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Structural Relationships of *Stemona* Alkaloids: Assessment of Species-Specific Accumulation Trends for Exploiting Their Biological Activities

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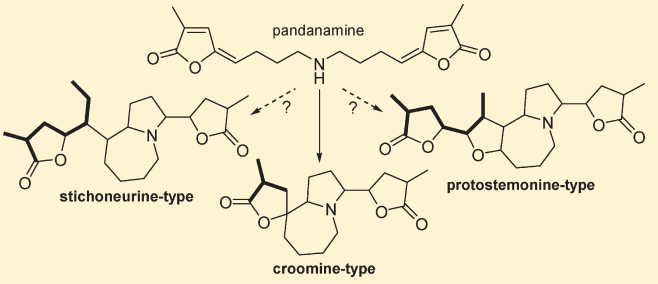
ABSTRACT: On the basis of a comparison of 42 *Stemona* samples, representing eight different species collected and cultivated in Thailand, species-specific accumulation trends of *Stemona* alkaloids were analyzed. An overview was achieved by comparative HPLC analyses of methanolic crude extracts of underground parts coupled with diode array or evaporative light scattering detectors. All major compounds were isolated and their structures elucidated by NMR and MS analyses. Protostemonine- and stichoneurine-type derivatives dominated, from which the latter characterize *S. tuberosa* and *S. phyllantha* accumulating species-specific isomers of tuberostemonine (**3**). The widespread *S. curtisii* and *S. collinsiae* clearly deviate by protostemonine-type derivatives dominated by stemofoline (**10**) and/or didehydrostemofoline (**11**). Further diversification within this structural type results from a mutual accumulation of derivatives with a pyrrolo- or pyridoazepine nucleus, leading to chemical variability in *S. curtisii* and *S. aphylla*.



Stemona alkaloids represent a unique chemical character of the small monocotyledonous family Stemonaceae consisting of the three genera, *Stemona*, *Stichoneuron*, and *Croomia*, comprising about 30 species. On the basis of more recent taxonomic treatments for the flora of Thailand the genus *Stemona* is represented by 11[1] and *Stichoneuron* by five species.[2] More than 130 alkaloids are known from *Stemona*,[3] whereas only two were reported from *Stichoneuron*, isolated as various isomers.[4,5] Generally, the highest concentration was found in the underground parts of *Stemona* species, consisting of tuberous roots and rhizomes.[6] With respect to biosynthetic considerations and their various distribution, they were classified into three skeletal types.[6,7] On the basis of purely chemical aspects an alternative classification into eight groups was suggested, where the name of the structurally simplest derivative was used for group denominations.[3]

A wide range of practical applications has led to an increasing interest in this class of alkaloids. Important activities were summarized in a previous review.[7] In the meantime further investigations supported their antitussive activity[8−13] and insect toxic properties.[14−16] Moreover, oxytocin antagonism,[17] nitric oxide inhibition,[18] and increasing chemosensitivity via P-glycoprotein-mediated multidrug resistance[19] revealed further pharmacological activities. Basically, most effects can be attributed to either tuberostemonine (**3**) or stemofoline (**10**) derivatives, representing two different skeletal types.[7] As shown in a previous overview, this chemical divergence also suggests a clear taxonomic segregation within the genus *Stemona*,[6] later confirmed by morphological characters[11,20] and microsatellite markers.[21] Further progress in exploiting the various biological activities is greatly hampered by using not properly identified and documented plant material or, even more, by purchasing mixtures of underground parts from local markets or medical plant suppliers originating from different localities or species.[7] Moreover, with respect to chemical instability of some *Stemona* alkaloids, the formation of artifacts may be expected, especially after extensive acid−base treatment.[5,7]

In the course of identifying the genetic diversity of 68 *Stemona* samples, collected from different geographical localities in Thailand, corresponding plant material was cultivated under field conditions in the Maize and Sorghum Research Centre "Rai Suwan" of Kasetsart University near Nakhon Ratchasima in east Thailand.[21] Besides morphological and anatomical studies,[20] this comprehensive collection also served for a broad-based phytochemical comparison to establish species-specific accumulation trends. Additional collections from natural habitats are included to complete this survey. Taking into account the chemical instability of some alkaloids and the difficulties in species delimitations, the present paper should provide a basis

