

Structural Relationships, Distribution and Biological Activities of *Stemona* Alkaloids

Harald Greger

Abstract

Stemona alkaloids represent a unique class of natural products exclusively isolated from the monocotyledonous family Stemonaceae comprising three genera mainly distributed in southeast Asia. Structurally the alkaloids are characterised by a pyrrolo[1,2-*a*]azepine nucleus usually linked with two carbon chains mostly forming terminal lactone rings. Based on biosynthetic considerations and their various distribution the present review describes 82 *Stemona* alkaloids grouped into three skeletal types. Due to different carbon chains attached to C-9 of the pyrroloazepine nucleus they were classified into stichoneurine-, protostemonine- and croomine-type alkaloids. The genera *Croomia* and *Stichoneuron* only accumulate croomine or stichoneurine derivatives, respectively, whereas the genus *Stemona* produces all three types of alkaloids. However, species-specific accumulation trends towards certain structural types represent valuable chemosystematic criteria. Bioassays with larvae of *Spodoptera*

littoralis exhibited very high insect toxicity for the roots of *Stemona* species containing certain protostemonine derivatives, especially didehydrostemofoline, whereas those with dominating stichoneurine or croomine derivatives showed low toxicity but sometimes remarkable repellence due to an accumulation of tuberostemonine. Tuberostemonine also showed effects on the motility of helminth worms and reduced the excitatory transmission at the crayfish neuromuscular junction. Significant antitussive activity was shown for the stereoisomeric neotuberostemonine in guinea-pig after cough induction by citric acid aerosol stimulation. Studies on structure-activity relationship with seven related compounds revealed that the saturated tricyclic pyrrolobenzazepine nucleus of tuberostemonines is the prerequisite for antitussive activity.

Key words

Stemona alkaloids · pyrrolo[1,2-*a*]azepine alkaloids · structural diversity · bioactivity · *Stemona* · *Croomia* · *Stichoneuron*

Introduction

Various species of the genus *Stemona* (Stemonaceae) are widely used in China and other countries of southeast Asia as an anti-cough remedy and for their antiparasitic properties. Especially, extracts from the tuberous roots of *S. tuberosa* Lour., *S. sessilifolia* (Miq.) Miq., and *S. japonica* (Bl.) Miq. have long been recommended in Chinese and Japanese traditional medicine for a broad range of applications [1], [2], [3]. Since they are also used as domestic insecticides against different pests together

with those of several other *Stemona* species, the underground parts, including roots and rhizomes, are widely offered for sale on local markets and herb shops. However, because of the similar shape of the fleshy tuberous roots, the same vernacular names such as “Bai Bu” in China, “Bach Bo” in Vietnam, or “Non Tai Yak” and “Pong Mot Ngam” in Thailand are often used for different species and sometimes even for representatives of other plant families. This uncertainty in purchasing properly identified plant material has already led to far-reaching confusions in the chemical and pharmaceutical literature. For instance, on the

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market in Bangkok the roots of the legume *Clitoria macrophylla* Wall. were also sold under the name “Non Thai Yak”, from which the two characteristic rotenoids stemonacetal and clitoriactal were isolated [4]. Stemonacetal was named after its first detection in the roots of *S. collinsae* Craib [5]. However, considering the biosynthesis of rotenoids as a typical chemical character of the Leguminosae and particularly the already known formation of stemonacetal in *C. macrophylla*, the occurrence of rotenoids in *S. collinsae* appears very doubtful. In fact, in subsequent investigations neither rotenoids nor any other isoflavonoid derivative has been detected in *S. collinsae* collected from different habitats [6], [7]. Moreover, the alkaloid asparagamine A was repeatedly reported for the tuberous roots of *Asparagus racemosus* Willd. [8], [9], but represents a typical *Stemona* alkaloid closely related to the widespread stemofoline (**45**), originally isolated from *S. japonica* [10]. In fact, asparagamine A was later repeatedly isolated as major compound from *S. collinsae* and named didehydro-stemofoline (**48**) [6], [11], [12]. The presumption that *Asparagus* has been confused with *Stemona* was supported by a colorimetric comparison of 9 *Asparagus* collections from different provinces of China, where no alkaloids could be detected [13]. Furthermore, the dihydrophenanthrene racemosol (= stemanthrene D) was also reported for *A. racemosus* [9], [14], which, however, was shown to be a typical stilbenoid of *S. collinsae* [7] and several other *Stemona* species [15]. Hence, to avoid further confusion only properly identified plant material should be used for chemical investigations from which voucher specimens have been deposited in internationally accessible herbaria, and corresponding images should now also be available via the Internet, e.g., <http://www.phytochemie.botanik.univie.ac.at/herbarium>.

Stemonaceae represent a small family consisting of the three genera *Stemona*, *Croomia*, and *Stichoneuron*, comprising about 30 species. The family takes a rather isolated position within the monocotyledons and is mainly distributed in southeast Asia but extends also to tropical Australia and, with one species of *Croomia*, even to southeast United States [16], [17], [18]. *Stemona* is the largest genus with about 25 species mainly occurring as twining herbs with perennial tuberous roots. Many species prefer a seasonal climate and occur in rather dry vegetation. In appearance they much resemble certain species of *Dioscorea*, but can be easily distinguished by tetramerous flowers characterised by four conspicuous stamens and the primary veins of the leaves being connected by numerous approximate transverse ones. In spite of the good delimitation of *Stemona* from *Croomia* and *Stichoneuron* and already existing revisionary treatments for the Flora Malesiana [16] and Flora of China [17], there are still many taxonomic problems at the species level that remain to be solved.

The formation of *Stemona* alkaloids constitutes a unique chemical feature of the Stemonaceae not detected so far in any other plant family. Due to their structural complexity and instability almost all structures could only be determined by X-ray crystallographic analysis before 1980. Structurally they are characterised by a pyrrolo[1,2-*a*]azepine core usually linked with two carbon chains mostly forming terminal lactone rings. The intricate structures of these alkaloids, sometimes leading to very complex cage-type molecules, have already attracted considerable synthetic interest. They served as a stimulus for the development of

new strategies for the construction of the skeleton as well as for successful total syntheses of a number of derivatives e.g., [19], [20], [21]. In a previous review 42 naturally occurring derivatives were listed mostly isolated from the tuberous roots of the genus *Stemona* [1]. By contrast, only four derivatives were reported for the other two genera *Croomia* and *Stichoneuron* [22], [23], [47]. So far the *Stemona* alkaloids were either classified into eight groups by Ye et al. [24], or five by Pilli and Ferreira de Oliveira [1]. However, in the meantime 82 derivatives have already been described suggesting a new classification into only three skeletal types.

Even though the genus *Stemona* has long been recognised for its broad range of bioactivities, chemical investigations have long been restricted to only a few species mainly focusing on the well-known representatives of the Traditional Chinese Medicine *S. tuberosa*, *S. japonica*, and *S. sessilifolia*. In that case interest was mainly centred towards structure elucidation and synthesis [1], [2], whereas phytochemical comparisons between different species, geographical provenances, and different tissues within the same individual were missing. Furthermore, only a few bioassays have been carried out to evaluate the various bioactivities from crude extracts and isolated pure compounds [25], [26], [27], [28]. Recently a broad-based phytochemical comparison of well documented plants from natural habitats was started to give an overview about the metabolic capacity of the family Stemonaceae [6], [7], [15], [23], [29], [30]. Based on these results it can now be stated that different types of pyrroloazepine alkaloids represent a typical chemical character of all genera of the family. They mainly accumulate in the roots accompanied by a number of different stilbenoids [7], [15], and frequently also by characteristic dehydrotocopherols (chromenols) [30]. Parallel bioassays with polyphagous larvae of *Spodoptera littoralis* (Lepidoptera, Noctuidae) and various phyto-pathogenic fungi showed that the pronounced insecticidal activities of some *Stemona* extracts could exclusively be attributed to the formation of alkaloids [6], [29], whereas the fungitoxic properties were caused by different types of stilbenoids [7], [15].

This review gives an overview about the structural diversity of *Stemona* alkaloids and suggests a new classification based on biosynthetic considerations and their various distribution in different *Stemona* species. It also reports on their different activities revealed by various biological and pharmacological tests, and attempts to shed some light on the confusing literature concerning their occurrence and distribution.

Structural Relationships

The formation of alkaloids containing a pyrrolo[1,2-*a*]azepine core mostly linked with one or two lactone rings represents a typical chemical character of the Stemonaceae not detected so far in any other plant family. Like the biosynthesis of pyrrolizidine alkaloids the pyrroloazepine derivatives were speculated to be derived from a spermidine precursor linked with isoprene units [12]. However, this hypothesis could not be confirmed so far. In the first review in 1973, only seven derivatives have been described with a defined structure, all related to the two compounds tuberostemonine (**1**) or protostemonine (**39**) [31]. Twen-

ty-seven years later, in a following review, Pilli and Ferreira de Oliveira [1] already listed 42 different structures which they separated into five groups largely according to the arrangements of Ye et al. [24], [32], [33]. Following their systematic studies the latter authors concluded that all alkaloids can be classified into seven [33] or eight structural groups [24] according to the sites of connection between the basic pyrroloazepine skeleton and the side-chain at C-9. The deviating group denominations in the previous review [1] were explained by using the name of the structurally simplest derivative of each group as the parent name. In the meantime the number of *Stemona* alkaloids has been nearly doubled, now containing 82 derivatives. With the exception of the four alkaloids croomine (75), bisdehydrocroomine (76), and stichoneurines A (30) and B (31), isolated from *Croomia* [22], [47], and *Stichoneuron* [23], respectively, all other derivatives were only known from *Stemona* species. Based on structural considerations and their various distribution in different species, they are now classified into three skeletal types: the stichoneurine- (tuberostemonine-), protostemonine-, and croomine-type alkaloids. As shown in Fig. 1 the three types can be distinguished by different carbon chains attached to C-9 of the pyrroloazepine nucleus. In the stichoneurine- and protostemonine-types these chains usually contain eight carbon atoms forming a terminal lactone ring, but differ among each other in the branching pattern. In the croomine-type, by contrast, the chain consists only of four carbon atoms forming a lactone ring directly attached to C-9 in a spiro system. The first two types contain the majority of compounds comprising nearly 40 derivatives each (see Fig. 2 and Fig. 4), whereas from the third type only eight derivatives are known so far (see Fig. 7).

