Available online at www.sciencedirect.com
SCIENCE DIRECT
Phytochemistry 67 (2006) 52–57
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Isolation and chemotaxonomic significance of tuberostemospironine-type alkaloids from *Stemona tuberosa*

Ren-Wang Jiang [a], Po-Ming Hon [a], Yan-Tong Xu [b], Yiu-Man Chan [a], Hong-Xi Xu [c], Pang-Chui Shaw [a,d,*], Paul Pui-Hay But [a,b,*]

[a] *Institute of Chinese Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, PR China*
[b] *Department of Biology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, PR China*
[c] *Hong Kong Jockey Club Institute of Chinese Medicine, Hong Kong Science Park, Shatin, New Territories, Hong Kong SAR, PR China*
[d] *Department of Biochemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, PR China*

Received 7 February 2005; received in revised form 16 August 2005
Available online 21 November 2005

Abstract

An alkaloid named 6α-hydroxycroomine (**1**) as well as the known croomine (**2**), both belonging to the tuberostemospironine-alkaloid type, were isolated from *Stemona tuberosa* as the major components. The structure of **1** was elucidated through extensive spectroscopic analyses. Comparison of the HPLC profiles of the total alkaloids and the crude methanol extract showed that both compounds are naturally occurring. The first isolation of **1** and **2** from *S. tuberosa* has chemotaxonomic significance, confirming the close relationship between *Stemona* and *Croomia*. The *trn*L sequences of plants from the four genera of Stemonaceae cluster together as a clade, further lending support to retaining them in a single family.

Keywords: *Stemona tuberosa*; *Croomia*; Stemonaceae; Croomine; 6α-Hydroxycroomine; *trn*L sequence

1. Introduction

Stemonaceae is a small family with four genera, namely, *Croomia*, *Pentastemona*, *Stemona* and *Stichoneuron*. *Stemona* is the largest genus with about 32 species, whereas the other genera each have one or two species. They are distributed in Asia, tropical Australia and North America. The taxonomy of Stemonaceae is not settled. Some experts have suggested moving *Croomia* and *Stichoneuron* to a separate family, i.e., Croomiaceae (Ji and Duyfjes, 1994). *Pentastemona*, on the other hand, was moved to its own family Pentastemonaceae (Duyfjes, 1991; Thorne, 2000). So far, no further classification has been reported on the chemotaxonomic relationship of these genera.

The chemistry of these genera has attracted much interest. So far, some over 40 alkaloids had been isolated from them (Pilli and Ferreira de Oliveira, 2000). However, only two alkaloids, i.e., croomine and didehydrocroomine, were characterized from *Croomia* (Noro et al., 1979; Lin et al., 1993) and both compounds were not previously found in *Stemona*.

Stemona tuberosa Lour is a species registered in the Chinese Pharmacopoeia (Pharmacopoeia of P. R. China, 2005). The water extracts of the root tuber of this plant are used in Chinese, Japanese and Korean traditional medicines to treat respiratory disorders, e.g., bronchitis, pertussis and tuberculosis, and also as an anthelmintic agent for domestic animals (Jiangsu New Medical College, 1977). During the course of our on-going investigation of the chemical diversity of *S. tuberosa*, seven stenine-type alkaloids were characterized (Jiang et al., 2002; Chung et al., 2003) from a sample of this herb purchased from a local herb shop. Further investigation of the same species from

* Corresponding authors. Tel.: +852 2609 6299/6803; fax: +852 2603 5646/5123.
E-mail addresses: pcshaw@cuhk.edu.hk (P.-C. Shaw), paulbut@cuhk.edu.hk (P. Pui-Hay But).

0031-9422/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.phytochem.2005.10.004

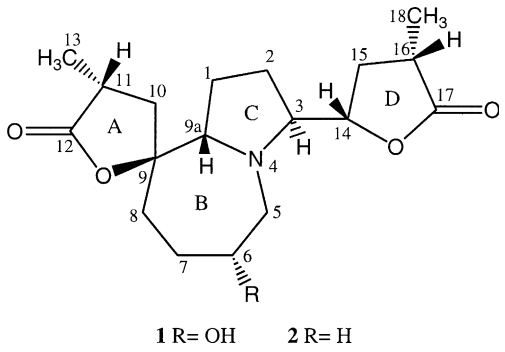

Fig. 1. Structural formulae of 6α-hydroxycroomine (**1**) and croomine (**2**).