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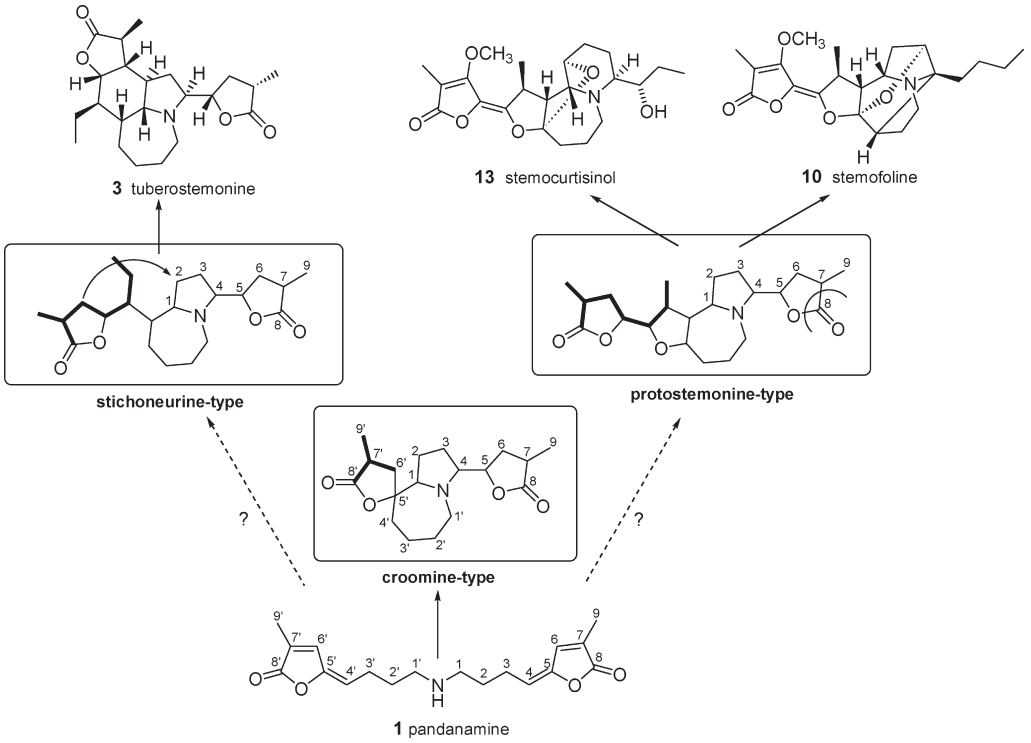

Figure 1. Proposed biosynthetic pathways of *Stemona* alkaloids.

for exploiting these compounds for further pharmaceutical development and pest control.

RESULTS AND DISCUSSION

Dried and powdered underground parts of 42 *Stemona* samples were extracted with methanol. According to a recent taxonomic revision,[1] they represent eight different species and two collections with uncertain taxonomic positions. In order to get an overview on characteristic alkaloid profiles, comparative HPLC analyses of the crude extracts were carried out coupled with diode array (DAD) or evaporative light scattering (ELSD). This method was validated to be sensitive and accurate to detect various types of *Stemona* alkaloids both with and without characteristic chromophoric groups.[22] Characteristic major compounds were isolated, and their structures elucidated by NMR and MS analyses. On the basis of these findings two clear-cut biosynthetic trends can be distinguished, either toward protostemonine- or stichoneurine-type derivatives (Figure 1). In contrast to reports on many Chinese collections[9,11,22−26] the croomine-type was represented only by croomine (**2**) itself in one sample of *S. tuberosa* Lour. (HG 890) collected in north Thailand (Figure 4).[6]

Because of far-reaching morphological similarities between the genera *Croomia* and *Stichoneuron*, Duyfjes recently pointed to a possible amalgamation of both.[2] So far only two species of *Stichoneuron*[4,5] and one of *Croomia*[27] have been subjected to chemical analysis. Of special chemotaxonomic importance was our recent discovery of the secondary amine pandanamine (**1**) in the rare *Stichoneuron calcicola* Inthachub, which was regarded as a plausible biogenetic precursor of *Stemona* alkaloids (Figure 1).[5] Interestingly, protostemonine-type alkaloids, otherwise representing the greatest diversity in the genus *Stemona*, could not be detected in the two genera. Instead, the isomers stichoneurines A and B were isolated from *Stichoneuron caudatum* Ridley,[4] and croomine (**2**) was isolated from *Croomia heterosepala* Okuyama.[27] Whereas the latter can structurally be directly derived from pandanamine (**1**), no explanation can be given for the formation of the stichoneurine- and protostemonine-type skeletons containing four additional carbon atoms (Figure 1).

In the present survey alkaloids with a stichoneurine-type skeleton were represented mainly by tuberostemonine (**3**) together with three isomers (**4**−**6**) showing a species-specific distribution. The formation of tuberostemonine A (**4**) appears to be typical for *S. phyllantha* Gagnep., whereas tuberostemonine N (**5**) together with neotuberostemonine (**6**) characterize *S. tuberosa* Lour. (Figures 2 and 4). Though this confirms our previous results,[6] it is in contrast to many phytochemical investigations in China, where in addition to isomers of tuberostemonine (**3**) a number of stemoninine (**7**) derivatives have been isolated (Figure 2).[10,24,26,28] Moreover, a series of oxidative modification products of tuberostemonine (**3**)[7] has been reported that could not be detected in the present study.

Most of the *Stemona* species in Thailand are characterized by alkaloids derived from a protostemonine-type skeleton (Figure 3). On the basis of the present survey protostemonine (**8**) itself plays a subsidiary role, mostly occurring in only small amounts. Major accumulation trends lead either to stemofoline (**10**) or stemocurtisinol (**13**) derivatives (Figure 1, Table 1). Both share an acyclic side chain and an oxygen bridge linking the pyrrolo- (**10**−**12**) or pyridoazepine rings (**13**, **14**, **16**), respectively. As outlined previously,[29] the formation of the six-membered piperidine ring can be explained by opening the five-membered pyrrolidine ring and incorporating C-18 of the butyl side chain (Figure 3). Stemofoline (**10**) represents a complex cage-type molecule with an oxygen bridge between C-2 and C-8 and an