Stichoneurine (tuberostemonine-)type alkaloids

Common to all derivatives of the stichoneurine-type is a carbon chain with an ethyl group (C-16–C-17) attached to C-10 (Fig. 1 and Fig. 2). Apart from the connection of the chain to C-9 additional linkages to the pyrroloazepine core and ether bridges lead to a variety of different structures within this type. As shown in Fig. 3 the recently described structures of stichoneurines A (30) and B (31) [23] may be regarded as common precursors. The well-known tuberostemonine (1) and its closely related derivatives tuberostemoenone (18), tuberostemoninol (19), oxotuberostemonine (24), and stenine (26) can be derived by linking C-12 of the lactone ring with C-1 of the pyrroloazepine core, whereas in parvineostemonine (38) an unusual linkage between C-11 and C-3 can be observed. The formation of an ether bridge between C-11 and C-8 leads to the spiro system of stemoninine (21), and another between C-12 and C-16 to the structure of parvistemonine (35). The unusual six-membered piperidone ring of tuberostemoninol (19) can be explained by opening the bond between C-1 and C-9a of tuberostemonine (1) and linking C-1 with C-9. The structure of tuberostemoenone (18) was speculated to be generated by opening the bond between C-9 and C-10 and closing a bond between C-10 and C-9a to form a five-membered ring [34]. Oxotuberostemonine (24) deviates from all other derivatives by a lactonisation between C-14 and C-1, and stenine (26) and parvineostemonine (38) by the lack of the methylated butyrolactone ring usually attached to C-3 (Fig. 3). With respect to the mass spectra of many *Stemona* alkaloids this lactone ring can easily be removed leading to a characteristic and dominant fragmentation peak ($M^+ - 99$) [23], [35]. Hence, it is tempting to ex-

plain the lack of the lactone ring at C-3 in a number of derivatives as a result of an oxidative cleavage, especially in those with a 3-carbonyl group in a lactam ring. The removal of this lactone ring from tuberostemonine (1) by permanganate oxidation and the transformation to 2-oxostenine (25) has already been described previously [36], [37]. In a more recent investigation 2-oxostenine (25) was also isolated from air-dried roots of *S. sessilifolia* cultivated and harvested in Shandong Province in China [38]. However, with regard to the rather harsh extraction conditions applied in that study, it cannot be excluded that 2-oxostenine (25) as well as the newly described sessilifoliamides A (29), B (32), C (33), D (34), and stemoninoamide (28), all possessing a 3-carbonyl group, represent extraction artifacts.

Formation of artifacts

Chemical stability plays an important role in evaluating biological activities of naturally occurring plant products. Their transformation during extraction and/or fractionation processes is often accompanied by a strong decrease or even loss of biological functions. Within *Stemona* alkaloids especially some derivatives of the stichoneurine-type can be regarded as artifacts. For instance, bisdehydrotuberostemonine (8) was already reported to be readily obtained, in good yield, from mother liquors of tuberostemonine (1) which had been exposed to air for some time [31]. In view of the extremely mild conditions under which the stable aromatic pyrrole system was formed, it can be expected that all stereoisomers of bisdehydrotuberostemonine (8–13) as well as the other bisdehydro derivatives were generated in a similar way. However, bisdehydrotuberostemonine B (10) and bisdehydrotuberostemonine C (11) were supposed to be “bio-generated” from tuberostemonine B (4) and tuberostemonine C (5), respectively [39]. Oxotuberostemonine (24) was obtained from tuberostemonine mother liquors which had been set aside for an extended period [31], and also by mercuric acetate oxidation of tuberostemonine (1) [40]. Hence, the possibility can also not

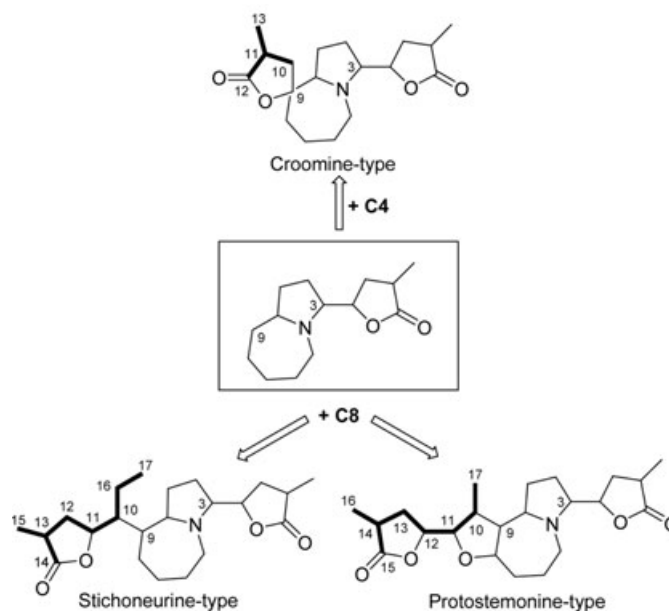


Fig. 1 Classification of *Stemona* alkaloids into three skeletal types based on different carbon chains attached to C-9 of the pyrroloazepine core.

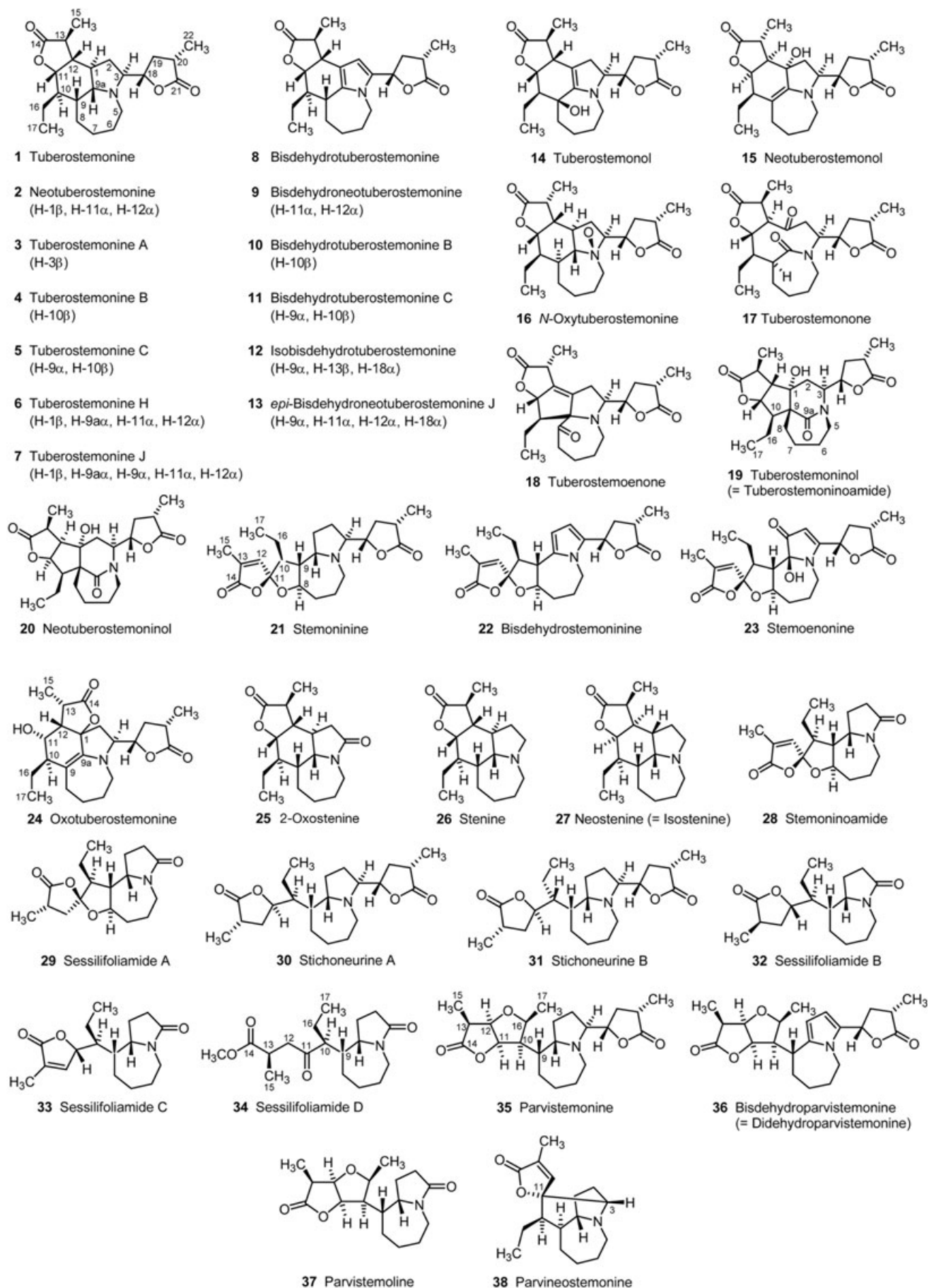


Fig. 2 Stichoneurine-type alkaloids.

