Structural Relationships, Distribution and Biological Activities of Stemona Alkaloids

Abstract

Stemona alkaloids represent a unique class of natural products exclusively isolated from the monocotyledonous family Stemonaecae 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-, protostemone-nine- and croinine-type alkaloids. The genera Croonia and Stichoneuron only accumulate croinine 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 didelystemolone, whereas those with dominating stichoneurine or croinine 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 stereosomeric 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 pyrroloazepine nucleus of tuberostemonesines is the prerequisite for antitussive activity.

Key words

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

Introduction

Various species of the genus Stemona (Stemonaecae) are widely used in China and other countries of southeast Asia as an antitussive 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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Bibliography

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 clitoriacetal 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 didehydrostemofoline (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 didehydrophenanthracenemotol (*=* stemanethrene 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.phytochemical.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 southeastern 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-α]azepine core mostly linked with one or two lactone rings representing a typical chemical character of the Stemonaceae not detected so far in any other plant family. Like the biosynthesis of pyrrolizidine alkaloids the pyrroleazepine 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].

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 pyrrolizidine 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-α]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 pyrroleazepine 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].

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**Review**

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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 alkaloïds can be classified into seven [33] or eight structural groups [24] according to the sites of connection between the basic pyrrolizidine 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 alkaloïds has been nearly doubled, now containing 82 derivatives. With the exception of the four alkaloïds cromine (75), bisdehydrocro-
mine (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 cro-
mine-type alkaloïds. As shown in Fig. 1 the three types can be distinguished by different carbon chains attached to C-9 of the pyrrolizidine nucleus. In the stichoneurine- and protostemony-
type these chains usually contain eight carbon atoms forming a terminal lactone ring, but differ among each other in the branching pattern. In the cromine-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 de-
rivatives 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 alkaloïds**

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 addi-
tional linkages to the pyrrolizidine core and ether bridges lead to a variety of different structures within this type. As shown in Fig. 3 the recently described structures of stichoneur-
ine 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), oxo-
tuberostemonine (24), and stenine (26) can be derived by linking C-12 of the lactone ring with C-1 of the pyrrolizidine core, whereas in parvinoestemonine (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 stemonine (21), and another between C-12 and C-16 to the structure of par-
vistenonime (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 der-
ivatives by a lactonisation between C-14 and C-1, and stenine (26) and parvinoestemonine (38) by the lack of the methylated butyro-
lactone ring usually attached to C-3 (Fig. 3). With respect to the mass spectra of many Stemona alkaloïds this lactone ring can easily be removed leading to a characteristic and dominant frag-
mentation 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. sessilian folia 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 stemonoinaide (28), all possessing a 3-carbonyl group, represent extraction artifacts.

**Formation of artifacts**

Chemical stability plays an important role in evaluating biologi-
ical activities of naturally occurring plant products. Their trans-
formation during extraction and/or fractionation processes is of-
ten accompanied by a strong decrease or even loss of biological functions. Within Stemona alkaloïds especially some derivatives of the stichoneurine-type can be regarded as artifacts. For in-
stance, bisdehydrotuberostemonine (8) was already reported to be readily obtained, in good yield, from mother liquors of tuber-
ostemonine (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 bisde-
hydrotuberostemonine 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 alkaloïds into three skeletal types based on different carbon chains attached to C-9 of the pyrrolizidine core.
Fig. 2  Stichoneurine-type alkaloids.

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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 stemeonine (23), can be regarded as artifacts has not been ascertained so far. Steric variations, e.g., from tuberostemonine (1–7) and bisdehydrotuberostemonine (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-9α to form a characteristic spirolactone system [39]. Stemonine (59), stemonamide (73), and the related parvistemoamidine (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), stemoamidine (73), and parvistemoamidine (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 stemofoamine (45) or its optical antipode parvistemonine (54). With respect to the high insecticidal activity of stemofoamine (45) and especially the related didehydrostemofoamine (asparagamine 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 butyro lactone ring followed by decarboxylation (Fig. 6). Eleven stemofoamine 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 1H-NMR analysis showed that this compound as well as stemofoamine (45), 2′-hydroxystemofoamine (47), and dihydrostemofoamine (52) were not produced via an acid-catalysed reaction during the acid extraction process [44].

