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Isolation and stereochemistry of two new alkaloids from *Stemona tuberosa*

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Dedicated to Professor Wei-Yuan Huang on the occasion of his 80th birthday

Abstract—Two new stenine-type alkaloids, neotuberostemonol (**3**) and neotuberostemoninol (**4**), along with the known compound neotuberostemonine (**2**), were isolated from *Stemona tuberosa* Lour. Their structures were characterized by X-ray crystallography in combination with spectroscopic methods, and their absolute configurations were inferred from the known configuration of tuberostemonine (**1**). In the crystalline state, weak C–H···O hydrogen bonds play an important role in the molecular packing of **3** and **4**. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

The herb *Radix Stemonae*, known as 'Bai-Bu' in Traditional Chinese Medicine, is derived from the root of *Stemona tuberosa* Lour (Stemonaceae family). It is often used as an anti-tussive drug to treat respiratory disorders, e.g. bronchitis, pertussis and tuberculosis, and also as an anthelmintic agent for domestic animals.[1] The crude extract of this plant was found to have anti-bacterial, anti-fungal, anti-viral and insecticidal activities.[2] The alkaloid tuberostemonine (**1**) from this species was reported to show inhibitory activity on the excitatory transmission at the crayfish neuromuscular junction.[3] The prominent clinical and pharmacological properties of this plant has prompted many phytochemical studies, and over a dozen stemona alkaloids isolated from this herb of different places of origin[4–6] can be structurally classified into three categories: (i) stenine-type, e.g. neotuberostemonine,[4b] (ii) stemoamide-type, e.g. stemoamide,[6b] and (iii) tuberostemospironine-type, e.g. tuberostemospironine.[6b] The complexity and structural diversity of this fascinating class of compounds have attracted considerable attention, and a number of ingenious strategies toward the total syntheses of stenine,[7] stemoamide[8] and the azepinoindole core[9] have appeared in recent years.

Our phytochemical studies on this herb from Hong Kong herb-shops resulted in the isolation of neotuberostemonine (**2**) and two new stenine-type alkaloids, namely neotuberostemonol (**3**) and neotuberostemoninol (**4**), respectively. Their molecular structures were determined by X-ray crystallography in combination with spectroscopic methods, and their absolute configurations were inferred with reference to the known configuration of tuberostemonine[10] (Fig. 1). The intermolecular interactions directing the molecular assembly of **3** and **4** were also investigated.

2. Results and discussion

A 95% EtOH extract of the herb was acidified with dilute HCl (4%), and the acid soluble fraction was adjusted to pH 9 with aqueous NH_3 and extracted with Et_2O. Crystals of **2** were deposited from the concentrated Et_2O solution. The mother liquor was combined to afford a mixture which was subjected to silica gel chromatography repeatedly to afford compounds **3** and **4**. The known compound **2** was identified by co-TLC with an authentic sample of neotuberostemonine and comparison of its physical and crystal data with the literature values.[4b,11]

2.1. Neotuberostemonol (3)

Compound **3** was obtained as colorless prismatic crystals, mp 195–197°C. Assignment of the molecular formula $C_{22}H_{31}NO_5$ was based on HRLSIMS (*m/z* $[MH]^+$ 390.2252,

Keywords: *Stemona tuberosa*; neotuberostemonol; neotuberostemoninol; neotuberostemonine; hydrogen bonds.

