 6.8	15.02, q
19	1.49, 1H, ddd, J = 12, H-19α 2.21, 1H, m, H-19β	34.19 t		
20	2.64, 1H, dq, J = 4.0, 6.8	35.31 d		
21		179.53 s		
22	1.23, 3H, d, J = 6.8	15.16 q		

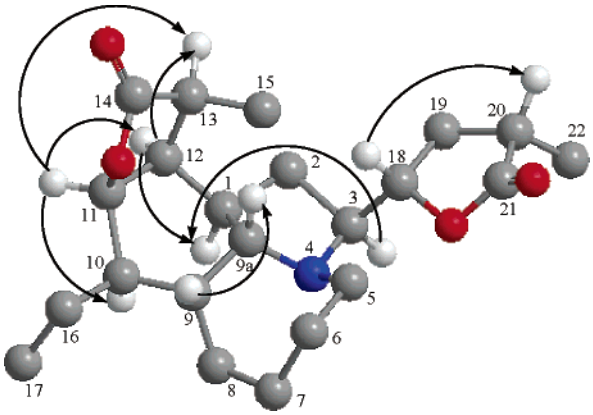

Figure 3. Key ROESY correlations of compound **1**. The three-dimensional structure is constructed by Chem3D Pro 9.0. Only hydrogen atoms binding to the chiral carbons are shown for clarity.

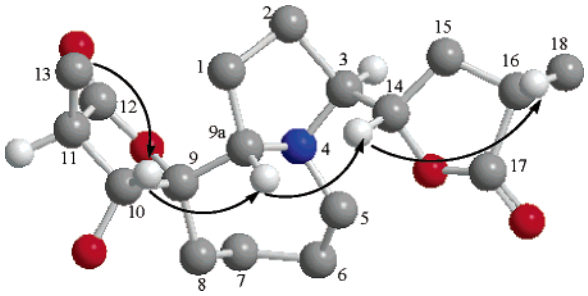

Figure 4. Key ROESY correlations of compound **2**. Only hydrogen atoms binding to the chiral carbons are shown for clarity.

Table 2. Sample List of Root Tubers from *Stemona tuberosa*

sample	location of collection	voucher number
1	Guangdong	ICM 2004-2540
2	purchased in Beijing	ICM 2005-2652
3	Guangxi	Woo 23973
4	Hong Kong	Hu & But 23960
5	Yunnan	ICM 2004-2541
6	purchased in Guangzhou	ICM 2005-2651
7	Shizhu, Sichuan	ICM 2005-2648
8	Masupo, Yunnan	ICM 2005-2650
9	Erbian, Sichan	ICM 2005-2649
10	Shanglin, Guangxi	ICM 2005-2646
11	Baoshan, Yunnan	ICM 2005-2645
12	Yudu, Jiangxi	ICM 2005-2647
13	Kunming Botanical Garden, Yunnan	ICM 2005-2644

m/z 238 $[M - C_5H_7O_2]^+$ in the EIMS spectrum indicated that compound **2** also has a typical α-methyl-γ-lactone ring annexed to C-3 of the perhydroazaazulene nucleus,[12] and the fragment 319 $[M - H_2O]^+$ reveals the presence of a hydroxyl group.

The ^{1}H NMR spectrum (Table 1) of **2** shows signals for two methines and two geminal protons attached to carbon atoms bearing a nitrogen functionality similar to those of **1** at δ_H 3.45 (1H, dd, J = 6.4, 6.8 Hz, H-3), 3.64 (1H, t, J = 8.4 Hz, H-9a), 3.50 (1H, dd, J = 1.0, 15.1 Hz, H-5α), and 3.27 (1H, dd, J = 11.7, 15.1 Hz, H-5β), which are characteristic of the perhydroazaazulene nucleus (rings B and C) of the *Stemona* alkaloids. The signal for the primary methyl group in **1** was not observed in **2**. The signals of two secondary methyl groups at δ_H 1.36 (3H, d, J = 6.8 Hz, H-13) and 1.20 (3H, d, J = 6.8 Hz, H-18) indicated the presence of two α-methyl-γ-lactone rings (rings A and D, respectively). This is confirmed by two pairs of lactone carbonyl and oxygenated carbon signals [ring A: δ 176.13 (C-12, s) and 90.53 (C-9, s); ring D: 179.59 (C-17, s) and 82.83 (C-14, d)]. The quaternary and downfield shifted C-9 in contrast to C-14 suggests that the former is a spiro-atom connecting the α-methyl-γ-lactone ring A and the perhydroazaazulene nucleus, which is further confirmed by the HMBC correlation of C-9 to H-10, H-9a, and H-8. The HMQC spectrum shows the cross-peak between δ 82.83 (C-14) and 4.18 (1H, ddd, J = 5.3, 6.8, 1.5 Hz, H-14), and the HMBC spectrum shows that H-14 is correlated to C-3, suggesting the location of another α-methyl-γ-lactone ring (ring D) at C-3. Thus compound **2** is a