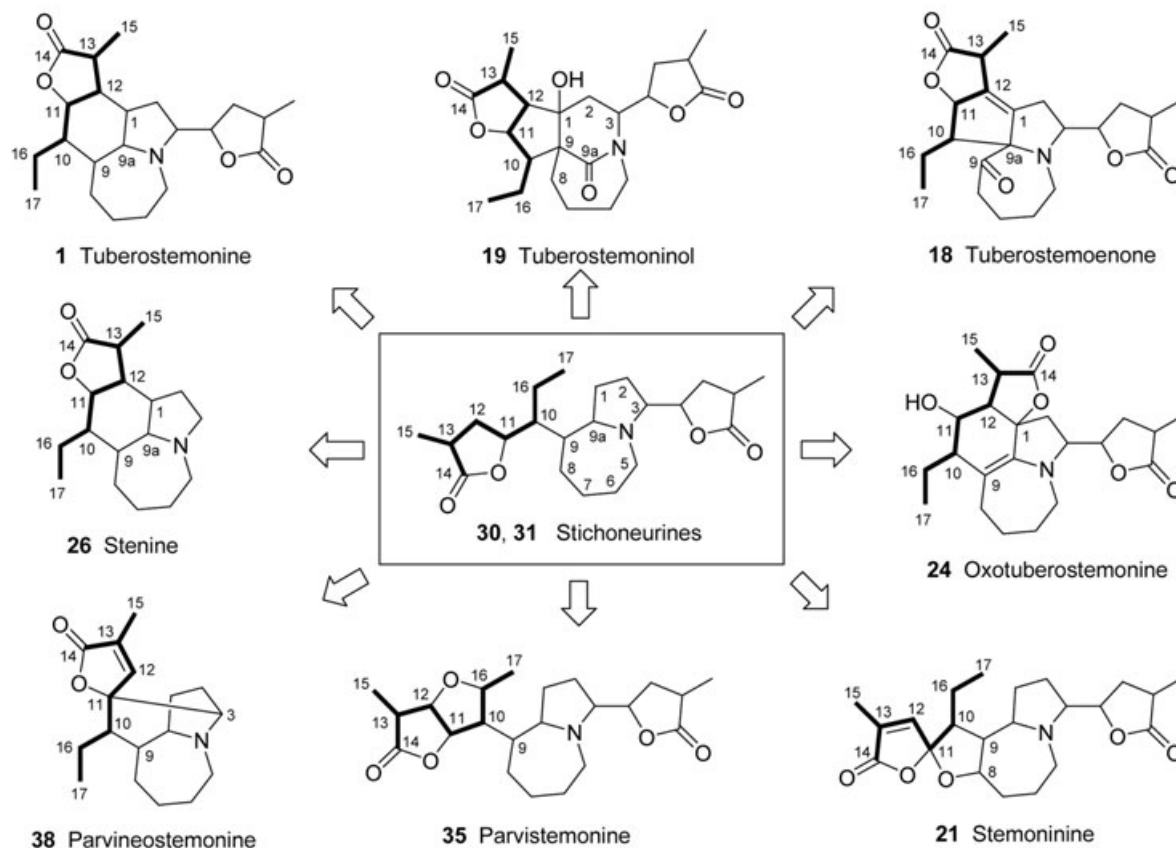


Fig. 3 Structural variation of stichoneurine-type alkaloids.

be excluded that compound **24** is actually an artifact, formed by air oxidation. To what extent other oxidation products of tuberostemonine (**1**), such as tuberostemonol (**14**), tuberostemonone (**17**) or stemoenone (**23**), can be regarded as artifacts has not been ascertained so far. Steric variations, e.g., from tuberostemonine (**1–7**) and bisdehydrostemonine (**8–13**) appear to be less subject to transformations during extraction and isolation. This has recently been confirmed for tuberostemonine A (**3**) differing from tuberostemonine (**1**) only by a β -orientated H-3 [23], and is also supported for the α - or β -orientation of H-11 and H-12 observed in different collections of *S. tuberosa*. For instance, in samples from Guangdong [41] and Hebei province [34] in China as well as from southeast Thailand [6], [23] all tuberostemonine derivatives showed β -orientations of H-11 and H-12 (**1**, **3**, **8**, **14**, **16**), whereas in those from Yunnan [32], Hongkong [42], and northern Vietnam [43] only corresponding derivatives with α -orientations (**2**, **9**, **20**, **15**, **27**), mostly indicated by the prefix “neo”, were detected.

Protostemonine type-alkaloids

Compounds of the protostemonine-type are characterised by a methyl group (C-17) at C-10 and the frequent formation of an unsaturated lactone ring linked to C-11 by a double bond (Fig. 4). As shown in Fig. 5, maistemonine (**66**) and the closely related stemonamine (**71**) can be derived from protostemonine (**39**) by opening the oxygen bridge between C-8 and C-11 and additionally linking C-12 of the unsaturated lactone ring to C-9a to form a characteristic spirolactone system [39]. Stemonine (**59**), stemoamide (**73**), and the related parvistemoamide (**74**) deviate from

protostemonine by the lack of the characteristic lactone ring shortening the chain from originally eight to three carbon atoms (Fig. 5). Since stemonine (**59**) could be obtained as a degradation product of protostemonine (**39**) by acid treatment [35], the lack of the unsaturated lactone ring could be the result of an oxidative cleavage. The absence of the other lactone ring at C-3 in neostemonine (**56**), stemonamine (**71**), stemoamide (**73**), and parvistemoamide (**74**) could be generated in a similar way as already discussed for some stichoneurine-type alkaloids (Fig. 3 and Fig. 5). The formation of an oxygen bridge between C-8 and C-2 within the pyrroloazepine nucleus accompanied by an additional C-C linkage between C-7 and C-3 leads to the complex cage-type structure of stemofoline (**45**) or its optical antipode parvistemonine (**54**). With respect to the high insecticidal activity of stemofoline (**45**) and especially the related didehydrostemofoline (= asparagine A) (**48**) [6], [11], the biosynthesis of these compounds deserves special interest. The butyl side chain attached to C-3 most likely can be regarded as a result of hydrolysis of the methylated butyrolactone ring followed by decarboxylation (Fig. 6). Eleven stemofoline derivatives (**45–55**) have already been described, differing by various substitution patterns of the butyl side chain and the formation of isomers. Stemoburkilline (**53**) deviates by opening the oxygen bridge between C-11 and C-8 accompanied by the formation of a hydroxy group at C-8 (Fig. 4). Examination of the crude ethanol extract by TLC and $^1\text{H-NMR}$ analysis showed that this compound as well as stemofoline (**45**), 2'-hydroxystemofoline (**47**), and dihydrostemofoline (**52**) were not produced via an acid-catalysed reaction during the acid extraction process [44].

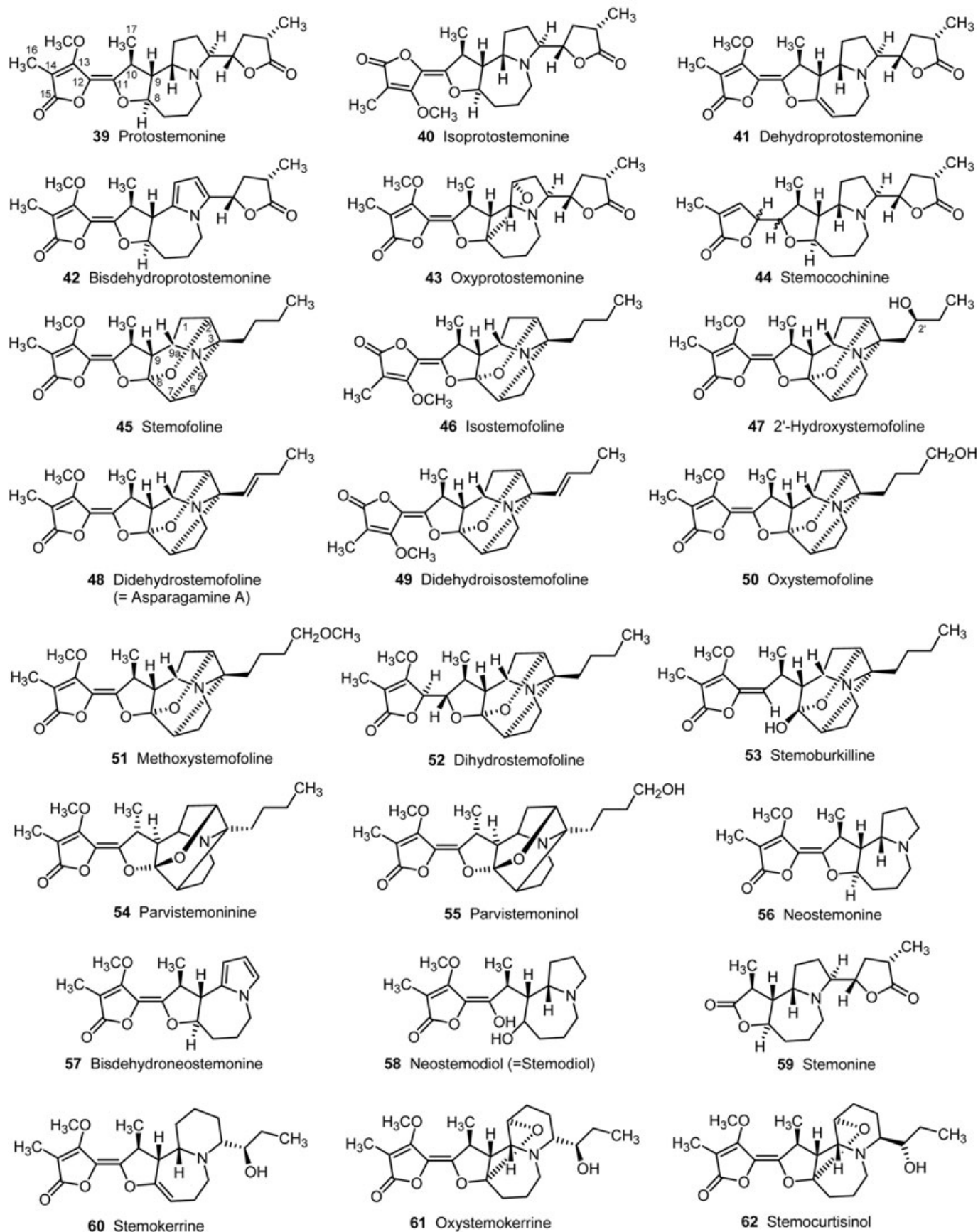


Fig. 4a Protostemonine-type alkaloids.