another source showed a completely different chemical pattern. Subsequent phytochemical studies on this sample resulted in the isolation of the new 6α-hydroxycroomine (**1**) and the known croomine (**2**) (Fig. 1). Though the structure of compound **2** was reported about three decades ago with its physical and chemical data, the NMR spectroscopic data were not clearly assigned (Noro et al., 1979). We report herein the isolation and structural elucidation of compound **1**. The NMR spectroscopic data of **2** were compared with those of **1**. The chemotaxonomic significance of the two compounds is also discussed briefly. The *trn*L region in the genomic DNA of plants from the four genera of Stemonaceae was also amplified and corresponding sequences were compared to further confirm the results from phytochemical study.

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, then extracted with Et_2O and CH_2Cl_2 successively. Compound **1** was obtained from the CH_2Cl_2 fraction by silica gel column chromatography and compound **2** was obtained directly from the Et_2O fraction.

The HRFAB mass spectrum of **1** indicated a quasimolecular ion $[MH]^+$ at *m*/*z* 338.1971, corresponding to a molecular formula $C_{18}H_{27}NO_5$ with six units of unsaturation. The ESI mass spectrum showed a $[MH]^+$ ion peak at *m*/*z* 338. The characteristic cleavage fragment *m*/*z* 238 $[M-C_5H_7O_2]^+$ in EIMS indicated that **1** has an α-methyl-γ-lactone ring (Lin et al., 1992, 1994). Its IR (KBr) spectrum showed bands at 3408 (hydroxyl), 1772 and 1745 cm^{-1} (two saturated γ-lactone).

The ^1H NMR spectrum (Table 1) indicates the presence of two secondary methyl groups at δ_H 1.18 (3H, *d*, $J = 6.9$ Hz, H-18) and δ_H 1.27 (3H, *d*, $J = 8.0$ Hz, H-13), two low-field protons attached to carbon atoms bearing an oxygen functionality at δ_H 4.31 (1H, *ddd*, $J = 5.0$, 8.5, 10.0 Hz, H-14) and δ_H 4.08 (1H, *m*, H-6), two methines and two geminal protons attached to carbon atoms bearing a nitrogen function at δ_H 3.35 (1H, *m*, H-3), δ_H 3.28 (1H, *dd*, $J = 6.8$, 7.0 Hz, H-9a), δ_H 3.57 (1H, *dd*, $J = 5.9$, 13.2 Hz, H-5α) and δ_H 3.34 (1H, *m*, *dd*, $J = 3.4$, 13.2 Hz, H-5β). The ^{13}C NMR and DEPT spectra of **1** show 18 carbon atoms: two lactonic carbonyl atoms at δ_C 179.58 (C-12, *s*) and 179.76 (C-17, *s*), three carbon atoms linked to a nitrogen atom at δ_C 69.52 (C-3, *d*), 58.01 (C-5, *t*) and 70.37 (C-9a, *d*), three carbon atoms bearing an oxygen functionality at δ_C 70.84 (C-6, *d*), 80.51 (C-14, *d*) and 88.68 (C-9, *s*), two additional methine carbons (δ_C 36.84 and 35.62), six additional methylene groups (δ_C 26.91, 28.01, 31.54, 35.45, 37.47 and 38.01) and two methyl groups (δ_C 15.40 and 18.34).