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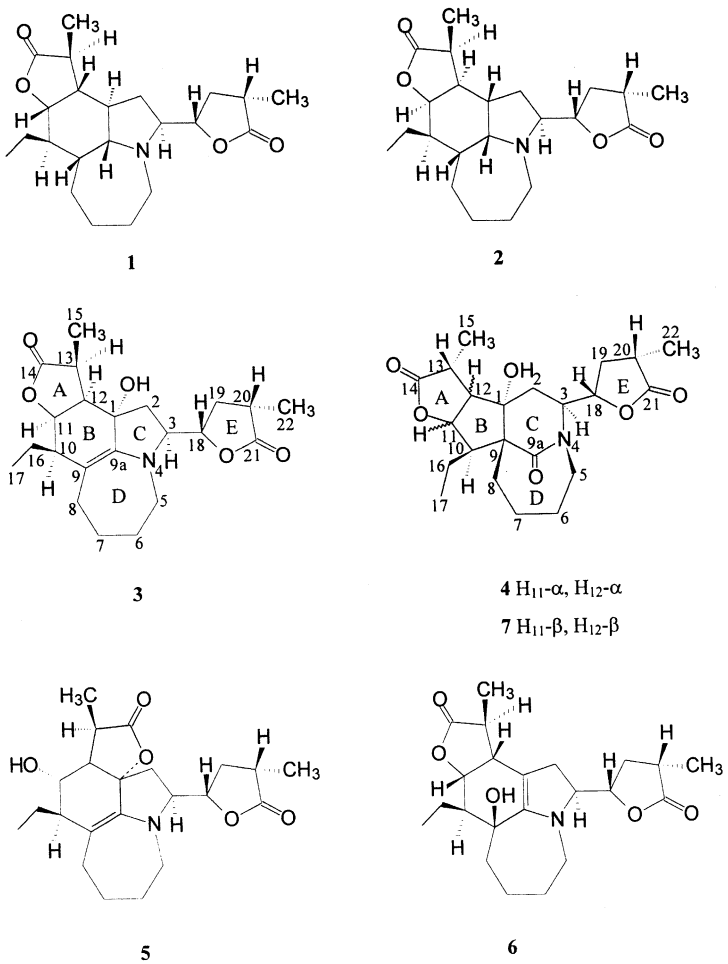

Figure 1. Structural formulae of tuberostemonine (**1**), neotuberostemonine (**2**), neotuberostemonol (**3**), neotuberostemoninol (**4**), oxotuberostemonine (**5**), tuberostemonol (**6**) and tuberostemoninol (**7**).

calculated 390.2271). The EI mass spectrum showed a $[MH]^+$ ion peak at *m/z* 390. The characteristic cleavage fragment *m/z* 291 $[MH-C_5H_7O_2]^+$ indicated that **3** has an α-methyl-γ-lactone ring.[4b,6b,c] Its IR (KBr) spectrum showed bands at 3409 (hydroxyl), 1770 and 1743 cm^{-1} (two saturated γ-lactone).

The ^1H NMR spectrum (Table 1) indicates the presence of a primary methyl group at δ_H 0.98 (3H, t, *J*=7.2 Hz, H-17) and two secondary methyl groups at δ_H 1.19 (3H, d, *J*=7.0 Hz, H-22) and δ_H 1.41 (3H, d, *J*=7.6 Hz, H-15), two low-field protons attached to carbon atoms bearing an oxygen function at δ_H 5.09 (1H, dd, *J*=4.0, 8.6 Hz, H-11) and δ_H 4.25 (1H, ddd, *J*=5.6, 7.4, 10.7 Hz, H-18), a methine and two geminal protons attached to carbon atoms bearing a nitrogen function at δ_H 3.69 (1H, dt, *J*=6.8, 10.7 Hz, H-3), δ_H 3.60 (1H, ddd, *J*=2.8, 4.8, 12.2 Hz, H-5β) and δ_H 2.90 (1H, dd, *J*=7.6, 12.2 Hz, H-5α). The ^{13}C NMR and DEPT spectra of **3** show 22 carbon atoms: two lactonic carbonyl atoms (δ_C 179.13 and 179.24), two olefinic carbon atoms conjugated with the nitrogen atom (δ_C 151.00 and 106.52), three carbon atoms bearing oxygen (δ_C 77.05, 80.37 and 83.37), seven methylene groups (δ_C 52.40, 38.63, 34.45, 30.09, 26.98, 26.59 and 21.99), three methyl groups (δ_C 13.11, 15.15 and 18.52) and four methine carbons (δ_C 51.51, 41.26, 38.59 and 35.18). These spectroscopic data are reminiscent of the tetracyclic stenine-type alkaloids bearing an α-methyl-γ-lactone ring annexed to C-3.[6b] The full assignments and connectivities are determined by $^1H-^1H$ COSY, HMQC and HMBC spectra. The $^1H-^1H$ COSY spectrum establishes spin systems involving H-10, H-11, H-12, H-13 and H_3-15, and H-2, H-3, H-18, H-19, H-20 and H_3-22. The HMQC spectrum revealed the signal at δ_H 5.09 (H-11) attached to a carbon at δ_C 80.37 (C-11), and the HMBC spectrum showed H-11 correlated to C-1, C-9, C-10, C-12, C-13 and C-14, suggesting that the γ-lactone was formed by ring closure involving the oxygen atoms bridged to C-11 and C-14. Similarly, the HMQC spectrum revealed the signal at δ_H 4.25 (H-18) attached to a carbon at δ_C 83.37 (C-18), and the HMBC spectrum showed H-18 correlated to C-2, C-3, C-19, C-20 and C-21, suggesting that another γ-lactone attached to C-3 was formed by ring closure involving the oxygen atoms bridged to C-18 and C-21.