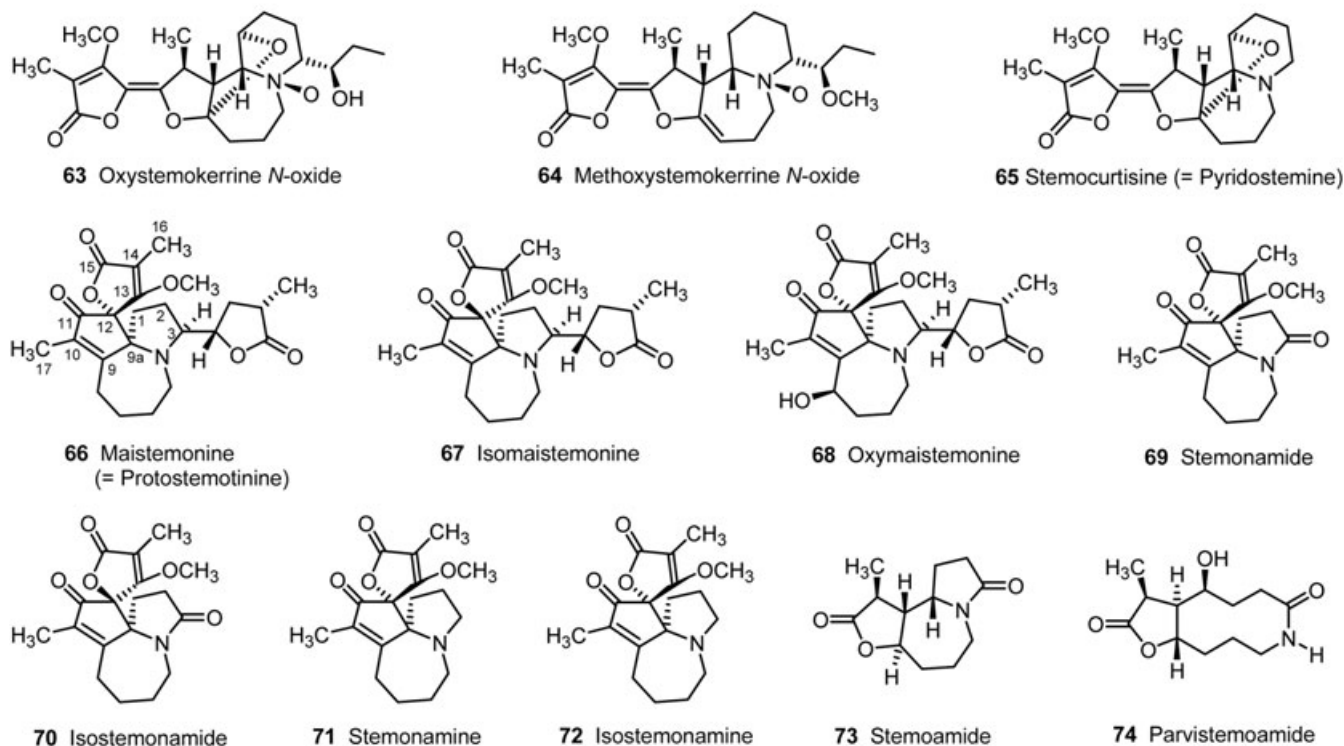


Fig. 4b Protostemonine-type alkaloids.

Recently a series of pyridoazepine alkaloids was reported [29], [45], [46], representing a new biosynthetic trait obviously also derived from a protostemonine-type precursor (Fig. 5 and Fig. 6). Starting with the formation of a butyl chain at C-3, already discussed for the stemofolines, the characteristic six-membered piperidine ring of the pyridoazepines was thought to emerge from a five-membered pyrrolidine ring by ring cleavage and incorporation of C-18 from the butyl side chain [29]. The remaining propyl chain is typical for stemokerrine (60) and related derivatives (61–64), and has obviously been lost in stemocurtisine (= pyridostemine) (65). Comparing the structures presented in Fig. 4 it becomes apparent that the additional formation of an oxygen bridge within the pyrrolo- or pyridoazepine core represents a frequent structural feature. However, in contrast to the stemofolines with an oxygen bridge between C-2 and C-8, the recently described oxyprotostemonine (43), oxystemokerrine (61), oxystemokerrine *N*-oxide (63) [29], stemocurtisine (65) [45], and stemocurtisinol (62) [46] deviate with a bridge between C-1 and C-8. Further protostemonine derivatives are produced by isomerisation of the characteristic double bond between C-11 and C-12 leading to the corresponding stereoisomers isoprotostemonine (40), isostemofoline (46), and didehydroisostemofoline (49), and by the formation of bisdehydro (= pyrrole) derivatives (42, 57), already mentioned for corresponding stichoneurine-type derivatives. The structurally simplest *Stemona* alkaloid, parvistemoamide (74), shows also close relations to the protostemonine group and may be directly derived from stemoamide (73) by opening the bond between the nitrogen atom and C-9a and additionally forming a hydroxy group (Fig. 5).

Croomine-type alkaloids

Up to now eight croomine-type alkaloids have been described. They can be clearly separated from the stichoneurine and protostemonine derivatives by only four carbon atoms linked to C-9 forming a lactone ring directly attached to the pyrroloazepine core in a spiro system (Fig. 1). The formation of a pyrrole system in bisdehydrocroomine (76) and the lack of the lactone ring usually attached to C-3 in tuberostemospirine (82), represent reaction steps already discussed for stichoneurine- and protostemonine-type alkaloids. As shown in Fig. 7, the remaining six derivatives differ in the oxygenation pattern mainly located in the seven-membered azepine ring. A characteristic oxygen bridge between C-6 and C-9a was found in stemotinine (80) and isostemotinine (81), whereas the two stereoisomers stemospirine (78) and stemonidine (79) have a methoxy group at C-8. More recently the structure of the new 6-hydroxycroomine (77) was published [23], suggesting close connections to the stemotinines. Tuberostemospirine (82) deviates from the other croomine-type alkaloids by the formation of a hydroxy group at C-10 in the spiro lactone system.

Accumulation and Distribution

Croomia and Stichoneuron

Comparing the literature and results from the author's laboratory it becomes apparent that most of the *Stemona* can be separated into two groups on the basis of different types of alkaloids. They were characterised by the predominant accumulation either of stichoneurine or protostemonine derivatives. In contrast, croomine-type alkaloids played an inferior role dominating

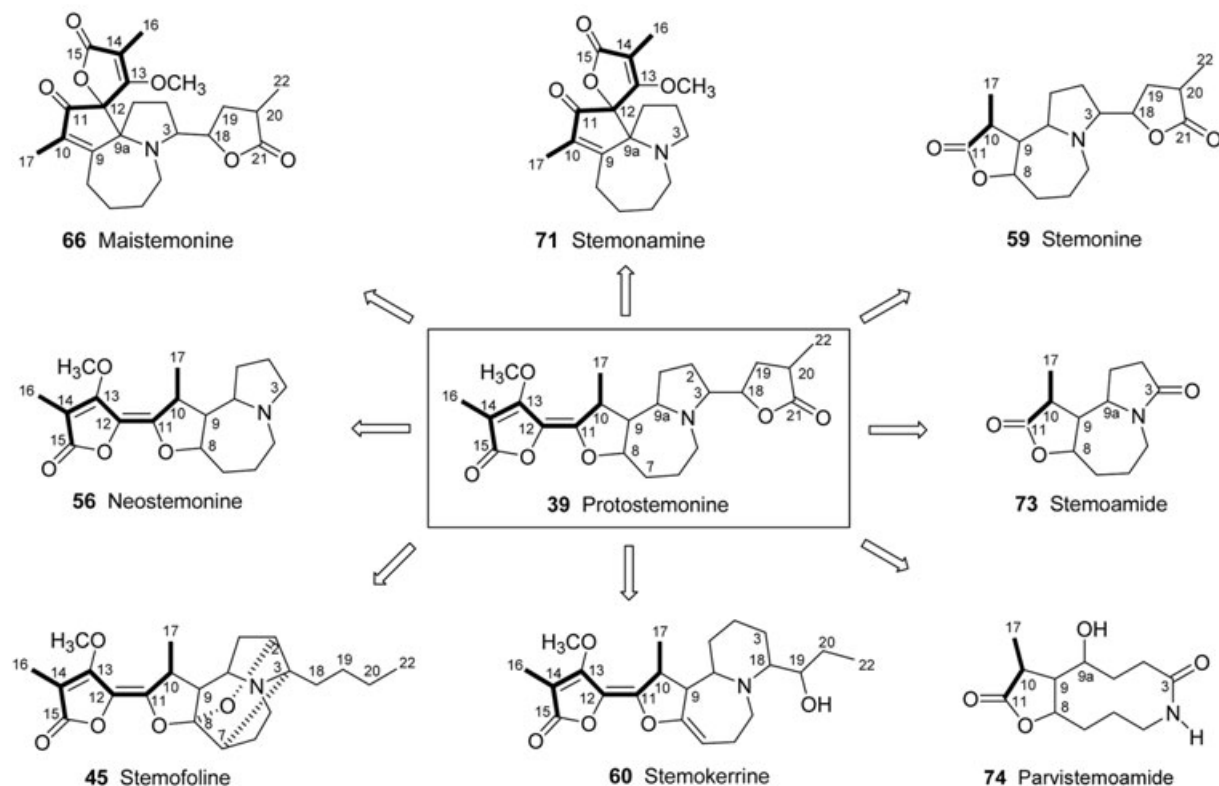


Fig. 5 Structural variation of protostemonine-type alkaloids.