The full assignments and connectivities were determined by ^1H–^1H COSY, HSQC and HMBC spectroscopic analyses. The ^1H–^1H COSY spectrum shows four spin systems: (i) H-10 → H-11 → H-13 (ring A); (ii) H-5 → H-6 → H-7 → H-8 (ring B), (iii) H-9a → H-1 → H-2 → H-3 (ring C) and (iv) H-14 → H-15 → H-16 → H-18 (ring D) indicating a tetracyclic structure, which plus the two lactonic carbonyls, account for the six units of unsaturation as revealed by the molecular formula. The HMBC correlations of C-17 to H-15, H-16 and H_3-18, and C-14 to H-15 and H-3 indicate that an α-methyl-γ-lactone ring is formed by ring closure involving the oxygen atom bridged to C-14 and C-17 and its location at C-3. The HMBC correlations of C-9 (oxygenated quaternary carbon) to H-10 and H-11 (ring A), H-7 and H-8 (ring B) and H-9a and H-1 (ring C) in combination with its more downfield nature relative to C-14 (oxygenated methine) indicate that C-9 is a spiro-atom connecting the lactone ring A and the azepinoindole nucleus. The HSQC spectrum revealed that the signal at δ_H 4.08 (H-6) is attached to a carbon at δ_C 70.84 (C-6), and the HMBC spectrum showed C-6 is correlated to H-5α, H-5β, H-7α and H-7β, suggesting that the hydroxyl group is attached to C-6, which is further confirmed by the appearance of the multiple complex NMR feature of H-6 and the double doublet feature of H-5α and H-5β. These spectroscopic data are thus reminiscent of the tetracyclic tuberostemospironine-type alkaloids bearing a croomine skeleton with an additional hydroxyl group attached to C-6.

The relative configuration of compound **1** was assigned from analysis of its ROESY spectrum. The proton H-6 shows a correlation with H-9a, and H-9a is correlated to H-14, which is further correlated to H-16. These results suggested that H-6, H-9a, H-14 and H-16 are on the same side of the molecule and are β-oriented. The α-methyl-γ-butyrolactone ring (18*S* and 20*S*) attached to C-3 in the pyrrolidine ring was widely observed in alkaloids from *Stemona* related species. Since the absolute configuration of croomine (**2**), sharing the same carbon skeleton as **1**, has been well established through X-ray analysis using the anomalous dispersion method (Noro et al., 1979), the absolute configurations of **1** can be inferred considering the biogenetic relationships in *Stemona* alkaloids as shown in Fig. 1. Accordingly, compound **1** is identified as 6α-hydroxycroomine.

Table 1
^{1}H NMR and ^{13}C NMR spectroscopic data of alkaloids **1** and **2**[a]

No.	6α-Hydroxycroomine (**1**)		Croomine (**2**)	
	δ_H(mult. *J* in Hz)	δ_CDEPT	δ_H(mult. *J* in Hz)	δ_CDEPT
1	1.53 (1H, *m*) 1.78–1.84 (1H, *m*)	28.01, *t*	1.42 (1H, *m*) 1.60 (1H, *m*)	27.80, *t*
2	1.36 (1H, *m*) 1.78-1.84 (1H, *m*)	26.91, *t*	1.39 (1H, *m*) 1.60 (1H, *m*)	26.83, *t*
3	3.35 (1H, *m*)	69.52, *d*	3.25 (1H, *dd*, 6.6,7.0)	69.52, *d*
5	3.57 (1H, *dd*, 5.9, 13.2) 3.34 (1H, *dd*, 3.4, 13.2)	58.01, *t*	3.09 (2H, *m*)	50.00, *t*
6	4.08 (1H, *m*)	70.84, *d*	1.68 (2H, *m*)	22.54, *t*
7	1.90 (1H, *m*) 2.02 (1H, *m*)	31.54, *t*	1.76 (1H, *m*) 1.80 (1H, *m*)	28.22, *t*
8	2.02 (1H, *m*) 1.87 (1H, *m*)	38.01, *t*	1.84 (1H, *m*) 1.76 (1H, *m*)	41.40, *t*
9		88.68, *s*		89.53, *s*
9a	3.28 (1H, *dd*, 6.8, 7.0)	70.37, *d*	3.39 (1H, *t*, 7.2)	67.31, *d*
10	2.47 (1H, *dd*, 13.5, 10.2) 1.64 (1H, *dd*, 13.5, 7.8)	37.47, *t*	2.33 (1H, *dd*, 10.8, 13.6) 1.50 (1H, *dd*, 13.8, 7.8)	37.80, *t*
11	2.82 (1H, *ddq*, 10.2, 7.8, 8.0)	36.84, *d*	2.77 (1H, *m*)	36.42, *d*
12		179.58, *s*		179.62, *s*
13	1.27 (3H, *d*, 8.0)	18.34, *q*	1.24 (3H, *d*, 7.5)	18.01, *q*
14	4.31 (1H, *ddd*, 8.5, 5.0, 10.0)	80.51, *d*	4.22 (1H, *ddd*, 6.6, 5.0, 10.0)	81.15, *d*
15	1.32 (1H, *m*) 2.15 (1H, *m*)	35.45, *t*	1.33 (1H, *m*) 2.14 (1H, *m*)	35.15, *t*
16	2.61 (1H, *dq*, 11.8, 7.8)	35.62, *d*	2.62 (1H, *dq*, 12.3, 7.2)	35.48, *d*
17		179.76, *s*		179.82, *s*
18	1.18 (3H, *d*, 6.9)	15.40, *q*	1.18 (3H, *d*, 6.9)	15.30, *q*