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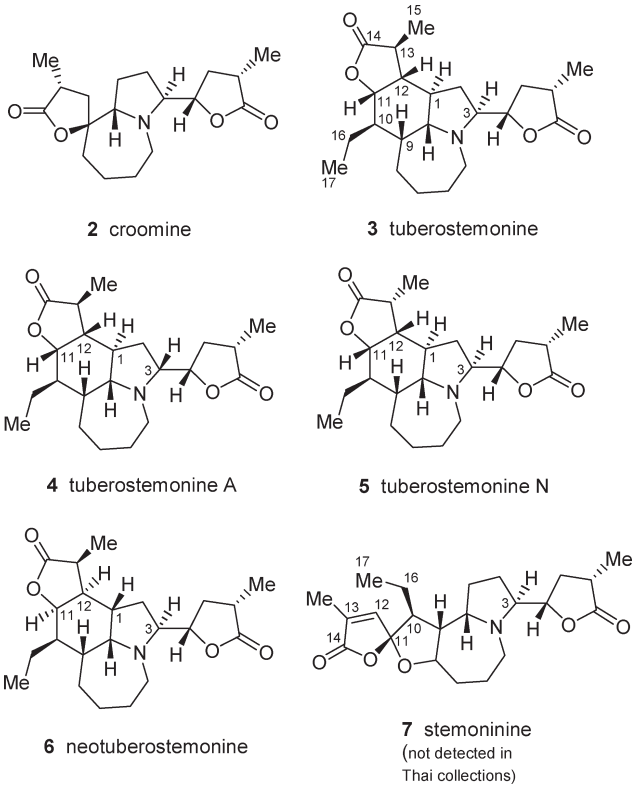

Figure 2. Alkaloids with a croomine (**2**)- or stichoneurine-type skeleton (**3**−**7**) detected by ELSD.

additional C−C linkage between C-3 and C-7. It is a major compound in many *Stemona* species, but has not been detected in members of the *S. tuberosa* group characterized by stichoneurine-type alkaloids (Figures 2 and 4). In some species stemofoline (**10**) was shown to co-occur with the pyridoazepine derivatives **13** and **15** and small amounts of oxyprotostemonine (**9**) (Table 1). In this case an infraspecific variation was observed, especially in *S. curtisii* Hook.f., a prominent species of south and southwest Thailand. From 11 different collections five were characterized by **10** as the major compound, two by additional small amounts of **13** and **15**, and four deviate by a preponderance of **13** and **15** (Table 1). These findings are in good agreement with our previous results, where six different collections of *S. curtisii* showed various accumulation trends either toward **10** or the pyridoazepine derivatives **13**−**15**.[6] However, it is interesting to note that in contrast to previous findings no oxystemokerrine *N*-oxide (**16**) was detected in the present analysis.

Unlike in *S. curtisii*, a far-reaching chemical uniformity was found in 11 different collections of *S. collinsiae* Craib, another well-known and widespread species of Thailand. All samples were characterized by a clear preponderance of didehydrostemofoline (**11**) accompanied by only small amounts of stemofoline (**10**) (Table 1). In spite of similarities with *S. collinsiae*, two collections from the provinces Lopburi and Nakhon Ratchasima in northeast Thailand were separated because of some morphological divergencies. In comparison to all other collections, characterized by yellowish white tepals and mostly erect habit, they deviate by pinkish tepals and a twining habit. Moreover, their stamens appear somewhat narrower and longer than those of *S. collinsiae*. This slight divergency is also underlined by a different chemical profile,

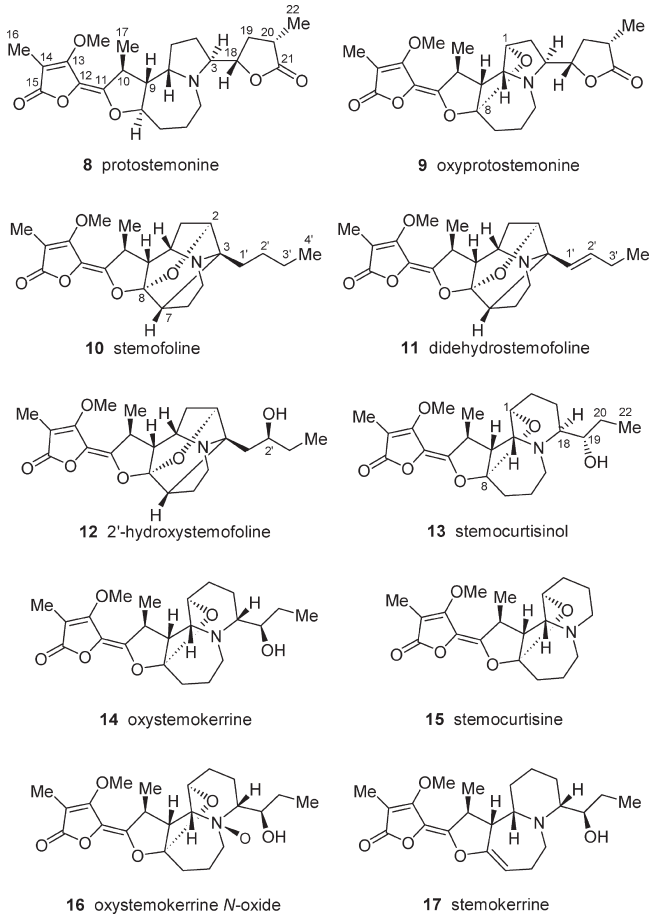

Figure 3. Pyrrolo- (**8**−**12**) and pyridoazepine alkaloids (**13**−**17**) with a protostemonine-type skeleton detected by DAD.

consisting of nearly equal amounts of stemofoline (**10**) and didehydrostemofoline (**11**) (Table 1).