A perspective view of the molecular structure of **3** is presented in Fig. 2. The presence of the $\Delta^{9,9a}$ double bond and the hydroxyl group at C-1 were confirmed by the shortened bond distances (1.348 and 1.338 Å, respectively) and the atom displacement parameters. The relative configurations of the chiral centers C-1, C-3, C-10, C-11, C-12, C-13, C-18 and C-20 were established to be *S*, *S*, *R*, *R*, *R*, *S*, *S* and *S*. The five-membered lactone rings A and E and

Table 1. ^{13}C NMR (100 MHz, C_5D_5N) and ^{1}H NMR (400 MHz, C_5D_5N) data

Position	3		4	
	δ_C DEPT	δ_H (mult., J (Hz))	δ_C DEPT	δ_H (mult., J (Hz))
1	77.05s		83.17s	
2	38.63t	1.72 (1H, m, 2-H^{α}) 2.33 (1H, m, 2-H^{β})	33.74t	2.17 (1H, m, 2-H^{α}) 2.49 (1H, m, 2-H^{β})
3	66.73d	3.69 (1H, dt, 6.8, 10.7)	59.79d	4.37 (1H, dt, 4.0, 10.0)
4				
5	52.40t	2.90 (1H, dd, 7.6, 12.2) 3.60 (1H, ddd, 2.8, 4.8, 12.2)	48.57t	3.31 (1H, dd, 7.6, 11.9) 3.91 (1H, dt, 6.8, 11.9)
6	26.98t	1.54 (1H, m, 6-H^{α}) 1.96 (1H, m, 6-H^{β})	18.85t	1.46 (1H, m, 6-H^{α}) 1.61 (1H, m, 6-H^{β})
7	26.59t	1.36 (1H, m, 7-H^{α}) 1.84 (1H, m, 7-H^{β})	27.92t	1.52 (1H, m, 7-H^{α}) 1.71 (1H, m, 7-H^{β})
8	30.09t	1.51 (1H, m, 8-H^{α}) 1.77 (1H, m, 8-H^{β})	27.52t	1.20 (1H, m, 8-H^{α}) 2.13 (1H, m, 8-H^{β})
9	106.52s		61.52s	
9a	151.00s		184.66s	
10	41.26d	3.22 (1H, dt, 4.0, 11.4)	48.29d	3.60 (1H, br d, 3.6)
11	80.37d	5.09 (1H, dd, 4.0, 8.6)	84.72d	5.15 (1H, dd, 5.6, 6.0)
12	51.51d	2.99 (1H, t, 8.6)	54.79d	3.56 (1H, dd, 6.8, 11.8)
13	38.59d	2.40 (1H, br q, 7.6)	37.89d	3.22 (1H, dq, 4.0, 7.6)
14	179.13s		179.04s	
15	18.52q	1.41 (3H, d, 7.6)	15.06q	1.57 (3H, d, 7.6)
16	21.99t	1.69 (2H, m)	33.77t	1.85 (1H, dq, 7.6, 14.8) 2.36 (1H, dq, 7.6, 14.8)
17	13.11q	0.98 (3H, t, 7.2)	13.01q	1.11 (3H, t, 7.6)
18	83.37d	4.25 (1H, ddd, 5.6, 7.4, 10.7)	77.51d	4.72 (1H, ddd, 4.8, 10.0, 10.4)
19	34.45t	1.47 (1H, m) 2.25 (1H, m)	36.14t	1.34 (1H, m) 2.45 (1H, m)
20	35.18d	2.68 (1H, dq, 7.0, 14.4)	35.52d	2.82 (1H, dq, 7.0, 14.4)
21	179.24s		178.63s	
22	15.15q	1.19 (3H, d, 7.0)	13.15q	1.13 (3H, d, 7.0)

pyrrole ring C all adopt the envelope conformation with C-11, C-3 and C-19 displaced by 0.153, 0.393 and 0.491 Å from the corresponding least-squares plane of the remaining four atoms, respectively. Both the six-membered ring B and the seven-membered ring D exist in a twist-boat conformation (Table 2).