[a] Deuteropyridine was selected as the solvent for measurement of NMR spectra of compounds **1** and **2**. The ^{1}H NMR of the molecules showed characteristic sharper signals in deuteropyridine than deuterochloroform and deuteromethanol (Jiang et al., 2002).

Compound **2** has been previously isolated from *Croomia heterosepala* (Noro et al., 1979) and *C. japonica* (Lin et al., 1993). The intriguing structure of **2** has attracted a number of ingenious strategies toward its total syntheses (Williams et al., 1989; Martin et al., 1999). The NMR spectroscopic data of **2** had been listed before but not assigned. Thus the spectroscopic data of **2** were compared with those of **1** as shown in Table 1.

In order to investigate if the two alkaloids were artifacts by acid (HCl) and base (NH_3) process during purification, the HPLC chromatograms of the total alkaloids obtained after acid and base treatment and of the crude methanol extract were compared as shown in Figs. 2A and C, respectively. It should be noted that these alkaloids are non-chromophoric, and thus are not sensitive under direct UV detection (Fig. 2B). Thus evaporative

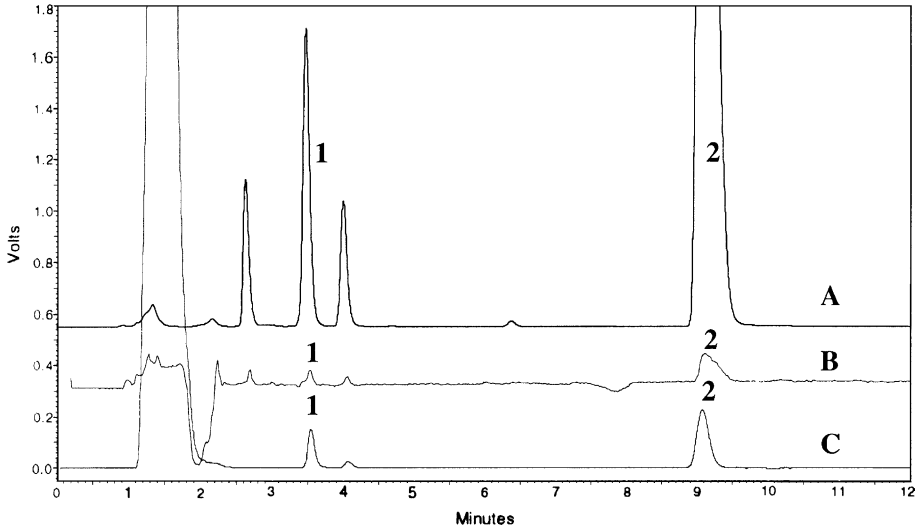

Fig. 2. HPLC chromatograms for the *Stemona tuberosa*: ELSD detection of total alkaloids (A), UV detection of the total alkaloids at 215 nm with low signal and large noise (B), and ELSD detection of crude methanol extract (C). Compound **2** is the predominant component and compound **1** is the second major one. No other significant peak is detected when the analysis time is extended to 30 min.

light scattering detection (ELSD) (Lin et al., 2001) depending on the size, shade and number of eluate particles was used in conjuction with HPLC. The chromatograms showed that compounds **1** ($t_R = 3.55$ min) and **2** ($t_R = 9.26$ min) exist both in the total alkaloid and the crude methanol extract. Thus both compounds are natural occurring alkaloids.