S. cochinchinensis Gagnep. can be readily distinguished from other *Stemona* species by its characteristic stamens, showing an elongated appendage of the connective narrower than the anther.[1] The HPLC profile in the present study suggests an infraspecific chemical variability. Whereas previous findings from the Nong Khai Province in northeast Thailand (HG 884) were characterized by a predominance of stemofoline (**10**) accompanied by small amounts of 2′-hydroxystemofoline (**12**),[6] the present results show a reduced alkaloid pattern represented by small amounts of protostemonine (**8**). Stemofoline (**10**) was previously also reported as the major compound for three different samples of *S. burkillii* D. Prain together with its 2′-hydroxy derivative **12** and small amounts of closely related derivatives.[6,30] They were collected in the mountains of the provinces Lamphun and Chiang Mai in north Thailand (HG 946, HG 887).[6] However, in the present study no plant material was available for reinvestigation.

S. aphylla Craib and the recently described *S. involuta* Inthachub appear to be closely interrelated and are mainly distributed in the dry and open habitats in north, northeast, and east Thailand.[1] Differences are mainly based on stamen morphology as well as on size and color of the flowers. The chemical profiles of *S. aphylla*, collected in different localities, show some variability. In two samples, originating from the provinces Mahasarakham and Ubon Ratchathani in northeast Thailand, the alkaloid formation

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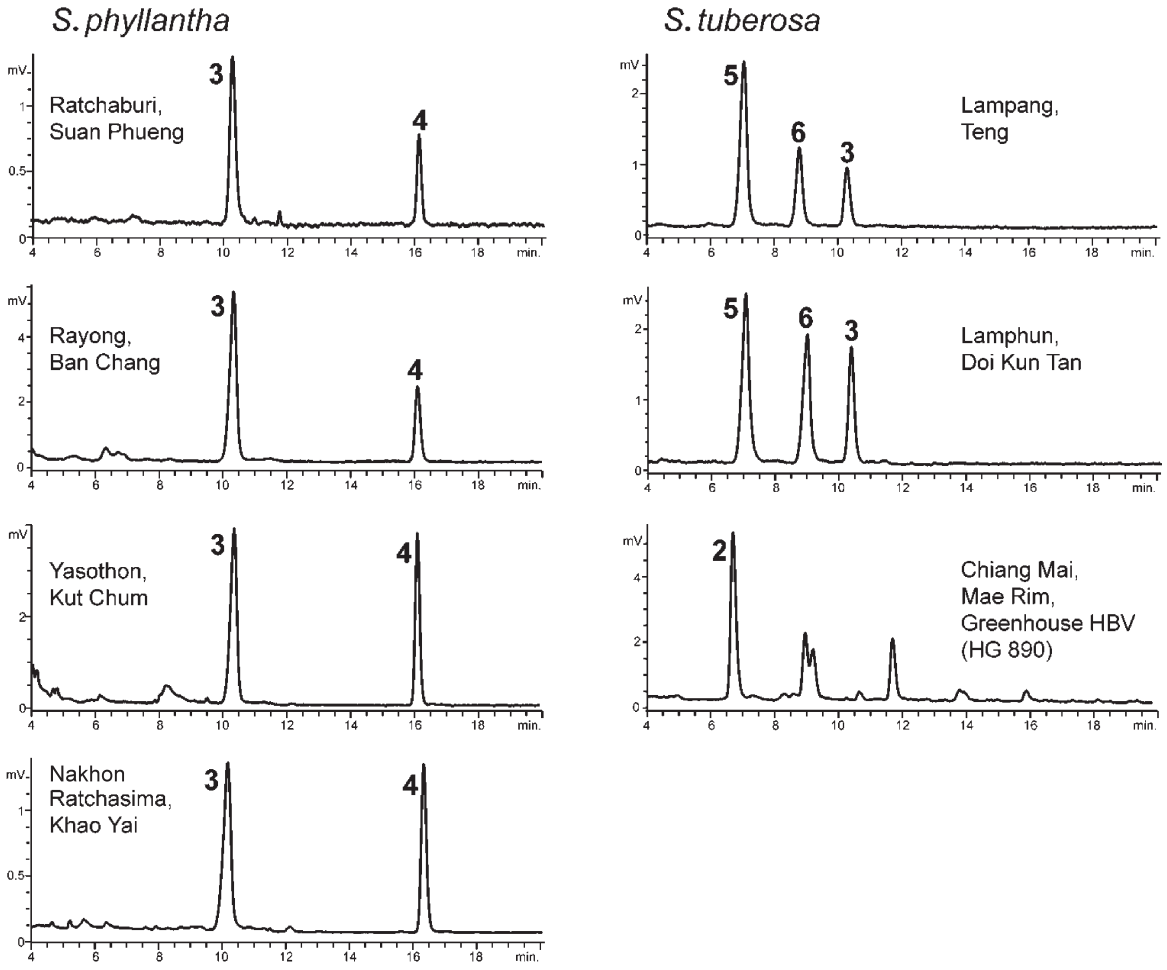

Figure 4. HPLC-ELSD comparison of methanolic crude extracts of underground parts of *S. phyllantha* accumulating tuberostemonine (**3**) and tuberostemonine A (**4**), and *S. tuberosa* accumulating tuberostemonine (**3**), tuberostemonine N (**5**), neotuberostemonine (**6**), and croomine (**2**).