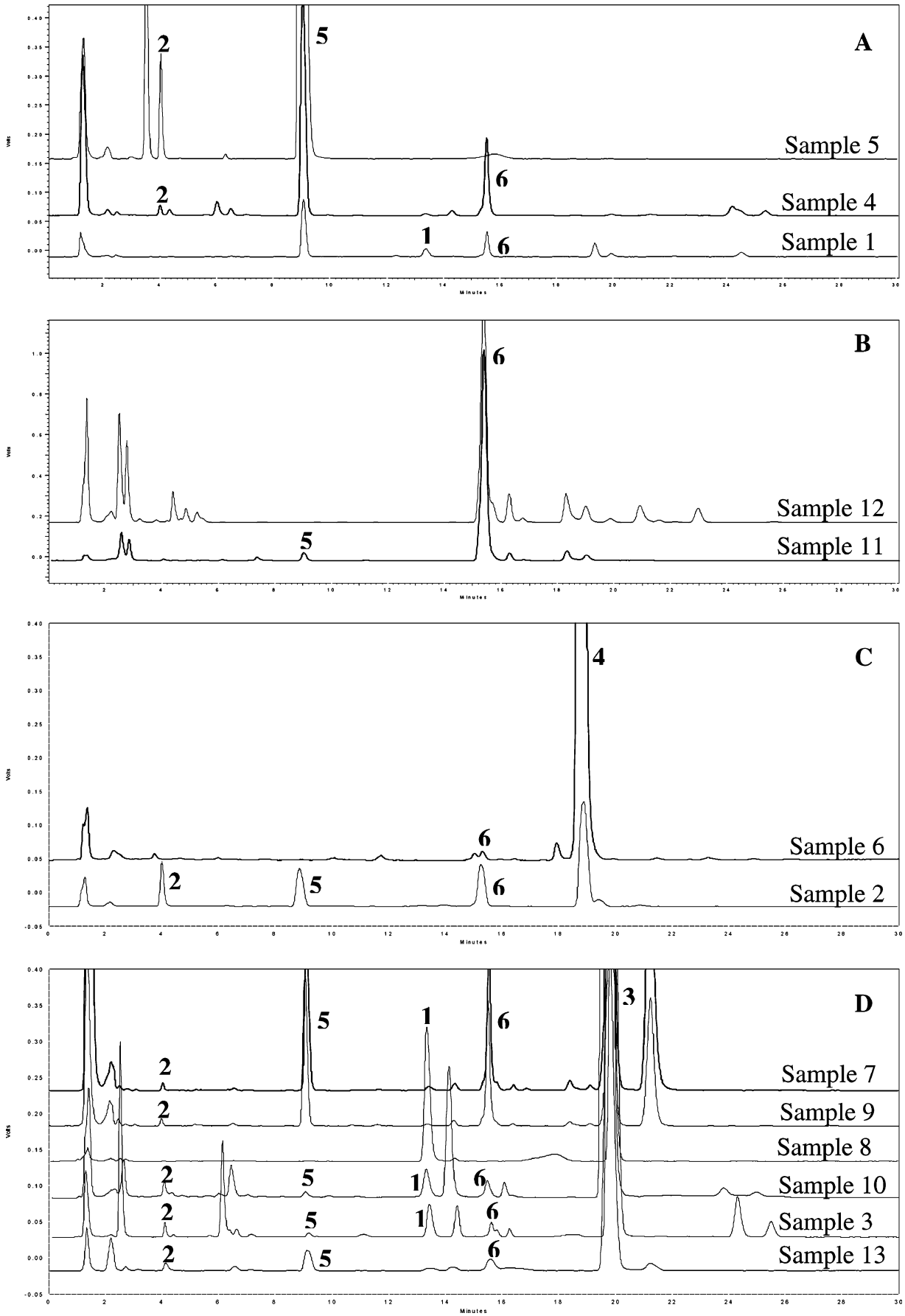

Figure 5. HPLC-ELSD chromatograms of *S. tuberosa*. The 13 sources fall into four types: type A with the major component being croomine (**5**, t_R = 9.0 min), type B with the major component being stemoninine (**6**, t_R = 15.7 min), type C with the major component being neotuberostemonine (**4**, t_R = 19.0 min), and type D with the major component being tuberostemonine (**3**, t_R = 19.7 min). Compounds **1** and **2** represent tuberostemonine K and tuberospironine, respectively.

tetracyclic ring structure, which in addition to the two carbonyls accounts for the six degrees of unsaturation.

Because the two C−O signals (δ_C 82.83 and 90.53) were assigned to the lactone functionalities, the remaining one (δ 78.79, d) must be due to a hydroxyl group. The HMQC spectrum revealed that the signal at δ 78.79 is correlated to the proton at δ 4.09 (H-10, d, J = 10.3 Hz), and the HMBC spectrum showed that H-10 is correlated to carbons C-8, C-9, C-9a, C-11, and C-13, suggesting that the hydroxyl group is located at C-10. This is confirmed by a well-defined H-10 doublet with a large coupling constant, $J_{10,11}$ = 10.3 Hz. These spectroscopic data are reminiscent of tuberostemospironine,[15] with an α-hydroxyl group at C-10 and an additional α-methyl-γ-lactone ring attached to C-3. The full assignments and connectivities are determined by ^{1}H−^{1}H COSY, HMQC, and HMBC spectra.