only in the genus *Croomia* [22], [47] and some provenances of *S. tuberosa* [23], [48]. It was of special chemosystematic interest, that *Stichoneuron* and *Croomia* accumulated only the structurally simple key compounds stichoneurines A (**30**) and B (**31**) [23] or croomine (**75**) [22], respectively, whereas the genus *Stemona* produced various derivatives of all three skeletal types (Fig. 1). However, different accumulation trends towards stichoneurine or protostemonine type derivatives contributed to an infrageneric grouping of *Stemona* which appeared to be well in line with morphological characters.

Stemona sessilifolia, *S. japonica*, and *S. mairei*

Most of the chemical reports available so far focused on the alkaloids of the three well-known representatives of the Traditional Chinese Medicine *S. tuberosa*, *S. japonica*, and *S. sessilifolia*. However, as already pointed out by Xu [2], the species were not al-

ways properly identified. Especially *S. sessilifolia* sometimes appeared to have been confused with *S. tuberosa* and was erroneously reported to produce predominantly stichoneurine derivatives such as tuberostemonine (**1**), oxotuberostemonine (**24**), tuberostemonine A (**3**) [36], [40], and stemoninine (**21**) [49], as well as stemoninoamide (**28**), neotuberostemonol (**15**), tuberostemonone (**17**), stenine (**26**), 2-oxostenine (**25**), and the sessilifoliamides A (**29**), B (**32**), C (**33**), and D (**34**) [38]. However, in accordance with preliminary results of the author's laboratory and personal communications from Prof. Yang Ye from the State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences and Dr. Ren-Wang Jiang from the Department of Chemistry, The Chinese University of Hong Kong, no stichoneurine (tuberostemonine)-type derivatives could be detected in *S. sessilifolia*. Instead, protostemonine (**39**) and stemonine (**59**) together with related derivatives such

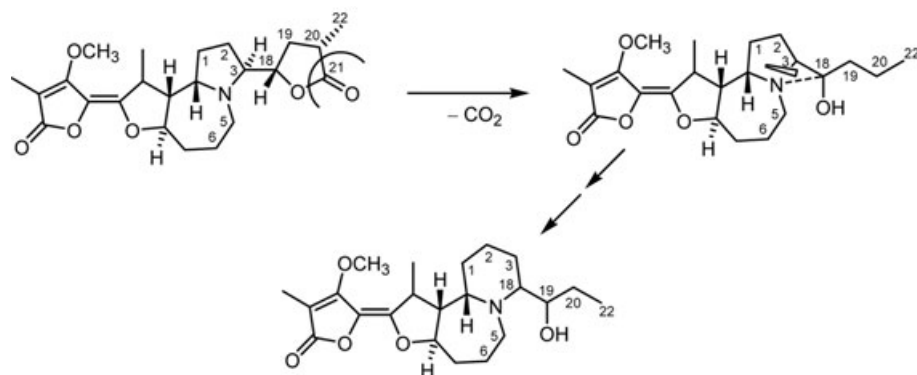


Fig. 6 Proposed biosynthetic connections between pyrrolo- and pyridozepines.

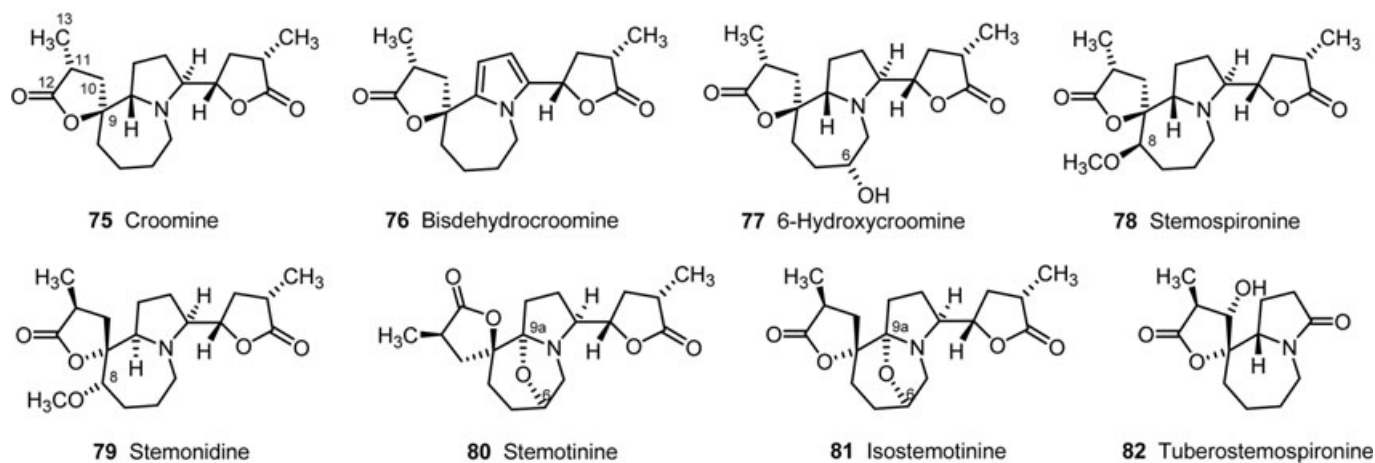


Fig. 7 Croomine-type alkaloids.

as isoprotostemonine (**40**), bisdehydroprotostemonine (**42**), and maistemonine (**66**) were found in this species [2]. This is in good agreement with the chemical patterns of *S. japonica* and *S. mairei* which also showed many morphological similarities to *S. sessilifolia* (Table 1).

The most prominent alkaloid of the roots of *S. japonica* is protostemonine (**39**) whose structure was described by Irie et al. [35]. However, from the stems and leaves of the same species these workers isolated for the first time stemofoline (**45**) as the major compound and established its structure by X-ray crystallographic analysis [10]. More detailed investigations by Ye et al. [33] with 10 kg roots of a Chinese provenance of *S. japonica* confirmed the accumulation trend towards protostemonine (**39**) and additionally led to the isolation of stemonine (**59**) as the second major compound together with bisdehydroprotostemonine (**42**), isoprotostemonine (**40**), neostemonine (**56**), and bisdehydroneostemonine (**57**). A further structural variation of protostemonine (**39**) was found in *S. japonica* leading to the two isomers stemonamine (**71**) and isostemonamine (**72**) (Fig. 5). Their isomeric spiro structures were expected to be interconvertible in acid or base through an intermediate [50]. In addition to the already mentioned derivatives of the Chinese provenance of *S. japonica*, Ye et al. [24] could isolate a series of five further protostemonine-type alkaloids which all were characterised by a spiro system. Besides the already known isomers stemonamine (**71**) and isostemonamine (**72**), they also found a new pair of isomers named stemonamide (**69**) and isostemonamide (**70**) together with maistemonine (**66**) as major derivative. The latter compound was originally described for *S. mairei* (H. Léveillé) K. Krause, from where it was isolated together with oxymaistemonine (**68**), stemonamine (**71**), and protostemonine (**39**) [51], [52]. Since neither in *S. japonica* nor in the probably related *S. mairei* could the corresponding isomer of the major alkaloid maistemonine (**66**) be detected, Ye et al. [24] questioned the conversion of the isomeric spiro structures during acid or base treatment and concluded that they are both naturally occurring compounds. Maistemonine (**66**) was later also reported for the roots of *S. sessilifolia*, but was erroneously described as a new alkaloid and named protostemotinine [53]. With respect to the data discussed so far, all alkaloids of *S. japonica*, *S. mairei* and *S. sessilifolia* belong to the protostemonine-type. However, it should be pointed

out that Sakata et al. [25] also found the new stemospironine (**78**) together with the dominating stemofoline (**45**) in the leaves and stems of *S. japonica*, indicating an additional accumulation trend towards croomine-type alkaloids (Fig. 7). The co-occurrence of the protostemonine derivative isomaistemonine (**67**) as major compound of the roots of *S. japonica* with a series of stichoneurine (tuberoSTEMOSPIRONINE) derivatives such as tuberoSTEMOSPIRONINE B (**4**), C (**5**), bisdehydroTUBEROSTEMOSPIRONINE B (**10**), and C (**11**) is surprising and should be rechecked with carefully selected plant material (Table 1); all the more, as different species names were used in that report for the title and legend of the figure on the one hand, and for the experimental part on the other [39].