is strongly reduced, showing only small amounts of protostemonine (**8**) (Table 1). In addition, both of the corresponding HPLC profiles are characterized by an accumulation of stilbenoids (Figure 5).[31] A similar chemical trend has been reported for two other collections from Ubon Ratchathani and Kanchanaburi, previously published as unidentified species HG 912 and HG 913.[6] In order to gain more insight into the chemical variability of *S. aphylla*, extracts of two further collections, originating from the provinces of Udon Thani (HG 915) and Sukhothai (HG 893), were reinvestigated. Both have been published previously as unidentified species.[6] They are characterized by pyridoazepines, either by stemocurtisine (**15**) and oxystemokerrine (**14**) (HG 915) or by oxystemokerrine (**14**) together with its *N*-oxide (**16**) (HG 893), respectively (Figure 5 A). More detailed comparative analyses between extracts of different individuals from the same locality near Sukhothai (HG 893) show different accumulation trends toward either the pyridoazepines **14** and **16** or a series of stilbenoids (Figure 5 B, individuals 1−4).[31] This vicariance between alkaloid and stilbenoid accumulation was observed in the protostemonine (**8**)-containing collections mentioned before. The closely related *S. involuta* was not analyzed in the present study, but results from our previous investigation can be used for comparison. In this case the corresponding collection has been published as unidentified species HG 943.[6] It originated from "*locus classicus*" near Nakhon Ratchasima and was later used as specimen for the description of the new species.[1] Its HPLC profile was characterized by oxystemokerrine (**14**) as the major compound together with its *N*-oxide **16** and small amounts of protostemonine (**8**).[6]

S. rupestris Inthachub represents another newly described species endemic to northeast Thailand. It is closely related to *S. pierrei* Gagnep., but differs by longer tepals and shorter appendages of the thecae.[1] Accordingly, its chemical profile is similar to that of *S. pierrei*, which has been published previously.[6,32] It shows also a strongly reduced alkaloid formation, consisting only of traces of protostemonine (**8**), but is accompanied by a prominent pattern of stilbenoids. However, on the basis of comparative HPLC-DAD analyses, the stilbenoids differ from those of *S. aphylla* most likely representing dihydrophenanthrenes (stemanthrenes) as reported for *S. pierrei*.[32]

Two different samples of *S. kerrii* Craib, collected in the mountains of the provinces of Lamphun and Chiang Mai in north Thailand, exhibited closely related HPLC profiles. In accordance with previous findings[6,29] they are characterized by stemokerrine (**17**) as the major compound accompanied by small amounts of oxystemokerrine (**14**) and oxyprotostemonine (**9**). This confirms a recent taxonomic statement that *S. kerrii* represents a rather homogeneous species.[1] However, in contrast to previous results no oxystemokerrine *N*-oxide (**16**) was detected.[6,29]

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Table 1. Distribution of Protostemonine-Type Alkaloids in Thailand[a]

	pyrroloazepines					pyridoazepines			
geographical distribution	8	9	10	11	12	13	14	15	17
S. curtisii									
Surat Thani, Kanchanadit			●						
Krabi, Ao Luek			●						
Krabi, Muang			●						
Songkhla, Na Tha Wi			●						
Songkhla, Hat Yai			●						
Trang, Ratsada			●		+	+		○	
Phattalung, Muang			●		+	+		+	
Prachuap Khiri Khan, Bang Saphan Noi		+				○		●	
Prachuap Khiri Khan, Sam Roi Yot		+				+		●	
Prachuap Khiri Khan, Thap Sakae	+	○				○		●	
Nakhon Si Thammarat, Thung Song		○						●	
S. collinsiae									
Chonburi, Si Racha			+	●					
Saraburi, Kaeng Khoi			+	●					
Nakhon Nayok, Pak Phli			+	●					
Prachinburi, Kabin Buri			+	●					
Nakhon Ratchasima, Wang Nam Khieo			+	●					
Nakhon Ratchasima, Tup Lan			+	●					
Nakhon Ratchasima, Khao Yai			+	●					
Phitsanulok, Phu Hin Rong Khla			+	●					
Loei, Phu Kradueng			+	●					
Ubon Ratchathani, Mueang			+	●					
Ubon Ratchathani, Si Mueang Mai			+	●					
unidentified species									
Nakhon Ratchasima, Pak Chong			●	○					
Lopburi, Chai Badan			●	●					
S. aphylla									
Mahasarakam, Na Dun	+								
Ubon Ratchathani, Khong Chiam	+								
Udon Thani, Nong Wua So (HG915)							○	●	
Sukhothai, Khiri Mat (HG893)							●		
S. rupestris									
Yasothon, Loeng Nok Tha	+								
S. cochinchinensis									
Surin, Rattanaburi	+								
S. kerrii									
Chiang Mai, Mae Rim		○					○		●
Lamphun, Doi Khun Tan							+		●

[a] Symbols correspond to peak sizes of HPLC-DAD profiles (compare Figure 5). ● = major compound, ○ = minor compound, + = traces.