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Fig. 4a  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 stemokermine (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), oxystemokermine (61), oxystemokermine N-oxide (63) [29], stemocurtisine (65) [45], and stemocurtisinosol (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 didydrocortisinosmethyline (49), and by the formation of bisdehydro (= pyrrole) derivatives (42, 57), already mentioned for corresponding stichonene-type derivatives. The structurally simplest Stemonam, parvistemoam (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).

**Cromine-type alkaloids**

Up to now eight cromine-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 bisdehydrocromine (76) and the lack of the lactone ring usually attached to C-3 in tuberostemopirone (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 stemonine (80) and isostemotinine (81), whereas the two stereoisomers stemopirone (78) and stemonide (79) have a methoxy group at C-8. More recently the structure of the new 6-hydroxy-cromine (77) was published [23], suggesting close connections to the stemotinines. Tuberostemopirone (82) deviates from the other cromine-type alkaloids by the formation of a hydroxy group at C-10 in the spiro lactone system.

**Accumulation and Distribution**

**Cromina and Stichonemon**

Comparing the literature and results from the author’s laboratory it becomes apparent that most of the Stemonaecae 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, cromine-type alkaloids played an inferior role dominating...
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 croamine (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 always 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 stemonine (21) [49], as well as stemoninaamide (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

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**Fig. 5** Structural variation of protostemonine-type alkaloids.

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**Fig. 6** Proposed biosynthetic connections between pyrrolo- and pyridoazepines.
as isoprotostemone (40), bisdehydroprotostemone (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 protostemone (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 stemonofoline (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 protostemone (39) and additionally led to the isolation of stemonine (59) as the second major compound together with bisdehydroprotostemone (42), isoprotostemone (40), neostemonine (56), and bisdehydro- neostemonine (57). A further structural variation of protostemone (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 protostemone-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 protostemone (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 protostemone-type. However, it should be pointed out that Sakata et al. [25] also found the new stemosporinine (78) together with the dominating stemonofoline (45) in the leaves and stems of S. japonica, indicating an additional accumulation trend towards cromoine-type alkaloids (Fig. 7). The co-occurrence of the protostemone derivative isomaistemonine (67) as major compound of the roots of S. japonica with a series of stichoneurine (tuberostemone) derivatives such as tuberostemone B (4), C (5), bisdehydrotauberostemone 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].

Stemonoa collinsae and S. curtisi

A clear accumulation trend towards protostemone-type alkaloids was also observed in S. collinsae Craib and S. curtisi Hook. f., two prominent species of Thailand. However, all provenances of S. collinsae investigated so far were clearly characterised by the predominance of stemonofoline derivatives [6], [11], whereas those of S. curtisi showed remarkable variation either towards stemonofoline (45), or protostemone (39), or the pyridoazepines oxy-stemokerrine (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 dehydrostemonofoline (= asparagamine A) (48) which was shown to be accompanied by stemofoline (45), 2’-hydroxystemofoline (47), and small amounts of dehydrooxygenstemonofoline (49) [6, 11]. Regarding that clear preference towards stemonofoline derivatives, the accumulation of the stichoneurine type alkaloids neostemine (= isostemine) (27), neotuberostemone (2), and bisdehydrooetuberostemone (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. curtisi, collected in Satun province of south Thailand, was reported to accumulate mainly stemonofoline (45) together with 2’-hydroxystemofoline (47) and small amounts of oxy-stemokerrine (61), protostemone (39), dehydroprotostemone (41), oxyprotostemone (43), and stemochinin (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 oxyprotostemone (43) [46]. Based on un-
published results from the author’s laboratory a third chemotype of S. curtisi 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].

Stemono kerrii, S. burkili, and S. saxorum
S. kerrii Crab and S. burkili 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 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 stemochonine (44) [29]. By contrast, S. burkili 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), bisdehydroestemofoline (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.