In the solid state, the intermolecular hydrogen bond O-5–H···O-4 (2.894 Å, x, $y-1$, z) links the molecules into a chain running parallel to the b-axis. Two adjacent chains related by 2_1 are connected by C-18–H···O-2 (3.279 Å, $1-x$, $0.5+y$, $1-z$) hydrogen bonds to form a scaffold-like double chain. Adjacent double chains are further linked by C-5–H···O-4 (3.315 Å, x, $y-1$, z) hydrogen bonds to form a two-dimensional network (Fig. 3).

Stemona alkaloids bearing a double bond located at C-1–C-9 or two double bonds located at C-1–C-9a and C-2–C-3 to form a pyrrole ring are common;[4b,5b,12] However, the migration of the C-1–C-9a double bond to C-9–C-9a is quite rare. There is only one example, i.e. oxotuberostemonine

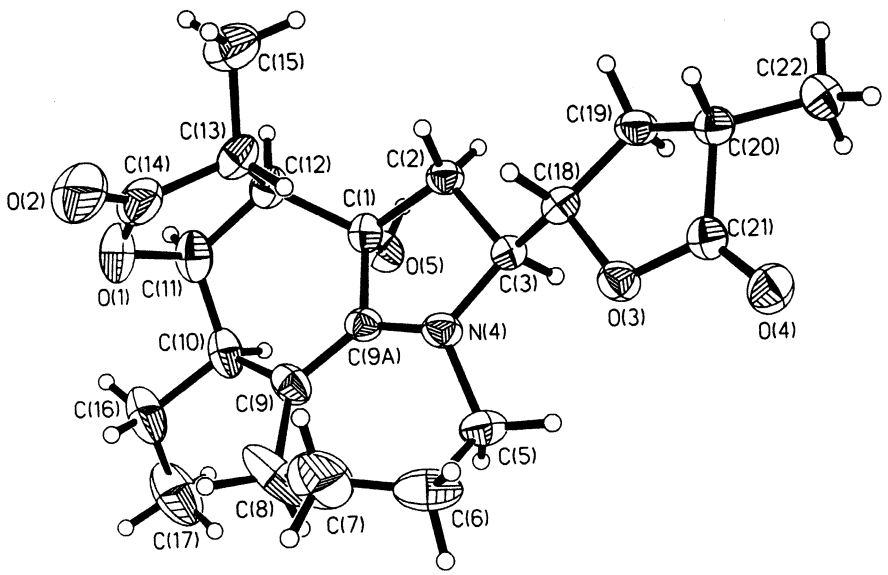

Figure 2. Molecular structure of neotuberostemonol (**3**) showing the atom labeling scheme. The C, N and O atoms are drawn as 30% thermal ellipsoids.

Table 2. Crystal data and structure refinement for compounds **3** and **4**·H_2O

Compound	**3**	**4**·H_2O
CCDC deposit no.	175170	175171
Color/shape	Colorless/prism	Colorless/prism
Crystal dimensions (mm^3)	0.24×0.35×0.56	0.18×0.27×0.60
Chemical formula	$C_{22}H_{31}NO_5$	$C_{22}H_{31}NO_6{\cdot}H_2O$
Formula weight	389.48	423.49
Temperature (K)	293(2)	293(2)
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1$ (No. 4)	$P2_1$ (No. 4)
Unit cell dimensions	a=9.7090(7) Å	a=8.0436(7) Å
	b=8.7904(6) Å	b=6.6308(5) Å
	c=12.2457(9) Å	c=19.797(1) Å
	β=98.548(2)°	β=91.284(2)°
Volume ($Å^3$)	1033.51(13)	1055.62(15)
Z	2	2
Density (calculated) (mg m^3)	1.252	1.332
Absorption coefficient (mm^{-1})	0.088	0.099
Diffractometer/scan	Bruker SMART CCD/ϖ	Bruker SMART CCD/ϖ
θ range (°)	1.68–26.02	1.03–25.06
Reflections measured	6282	5960
Independent reflections (R_{int})	3832 (0.0239)	3714 (0.0388)
Observed reflections	2820	2784
Data/restrains/parameters	3832/1/256	3714/1/282
Extinction coefficient	0.010(4)	0.004(1)
Goodness of fit on F^2	1.077	1.006
Final R indices [$I>2\sigma I$]	0.0565	0.0424
R indices (all data)	0.0775	0.0643

(**5**), but it was presumed to be an artifact formed by air oxidation of tuberostemonine.[13] Thus compound **3** represents the first example of a natural stemona alkaloid bearing a double bond between C-9 and C-9a. The closest known alkaloid related to **3** is tuberostemonol (**6**)[6b] which was isolated from the same species collected from Guangdong Province, China. Compound **3** differs from **6** at the positions of the hydroxyl group and the double bond.