The relative configuration of compound **2** was determined by a ROESY spectrum (Figure 4). The correlations of H-14/H-16, H-14/H-9a, and H-9a/H-10 indicate that these four protons are *cis*-related and *β*-oriented. The cross-peak H-10/H$_3$-13 and absence of correlation between H-10 and H-11 prove the *cis* arrangement of H-10 with the adjacent methyl group and indicate that H-11 is α-oriented. Results of the ROESY analysis allowed assignment of the relative configurations of all chiral centers as *rel*-(3*S*, 9*S*, 9a*S*, 10*S*, 11*S*, 14*S*, and 16*S*). The similar coupling constants of H-10 and H-11 in **2** as compared with those in tuberostemospironine[15] also suggested that these two compounds possess the same configurations at C-10 and C-11. Accordingly, the structure of **2** was established as tuberospironine.

Both compounds **1** and **2** possess an α-methyl-γ-butyrolactone ring (18*S* and 20*S*) attached to C-3 in the perhydroazaazulene ring, a common feature in alkaloids from *Stemona* species. Since the absolute configuration of tuberostemonine (**3**) sharing the same α-methyl-γ-butyrolactone ring as **1** and **2** has been established through X-ray analysis on its methobromide dihydrate using the anomalous dispersion method,[9] the absolute configurations of **1** and **2** can be tentatively inferred considering the biogenetic relationships in *Stemona* alkaloids as shown in Figure 1.