Stemono cochinichensis, S. pierre, and S. parviflora
S. cochinichensis Gagnep. and S. pierre 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. cochinichensis was characterised by a preponderance of stemofoline (45) accompanied by small amounts of 2-hydroxystemofoline (47), stemochonine (44), and protostemonine (39) [29], whereas S. pierre accumulated mainly protostemonine (39) together with small amounts of stemofoline (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 stichonurine 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 parvistemonine (54) and parvisstemoninol (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 Stemono alkaloid parvistemomamide (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 stichonurine derivatives and are represented by parvistemonine (35) [58] and the related derivates parvisstemone (37) and bisdehydroparvistemonine (= di-dehydroparvistemonine) (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 parvistemonine (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 parvistemone was accompanied by the pyridoazepine derivatives oxystomokerrin (61) and stemocurisine (= pyridostemine) (65) [29].

**Stemona tuberosa complex**

The best known Stemona alkaloid is tuberostemone (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, tuberostemone (1) can be regarded as a derivative of stichonene (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 tuberostemone (1) and related derivatives (Fig. 2). For instance, the roots of a collection from Yunnan province in southwest China accumulated mainly neotuberostemone (2) which was accompanied by small amounts of bisdehydrooxotuberostemone (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 tuberostemone H (6), tuberostemone J (7), epi-bisdehydrooxotuberostemone J (13), neotuberostemol (15), neotuberostemoninol (20), and neostemone (= isostemone) (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 beside the prevailing tuberostemone (1) a series of corresponding derivatives such as bisdehydrooxotuberostemone (8), isobisdehydrooxotuberostemone (12), tuberostemol (14), N-oxytuberostemone (16) and tuberostemoninol (19) was found. In addition, a number of further related derivatives were also isolated as minor compounds and identified as tuberostemone (17), tuberostemone (18), and oxotuberostemone (24). This stereochemical trend was recently also described for the roots of *S. tuberosa* from southeast Thailand characterised by tuberostemone (1) and tuberostemone A (3) [23]. All these provenances were dominated by derivatives showing a close structural relationship to tuberostemone (1). Beyond that, a series of spiro derivatives was also described [2], which were closely related to stemonine (21) (Fig. 3). They were named bisdehydrostemonine (= didehydrostemonine) (22), stemonenone (23) [2] and stemoninoamide (28) [64]. The original description of stemonine (21) for *S. sessilifolia* [49], [67] can most likely be explained by a confusion with *S. tuberosa* [2].

Apart from the predominance of stichonene type alkaloids in *S. tuberosa*, some remarkable exceptions were reported, where this accumulation trend was replaced by croomine-type derivaties (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-hydroxyCroomeine (77) [23]. Tuberostemopirine (82) was also detected in a provenance from south China as minor compound besides dominating tuberostemone derivatives [41]. Interestingly, in that provenance the structurally simple stemoamide (73) was also found in trace amounts, which was regarded as a protostemone derivative (Fig. 5).

Summarising the data presented in Table 1 it became apparent that no protostemone-type alkaloids were detected in the two genera *Stichoneuron* and *Croomeia*, but they were otherwise dominating in most of the *Stemona* species. By contrast, stichonene derivatives represent the major alkaloids in the taxonomically complex *S. tuberosa* group, and stichonene 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. collinsiae* [43]. However, as already mentioned above, the accumulation of stichonene 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 *Croomeia*, but related derivatives (77, 80, 81) were described as major compounds for some provenances of *S. tuberosa*, obviously replacing the stichonene derivatives [23], [48]. In this connection it is noteworthy that tuberostemopirine (82) was also detected in a collection of *S. tuberosa* as minor compound together with dominating stichonene derivatives [41]. Interestingly, a croomine-type alkaloid (78) was also accumulated together with dominating protostemone 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 protostemone-type alkaloids can additionally be classified according to three different accumulations trends leading a) to protostemone (39) itself and closely related compounds, or b) to structures derived from stemofoline (45), or c) to pyridozepine alkaloids (Fig. 5). Whereas all three trends were alternately found in different provenances of *S. Curtisii* collected in south Thailand, mainly protostemone (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. cochinichinensis* and *S. collinsiae*, from which especially didehydrostemonadine (= asparagine A) (48) was shown to be a typical chemical character of *S. collinsiae*. A preferred formation of pyridoazepine-type alkaloids was found in different provenances of *S. kerrii* leading to the major compounds stemokerrine (68) and oxystemokerrine (61).
Table 2  Insecticidal (LC50) and growth inhibitory activities (EC50) of Stemona alkaloids compared with commercial azadirachtin against neonate larvae of Spodoptera littoralis

