

Feeding Deterrence and Contact Toxicity of *Stemona* Alkaloids—A Source of Potent Natural Insecticides

BRIGITTE BREM,[†] CHRISTOPH SEGER,^{‡,§} THOMAS PACHER,[†] OTMAR HOFER,[‡]
 SRUNYA VAJRODAYA,^{||} AND HARALD GREGER^{*,†}

Comparative and Ecological Phytochemistry Department, Institute of Botany,
 University of Vienna, Rennweg 14, A-1030 Wien, Austria;
 Institute of Organic Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Wien, Austria; and
 Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

On the basis of chronic feeding bioassays with neonate larvae of *Spodoptera littoralis* reared on an artificial diet, the methanolic leaf and root extracts from *Stemona collinsae* displayed very high insect toxicity compared to those of two *Aglaia* species, a commercial Pyrethrum extract, and azadirachtin, whereas *S. tuberosa* extracts demonstrated low activity in roots and no activity in leaves. Beyond that, in leaf disk choice tests against fifth instar larvae, *S. collinsae* showed strong antifeedant activity, whereas *S. tuberosa* was characterized by remarkable repellency. The anti-insect properties of both species were based on pyrrolo[1,2-*a*]azepine alkaloids, from which didehydrostemofoline (asparagamine A) was the major compound of the roots of *S. collinsae*, exhibiting the highest toxicity in feeding assays. Saturation and hydroxylation of the side chain in the co-occurring stemofoline and 2'-hydroxystemofoline, respectively, led to an increasing loss of activity. Contact toxicity tests with stemofoline and didehydrostemofoline exhibited even higher activities than those of Pyrethrum extract. Tuberostemonine was the dominating alkaloid in the roots of *S. tuberosa*, showing outstanding repellency but no toxic effects.

KEYWORDS: Pyrrolo[1,2-*a*]azepine alkaloids; didehydrostemofoline; stemofoline; 2'-hydroxystemofoline; tuberostemonine; Stemonaceae; *Stemona collinsae*; *Stemona tuberosa*; insecticidal activity; antifeedant; repellent; contact toxicity; *Spodoptera littoralis*

INTRODUCTION

Stemonaceae represent a rather isolated family within the monocotyledons consisting of 3 genera and about 32 species. Their range of distribution is centered in southeast Asia but extends also to tropical Australia and with one species even to southeast United States (1, 2). *Stemona* is the largest genus with about 25 species occurring as subshrubs or twining herbs, mostly with perennial tuberous roots. Many species prefer a seasonal climate and occur in rather dry vegetation. The roots of *Stemona tuberosa* Lour., *S. japonica* (Bl.) Miq., and *S. sessilifolia* (Miq.) Miq. have long been recommended in Chinese and Japanese traditional medicine for the treatment of respiratory diseases and against enteric helminths and ectoparasites on humans and cattle (3–6). Moreover, extracts from *Stemona* species were also used against insect pests (7–10).

Even though the genus *Stemona* has long been recognized for its broad range of bioactivities, phytochemical investigations

have been restricted to only a few species (for a recent review see ref 11). Further progress was mainly hampered by using plant material which often was not properly identified. This was caused by the popular use of the tuberous fleshy roots of different species purchased on the markets under the same vernacular names such as, for example, “Bai Bu” in China, “Bach Bo” in Vietnam, or “Non Tai Yak” and “Pong Mot Ngam” in Thailand. Sometimes even representatives from different families were equally named because of the similar shape of the roots. In this context Taguchi et al. (12) already pointed out that the rotenoids reported for *Stemona collinsae* Craib (13) most likely originated from roots of the legume *Clitoria macrophylla* Wall. (see also refs 1 and 14). Later, the new alkaloid asparagamine A was reported for the tuberous roots of *Asparagus racemosus* Willd. (Asparagaceae) (15), which, however, represented a typical *Stemona* alkaloid closely related to the well-known stemofoline (16). In fact, we found large amounts of asparagamine A in *S. collinsae*, which was also isolated from the same species in a parallel investigation and named didehydrostemofoline (17). Moreover, on the basis of a colorimetric comparison of 44 *Stemona* and 9 *Asparagus* samples from different provinces in China, no alkaloids could be detected in the latter genus (18). The presumption that

* Corresponding author. Telephone: +43-1-4277-54070. E-mail: harald.greger@univie.ac.at.