Stemona collinsae and *S. curtisii*

A clear accumulation trend towards protostemonine-type alkaloids was also observed in *S. collinsae* Craib and *S. curtisii* Hook. f., two prominent species of Thailand. However, all provenances of *S. collinsae* investigated so far were clearly characterised by the predominance of stemofoline derivatives [6], [11], whereas those of *S. curtisii* showed remarkable variation either towards stemofoline (**45**), or protostemonine (**39**), or the pyridoazepines oxySTEMOKERRINE (**61**), stemocurtisinol (**62**), and stemocurtisine (= pyridostemine) (**65**) [29], [45], [46]. A typical chemical character of all root extracts of *S. collinsae*, collected in southeast and east Thailand, was the predominance of didehydroSTEMOFOLINE (= asparagine A) (**48**) which was shown to be accompanied by stemofoline (**45**), 2'-hydroxystemofoline (**47**), and small amounts of didehydroisostemofoline (**49**) [6], [11]. Regarding that clear preference towards stemofoline derivatives, the accumulation of the stichoneurine type alkaloids neostenine (= isostenine) (**27**), neotuberoSTEMOSPIRONINE (**2**), and bisdehydroneotuberoSTEMOSPIRONINE (**9**) in a Vietnamese collection of *S. collinsae* appears doubtful and might be explained by a confusion with *S. tuberosa* [43]. The root extract of *S. curtisii*, collected in Satun province of south Thailand, was reported to accumulate mainly stemofoline (**45**) together with 2'-hydroxystemofoline (**47**) and small amounts of oxySTEMOKERRINE (**61**), protostemonine (**39**), dehydroprotostemonine (**41**), oxyprotostemonine (**43**), and stemocochinine (**44**) [29]. By contrast, another collection from Trang province of south Thailand was shown to accumulate mainly the pyridoazepines stemocurtisine (**65**) [45] together with stemocurtisinol (**62**) and oxyprotostemonine (**43**) [46]. Based on un-

Table 1 Distribution of different types of alkaloids in Stemonaceae

<i>Stemonaceae</i> genera and species	Protostemonine-type	Stichoneurine-type	Croomine-type	References
<i>Stichoneuron caudatum</i>		30, 31		[23]
<i>Croomia japonica</i>			75, 76	[22], [47]
<i>Stemona sessilifolia</i>	39, 40, 42, 59, 66	^a 1, 3, 15, 17, 21, 24, 25, 26, 28, 29, 32, 33, 34	79	[2], [36], [38], [40], [49], [53], [67]
<i>S. japonica</i>	39, 40, 42, 45, 56, 57, 59, 66, 67, 69, 70, 71, 72	^a 4, 5, 10, 11	78	[10], [24], [25], [33], [35], [39], [50]
<i>S. mairei</i>	39, 66, 68, 71			[51], [52]
<i>S. collinsae</i>	39, 45, 47, 48, 49	^a 2, 9, 27		[6], [11], [43],
<i>S. curtisii</i>	39, 41, 43, 44, 45, 47, 59, 61, 62, 63, 65			[29], [45], [46], [54]
<i>S. kerrii</i>	39, 41, 43, 44, 60, 61, 63, 64			[29]
<i>S. burkillii</i>	45, 47, 52, 53			[44]
<i>S. saxorum</i>	39, 42, 56, 57			[55]
<i>S. cochinchinensis</i>	39, 45, 44, 47			[29]
<i>S. pierrei</i>	39, 59			[15]
<i>S. parviflora</i>	45, 50, 51, 54, 55, 74	35, 36, 37, 38		[56], [57], [58], [59], [60]
undet. spec. HG 915	61, 65	35		[29]
<i>S. tuberosa</i>	73	1, 2, 3, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 28	75, 77, 80, 81, 82	[2], [23], [32], [34], [41], [42], [61], [62], [63], [64], [65], [66]

^a Compounds were isolated from plants with uncertain identity.

published results from the author's laboratory a third chemotype of *S. curtisii* from south Thailand was also found, which was characterised by a predominant accumulation of protostemonine (39) itself accompanied by stemonine (59). However, in this case the rhizome clearly deviated from the roots containing mainly stemofoline (45) together with small amounts of 2'-hydroxystemofoline (47) and oxystemokerrine *N*-oxide (63) [54].

Stemona kerrii, *S. burkillii*, and *S. saxorum*

S. kerrii Craib and *S. burkillii* D. Prain were collected in the mountainous forests of north and northwest Thailand. Their chemical profiles can be distinguished by different accumulation trends towards pyridoazepine derivatives in the former [29] and stemofoline derivatives in the latter [44]. Detailed chemical comparisons between two different geographical provenances of *S. kerrii* displayed a clear preponderance of stemokerrine (60) and oxystemokerrine (61) accompanied by oxystemokerrine *N*-oxide (63), methoxystemokerrine *N*-oxide (64), protostemonine (39), and dehydroprotostemonine (41) as well as small amounts of oxyprotostemonine (43) and stemocochinine (44) [29]. By contrast, *S. burkillii* was characterised by stemofoline (45) as major compound together with 2'-hydroxystemofoline (47) and small amounts of the two rare derivatives dihydrostemofoline (52) and stemoburkilline (53) [44]. In the Flora of China *S. saxorum* Gagnep. was included in *S. kerrii* as a synonym [17], but its alkaloid profile clearly deviated by the formation of neostemonine (56), bisdehydroneostemonine (57), protostemonine (39) and bisdehydroprotostemonine (42) [55]. Hence, a critical re-investigation of corresponding voucher specimens would help to clarify the taxonomic position of that collection.

Stemona cochinchinensis, *S. pierrei*, and *S. parviflora*

S. cochinchinensis Gagnep. and *S. pierrei* Gagnep. were collected in the dry open habitats of east Thailand. They also showed a clear preference towards protostemonine-type alkaloids. In this case the root extract of *S. cochinchinensis* was characterised by a preponderance of stemofoline (45) accompanied by small amounts of 2'-hydroxystemofoline (47), stemocochinine (44), and protostemonine (39) [29], whereas *S. pierrei* accumulated mainly protostemonine (39) together with small amounts of stemonine (59) [15]. A somewhat isolated position was taken by *S. parviflora* C. H. Wright, known only from Hainan island of south China, where it occurs on streamsides, rock crevices in valleys and waste places [17]. In this species protostemonine-type alkaloids were shown to co-occur with a series of compounds listed in this review as stichoneurine derivatives on the basis of their characteristic branching pattern of the carbon chain attached to C-9. The protostemonine derivatives were represented by stemofoline structures from which parvistemoninine (54) and parvistemoninol (55) were described as optical antipodes of stemofoline (45) and oxystemofoline (50) [56]. The latter two compounds were also isolated from that species together with methoxystemofoline (51) as minor components [57]. Of special structural interest was the isolation of the structurally simplest *Stemona* alkaloid parvistemoamide (74) [59], which most likely can be directly derived from stemoamide (73) isolated in trace amounts from *S. tuberosa* [41]. The most characteristic alkaloids of *S. parviflora* are classified as stichoneurine derivatives and are represented by parvistemonine (35) [58] and the related derivatives parvistemoline (37) and bisdehydroparvistemonine (= dihydroparvistemonine) (36) [59]. Moreover, from the stems and leaves of this species an alkaloid with a novel structure (Fig. 3) could be isolated, which was named parvineostemonine (38) [60]. Interestingly, the rare parvistemonine (35) was recent-

ly also found as major compound in the root extract of an as yet unidentified *Stemona* species (HG 915) collected in northeast Thailand which, however, did not show any morphological similarities with *S. parviflora*. In this case parvistemonine was accompanied by the pyridoazepine derivatives oxystemokerrin (**61**) and stemocurtisine (= pyridostemine) (**65**) [29].

Stemona tuberosa complex

The best known *Stemona* alkaloid is tuberostemonine (**1**) isolated from many different provenances and/or varieties of *S. tuberosa* which were mainly collected in different provinces of China, Vietnam, and Thailand. In spite of the good delimitation of the *S. tuberosa* complex from the other *Stemona* species and an already existing preliminary proposal for an infraspecific grouping [16], there are still many taxonomic problems that remain to be solved. As shown in Fig. 3, tuberostemonine (**1**) can be regarded as a derivative of stichoneurine (**30**) and, accordingly, is frequently accompanied by corresponding derivatives. However, as mentioned above and already pointed out by Kondo et al. [61], chemical instability makes it difficult to preserve the free base and can lead to the formation of artifacts during isolation and fractionation. Apart from that, variation of the alkaloid profiles was created by different stereoisomers of tuberostemonine (**1**) and related derivatives (Fig. 2). For instance, the roots of a collection from Yunnan province in southwest China accumulated mainly neotuberostemonine (**2**) which was accompanied by small amounts of bisdehydroneotuberostemonine (**9**) [32]. In this case both compounds were characterised by an α -orientation of H-11 and H-12. The same stereochemical trend was also observed in provenances from north Vietnam [43], [62] and Hongkong, where in the latter collection additionally the corresponding derivatives tuberostemonine H (**6**), tuberostemonine J (**7**), *epi*-bisdehydroneotuberostemonine J (**13**), neotuberostemonol (**15**), neotuberostemoninol (**20**), and neostenine (= isostenine) (**27**) were isolated, all showing the same orientation of H-11 and H-12 [42], [63]. By contrast, a dominating β -orientation of H-11 and H-12 was observed in *S. tuberosa* from south [41], [64] and north China [34], [65], [66], where besides the prevailing tuberostemonine (**1**) a series of corresponding derivatives such as bisdehydrotuberostemonine (**8**), isobisdehydrotuberostemonine (**12**), tuberostemonol (**14**), *N*-oxytuberostemonine (**16**) and tuberostemoninol (**19**) was found. In addition, a number of further related derivatives were also isolated as minor compounds and identified as tuberostemonone (**17**), tuberostemonone (**18**), and oxotuberostemonine (**24**). This stereochemical trend was recently also described for the roots of *S. tuberosa* from southeast Thailand characterised by tuberostemonine (**1**) and tuberostemonine A (**3**) [23]. All these provenances were dominated by derivatives showing a close structural relationship to tuberostemonine (**1**). Beyond that, a series of spiro derivatives was also described [2], which were closely related to stemoninine (**21**) (Fig. 3). They were named bisdehydrostemoninine (= didehydrostemoninine) (**22**), stemononine (**23**) [2] and stemoninoamide (**28**) [64]. The original description of stemoninine (**21**) for *S. sessilifolia* [49], [67] can most likely be explained by a confusion with *S. tuberosa* [2].