Simultaneous isolation of **1** and **2** from *S. tuberosa* as its major components not only increases the chemical diversity but also shows that similar components can be found in both genera of *Stemona* and *Croomia*. The stemona alkanoids, possessing the pyrrolo- or pyrido(1,2-α) azepine nucleus (Kaltenegger et al., 2003; Mungkornasawakul et al., 2004) as the smallest common structural core, are characteristic components in the family Stemonaceae (Pilli and Ferreira de Oliveira, 2000). So far, no such alkaloid has been discovered outside the family Stemonaceae in the plant kingdom. Though the same nucleus has been found recently in skins of the Colombian poison frog *Dendrobates lehmanni* (Garraffo et al., 2001), the auxiliary ring systems and substitution patterns are significantly different. Due to the occurrence of common chemical components, it would be better to retain these two genera in the family Stemonaceae.

The chemical results also coincided with those of DNA study. The genomic DNA of plants from genera *Croomia*, *Pentastemona*, *Stemona* and *Stichoneuron* (Table 2) was extracted, and then the *trn*L region was amplified and submitted to direct sequencing. The resulting sequences were compared with the *trn*L DNA derived from plants in families Liliaceae, Pandanaceae, Dioscoreaceae and Asparagaceae which were deposited in the GenBank (Table 3). Full sequences can be accessed in Supplementary material (see Appendix 1). Selection of these four families is due to their close relationship to Stemonaceae (Krause, 1930; Dahlgren et al., 1985; Huber, 1991; Chase et al., 1995). The consensus parsimony tree based on *trn*L intron of these families is shown in Fig. 3, which revealed that *Stemona* and *Croomia* together with *Pentastemona* and *Stichoneuron* settle in the same clade (Chan, 2004), and Stemonaceae was found to be closer to Pandanaceae than to the other three families. Thus our cladistic study, based on parsimonious analysis of *trn*L DNA sequences, confirmed the close relationship of the four genera and further favored retaining them in the same family.

Table 3
*trn*L DNA sequences from NCBI GenBank

Species	Family	NCBI Accession No.
Asparagus acutifolius	Asparagaceae	**AJ441168**
Asparagus falcatus	Asparagaceae	**AF508514**
Asparagus officinalis	Asparagaceae	**AJ441164**
Dioscorea opposita	Dioscoreaceae	**D89701**
Dioscorea rotundata	Dioscoreaceae	**D89695**
Dioscorea trifida	Dioscoreaceae	**D89682**
Freycinetia funicularis	Pandanaceae	**AY337702**
Lilium catesbaei	Liliaceae	**AF303701**
Martellidendron masoalense	Pandanaceae	**AY337709**
Pandanus odoratissimus	Pandanaceae	**AY337693**
Calochortus greenei	Liliaceae	**AY430569**
Chloranthus angustifolius	Chloranthaceae	**AF364600**

3. Experimental

3.1. General

ORD were recorded on a Perkin–Elmer 341 Polarimeter in MeOH solution. The UV spectra were obtained on an online Beckman 168 DAD spectrophotometer. IR spectra were recorded on a Nicolet Impact 420 FT-IR spectrometer. ESIMS were recorded on a Finnigan MAT TSQ 7000 instrument. HRFABMS measurements were made 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, respectively. 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.

3.2. Plant material

The herb sample of *S. tuberosa* used for phytochemical study was collected in Yunnan Province, China in September 2003. Both *trn*l and 5S-rRNA sequences were used to identify the botanical origin of this herb. A specimen (ICM 20042541) is kept in the Herbarium, Institute of Chinese Medicine, The Chinese University of Hong Kong. Details of samples used for DNA study are shown in Table 2.

Table 2
Samples list for *trn*L DNA study

Plants	Sources	Specimens No.	NCBI Accession No.
Stemona parviflora	Hainan, China	Ma 9066	**DQ085268**
Stemona tuberosa	Yunan, China	ICM 20042541	**DQ085269**
Stemona japonica	Nanjing Institute of Botany, Nanjing, China	Hu & But 23971	**DQ085270**
Stemona sessilifolia	Institute of Medicinal Plant, Beijing, China	Hu & But 23972	**DQ085271**
Stichoneuron caudatum	Royal Botanic Garden, Kew, UK	Leiden B.G. 910654	**DQ085273**
Pentastemona sumatrana	Royal Botanic Garden, Kew, UK	Leiden B.G. 910375	**DQ085274**
Croomia japonica	Asiatica Nursery, USA	Hu & But 24033	**DQ085275**
Croomia pauciflora	Royal Botanic Garden, Kew, UK	Gholson 10360	**DQ085276**