[†] Comparative Phytochemistry Department, University of Vienna.

[‡] Institute of Organic Chemistry, University of Vienna.

[§] Present address: Institute of Pharmaceutical Chemistry and Pharmaceutical Technology, University of Graz, A-8010 Graz, Austria.

^{||} Kasetsart University.

Stemona has been confused with *Asparagus* was further supported by the isolation of a new dihydrophenanthrene from *A. racemosus* (19), a compound which was recently also isolated from *S. collinsae* together with related stilbenes in our laboratory (20).

Despite the well-known insect toxicity of crude extracts of some *Stemona* species, only a few investigations were carried out to determine the causative compounds. In that case they could be attributed to alkaloids uniformly characterized by a pyrrolo[1,2-*a*]azepine nucleus (7, 17, 21). However, because of the unusual molecular architecture of this class of alkaloids, most of the chemical investigations so far only reported on structure elucidation and synthesis of this family specific class of compounds (for further literature see ref 11). Detailed phytochemical comparisons between different species and geographical provenances, and bioassay of activities from various plant parts, are still lacking.

To contribute to a better understanding of the insecticidal capacities of *Stemona* alkaloids, we now compare the activities of methanolic crude extracts from the roots and leaves of *S. tuberosa* and *S. collinsae* with those of two very active *Aglaia* species (Meliaceae) using a commercial Pyrethrum extract (Fluka, Switzerland) and azadirachtin (Roth, Germany) as appropriate standards. Both standards are well-known for their feeding deterrent properties as well as insecticidal activities (22–24). In the present paper we describe the different effects of the root and leaf extracts from *S. collinsae* and *S. tuberosa* as well as from the isolated pure alkaloids against larvae of *Spodoptera littoralis* Boisduval (Lepidoptera, Noctuidae).

MATERIALS AND METHODS

General Experimental Procedures. NMR: Bruker DRX400 WB. FD-MS: Finnigan MAT 900 S. Optical rotation: Perkin-Elmer polarimeter 241. IR: Perkin-Elmer 16 PC FT-IR. UV: Hewlett-Packard, 8452A diode array spectrophotometer. HPLC: Hewlett-Packard 1090 II, UV diode array detection at 300 nm, column 250 mm × 4 mm, Hypersil BDS C-18, 5 μm, mobile phase MeOH (gradient 0–40%) in aqueous buffer (0.015 M *o*-phosphoric acid, 0.0015 M tetrabutylammonium hydroxide, pH 3), flow rate 1 mL/min.

Plant Material. *S. collinsae* (HG840) and *S. tuberosa* (HG851) originated from natural habitats in southeast Thailand: (a) *S. collinsae* from Khao Khieo near Chonburi and (b) *S. tuberosa* from Khao Chamao near Rayong. *Aglaia* species were collected in southwest Thailand near Prachuap Khiri Khan: (a) *A. edulis* (HG515) from Tap Sakae (25) and (b) *A. odorata* (HG516) from the Huay Yang waterfall. Leaves and roots (including rhizomes) from *Stemona* species were collected in flowering stage in February 2000. Voucher specimens are deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