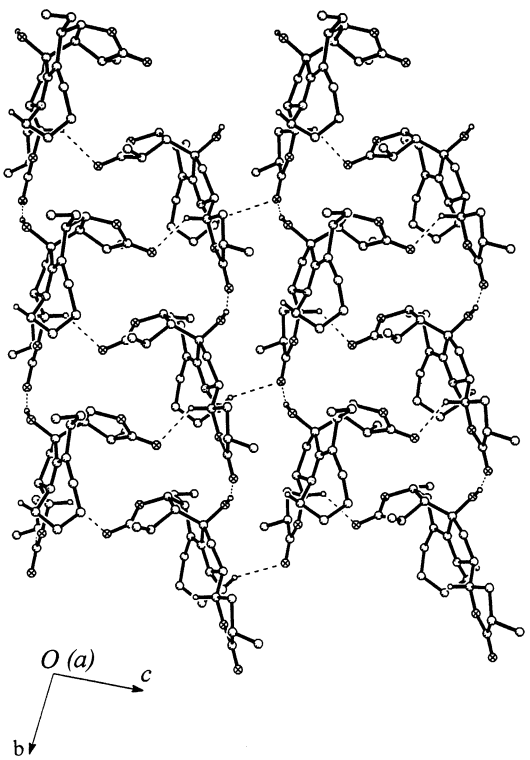

Figure 3. Packing diagram viewed down the *a*-axis, showing the two-dimensional network of **3**. The dashed lines indicate intermolecular O–H···O hydrogen bonds and C–H···O interactions. Selected H-atoms highlighting the hydrogen bonding are shown.

2.2. Neotuberostemoninol (4)

Compound **4** was obtained as colorless prismatic crystals, mp 190–192°C. The HRLSI-mass spectrum of **4** indicated a peseudomolecular ion $[MH]^+$ at *m/z* 406.2224 corresponding to $C_{22}H_{31}NO_6$. The IR spectrum showed a characteristic band for the hydroxyl group at 3482 cm^{-1} and bands in the carbonyl region at 1763, 1750 and 1682 cm^{-1} for two lactones and one lactam carbonyls, respectively. The observed ^1H and ^{13}C NMR spectra are unlike those of **3**; however, they are comparable to those obtained for tuberostemoninol (**7**),[6c] which had also been isolated from this species originated in Guangdong Province of China and had its relative configuration (H-10α, H-11β and H-12β) confirmed by X-ray diffraction. However, the NOE observed at H-10 (5.0%) and H-12 (6.2%) when H-11 was irradiated indicated that these three protons are all on the same side and that they are α-oriented because the H-10α configuration has been observed for all the stenine-type alkaloids previously isolated from related species of *Stemona*. Thus **4** differs from **7** by the configurations of these chiral centers. Similar to **3**, the full assignment of the NMR data of **4** (Table 1) was confirmed by ^1H–^1H COSY, HMQC and HMBC spectra. It is noteworthy that the six-membered ring B and five-membered ring C in **3** are changed into a five-membered and a six-membered ring in **4**, respectively. These changes are confirmed by the key HMBC correlations: H-8→C-1 band H-10→C-1. In contrast, such correlations cannot be found in the HMBC spectrum of **3**.