The present survey of 42 different *Stemona* samples collected in Thailand confirms to a large extent our previous findings based on 39 collections.[6] It shows again a clear chemical segregation of *S. tuberosa* and *S. phyllantha*, exclusively characterized by tuberostemonine derivatives with a stichoneurine-type skeleton (**3**–**6**) (Figures 2, 4). The reinvestigation of *S. tuberosa*, originating from the mountains near Chiang Mai in north Thailand (HG 890),[4,6] cultivated in the Botanical Garden of the University of Vienna, shows again a preponderance of croomine (**2**). This skeletal type, also classified as tuberostemospironine type,[23] appears to be more widespread in samples of *S. tuberosa* originating from the mountains of south China[9,11,22−26] and Vietnam,[6] but was also found in *S. phyllantha* from Indonesia, originally published as *ex aff. S. tuberosa* (HG 918).[6]

On the basis of previous reports the well-known antitussive properties of *Stemona* extracts were thought to be restricted to stichoneurine- and croomine-type alkaloids of the *S. tuberosa* group.[7,9−11,33] However, recent findings also exhibited significant activities in derivatives with a protostemonine-type skeleton isolated from the Chinese *S. sessilifolia* (Miq.) Miq.[13] More detailed experiments with the citric acid-induced guinea pig cough model combined with electrical stimulation of the superior laryngeal nerve showed that the stichoneurine-type derivatives tuberostemonine (**3**), neotuberostemonine (**6**), and stemoninine (**7**) target the peripheral cough reflex pathway, whereas croomine (**2**) acts on central sites. The latter also showed central depressant effects, which may partly account for the side effects of some *Stemona* extracts, leading to nausea, vomiting, delirium, and headache.[12]

The strong insecticidal activity of stemofoline (**10**) was first detected in the leaf extract of *S. japonica*.[34] This was later confirmed by more detailed electrophysiological in vitro tests on the insect nicotinic acetylcholine receptor and by in vivo screenings against relevant agricultural insect pests.[15] Stemofoline (**10**) represents the major compound in the underground parts of many collections of *S. curtisii* (Table 1). It was also reported to dominate in *S. burkillii*[6,19,30] and in one collection of *S. cochinchinensis*.[6,29] In *S. burkillii* it was shown to increase the chemosensitivity via P-glycoprotein-mediated multidrug resistance.[19] Moreover, in *S. javanica* (Kunth) Engl., collected in east Java, Indonesia, it was reported to inhibit nitric oxide production (NO) in a mouse macrophage-like cell line stimulated by lipopolysaccharide.[18] The closely related didehydrostemofoline (**11**) also showed pronounced insect toxic effects against larvae of the two well-known pest insects *Plutella xylostella*[35] and *Spodoptera littoralis*.[36] This compound, previously named asparagamine A, also exhibited an oxytocin antagonistic effect by delaying parturition in Wistar rats.[17] As shown in Table 1 didehydrostemofoline (**11**) represents a typical chemical marker of *S. collinsiae*, where it accumulated in all collections and was also found in two probably closely related, but as yet unidentified samples. Stemofoline derivatives with their high insecticidal activity already inspired as lead structure the development of synthetic cyanotropanes, a novel class of commercial insecticides interacting at the nicotinic acetylcholine receptor.[37]

Apart from accumulation trends toward alkaloids with different skeletal types (Figure 1) the present survey repeatedly shows a general reduction of alkaloid formation, mainly in *S. rupestris* and *S. cochinchinensis*, but also in some collections of *S. aphylla* (Figure 5; Table 1; ref 6), originating from periodically dry and hot habitats. In this case only small amounts of protostemonine (**8**) were detected accompanied by an accumulation of stilbenoids. It is tempting to interpret this metabolic trend as a loss of enzymatic activity, and protostemonine (**8**) as the remaining biosynthetic precursor, usually leading to the formation of stemofoline (**10**) or stemocurtisinol (**13**) derivatives (Figure 1).

To establish species-specific accumulation trends, only compounds occurring in higher concentration were considered for the present comparison. Inspection of literature data showed an additional number of minor constituents that were detected only in extracts obtained from larger quantities of plant material. They were shown to represent mostly closely related derivatives of the