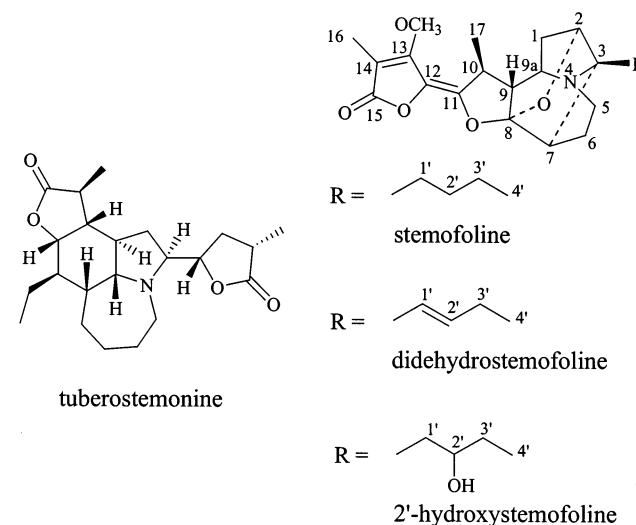
Extraction, Isolation, and Identification. The roots and rhizomes of *S. collinsae* were air-dried for 3 weeks (74 g), homogenized, and extracted twice with MeOH at room temperature for 5 days, filtered, and concentrated. The CHCl₃ fraction (750 mg) from the aqueous solution was evaporated to dryness under reduced pressure and used for comparative HPLC analyses and insect bioassays. The lipophilic crude extract was separated by column chromatography (Merck silica gel 60, 35–70 mesh) with hexane, EtOAc, and MeOH. The fractions eluted with pure EtOAc and 25% MeOH in EtOAc were combined (370 mg) and further separated by preparative MPLC (400 mm × 38 mm column, Merck LiChroprep Si 60, 25–40 mesh, UV detection, 254 nm) starting with 80% EtOAc in hexane. The fraction eluted with 100% EtOAc afforded impure dihydrostemofoline (140 mg) which yielded 20 mg of pure material by crystallization in MeOH. The MPLC fraction eluted with 100% MeOH afforded a mixture (18 mg) which was further separated by preparative TLC (Merck silica gel 60, F₂₅₄; CH₂Cl₂/EtOAc/MeOH = 70:25:5) to give 3.5 mg of stemofoline and 2.5 mg of 2'-hydroxystemofoline. Roots and rhizomes of *S. tuberosa* (550 g) were extracted as mentioned above. Impure tuberostemonine

Table 1. ¹³C NMR Data of Stemofoline Derivatives (CDCl₃, δ/ppm, TMS, 400 MHz)

position	stemofoline		dihydrostemofoline		2'-hydroxystemofoline	
1	33.3	t	32.9	t	33.7	t
2	78.6	d	79.7	d	78.7	d
3	82.8	s	83.1	s	82.7	s
5	47.6	t	48.0	t	47.1	t
6	27.3	t	26.9	t	26.6	t
7	49.9	d	51.2	d	52.7	d
8	112.7	s	112.8	s	112.0	s
9	47.6	d	47.6	d	47.6	d
9a	60.9	d	60.9	d	60.8	d
10	34.6	d	34.6	d	34.4	d
11	148.4	s	148.4	s	147.8	s
12	127.9	s	128.0	s	128.0	s
13	162.8	s	162.8	s	162.7	s
14	98.6	s	98.6	s	98.8	s
15	169.7	s	169.7	s	169.6	s
16	9.2	q	9.2	q	9.2	q
17	18.3	q	18.3	q	18.3	q
1'	26.7	t	126.5	d	36.1	t
2'	31.6	t	133.4	d	71.2	d
3'	23.1	t	25.3	t	30.6	t
4'	14.0	q	13.5	q	9.7	q
OCH ₃	58.8	q	58.8	q	58.8	q

(968 mg), already obtained from the CHCl₃ fraction by crystallization in MeOH, was further separated by column chromatography using mixtures of hexane, EtOAc, and MeOH as solvent. From the fraction eluted with 10% MeOH in EtOAc (144 mg) was obtained 10 mg of pure tuberostemonine by crystallization in MeOH.

Comparison of NMR spectra with literature data confirmed the structures of tuberostemonine (33), stemofoline, and dihydrostemofoline (15, 17). The structure of 2'-hydroxystemofoline was determined in our laboratory: [α]_D²⁰ = +197° (MeOH, c = 0.5); FD-MS *m/z* =



403 (M⁺); ¹H NMR (CDCl₃, δ/ppm, TMS, 400 MHz) 6.60 (br s, 1H, OH), 4.36 (br s, 1H, 2-H), 4.14 (s, 3H, 13-OMe), 3.63 (m, 1H, 2'-H), 3.34 (br s, 1H, 9a-H), 3.25 (ddd, 1H, *J* = 13.7, 10.6, 5.3 Hz, 5-H_α), 3.11 (dq, 1H, *J* = 9.8, 6.5 Hz, 10-H), 3.02 (ddd, 1H, *J* = 13.7, 8.8, 4.8 Hz, 5-H_β), 2.65 (s, 1H, 7-H), 2.08 (s, 3H, 14-Me), 2.05 (d, 1H, *J* = 15.0 Hz, 1-H_α), 1.97 (m, 1H, 6-H_α), 1.89 (m, 1H, 6-H_β), 1.85 (m, 1H, 1-H_β), 1.82 (dd, 1H, *J* = 9.8, 3.6 Hz, 9-H), 1.74 (dd, 1H, *J* = 14.2, 2.0, 1'-H_α), 1.64 (dd, *J* = 14.2, 10.6 Hz, 1'-H_β), 1.52 (m, 1H, 3'-H_α), 1.43 (m, 1H, 3'-H_β), 1.38 (d, 3H, *J* = 6.5 Hz, 10-Me), 0.95 (t, 3H, *J* = 7.3 Hz, 4'-Me). For ¹³C NMR data of the stemofolines see Table 1.