In order to confirm the relative configuration of **4**, an X-ray crystallographic analysis was undertaken with a single crystal obtained by slow evaporation from a mixture of

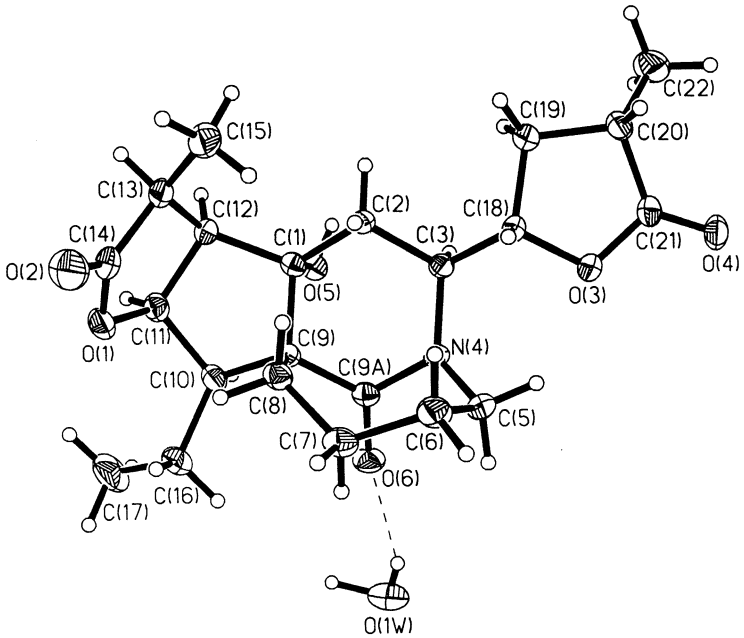

Figure 4. Molecular structure of the 1:1 hydrate of neotuberostemoninol (**4**) and the atom labeling scheme. The C, N and O atoms are drawn as 30% thermal ellipsoids. The intermolecular hydrogen bond in the asymmetric unit is indicated by a dashed line.

hexane and ethyl acetate. Results of the X-ray structure elucidation (Fig. 4) allowed definitive assignment of all chiral centers as 1*S*, 3*S*, 9*R*, 10*R*, 11*R*, 12*R*, 13*S*, 18*S* and 20*S*. The conformations of five-membered rings A and E are the same as **3**. The five-membered ring B and six-membered ring C show envelope and chair conformations, respectively. The flexible seven-membered ring D adopts a chair conformation as compared with the twist-boat found in **3**. Differences between the two conformations of ring D in **3** and **4** can be observed in the torsion angles (Table 3). Compound **4** can be related to the known **2**, which is the main constituent of the roots of *S. tuberosa*, by the oxidative cleavage of the C-1–C-9 bond to form a dicarbonylic system, followed by a Michael addition, i.e. nucleophilic attack by the α-proton (H-9) of the carbonyl group (C-9a) at the carbonyl group at C-1.

The asymmetric unit of **4** consists of one independent molecule and one water molecule. Compound **4** is the first example that crystallizes as a hydrate among the stemona alkaloids. It is of interest to examine the three-dimensional packing of the molecules of **4** within the crystalline lattice. Compound **4** forms hydrogen-bonded chains running parallel to the *c*-axis with the molecules linked by C–H···O interactions. Within an individual chain, the molecules of **4** form pairs through two C–H···O interactions (C-3–H···O-4, D=3.383 Å, d=2.50 Å, θ=150°, $-x$, 0.5+y, 1−z, and C-20–H···O-3, D=3.289 Å, d=2.57 Å, θ=134°, $-x$, −0.5+y, 1−z) in a tail-to-tail mode (ring E overlap), and two adjacent molecular pairs are cross-linked through the C-13–H···O-2 interaction (D=3.167 Å, d=2.43 Å, θ=132°, $-x$, 0.5+y, $-z$) in a head-to-head mode (ring A contact). The adjacent chains are further cross-linked through water molecules involving the carbonyl group at C-9a and the hydroxyl group at C-1 (O-1w–H···O-6 2.860 Å, x, y, z; O-5–H···O-1w 2.835 Å,

Table 3. Selected torsion angles (°) of compounds **3** and **4**·H_2O

Torsion angles	**3**	**4**·H_2O
C(9A)–N(4)–C(5)–C(6)	−81.0(4)	−68.3(3)
N(4)–C(5)–C(6)–C(7)	40.9(6)	53.8(3)
C(5)–C(6)–C(7)–C(8)	41.8(7)	−75.2(3)
C(6)–C(7)–C(8)–C(9)	−72.7(8)	72.7(4)
C(5)–N(4)–C(9A)–C(9)	36.6(5)	108.7(3)
C(7)–C(8)–C(9)–C(9A)	23.0(8)	−9.5(3)
C(8)–C(9)–C(9A)–N(4)	−4.1(6)	−72.7(3)