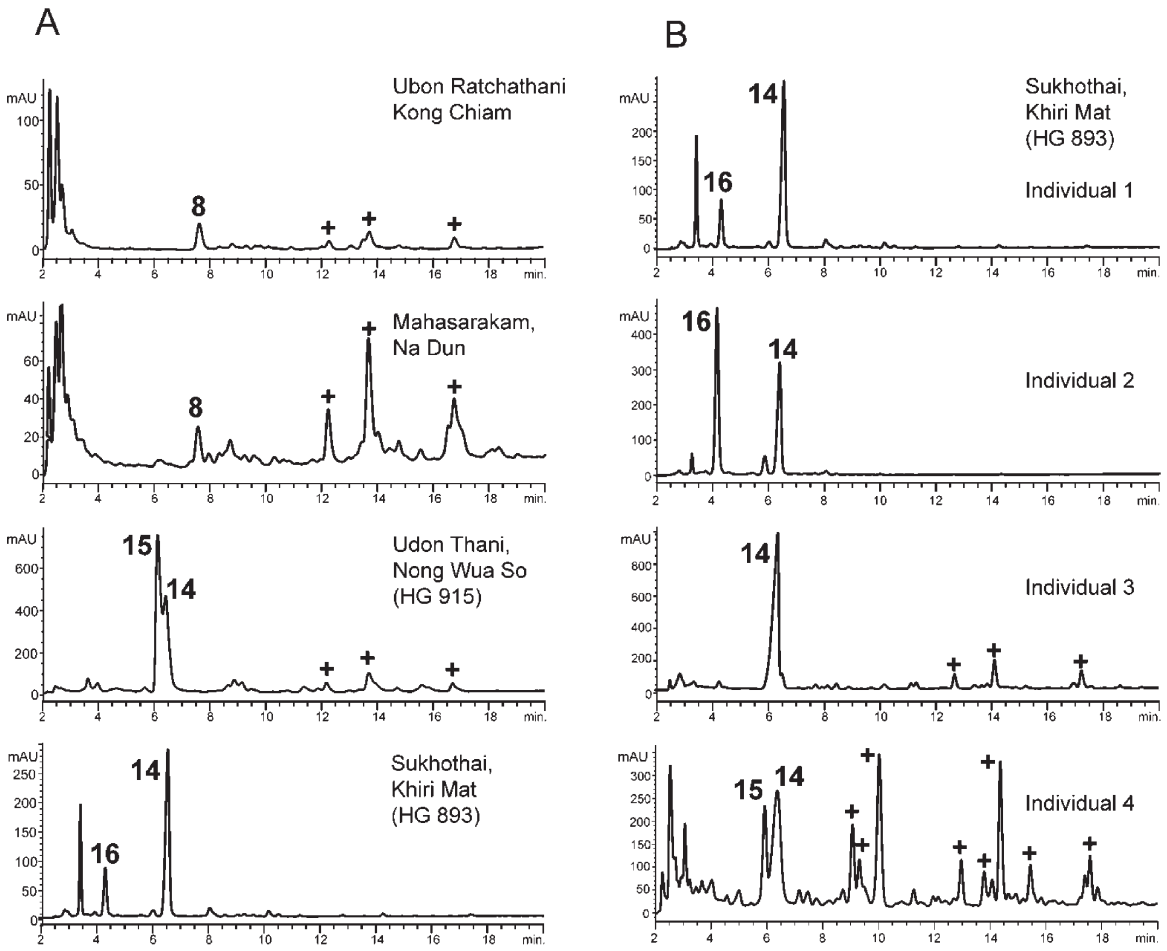

Figure 5. HPLC-DAD comparison of methanolic crude extracts of underground parts of *S. aphylla* demonstrating chemical variability between different localities (A) and interindividual differences within the same population (B). + = stilbenoids, UV detection at 280 nm.

major compounds with the same basic structure. This has been exemplified in extracts with dominating stemokerrine (**17**)[38] or stemofoline (**10**) alkaloids.[15,30,39,40] Moreover, the formation of artifacts should also be taken into consideration. For example the 11*E*-isomer of didehydrostemofoline (**11**) is most likely formed by photoisomerization.[35,39] In a previous review it has been speculated that the frequently published bisdehydro derivatives of all three skeletal types with a stable aromatic pyrrole system possibly represent artifacts.[7] This hypothesis seems to be confirmed by the present comprehensive survey, where no accumulation of these compounds was detected. That possibly also applies to a series of oxidative modification products of tuberostemonine (**3**) reported for Chinese collections of *S. tuberosa*.[7,9,26] The absence of *N*-oxides, formerly frequently found in different species,[6,38,39] might be explained by different ways of sample preparation. Whereas in the present study the underground parts were rapidly dried at 50 °C, ground, and stored in a gastight plastic container, the plant material of previous investigations, indicated by HG numbers (Figures 4 and 5; Table 1), has been slowly dried as a whole at room temperature and was immediately extracted after grinding.[6]

In conclusion, only derivatives of the stichoneurine or protostemonine skeletal type play a prominent role in Thai *Stemona* species. Within the stichoneurine group it is interesting to note that no stemoninine (**7**) derivatives were detected, which otherwise were frequently reported as dominating compounds in some Chinese collections. On the other hand, tuberostemonine A (**4**), frequently found in Thailand, was not detected so far in China.[9−11,22,24−26] This isomer was originally identified in the roots of *S. sessilifolia*, obtained from Mikuni and Co., Tokyo, which was most likely confused with *S. tuberosa*.[41] Regarding the protostemonine group, it should be pointed out that no maistemonine derivatives with a spiro system were detected in the present study, but were frequently accumulated as major compounds in collections from China.[7,25] Remarkable differences were also observed in the distribution of the biologically highly active stemofoline (**10**). Although originally isolated from the leaf extract of *S. japonica*, it represents a typical and widespread chemical character of the underground parts of various *Stemona* species in Thailand, but is only rarely reported so far for Chinese collections.

EXPERIMENTAL SECTION

General Experimental Procedures. HPLC: Agilent 1100 series, UV diode array detection at 280 nm. ELSD: Agilent 1260 infinity, temp 40 °C, nitrogen flow 3.6 L/min. Hypersil BDS-C_{18} column (4.6 mm i.d. × 25 cm, 5 μm), mobile phases: (A) 10 mM NH_4OAc in H_2O and (B) MeOH. Gradient elution was used from 55% to 90% B in A for 19 min, 90% B in A to 100% B for 1 min, and 100% B for 10 min; flow rate was

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1 mL/min and injection volume 10 μL. The separation of stemofoline (**10**) from didehydrostemofoline (**11**) was achieved by changing the mobile phase A to 1 mM NH_4OAc in H_2O. TLC was performed on silica gel 60 F_{254} plates (0.2 mm, Merck) with CH_2Cl_2/EtOAc/MeOH/NH_4OH (70:25:5:1) for protostemonine- and CH_2Cl_2/EtOAc/MeOH/NH_4OH (50:45:5:1) for stichoneurine-type alkaloids, respectively, and sprayed with Dragendorff's reagent. The concentration of samples subjected to HPLC and TLC was 10 mg/mL for crude extracts and 0.2 mg/mL for pure compounds.