Feeding Bioassays with Artificial Diet. Chronic feeding bioassays were carried out with neonate larvae of the polyphagous pest insect *Spodoptera littoralis* Boisduval (Lepidoptera, Noctuidae). The larvae

Table 2. Insecticidal (LC₅₀) and Growth-Inhibitory (EC₅₀) Activities of Lipophilic Crude Extracts from *S. collinsae* and *S. tuberosa* against *Spodoptera littoralis* Compared to Those of Two Highly Active *Aglaia* Species (Meliaceae) and Commercial Pyrethrum Extract^a

plants	tissue	LC ₅₀ (ppm)	EC ₅₀ (ppm)
<i>S. collinsae</i>	leaves	15	3
	root	3	1
<i>S. tuberosa</i>	leaves	>1000	>1000
	root	581	158
<i>A. edulis</i>	leaves	>250	200
	root	33	4
<i>A. odorata</i>	leaves	130	10
	root	121	4
Pyrethrum extract		58	18

^a Chronic feeding experiments were conducted with neonate larvae ($n = 20$ for each treatment) that were forced to feed on an artificial diet spiked with six different concentrations of the tested plant extracts (0.5–50 ppm for *S. collinsae*, 50–1000 ppm for *S. tuberosa*, 1–250 ppm for *A. edulis*, 1–300 ppm for *A. odorata*, 1–100 ppm for Pyrethrum extract). After 5 days of exposure, survival and weight of the surviving larvae were determined and compared to those of controls that had been exposed to a diet treated with solvent (MeOH) only. From the dose–response curves, LC₅₀ and EC₅₀ values were calculated by probit-log analysis.

Table 3. Insecticidal (LC₅₀) and Growth-Inhibitory (EC₅₀) Activities of Four *Stemona* Alkaloids and Commercial Azadirachtin against Neonate Larvae of *Spodoptera littoralis*^a

compound	LC ₅₀ (95% FL ^b) (ppm)	EC ₅₀ (95% FL ^b) (ppm)
didehydrostemofoline	0.84 (0.66–1.06)	0.46 (0.32–0.62)
stemofoline	2.04 (1.63–2.56)	1.46 (1.33–1.60)
2'-hydroxystemofoline	30.33 (26.63–34.69)	38.47 (7.29–182.17)
tuberostemonine	>500	~500
azadirachtin	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 nonchoice test with eight different concentrations of isolated compounds (0.1–10 ppm for didehydrostemofoline and stemofoline, 10–200 ppm for hydroxystemofoline, 100–500 ppm for tuberostemonine, and 0.025–10 ppm for azadirachtin). After 5 days of exposure, survival and weight of the surviving larvae were determined and compared to those of controls that had been exposed to a diet treated with solvent (MeOH) only. From the dose–response curves, LC₅₀ and EC₅₀ values were calculated by probit-log analysis. ^b Fiducial limits.

were from a laboratory colony reared on an artificial diet under controlled conditions at 26 °C, as described previously (26). Incorporation of crude extracts or compounds into the artificial diet was performed according to standard procedures (27). The test insects ($n = 20$) were kept on an artificial diet spiked with six different concentrations of plant extracts (0.5–1000 ppm) or with eight different concentrations of the pure alkaloids (0.1–500 ppm), which were applied with MeOH. After 5 days (moist chamber, darkness, 26 °C) the survival rate and the growth of the surviving larvae were monitored in comparison to those of controls that had been exposed to diet treated with solvent only. Bioassays were conducted in duplicate (crude extracts) or in triplicate (pure compounds). From the dose–response curves, LC₅₀ and EC₅₀ (effective concentration corresponding to 50% growth-inhibition) values were calculated by probit-log analysis (28) (Tables 2 and 3).