Apart from the predominance of stichoneurine type alkaloids in *S. tuberosa*, some remarkable exceptions were reported, where this accumulation trend was replaced by croomine-type deriva-

tives (Fig. 1 and Fig. 7). A collection from Wenshan district, Yunnan province, in China was characterised by a preponderance of stemotinine (**80**) accompanied by isostemotinine (**81**) [48], whereas another collection from the mountains of north Thailand was shown to accumulate exclusively croomine (**75**) and the new 6-hydroxycroomine (**77**) [23]. Tuberostemospiroline (**82**) was also detected in a provenance from south China as minor compound besides dominating tuberostemonine derivatives [41]. Interestingly, in that provenance the structurally simple stemoamide (**73**) was also found in trace amounts, which was regarded as a protostemonine derivative (Fig. 5).

Summarising the data presented in Table 1 it became apparent that no protostemonine-type alkaloids were detected in the two genera *Stichoneuron* and *Croomia*, but they were otherwise dominating in most of the *Stemona* species. By contrast, stichoneurine derivatives represent the major alkaloids in the taxonomically complex *S. tuberosa* group, and stichoneurine A (**30**) itself together with its stereoisomer B (**31**) were detected as the sole alkaloids in the genus *Stichoneuron*. Derivatives of both skeletal types were found to co-occur in *S. parviflora* and an unidentified species (HG 915) of northeast Thailand, but were also reported for some collections of *S. sessilifolia* [38], [40], [49], *S. japonica* [39], and *S. collinsae* [43]. However, as already mentioned above, the accumulation of stichoneurine derivatives in the latter three species can most likely be explained by the use of not properly identified plant material (Table 1). From the croomine-type structures croomine (**75**) itself and its bisdehydro (= pyrrole) derivative (**76**) were the only alkaloids detected in the genus *Croomia*, but related derivatives (**77**, **80**, **81**) were described as major compounds for some provenances of *S. tuberosa*, obviously replacing the stichoneurine derivatives [23], [48]. In this connection it is noteworthy that tuberostemospiroline (**82**) was also detected in a collection of *S. tuberosa* as minor compound together with dominating stichoneurine derivatives [41]. Interestingly, a croomine-type alkaloid (**78**) was also accumulated together with dominating protostemonine derivatives in *S. japonica* [25], and the corresponding isomer (**79**) was reported for *S. sessilifolia* [2], [48].

Stemona root extracts characterised by a preponderance of protostemonine-type alkaloids can additionally be classified according to three different accumulations trends leading a) to protostemonine (**39**) itself and closely related compounds, or b) to structures derived from stemofoline (**45**), or c) to pyridoazepine alkaloids (Fig. 5). Whereas all three trends were alternately found in different provenances of *S. curtisii* collected in south Thailand, mainly protostemonine (**39**) itself together with stemonine (**59**) were typical for *S. pierrei*, *S. japonica*, and *S. sessilifolia*. The latter two species together with the probably closely related *S. mairei* were additionally characterised by the formation of spiro structures derived from maistemone (**66**). The pronounced accumulation of stemofoline (**45**) and related derivatives was typical for *S. burkillii*, *S. cochinchinensis* and *S. collinsae*, from which especially didehydrostemofoline (= asparagine A) (**48**) was shown to be a typical chemical character of *S. collinsae*. A preferred formation of pyridoazepine-type alkaloids was found in different provenances of *S. kerrii* leading to the major compounds stemokerrine (**60**) and oxystemokerrine (**61**).

Table 2 Insecticidal (LC_{50}) and growth inhibitory activities (EC_{50}) of *Stemona* alkaloids compared with commercial azadirachtin against neonate larvae of *Spodoptera littoralis*^a [6], [29]

Alkaloids	LC_{50}	(95% FL ^b) ppm	EC_{50}	(95% FL ^b) ppm
didehydrostemofoline (48)	0.8	(0.7–1.1)	0.5	(0.3–0.6)
stemofoline (45)	2.0	(1.6–2.6)	1.5	(1.3–1.6)
oxystemokerrine (61)	5.9	(4.2–9.1)	0.7	(0.1–1.3)
dehydroprotostemonine (41)	6.1	(4.3–9.1)	0.8	(0.4–1.3)
oxystemokerrine N-oxide (63)	12.5	(7.2–22.5)	0.4	(0.1–0.9)
protostemonine (39)	17.7	(13.2–24.8)	2.2	(1.5–2.9)
2'-hydroxystemofoline (47)	30.3	(26.6–34.7)	38.5	(7.3–182.2)
stemokerrine (60)	58.4	(48.0–73.0)	14.1	(12.0–16.3)
croomine (75)	~ 120		~ 20	
methoxystemokerrine N-oxide (64)	~ 150		16.3	(10.0–27.3)
stemocurtisine (65)	148.9	(92.8–336.7)	96.1	(61.3–218.7)
oxyprotostemonine (43)	159.0	(99.2–838.8)	46.9	(29.9–75.2)
stemocochinine (44)	170.4	(150.4–199.5)	60.9	(37.9–95.8)
parvistemonine (35)	~ 350		162.7	(133.3–233.5)
tuberostemonine (1)	> 500		~ 500	
neotuberostemonine (2)	> 500		> 500	
azadirachtin ^c	8.2	(6.6–11.6)	0.04	(0.02–0.07)

^a Feeding studies were conducted with neonate larvae ($n = 20$ for each treatment) in a non-choice test with different concentrations of isolated compounds. After 5 days of exposure, survival and weight of the surviving larvae were determined and compared to controls that had been exposed to diet treated with solvent (MeOH) only. From the dose-response curves LC_{50} (lethal concentration) and EC_{50} (effective concentration) values were calculated by probit-log analysis.

^b Fiducial limits.

^c Purchased from Roth, Karlsruhe, Germany.

Regarding the possible formation of artifacts of some stichoneurine-type alkaloids, conclusions about varying accumulation trends within the *S. tuberosa* complex are premature. However, as already pointed out above, different geographical provenances were characterised by stereochemical trends towards the formation of α - or β -orientated H-11 and H-12. Moreover, the formation of spiro structures related to stemonine (21) (Fig. 3) led to a further structural variation of stichoneurine derivatives [2]. Parvistemonine (35) and related derivatives (36, 37) as well as parvineostemonine (38) represent unique structures also regarded in this review as derived from stichoneurines (Fig. 3). So far they were only found in *S. parviflora* from Hainan island of south China and in an unidentified species (HG 915) from northeast Thailand [29], [58], [59].

The general survey presented in Table 1 let us expect that in principle all three types of alkaloids can be produced in *Stemona* species, but species specific accumulation trends towards certain structural types represent valuable chemosystematic criteria.

Biological Activities

The tuberous roots of *S. japonica*, *S. sessilifolia*, and *S. tuberosa*, known as “Radix Stemonae”, have long been recommended in Chinese, Japanese, and Vietnamese traditional medicine to relieve cough and asthma, and were also used against enteric helminths and ectoparasites on humans and cattle [1], [2], [3], [68], [69], [70]. In southeast Thailand roots and leaves of “Non Tai Yak”, most probably derived from *S. collinsae*, but published as *S. curtisii*, were used to protect pepper plants against insect attacks. In central Thailand “Non Tai Yak” is known to prevent the

infestation of anchovy sauce “Ka Pi” by housefly larvae, and traditional medical practitioners in south Thailand recommend it as scabicide, pediculocide, and against helminth worms, in spite of the fact that ingestion of too much is potentially fatal. There is also reliable information that laymen used “Non Tai Yak” to alleviate toothache [27]. However, due to the use of the same vernacular names such as “Non Tai Yak” in Thailand, “Bai Bu” in China, and “Bach Bo” in Vietnam, for different *Stemona* species, it is very difficult to predict and allocate specific effects to certain species and active compounds. All the more, as some species like, e.g., *S. curtisii* or *S. tuberosa*, are known to occur as various chemotypes.