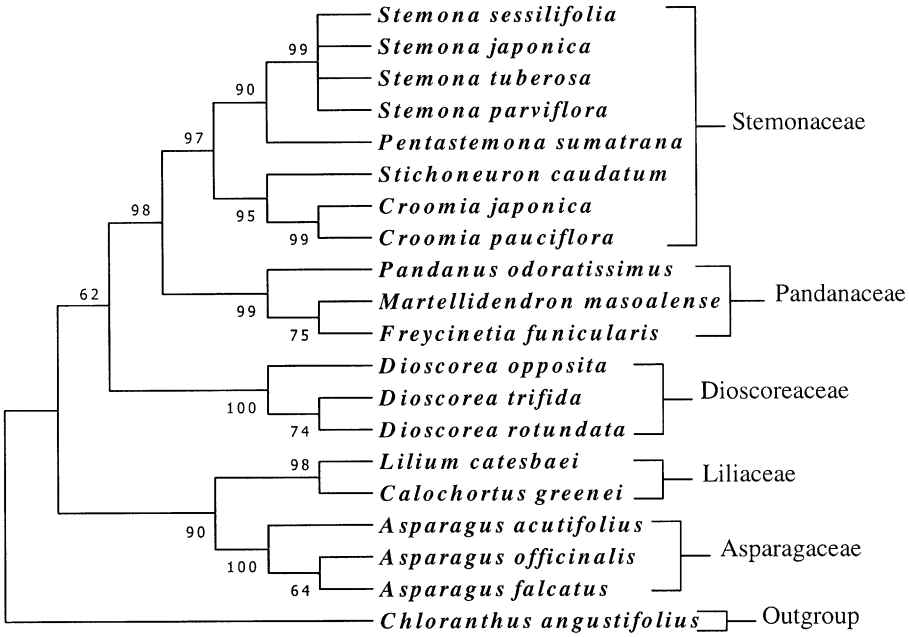

Fig. 3. Consensus parsimony tree based on *trn*L intron of plants from Stemonaceae, Pandanaceae, Dioscoreaceae, Liliaceae and Asparagaceae (20 sequences). The tree is rooted through an outgroup *Chloranthus angustifolius*. Bootstrap values based on 1000 replications are shown on branches.

3.3. Extraction and isolation

The dried roots (1 kg) were chopped into small pieces (2–4 mm) and refluxed with 95% ethanol (4 L) for 2 h. This extraction was repeated two more times. The extracted portions were combined and concentrated by evaporation under reduced pressure at 45 °C to give a syrup extract, which was then dissolved with 4% HCl (400 ml). After filtration, the filtrate was adjusted to pH 9 with aqueous NH_3 (35%). The basic aqueous solution was extracted with Et_2O (300 ml × 2) and CH_2Cl_2 (300 ml × 2).

The combined Et_2O solution was washed with H_2O and then evaporated under reduced pressure to give an oil containing compound **2** (800 mg). The CH_2Cl_2 extract solution was evaporated to dryness to give a residue, which was subjected to silica gel column chromatography eluted with a mixture of hexane and EtOAc (1:1). Based on TLC, the eluates were combined and evaporated to afford compounds **1** (400 mg) and **2** (40 mg).

6α-hydroxycroomine (***1***): white powder, formula $C_{18}H_{27}NO_5$, $[\alpha]_D^{20}$ +24.6° (MeOH; c0.5); ESIMS *m/z*: $[MH]^+$ 338 (100), EIMS $[M-C_5H_7O_2]^+$ 238 (100), positive HRFABMS *m/z* $[MH]^+$ 338.1971, calculated 338.1967. For ^1H and ^{13}C NMR spectra, see Table 2.

3.4. HPLC analysis

Sample preparation. A small amount of chopped roots (5 g) of *S. tuberosa* was refluxed with 95% ethanol (100 ml) for an hour. 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% NH_4OH solution to pH 9 and extracted with CH_2Cl_2. The extract was condensed and dissolved in methanol (2 ml) to afford the total alkaloid solution. For the methanol crude extract, the chopped roots (2 g) was extracted with methanol (5 ml) under ultrasonic conditions for an hour. Both final solutions were filtered through a 0.22 μm PTFE syringe filter, with an aliquot of each filtrate (10 μl) subjected to HPLC analysis.