Leaf Disk Choice Test. The leaf disk assays were carried out with fifth instar larvae of *S. littoralis* (average weight = 70 mg/larva). Leaf disks of a standard size (1.33 cm²) were cut from lettuce leaves (*Lactuca sativa* L.) with a cork borer and used as substrates for the presentation of test extracts and controls to the test insects. The leaf disks were treated on their upper surface with either 20 μL of test solutions of plant extracts at concentrations from 75 to 0.5 μg/cm² or 1–0.001 μg/cm² of pure compounds. Control leaf disks were treated with the same volume of the solvent only (MeOH, acetone in Pyrethrum extract). After the solvent was evaporated, two treated and two control leaf disks were placed on moist filter paper in a Petri dish and presented to two larvae.

Table 4. Feeding Inhibitory Activities of Lipophilic Crude Extracts from *S. collinsae* and *S. tuberosa* against *Spodoptera littoralis* Compared to Those of Commercial Pyrethrum Extract^a

plant	tissue	activity at the following concentrations of crude extract (μg/cm ² leaf disk)					
		75	40	20	10	5	0.5
<i>S. collinsae</i>	leaves	A	A	A	A	a	–
	roots	A	A	A	A	a	–
<i>S. tuberosa</i>	leaves	R	A	a	–	–	–
	roots	R	R	R	R	A	–
Pyrethrum extract		R	R	R	R	A	–

^a The feeding preference of fifth instar larvae of *S. littoralis* was observed in a leaf disk choice bioassay in order to assess repellent or antifeedant properties. The assays were conducted in triplicate. To avoid a nonchoice situation, results were taken when approximately 50% of the total area of control disks in each Petri dish was eaten. R: repellent activity, feeding inhibition without tasting treated leaf disks. A: strong antifeedant activity, less than 5% of the total area of treated leaf disks in each Petri dish was consumed. a: antifeedant activity, 5–20% of the total area of treated leaf disks in each Petri dish was consumed. –: inactive.

Table 5. Feeding Deterrence of Tuberostemonine Compared to Commercial Azadirachtin^a

test compound	feeding deterrence at the following concentrations of crude extract (μg/cm ² leaf disk)			
	1	0.1	0.01	0.001
tuberostemonine	R	R	a	–
azadirachtin	R	R	R	A

^a The feeding preference of fifth instar larvae of *S. littoralis* was observed in a leaf-disk choice bioassay in order to assess repellent or antifeedant properties. The assays were conducted in triplicate. To avoid a nonchoice situation, results were taken when approximately 50% of the control disks in each Petri dish was eaten (0.665 cm²). R: repellent activity, feeding inhibition without tasting treated leaf disks. A: strong antifeedant activity, less than 5% of the total area of treated leaf disks in each Petri dish was consumed. a: antifeedant activity, 5–20% of the total area of treated leaf disks in each Petri dish was consumed. –: inactive.

To avoid a nonchoice situation, results were taken when approximately 50% of controls (0.665 cm²) were eaten after a maximum of 24 h. On the basis of three independent trials, each experiment was conducted in duplicate, leading to six replicates with three stock solutions (Tables 4 and 5).

Contact Toxicity. The inner walls of glass vials (area = 0.75 dm²) were coated with a solution of the test compounds in MeOH or acetone (Pyrethrum extract) using concentrations from 13 to 0.1 μg/dm² or 133 μg/dm² in the cases of tuberostemonine and azadirachtin, respectively. After evaporation of the solvent, 20 neonate larvae of *S. littoralis* were introduced into the vial for a 3h period followed by administration of an artificial diet. After 48 h the survival rate was monitored and LC₅₀ values were calculated by probit-log analysis (28). Bioassays were conducted in triplicate or duplicate (tuberostemonine and azadirachtin) (Table 6).

Direct Injection into the Hemolymph. Fifty and one hundred micrograms of tuberostemonine, dissolved in 1 μL of MeOH, were injected directly into the hemolymph of sixth instar larvae (450 mg/larva, 10 larvae for each concentration), whereas controls were treated with 1 μL of MeOH only. A microsyringe (Hamilton, 701 RN, needle: 51 mm, gauge 34, pst 4) was used to inject the sample through the cuticle into the ventral abdomen without hurting the digestive tract. After 48 h, the survival rates were evaluated.