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>LC50 (95% FI) ppm</th>
<th>EC50 (95% FI) ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>didehydrostemofoline (48)</td>
<td>0.8 (0.7 – 1.1)</td>
<td>0.5 (0.3 – 0.6)</td>
</tr>
<tr>
<td>stemofoline (45)</td>
<td>2.0 (1.6 – 2.6)</td>
<td>1.5 (1.3 – 1.6)</td>
</tr>
<tr>
<td>oxystemokerine (61)</td>
<td>5.9 (4.2 – 9.1)</td>
<td>0.7 (0.1 – 1.3)</td>
</tr>
<tr>
<td>dehydroprotostemonine (41)</td>
<td>6.1 (4.3 – 9.1)</td>
<td>0.8 (0.4 – 1.3)</td>
</tr>
<tr>
<td>oxystemokerine N-oxide (63)</td>
<td>12.5 (7.2 – 22.5)</td>
<td>0.4 (0.1 – 0.9)</td>
</tr>
<tr>
<td>protostemonine (39)</td>
<td>17.7 (13.2 – 24.8)</td>
<td>2.2 (1.5 – 2.9)</td>
</tr>
<tr>
<td>2’-hydroxystemofoline (47)</td>
<td>30.3 (26.6 – 34.7)</td>
<td>38.5 (7.3 – 182.2)</td>
</tr>
<tr>
<td>stemokernine (60)</td>
<td>58.4 (48.0 – 73.0)</td>
<td>14.1 (12.0 – 16.3)</td>
</tr>
<tr>
<td>croxine (79)</td>
<td>–120</td>
<td>–20</td>
</tr>
<tr>
<td>methoxystemokernine N-oxide (64)</td>
<td>–150</td>
<td>16.3 (10.0 – 27.3)</td>
</tr>
<tr>
<td>stemocurarine (65)</td>
<td>148.9 (92.8 – 336.7)</td>
<td>96.1 (61.3 – 218.7)</td>
</tr>
<tr>
<td>oxyprotostemonine (43)</td>
<td>159.0 (99.2 – 838.8)</td>
<td>46.9 (29.9 – 75.2)</td>
</tr>
<tr>
<td>stemocurarine (44)</td>
<td>170.4 (150.4 – 199.5)</td>
<td>60.9 (37.9 – 95.8)</td>
</tr>
<tr>
<td>parvistemonine (35)</td>
<td>–350</td>
<td>162.7 (133.3 – 233.5)</td>
</tr>
<tr>
<td>tuberostemonine (1)</td>
<td>&gt; 500</td>
<td>–500</td>
</tr>
<tr>
<td>neotuberostemonine (2)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>azadirachtin^a</td>
<td>8.2 (6.6 – 11.6)</td>
<td>0.04 (0.02 – 0.07)</td>
</tr>
</tbody>
</table>