The *Stemona* alkaloids are characterized by the main pyrrolo- or few pyrido-(1,2-α) azepine skeleton.[16,17] So far, this kind of alkaloid is restricted to the family Stemonaceae. Although the same nucleus was found in the skins of a Colombian frog, the auxiliary ring system and substitution pattern are significantly different.[18] However, significant chemical variation was observed in *S. tuberosa* regarding the alkaloidal composition and corresponding contents. The HPLC chromatograms of the total alkaloids of this herb from 13 sources (Table 2) were compared as shown in Figure 5A−D. It should be noted that most of the *Stemona* alkaloids are not sensitive under direct UV detection due to the absence of conjugated functional groups.[19] Thus ELSD[20] depending on the size, shape, and number of eluate particles was hyphenated with HPLC. The HPLC profiles of these samples can be divided into four types, A−D. Type A included samples 1, 4, and 5 with the major component being peak **5** (Figure 5A, t_R = 9.0 min), which was identified as croomine (**5**).[21] Type B was represented by samples 11 and 12 with the major component being peak **6** (Figure 5B, t_R = 15.7 min), which was identified as stemoninine (**6**).[22] Type C was found in samples 2 and 6 with the major component being peak **4** (Figure 5C, t_R = 19.0 min), which was identified as neotuberostemonine (**4**).[6] Type D appeared more common, in samples 3, 7, 8, 9, 10, and 13, with the major component being compound **3** (Figure 5D, t_R = 19.7 min), which was identified as tuberostemonine. Identification of compounds **3**−**6** was based on the comparison of their mass spectrum and retention time with authentic samples, which were isolated and identified in our laboratory. The retention time of **4** is close to that of **3**; thus compound **4** was further crystallized as colorless blocks whose structure was confirmed by X-ray analysis and compared with reported data.[13] Compound **1** was detected in samples 1, 3, 8, and 10, while compound **2** was observed in samples 2, 3, 4, 5, 7, 9, 10, and 13, indicating that different types also possessed some common components. The distribution of compounds **3**−**6** is shown in Figure 5.

Plants produce secondary metabolites that may contribute to the species fitness of survival against the environment.[23] In the present investigation, four chemical profiles were found in the 13 samples of the same species. Thus *S. tuberosa* represents an interesting example with significant chemical diversity in relation to differences in collection sources. We have also compared the HPLC chromatograms of the total alkaloids of sample 5 obtained after acid and base processes and of the crude MeOH extract under ultrasonic conditions, and found that they are stable in this process.[19] This chemical variability may involve genetic parameters, physiological conditions, such as differences in age and harvest time, and environmental factors, such as geographic distribution and soil conditions. Both tuberostemonine[4] and neotuberostemonine[5] were reported to possess potent biological activities. However, these two isomers were not universally present in all samples of *S. tuberosa*. The chemical diversity and variability of this herb suggest that careful chemical authentication is needed to provide a guarantee of the consistency and efficacy in its ethnomedical applications.

Results of this study provide a qualitative survey of different chemical types in *S. tuberosa*. Further investigation of the causes of such diversity is warranted so as to minimize the chemical variations through proper agricultural practices.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter in MeOH solution. ESIMS were recorded on a Finnigan MAT TSQ 7000 instrument. HRFABMS measurements were done on an API QSTAR Pulsar i system Q-TOF mass spectrometer. NMR spectra were obtained (^{1}H, ^{13}C, DEPT, ROESY, ^{1}H−^{1}H COSY, HMQC, and HMBC) on a Bruker spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts are reported in ppm with reference to C_5D_5N, and coupling constants are in Hz. Column chromatography was performed with silica gel (Merck, Germany); TLC was performed on precoated Si gel 60 F_{254} plates (0.2 mm thick, Merck), and spots were detected by spraying with Dragendorff's reagent.

Plant Materials. The herb samples of *S. tuberosa* were collected in different places in China. The root tubers of this species are larger in size than those of the other two species recorded in the Chinese Pharmacopoeia, and thus this species is the main commodity on the market. These herbs were properly identified by comparing with authentic plants cultivated to flower at the greenhouse of our Department of Biology and were confirmed to show the same morphological and anatomical characteristics.[24] Details of the voucher specimens are presented in Table 2.

Isolation of Compounds 1−3 from *S. tuberosa* in Guangxi Province (sample 3). The root tubers (1.5 kg) were chopped and percolated at room temperature with 95% EtOH until alkaloids were exhaustively extracted. The solvent was removed by evaporation under reduced pressure to give a thick syrup. The syrup was acidified with 4% HCl (500 mL) and filtered. The filtrate was washed with Et_2O (300 mL × 2). The H_2O layer was basified to pH = 9 with aqueous ammonia (35%) and then extracted with Et_2O (200 mL × 3) and CH_2Cl_2 (220 mL × 2) successively. The Et_2O extract was concentrated and recrystallized from MeOH to give colorless crystals (550 mg), which were identified as tuberostemonine (**3**) by X-ray analysis. The mother liquor was evaporated to dryness and subjected to column chromatography on silica gel eluted with $CHCl_3$−MeOH−NH_4OH (98:3:0.5) to afford compound **1** (70 mg). The CH_2Cl_2 extract was concentrated and chromatographed on silica gel using a mixture of CH_2Cl_2−MeOH−NH_4OH (35%) (97:3:0.5) as the eluant to yield compound **2** (15 mg).