HPLC conditions. Analytical HPLC was performed on a Beckman System Gold instrument equipped with a 125 solvent module, a 168 photo diode array detector and a 508 autosampler, and coupled to an Alltech 500 ELSD detector (Alltech, Deerfield, IL, USA) and a nitrox nitrogen generator. Chromatographic separations were carried out using a C18 column (150 × 4.6 mm, 3 μm; Alltech, USA), eluted with an isocratic solvent system comprised of CH_3CN–H_2O (4:6, v/v) containing 0.12% Et_3N at a flow rate of 1.0 ml/min. UV detection was at 215 nm. Temperature for the ELSD drift tube was set at 97 °C and the nitrogen flow was 2.6 SLPM (standard liters per minute).

3.5. DNA extraction, PCR and sequencing

DNA extraction. The DNA extraction was performed in a microcentrifuge tube (1.5 ml) using the modified Kang's method (Kang et al., 1998) described previously for extraction of DNA from single dried seed specimens. All the centrifuge experiments were done at room temperature. In the final step, the DNA pellet was resuspended in 50 μl double distilled water instead of the TE buffer in Kang's method.

Polymerase chain reaction (PCR). Primers Tab C (5′-CGA AAT CGG TAG ACG CTA CG-3′) and Tab D (5′-GGG GAT AGA GGG ACT TGA AC-3′) (Taberlet et al., 1991) were used for amplification of *trn*L region.

PCR was performed in a mixture containing 15.3 μl autoclaved double distilled water, 2.5 μl of 10× PCR buffer, 2 μl of 2.5 mM dNTP, 2 μl of 25 mM $MgCl_2$, 1 U *Taq* polymerase, 1 μl (10 mM) of both primers and 1 μl template DNA. Thermal cycling was performed in a MJ-PTC100 thermocycler and carried out as follows: one cycle of 95 °C for 5 min; then 20 cycles of 95 °C for 20 s, 56 °C for 30 s and 72 °C for 1.5 min; and a final extension at 72 °C for 5 min.

Sequencing. Direct sequencing was performed on purified PCR products of *trn*L using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer's instructions. The resulting DNA sequences were deposited in the NCBI GenBank. The accession numbers are shown in Table 2; full sequences are also provided in the Supplementary information (Appendices 1 and 2). Alignment and comparison of DNA sequences were performed by Clustalw from the European Bioinformatics Institute (EBI). The molecular evolutionary genetics analysis software (MEGA) version 2.1 (Kumar et al., 2001) was used for the construction of phylogenetic tree.

Acknowledgments

The research is funded by the Hong Kong Jockey Club Charities Trust. We thank the DNA bank of Royal Botanical Garden, Kew, for supplying us with DNA samples of *Croomia pauciflora*, *Pentastemona sumatrana* and *Stichoneuron caudatum*.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2005.10.004.

References

Chan, Y.M., 2004. Molecular authentication and taxonomy of radix stemonae. MPhil Thesis, The Chinese University of Hong Kong.

Chase, M.W., Duvall, M.R., Hills, H.G., Conran, J.G., Cox, A.V., Eguiarte, L., Hartwell, J., Fay, M.F., Caddick, L.R., Cameron, K.M., Hoot, S.B., 1995. Molecular phylogenetics of Lilianae. In: Rudall, P.J., Cribb, P.J., Cutler, D.F., Humphries, C.J. (Eds.), Monocotyledons: Systematics and Evolution. Royal Botanic Gardens, Kew, pp. 109–137.

Chung, H.S., Hon, P.M., Lin, G., But, P.P.H., Dong, H., 2003. Antitussive activity of Stemona alkaloids from *Stemona tuberosa*. Planta Med. 69, 914–920.

Committee for the Pharmacopoeia of PR China, 2005. Pharmacopoeia of PR China, Part I. Chemical Industry press, Beijing, China, p. 88.

Dahlgren, R.M.T., Clifford, H.T., Yeo, P.F., 1985. The Families of the Monocotyledons: Structure, Evolution, and Taxonomy. Springer, New York.

Duyfjes, B.E.E., 1991. Stemonaceae and Pentastemonaceae; with miscellaneous notes on members of both families. Blumea 36, 239–252.

Garraffo, H.M., Jain, P., Spande, T.F., Daly, J.W., Jones, T.H., Smith, L.J., Zottig, V.E., 2001. J. Nat. Prod. 64, 421–427.