RESULTS AND DISCUSSION

Lipophilic crude extracts from the leaves and roots of *S. collinsae* and *S. tuberosa* as well as four isolated pyrrolo[1,2-*a*]azepine alkaloids were tested against larvae of *Spodoptera*

Table 6. Contact Toxicity against *Spodoptera littoralis* Compared to Commercial Pyrethrum Extract^a

compound	LC ₅₀ (95% FL) ^b (μg/dm ²)
didehydrostemofoline	1.01 (0.56–1.79)
stemofoline	0.59 (0.31–0.95)
Pyrethrum extract	4.21 (1.93–11.51)
tuberostemonine	n.a. ^c
azadirachtin	n.a. ^c

^aContact toxicity was assessed using glass vials (area = 0.75 dm²) coated with solutions of seven different concentrations of test compounds (13–0.1 μg/dm² for tuberostemonine and 133 μg/dm² for azadirachtin). After evaporation of the solvent, 20 neonate larvae were introduced into the vials, and survival rates were assessed after 48 h. From the dose–response curves, LC₅₀ values were calculated by probit-log analysis. Bioassays were conducted in triplicate or duplicate (tuberostemonine and azadirachtin). ^bFiducial limits. ^cNot active at the concentration 133 μg/dm².

littoralis for anti-insect activities. To compare the different bioactive properties, chronic feeding bioassays with an artificial diet, leaf disk choice tests, and contact toxicity bioassays were carried out. In diet feeding assays, the leaf and root extracts of *S. collinsae* (HG840) displayed the highest insecticidal activities with LC₅₀ values as low as 3 ppm for the roots and 15 ppm for the leaves. These activities are much higher than those of a commercial Pyrethrum extract purchased from Fluka Chemie (Switzerland) (**Table 2**). On the other hand, extracts from *S. tuberosa* were clearly less toxic, with LC₅₀ values of 581 ppm for the roots and >1000 ppm for the leaves. The extraordinarily high insecticidal activity of crude extracts from *S. collinsae* also became obvious when compared with those of two very active *Aglaiia* species. The genus *Aglaiia* of the Meliaceae family has recently attracted considerable attention because of the formation of genus specific flavaglines with pronounced insecticidal activities (e.g. 25, 29–32). Although the LC₅₀ and EC₅₀ values of the root extract from *A. edulis* were very low, in *S. collinsae* even the usually weaker leaf extract was more active (**Table 2**). Flavaglines from *Aglaiia* species are known as potent, but rather slow-acting, toxins, requiring 5 days for full toxicity to be expressed when incorporated into an artificial diet. In contrast, the extracts of *S. collinsae* as well as the corresponding pure alkaloids caused rapid reactions with neonate larvae: 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. In a few minutes the larvae lost the capability of coordinated locomotion and, as a consequence, any chance to leave the treated diet. Death occurred after a maximum of 1 day. Larvae tested at sublethal doses recovered from growth retarding effects and completed normal development including metamorphosis, mating, and oviposition.

In accordance with parallel findings (17), the high activity of *S. collinsae* could be attributed to the predominance of didehydrostemofoline, which was accompanied by smaller amounts of stemofoline. On the basis of our more detailed feeding experiments with an artificial diet, the LC₅₀ value at 0.84 ppm for didehydrostemofoline was clearly lower than that of stemofoline at 2.04 ppm (**Table 3**). In addition, we also isolated and identified the related 2'-hydroxystemofoline as a minor component, which was shown to be a hitherto undescribed compound. In contrast to the cases of the other two stemofoline derivatives, its insect toxicity was much lower with a LC₅₀ value at 30.33 ppm. The extract from *S. tuberosa* clearly deviated by

an accumulation of the well-known tuberostemonine (33), which could easily be obtained by crystallization of the corresponding column fractions. According to feeding assays in a nonchoice situation, tuberostemonine is only weakly active with LC₅₀ and EC₅₀ values around 500 ppm (**Table 3**). Within the stemofoline derivatives, structure–activity relationship became apparent: didehydrostemofoline, characterized by an unsaturated *n*-butenyl side chain, displayed the strongest insecticidal activity against neonate larvae of *Spodoptera littoralis*. With a LC₅₀ value at 0.84 ppm and EC₅₀ value at 0.46 ppm, the results were similar to those of azadirachtin from the neem tree *Azadirachta indica* A. Juss., one of the most promising natural insecticides investigated to date (**Table 3**). Whereas the saturated *n*-butyl side chain of stemofoline slightly diminished insecticidal properties, the free hydroxy group at C2' of hydroxystemofoline led to a significant decrease of toxicity (**Table 3**).