Tuberostemonine K (1): white powder, formula $C_{22}H_{33}NO_4$; $[\alpha]_D^{20}$ −12.6 (MeOH; *c* 0.1); ESIMS *m*/*z* $[MH]^+$ 376 (100); EIMS 375 $[M]^+$, 276 $[M - C_5H_7O_2]^+$ (100); positive HRFABMS *m*/*z* $[MH]^+$ 376.2476, calcd 376.2482; ^{1}H NMR, ^{13}C NMR, and DEPT, see Table 1.

Tuberospironine (2): white powder, formula $C_{18}H_{27}NO_5$; $[\alpha]_D^{20}$ −14.4 (MeOH; *c* 0.5); ESIMS *m/z* $[MH]^+$ 338 (100), 361 $[M + H + Na]^+$; EIMS *m/z* 337 $[M]^+$, 319 $[M - H_2O]^+$, $[M - C_5H_7O_2]^+$ 238 (100); positive HRFABMS *m/z* $[MH]^+$ 338.1970, calcd 338.1967; ^1H NMR, ^{13}C NMR, and DEPT, see Table 1.

HPLC Conditions. Analytical HPLC was performed on a Beckman System Gold instrument equipped with a 125 solvent module, a 168 PDA detector, and a 508 autosampler, and coupled with an Alltech 500 ELSD detector (Alltech, Deerfield, IL) and a nitrox nitrogen generator. Chromatographic separation was carried out on a C18 column (150 × 4.6 mm, 3 μm; Alltech), using a gradient solvent system comprised of H_2O (A) and CH_3CN (B) containing 0.12% Et_3N. Gradient profile: 0−10 min, isocratic 40% B; 11−12 min, linear 40% to 50% of B; 13−28 min, isocratic 50% B; 29−30 min, linear 50% to 40% of B, with a flow rate of 1.0 mL/min. Temperature for the ELSD drift tube was set at 97 °C, and the nitrogen flow was 2.6 SLPM (standard liters per minute).

Sample Preparation for HPLC. The chopped root tubers (5 g) of *S. tuberosa* samples were refluxed in 95% EtOH (100 mL) for 1 h. The extracted solution was condensed under reduced pressure to afford a residue, which was dissolved in 4% HCl (50 mL) and filtered. The filtrate was basified with 25% ammonia solution to pH 9 and extracted with Et_2O. The extract was condensed and dissolved in MeOH (2 mL). The final solutions were filtered through a 0.22 μm PTFE syringe filter, and an aliquot of each filtrate (10 μL) was injected in the HPLC instrument for analysis.

Crystallographic data for tuberostemonine (**3**) have been deposited with the Cambridge Crystallographic Data Centre as CCDC number 283572. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: (+44)1223-336033; e-mail: deposit@ccdc.cam.ac.uk].

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Supporting Information Available: ^{13}C and ^1H NMR spectra for tuberostemonine K (**1**), tuberospironine (**2**), and tuberostemonine (**3**) and crystal data of **3** are available free of charge via the Internet at http://pubs.acs.org.

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(7) $C_{22}H_{33}O_4 \cdot 0.4(CH_3OH)$, colorless blocks were recrystallized from MeOH solution, orthorhombic, $M_r = 391.52$, $P2_12_12_1$, $a = 9.3605(14)$ Å, $b = 9.6806(16)$ Å, $c = 25.174(4)$ Å, $V = 2281.2(6)$ Å^3, $Z = 4$, $d_x = 1.140$ g cm^{-3}, $F(000) = 852$, μ(Mo Kα) = 0.078 mm^{-1}. Data collection was performed on a SMART CCD using graphite-monochromated radiation ($\lambda = 0.71073$ Å); 4674 [R(int) = 0.0477] unique reflections were collected to $\theta_{max} = 26.4°$, in which 2629 reflections were observed [$F^2 > 4\sigma(F^2)$]. The crystal structure was resolved by direct methods using SHELXS-97. The methanol molecule is disordered and refined isotropically with an occupancy of 0.4. In the final stage, $R = 0.0685$ and $S = 1.010$. CCDC no. 283572.
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