Huber, H., 1991. Angiospermem: Leitfaden dyrch die Ordungen und Familien der Bedecktsamer. Gustav Fischer Verlag, Stuttgart.

Jiang, R.W., Hon, P.M., But, P.P.H., Chung, H.S., Lin, G., Ye, W.C., Mak, T.C.W., 2002. Isolation and stereochemistry of two new alkaloids from *Stemona tuberosa* Lour. Tetrahedron 58, 6705–6712.

Jiangsu New Medical College, 1977. Dictionary of Chinese Traditional Medicine. Shanghai People's Publishing House, P. R. China, pp. 858–861.

Ji, Z.H., Duyfjes, B.E.E., 1994. Stemonaceae. In: Wu, Z.Y., Raven, P.H. (Eds.), Flora of China, vol. 24. Science Press/Missouri Botanical Garden, Beijing/St. Louis, pp. 70–72.

Kaltenegger, E., Brem, B., Mereiter, K., Kalchhauser, H., Kahlig, H., Hofer, O., Vajrodaya, S., Greger, H., 2003. Insecticidal pyrido(1,2-α)azepine alkaloids and related derivatives from Stemona species. Phytochemistry 63, 803–816.

Kang, H.W., Cho, Y.C., Yoon, U.H., Eun, M.Y., 1998. A simple and rapid procedure for isolation of total DNA suitable for fingerprint analysis from shape amaranthus. Plant Mol. Biol. Rep. 16, 90–91.

Krause, K., 1930. Stemonaceae. In: Engler, A., Prantl, K. (Eds.), Die natürlichen Pflanzenfamilien, second ed., band 15a, pp. 224–227.

Kumar, D., Tamura, K., Jakobsen, I.J., Nei, M., 2001. MEGA2: Molecular Evolutionary Genetics Analysis software. Arizona State University, Tempe, AZ, USA.

Lin, W.H., Ye, Y., Xu, R.S., 1992. Chemical studies on new Stemona alkaloids, IV: studies on new alkaloids from *Stemona tuberosa*. J. Nat. Prod. 55, 571–576.

Lin, W.H., Cai, M.S., Ying, B.P., Feng, R., 1993. Chemical constituents of *Croomia japonica* Miq. Acta Pharm. Sin. (Yaoxue Xuebao) 28, 202–206.

Lin, W.H., Ma, L., Cai, M.S., Barnes, R.A., 1994. Two minor alkaloids from roots of *Stemona tuberosa*. Phytochemistry 36, 1333–1335.

Lin, G., Li, P., Li, S.L., Chan, S.W., 2001. Chromatographic analysis of Fritillaria isosteroidal alkaloids, the active ingredients of Beimu, the antitussive traditional Chinese medicinal herb. J. Chromatogr. A 935, 321–338.

Martin, S.F., Barr, K.J., Smith, D.W., Bur, S.K., 1999. Applications of vinylogous mannich reactions. Concise enantiospecific total syntheses of (+)-croomine. J. Am. Chem. Soc. 121, 6990–6997.

Mungkornasawakul, P., Pyne, S.G., Jatisatienr, A., Supyen, D., Jatisatienr, C., Lie, W., Ung, A.T., Skelton, B.W., White, A.H., 2004. Phytochemical and larvicidal studies on *Stemona curtisii*: structure of a new pyrido(1,2-α)azepine Stemona alkaloid. J. Nat. Prod. 67, 675–677.

Noro, T., Fukushima, S., Ueno, A., Miyase, T., Iitaka, Y., Saiki, Y., 1979. A new alkaloid, croomine, from *Croomia heterosepala* Okuyama. Chem. Pharm. Bull. 27, 1495–1497.

Pilli, R.A., Ferreira de Oliveira, M.C., 2000. Recent progress in the chemistry of the *Stemona* alkaloids. Nat. Prod. Rep. 17, 117–127.

Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17, 1105–1109.

Thorne, R.F., 2000. The classification and geography of the flowering plants: dicotyledons of the class angiospermae. Bot. Rev. 66, 441–647.

Williams, D.R., Brown, D.L., Benbow, J.W., 1989. Studies of Stemona alkaloids. Total synthesis of (+)-croomine. J. Am. Chem. Soc. 111, 1923–1925.