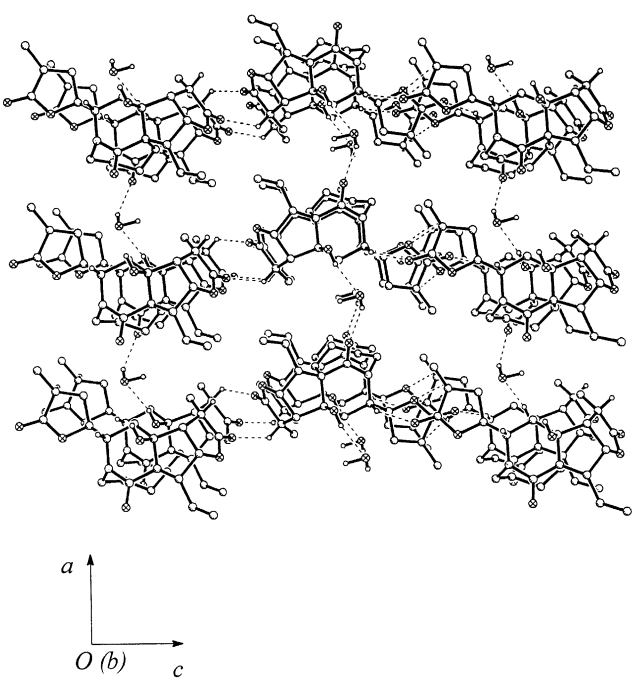

Figure 5. Packing diagram of **4**·H_2O viewed down the *b*-axis, the dashed lines indicate the intermolecular O–H···O hydrogen bonds and C–H···O interactions that connect the chains into a three-dimensional supramolecular assembly. Selected H-atoms highlighting the hydrogen bonding are shown.

$x-1$, y, z) with the water molecules serving both as hydrogen bond donors and acceptors, to form a three-dimensional supramolecular architecture (Fig. 5).

2.3. Discussion

Weak hydrogen bonds based on C–H donors are now well established[14] both as auxiliary binding, and in some cases, as the dominant factor in determining crystal packing[15] or molecular conformation,[16] and their importance in biological systems, e.g. nucleic acids and proteins, has received wide attention.[17] However, such weak hydrogen bonding in small molecules of natural products are less studied.[18] It can be seen that weak C–H···O hydrogen bonds also play an important role in directing the molecular assembly of **3** and **4** in the solid state. Natural products bearing a rich source of H-bond donors (CH, NH and OH) and acceptors (N and O) have proven to be an important origin of potential lead to drug development,[19] and hydrogen bonding may be important to maintain the bioactive conformation and recognize the binding site of the corresponding receptor.

Stemona alkaloids possessing the nucleus of pyrrolo[1,2-α]-azepine are the characteristic components of the family of Stemonaceae.[20] The same nucleus has been found recently in the skins of the Colombian poison frog *Dendrobates lehmanni*;[21] however, the auxiliary ring systems and substituents pattern are significantly different. Except for the simplest representative (stenine),[22] all stenine-type alkaloids from *Stemona* related species have an α-methyl-γ-butyrolactone ring (18*S* and 20*S*) attached to C-3 in the pyrrolidine ring. Since the absolute configuration of tuberostemonine, sharing the same carbon skeleton as **3** and **4**, has been well established through X-ray analysis using the anomalous dispersion method,[10] the absolute configurations of **3** and **4** can be inferred considering the biogenetic relationships in stemona alkaloids as shown in Fig. 1.

3. Conclusion

In the present investigation, neotuberostemonol (**3**) representing the first stemona alkaloid bearing a double bond between C-9 and C-9a and a hydroxyl group at C-1, and neotuberostemoninol (**4**) bearing a lactam functionality at C-9a, a hydroxyl group at C-1 and two α-oriented protons at C-11 and C-12, were isolated from the root of *S. tuberosa* and had their molecular structures established through a combination of spectroscopy and X-ray crystallography. 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. From this viewpoint, the absolute configurations of **3** and **4** were inferred by considering the biogenetic relationships. Stemona alkaloids are high hydrophobic; however, compound **4** represents the first example that crystallizes as a hydrate, and examination of its molecular packing highlights the important role of weak C–H···O hydrogen bonds in directing the molecular assembly in crystalline state.