Insecticidal activities

Despite the wide use of *Stemona* roots as bio-insecticides detailed comparative bioassays with extracts from different species, organs, and pure compounds are lacking. Mostly preliminary investigations have been reported without an accurate evaluation of the activities, e.g. [33], [34], and a number of different insects have been tested without knowing the active compounds of the plant extract [69]. Parallel to a recently started broad-based phytochemical comparison within the family Stemonaceae insect tests were carried out in the author's laboratory to give an overview about the various insecticidal potencies. On the basis of chronic feeding bioassays with neonate larvae of the cotton leaf worm *Spodoptera littoralis* Boisduval (Lepidoptera, Noctuidae) reared on artificial diet, methanolic leaf and root extracts of various *Stemona* species displayed dramatic differences in insecticidal activities [6]. Pronounced insect toxicity was determined in the leaves and especially in the roots of *S. collinsae*, *S. cochinchinensis*, and some provenances of *S. curtisii*, which was significantly higher than that of a commercial Pyrethrum extract. Even the roots of *S. kerrii* with a somewhat lower insecticidal ca-

capacity displayed higher activities [29]. By contrast, different provenances of *S. tuberosa* showed only very low activity in the roots and no activity in the leaves [6]. Moreover, in leaf disk choice tests against fifth instar larvae strong antifeedant activity was observed for the crude extract of *S. collinsae*, whereas *S. tuberosa* clearly differed by its low toxicity but remarkable repellence [6]. After bioassay-guided fractionations the high insect toxicities in the first three species could unambiguously be attributed to derivatives of the protostemonine type (Fig. 4.). As shown in Table 2 especially didehydrostemofoline (48), accumulated in *S. collinsae*, showed the highest activity with an LC₅₀ value as low as 0.8 ppm. Its insect toxicity was significantly higher than that of the well-known natural insecticide azadirachtin with an LC₅₀ of 8.2 ppm. In *S. collinsae* this compound was accompanied by smaller amounts of stemofoline (45) with an LC₅₀ of 2.0 ppm, and 2'-hydroxystemofoline (47) with 30 ppm, demonstrating structure-activity relationships (Table 2): didehydrostemofoline (48), characterised by an unsaturated *n*-butenyl side chain displayed the strongest insecticidal activity, whereas the saturated *n*-butyl side chain of stemofoline (45) diminished insecticidal properties. A significant decrease of toxicity, however, was caused by the free hydroxy group at C-2' of the side chain of 2'-hydroxystemofoline (47) [6]. The high insecticidal and antifeedant properties of stemofoline derivatives were also supported by biotests against the diamondback moth *Plutella xylostella* L. using the leaf disk assay [11]. In that investigation again didehydrostemofoline (48) exhibited the highest activity, even higher than the well-known naturally occurring insecticide rotenone. The corresponding saturated derivative stemofoline (45) was significantly weaker [11]. In a previous communication Sakata et al. [25] reported on marked insecticidal activity of fresh leaves of *S. japonica* against fourth instar larvae of the silkworm *Bombyx mori* L. In this case stemofoline (45) was the most active derivative in feeding experiments with artificial diet, being about 10⁴ times as toxic as stemospironine (78) with a croomine-type structure (Fig. 7). The pronounced insecticidal activities of the crude extract of *S. curtisii* and *S. cochinchinensis* could also be attributed to stemofoline (45) which was accumulated as major compound [29]. With respect to this marked insecticidal properties it was surprising that this compound was completely inactive against fifth instar larvae of the cabbage army worm *Mamestra brassicae* L. [25].

Apart from the high insecticidal properties of some stemofoline derivatives very strong activities were also observed for the related pyridoazepine derivative oxystemokerrine (61) with an LC₅₀ value of 5.9 ppm (Table 2). This compound was found in *S. kerrii*, *S. curtisii*, and in an unidentified *Stemona* species (HG 915) [29]. In the related stemokerrine (60) the lack of an oxygen bridge and the formation of a double bond between C-8 and C-9 obviously diminished activity leading to an LC₅₀ of 58.4 ppm. A more significant decrease of activity, however, was caused by the loss of the propyl side chain in stemocurtisine (= pyridostemine) (65) with an LC₅₀ of 148.9 ppm (Table 2). However, the formation of the open side chain and oxygen bridge of stemofolines and stemokerrines appeared not to be a general prerequisite for high insect toxicity: dehydroprotostemonine (41) with a lactone ring attached at C-3 and without an oxygen bridge also displayed high activity with an LC₅₀ of 6.1 ppm, comparable with that of oxystemokerrine (61). The corresponding saturated protostemo-

nine (39) was somewhat weaker with 17.7 ppm. Surprisingly, a significant decrease was observed in oxyprotostemonine (43), characterised by an oxygen bridge between C-1 and C-8 (Table 2) [29]. However, it should be pointed out that this compound was shown to possess a significant larvicidal activity on mosquito larvae of *Anopheles minimus* with an LC₅₀ of 4 ppm, ranging before stemocurtisine (65) with 18 ppm, and stemocurtisinol (62) with 39 ppm [46]. Comparing the insecticidal and growth inhibitory activities known so far from the alkaloids listed in Table 2 it became apparent, that the unsaturated lactonic 4-methoxy-3-methyl-2-furanone unit plays a crucial role. In fact, very weak or even no activity was observed in tuberostemonine (1) and neotuberostemonine (2) as well as in stemocochinine (44), and parvistemonine (35), where that ring was either modified or lacking.

To date only a few derivatives of the stichoneurine-type (Fig. 2) have been tested in bioassays. This may be partly due to the chemical instability of some major derivatives. However, leaf disk choice tests with crude extracts of *S. tuberosa* showed strong feeding inhibitory properties against fifth instar larvae of *Spodoptera littoralis*, similar to those of Pyrethrum extract [6]. In view of the low toxicity this high repellent activity was surprising: the larvae preferred controls even without tasting the treated disks. The bioactive principle responsible for the high repellence proved to be tuberostemonine (1) demonstrating activity levels comparable with those of azadirachtin from the neem tree, *Azadirachta indica* A. Juss., Meliaceae. At 0.1 µg of tuberostemonine/cm² the fifth instar larvae did not even taste the treated disk, whereas the Pyrethrum extract, often described as a repellent agent in patent specifications, showed no activity at the 5-fold higher concentration of 0.5 µg/cm²[6].

Comparing all results obtained so far from chronic feeding bioassays with *Stemona* alkaloids against *Spodoptera littoralis*, different modes of action could be observed. Apart from the different activities of tuberostemonine (1) mentioned above, the stemofoline derivatives (45, 48) caused rapid reactions with neonate larvae [6]. Only a few minutes after placing the larvae on the treated diet, the larvae completely ceased any further intake of food. This effect is apparently due to toxicity and not simply to feeding inhibition, because cessation of food intake is accompanied by vomiting and trembling of the mouthparts, legs and pseudolegs. Death occurred after a maximum of one day. Larvae tested at sublethal doses recovered from growth retarding effects and completed normal development including metamorphosis, mating, and oviposition [6]. Whereas these effects were assumed to be caused by neurotoxic interactions resulting in uncontrolled hyperactivity of larvae, the extracts from *S. kerrii* and the unknown species HG 915 were characterised by a delayed entrance of death accompanied by softening of the larval bodies [29]. Similar symptoms were already described for the insecticidal activity of stemospironine (78) [25].

Medicinal properties

To reveal the mechanism of the larvicidal activity of the root extract of *S. curtisii* as well as its toothache relieving property Prucksunand et al. [27] investigated its effect on the action potential of isolated frog sciatic nerves. Using a cathode-ray oscilloscope they observed a significant decrease of the heights of nerve potential and interpreted it as result of an inhibition of motor

nerve conduction. Whereas in that study no isolated alkaloids could be tested, Terada et al. [26] investigated the effects of tuberostemonine (**1**) on the motor activity of helminth worms. They demonstrated that this alkaloid paralysed the motility of *Angiostrongylus cantonensis*, the isolated mouse ileum, and the isolated frog rectus. Moreover, it showed contractive effects on the motility of *Dipylidium caninum* and *Fasciola hepatica*. Stimulated by these results Shinozaki and Ishida [28] investigated the possible action of tuberostemonine (**1**) on neuromuscular transmission in crayfish. In this case the amplitude of the excitatory junctional potential and the glutamate response was reduced in a dose-dependent manner at concentrations above 0.1 mmol. However, in binding experiments this alkaloid did not show a significant affinity towards ³H-labelled glutamate ligands in the mammalian central nervous system [71].

Of special medicinal importance in China was at all times the antitussive activity of *Radix Stemonae*, “Bai Bu”, consisting of the roots of *S. tuberosa*, *S. sessilifolia*, and *S. japonica*. It has been used in the treatment of chronic or acute cough, pulmonary tuberculosis, whooping cough and oxyuriasis. Liao et al. [72] examined the spasmolytic effect of a water extract of “Bai Bu” on the guinea-pig tracheal smooth muscle *in vitro*. They showed that the effect was not due to an activation on β -adrenoceptors. Receptor binding assays indicated that the extract interacted with the muscarinic receptors and the dihydropyridine binding site of L-type Ca²⁺ channels, but not with the histamine H₁ receptors [72]. However, in that study no determination of isolated active compounds has been carried out. In a more recent investigation bioactivity-guided fractionation of the crude extract of *S. tuberosa* led to the isolation and identification of the five stichoneurine-type alkaloids neotuberostemonine (**2**), tuberostemonines H (**6**) and J (**7**), *epi*-bisdehydroneotuberostemonine J (**13**), and neostenine (= isostenine) (**27**). These compounds were examined for antitussive activity in guinea-pigs after cough induction by citric acid aerosol stimulation. In this study neotuberostemonine (**2**) and neostenine (**27**) were shown to possess significant antitussive activities comparable with codeine but not involving the opioid receptors [63]. Further studies of the structure-activity relationship on these five isolated alkaloids and two synthetic analogues revealed that the saturated tricyclic pyrrolobenzazepine nucleus is the primary key structure contributing to the antitussive activity. Furthermore, all *cis* configurations at the three ring junctions are the optimal structure for the antitussive activity of that type of alkaloids [63].

In preliminary anti-tumour tests crude extracts of *S. tuberosa* and *S. collinsae* were compared for their effects on medullary thyroid carcinoma cells. Both extracts altered the phenotype of the cells from originally aggregating cells towards single-cell suspensions. However, the extract of *S. tuberosa* considerably enhanced apoptosis, whereas *S. collinsae* only moderately increased the apoptotic effect. Since this type of cancer cell is known to be relatively insensitive to chemo- or radiation therapy this marked activity could offer a new approach towards successful chemotherapy [73]. Further studies will have to show to what extent *Stemona* alkaloids were involved in that effect of the root extracts.

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