^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 LC50 (lethal concentration) and EC50 (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 parvinoestemonine (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 Stemoneae”, 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 helmith worms, in spite of the fact that ingestion of too much is potentially fatal. There is also reliable information that laiyen 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. cochinichinensis, 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-
pacity displayed higher activities [29]. By contrast, different pro-
veniences 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. collinsiae*, whereas *S. tuberosa* clearly differed by its low toxicity but remarkable repel-
rence [6]. After bioassay-guided fractionations the high insect toxici-
ties in the first three species could unambiguously be at-
tributed to derivatives of the protostemone type (Fig. 4). As
shown in Table 2 especially dihydrostemonoline (48), accumu-
lated in *S. collinsiae*, showed the highest activity with an LC50 val-
ue 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. collinsiae* this compound was ac-
 companied by smaller amounts of stemonoline (45) with an LC50
of 2.0 ppm, and 2′-hydroxystemonoline (47) with 30 ppm, de-
monstrating structure-activity relationships (Table 2): dihy-
drostemonoline (48), characterised by an unsaturated n-butenyl
side chain displayed the strongest insecticidal activity, whereas
the saturated n-butyl side chain of stemonoline (45) diminished
insecticidal properties. A significant decrease of toxicity, how-
ever, was caused by the free hydroxy group at C-2′ of the side
chain of 2′-hydroxystemonoline (47) [6]. The high insecticidal
and antifeedant properties of stemonoline derivatives were also
supported by biotests against the diamondback moth *Plutella
dylostella* L. using the leaf disk assay [11]. In that investigation
again dihydrostemonoline (48) exhibited the highest activity,
even higher than the well-known naturally occurring insecticide
rotenone. The corresponding saturated derivative stemonoline
(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 stemonoline (45) was the most active
derivative in feeding experiments with artificial diet, being about
104 times as toxic as stemospironine (78) with a croonine-type
structure (Fig. 7). The pronounced insecticidal activities of the
crude extract of *S. curtisi* and *S. cochinchinensis* could also be at-
tributed to stemonoline (45) which was accumulated as major
compound [29]. With respect to this marked insecticidal proper-
ties it was surprising that this compound was completely inac-
tive against fifth instar larvae of the cabbage army worm
*Mamestra brassicae* L. [25].

Apart from the high insecticidal properties of some stemonoline
derivatives very strong activities were also observed for the
related pyridoazepine derivative oxyostemokerrine (61) with an
LC50 value of 5.9 ppm (Table 2). This compound was found in *S.
kerrii, S. curtisi*, and in an unidentified *Stemona* species (HG
915) [29]. In the related stemonkerrine (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 (= pyrido-
stemonine) (65) with an LC50 of 148.9 ppm (Table 2). However, the
formation of the open side chain and oxygen bridge of stemonolines
and stemonkerrines appeared not to be a general prerequisite for
high insect toxicity: dehydroprostemonoline (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 oxyprostemonone (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 lar-
vae 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 be-
came apparent, that the unsaturated lactonic 4-methoxy-3-meth-
ethyl-2-furanone unit plays a crucial role. In fact, very weak or
even no activity was observed in tuberostemone (1) and neo-
tuberostemone (2) as well as in stemocochinoline (44), and par-
vistemonine (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 chem-
ical 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 sur-
pising: the larvae preferred controls even without tasting the
treated disks. The bioactive principle responsible for the high re-
pellence proved to be tuberostemone (1) demonstrating activity
levels comparable with those of azadirachtin from the neem
tree, *Azadirachta indica* A. Juss., Meliaceae. At 0.1 μg of tuberoste-
monine/cm2 the fifth instar larvae did not even taste the treated
disk, whereas the Pyrethrum extract, often described as a repel-
ent 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 bioas-
says with *Stemon* alkaloids against *Spodoptera littoralis*, differ-
ent modes of action could be observed. Apart from the different
activities of tuberostemone (1) mentioned above, the stemono-
line derivatives (45, 48) caused rapid reactions with neonate lar-
vae [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 in-
hibition, because cessation of food intake is accompanied by vo-
miting 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, mat-
ing, 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 un-
known species HG 915 were characterised by a delayed entrance
of death accompanied by softening of the larval bodies [29]. Sim-
ilar symptoms were already described for the insecticidal activ-
ity of stemospironine (78) [25].

**Medicinal properties**

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

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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 \(^{3}H\)-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 Stemonaæ, "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 spasmodytic 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 \(\beta\)-adrenoceptors. Receptor binding assays indicated that the extract interacted with the muscarinic receptors and the dihydropyridine binding site of L-type Ca\(^{2+}\) channels, but not with the histamine H\(_{1}\) 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 sticho-neurine-type alkaloids neotuberostemonine (2), tuberostemonines H (6) and J (7), epi-bisdihydropyridine-tuberostemonine J (13), and neostenine ( iso istenine) (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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