4. Experimental

4.1. General procedures

Melting points were determined using a Fisher Scientific instrument and were uncorrected. IR spectra were recorded on a Nicolet Impact 420 FT-IR spectrometer. The NMR spectra (^{1}H, ^{13}C, DEPT, NOE, ^{1}H–^{1}H COSY, HMQC and HMBC) were obtained on a Bruker 400 MHz spectrometer with chemical shifts reported in δ (ppm) using TMS as an internal standard. HRLSIMS measurements were made on an APEX 47e FTMS spectrometer. Column chromatography was performed with silica gel (Qingdao Haiyang Chemical Group Co. Ltd, China). 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.

4.2. Plant material

The herbal sample of *S. tuberosa* Lour was purchased in Hong Kong SAR, P. R. China in September, 2000. The material was identified at the Institute of Chinese Medicine, The Chinese University of Hong Kong, where a voucher specimen (No. 99-2300) is kept.

4.3. Extraction and isolation

Dry ground herbal sample (6 kg) was refluxed with 95% ethanol. After evaporation of most of the solvent, the residue was acidified with dilute HCl (4%) and centrifuged at 5°C, 3000 rpm for 40 min. The supernatant was adjusted to pH 9 with aqueous NH_3 and extracted with Et_2O. Crude **2** crystallized from the concentrated Et_2O solution and further recrystallization from EtOH (neotuberostemonine, 2.3 g). The mother liquor was combined to afford a mixture which was subjected to silica gel column chromatography and eluted with a gradient solvent system of $CHCl_3$/MeOH/NH_4OH. The fraction eluted by the solvent ratio of 92:8:0.05 was further subjected to column chromatography and eluted with another solvent system of hexane/EtOAc (35:65) to give **3** (22 mg) and **4** (9 mg). Colorless prisms of **3** and **4** were obtained through slow evaporation from hexane/ethyl acetate at molar ratios of 6:1 and 8:1, respectively.

4.3.1. Neotuberostemonol (3). $C_{22}H_{31}NO_5$, mp 195–197°C; IR (KBr) 3409 (hydroxyl), 1770 and 1743 cm^{-1} (two saturated γ-lactone); HRLSIMS *m/z* $[MH]^+$ 390.2252, calculated 390.2271; EIMS, *m/z* (rel. int.) 390 $[MH]^+$ (81), 360 $[M-C_2H_5]^+$ (100), 291 $[MH-C_5H_7O_2]^+$ (92), 263 $[MH-C_5H_7O_2-CO]^+$ (12); ^{1}H and ^{13}C data, see Table 1.

4.3.2. Neotuberostemoninol (4). $C_{22}H_{31}NO_6$, mp 190–192°C; IR (KBr) 3482 cm^{-1} (hydroxyl), 1763 (saturated γ-lactone), 1750 (saturated γ-lactone) and 1682 cm^{-1} (lactam); HRLSIMS (*m/z* $[MH]^+$, 406.2224, calculated 406.2220, EIMS, *m/z* (rel. int.) 406 $[MH]^+$ (3), 362 $[MH-CH_3-C_2H_5]^+$ (52), 307 $[MH-C_5H_7O_2]^+$ (10), 289 $[MH-C_5H_7O_2-H_2O]^+$ (38), 263 $[MH-CH_3-C_2H_5-C_5H_7O_2]^+$ (100); 261 $[MH-C_5H_7O_2-H_2O-CO]^+$ (9), ^{1}H and ^{13}C data, see Table 1.

4.4. X-Ray crystallography of 3 and 4

X-Ray intensities were measured on a Bruker SMART 1000 CCD diffractometer using graphite-monochromated radiation (λ=0.71073 Å). The reflections cover a hemisphere of reciprocal space and the data reduction was performed using SAINT-PLUS software.[23] The crystal structures were solved by direct methods using the SHELXS-97 program package[24] and refined by full-matrix least-squares on F^2. The non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms bonded to carbons were placed at their geometrically ideal positions. Hydrogen atoms bonded to oxygen were located on difference Fourier maps and included in the calculation of structure factors with isotropic temperature factors. The crystal data of **3** and **4** are shown in Table 2.

Complete lists of refined atomic coordinates, thermal parameters, structure factors, bond lengths, bond angles and torsion angles in standard CIF format for **3** and **4**·H_2O have been deposited at the Cambridge Crystallographic Data Centre as CCDC Ref. No. 175170 and 175171, respectively. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ UK (fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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