NMR and MS: The compounds were dissolved in 99.98% $CDCl_3$ or in 99.8% methanol-d_4 (ca. 5 mg in 0.7 mL) and transferred into 5 mm NMR sample tubes (Promochem, Wesel, Germany), respectively. Spectra were recorded by the Bruker Topspin 1.3 software on a Bruker DRX-600 AVANCE spectrometer (Bruker, Rheinstetten, Germany) at 600.13 MHz (^{1}H) or 150.90 MHz (^{13}C) at 298.0 $\pm$ 0.1 K. For 1D spectra 64k data points were measured using a 1.0 s relaxation delay. Fourier transformation led to spectra with a range of 8000 Hz (^{1}H) and 32000 Hz (^{13}C). 2D COSY, TOCSY, NOESY, HMQC, and HMBC spectra were measured by 128 to 256 experiments with 1024 to 2048 data points each. Linear forward prediction to 4k and 512 data points in the f_1 and f_2 dimension, respectively, sinusoidal multiplication in both dimensions, and Fourier transformation produce 2D spectra with a range of ca. 6000 and 32 000 Hz for ^{1}H and ^{13}C NMR, respectively. In methanol-d_4 small amounts of methanol-d_1 were used as internal standard for ^{1}H (δ_H 3.340) and methanol-d_4 for ^{13}C (δ_C 49.86) NMR measurements. In $CDCl_3$ residual $CHCl_3$ (δ_H 7.240) and $CDCl_3$ (δ_C 77.02) were used as internal standards for ^{1}H and ^{13}C NMR measurements, respectively. Structure determination: Combined SPECINFO and CSEARCH databases running under the CSEARCH program have been used for spectral similarity searches and prediction of ^{13}C NMR spectra.[42−44] Mass spectra were recorded on a 900S (Finnigan MAT) by direct infusion ESI in positive and negative mode, respectively.

Plant Material. Forty-two samples representing *S. aphylla*, *S. cochinchinensis*, *S. collinsiae*, *S. curtisii*, *S. kerrii*, *S. phyllantha*, *S. ruprestis*, *S. tuberosa*, and two unidentified samples were collected from various localities in Thailand (Table 1, Figures 4 and 5). Voucher specimens are deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, and those with HG numbers at the Herbarium of the Institute of Botany, University of Vienna. To facilitate the recognition of different species, pictures of voucher specimens and living plants are presented at http://www.phytochemie.botanik.univie.ac.at/herbarium/ and http://herbarium.univie.ac.at/database/search.php. Moreover, photographs of relevant Thai representatives have been published previously.[6,29]

Extraction and Isolation. Underground parts were dried in an oven at 50 °C, ground, and passed through a sieve (60 mesh). Each sample was stored separately in an airtight plastic container. Collections with HG numbers were slowly dried at room temperature as a whole and immediately extracted after grinding.[29] For HPLC-DAD or -ELSD analyses ca. 10 g of each sample was extracted with MeOH and partioned between $CHCl_3$ and H_2O, and the lipophilic layer was used for comparison (Figures 4 and 5). For preparative isolation the $CHCl_3$ fractions were roughly separated by CC (Merck silica gel 60, 0.2−0.5) with solvent mixtures of petroleum ether, EtOAc, and MeOH or petroleum ether, $CHCl_3$, and MeOH with increasing polarity. The alkaloid-containing fractions, monitored by TLC sprayed with Dragendorff's reagent, were pooled and further separated by MPLC (Merck silica gel 60, 40−60 μm) with mixtures of EtOAc and MeOH as described previously.[29] Final purification was achieved by isocratic CC using Sephadex LH-20 with MeOH.

Dried and ground underground parts of *S. tuberosa* (HG 890, 36 g) gave a $CHCl_3$ fraction (197 mg) affording purified croomine (**2**) (55 mg). *S. phyllantha* (Ratchaburi, Suan Phueng, 120 g) gave a $CHCl_3$ fraction (1.27 g) affording tuberostemonine (**3**) (152 mg) and tuberostemonine A (**4**) (270 mg). *S. tuberosa* (Lamphun, Doi Kun Tan, 80 g) gave a $CHCl_3$ fraction (2.04 g) affording tuberostemonine N (**5**) (90 mg) and neotuberostemonine (**6**) (98 mg). *S. kerrii* (Chiang Mai, Mae Rim, 100 g) gave a $CHCl_3$ fraction (510 mg) affording oxyprotostemonine (**9**) (5.3 mg), oxystemokerrine (**14**) (5.4 mg), and stemokerrine (**17**) (17.4 mg). *S. curtisii* (Songkhla, Na Tha Wi, 300 g) gave a $CHCl_3$ fraction (2.5 g) affording stemofoline (**10**) (49 mg). *S. collinsiae* (Nakhon Ratchasima, Wang Nam Kiao, 400 g) gave a $CHCl_3$ fraction (2.2 g) affording didehydrostemofoline (**11**) (121 mg). *S. curtisii* (Prachuap Khiri Khan, Bang Saphan Noi, 200 g) gave a $CHCl_3$ fraction (1.13 g) affording stemocurtisinol (**13**) (40 mg) and stemocurtisine (**15**) (78.5 mg). Protostemonine (**8**) was not isolated in the present study. An authentic sample, originating from our previous work on *S. pierrei*,[32] was used for chromatographic comparison.

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