In addition to insecticidal activities after oral administration, the crude extracts from both *Stemona* species also showed strong feeding inhibitory properties against fifth instar larvae of *Spodoptera littoralis* in a leaf disk choice test determined in triplicate (**Table 4**). In view of the low toxicity observed for *S. tuberosa*, the high repellent activity was surprising: the larvae preferred controls even without tasting treated disks. As shown in **Table 4**, the repellency of the root extract was comparable with the activity of the Pyrethrum extract, exhibiting feeding inhibition at concentrations as low as 10 μg/cm². The extract from the dried leaves was shown to be less active, leading to feeding inhibition only at 75 μg/cm². By contrast, the extracts from *S. collinsae* clearly differed by displaying strong antifeedant properties (**Table 4**). The rapid reactions observed with neonate larvae of *Spodoptera littoralis* suggest strong toxic effects rather than simple feeding inhibition.

The bioactive principle responsible for the high repellency of *S. tuberosa* proved to be tuberostemonine, showing activity levels comparable with those of azadirachtin (**Table 5**). At 0.1 μg of tuberostemonine/cm² the fifth instar larvae did not even taste the treated disks, whereas the Pyrethrum extract, often described as a repellent agent in patent specifications (e.g. ref 22), showed no activity at the 5-fold higher concentration of 0.5 μg/cm² (**Table 4**). Because of the instability of pure tuberostemonine (Greger et al., unpublished), we suggested that its inactivity after oral administration in our nonchoice feeding experiment might be explained by higher temperatures used during preparation of the artificial diet. To avoid higher temperatures, tuberostemonine was directly injected into the hemolymph of sixth instar larvae. Even at concentrations of 100 μg/larva, the survival rate was 100%, leading to the conclusion that tuberostemonine acts as a potent repellent without toxic effects.

In a contact toxicity bioassay with neonate larvae in glass vials coated with solutions of seven different concentrations of test compounds, stemofoline and didehydrostemofoline again displayed very high activity (**Table 6**). The symptoms were similar to those after oral intake with a time delay of about 1 h. However, in contrast to the case of feeding bioassays (**Table 3**), stemofoline exhibited a somewhat higher activity than didehydrostemofoline. The insect-toxic potencies of both alkaloids exceed even those of a Pyrethrum extract, as yet probably the most frequently used commercial natural product in crop protection. By contrast, tuberostemonine and azadirachtin proved to be inactive (**Table 6**).

The four tested compounds belong to the class of pyrrolo-[1,2-*a*]azepine alkaloids only known so far from *Stemoneaceae*. Besides the well-known tuberostemonine (33), the three

stemofoline derivatives are characterized by additional C—C and oxygen bridges between the pyrrol and the azepine rings forming a cage-type molecule. An open *n*-butyl side chain is common to the stemofolines. They differ from each other by additional oxygen substituents and the formation of a double bond. Stemofoline was first isolated from *S. japonica* and described by Irie et al. (16) and was mainly characterized by X-ray analysis. The closely related dehydro derivative was later erroneously reported for *Asparagus racemosus* and named asparagamine A (15). Here again X-ray analysis was the conclusive tool for structure determination; however, no reference was given to the first publication concerning this type of compound 25 years ago. In both papers the ¹H and ¹³C NMR data were given only fragmentarily. Terminal side chain oxidation of stemofoline to 4'-hydroxy- and 4'-methoxybutyl derivatives, named oxystemofoline and methoxystemofoline, was reported for *S. parviflora* (34), and that of the (*E*)-alkene isomer isostemofoline, for various *Stemona* species (see ref 35). Corresponding to our results, stemofoline was isolated together with its dehydro derivative asparagamine A from *S. collinsae* by Jiwajinda et al. (17). In that report the complete ¹H NMR data were given for asparagamine A, which was designated as didehydrostemofoline without referring to its original description.

In the present investigation didehydrostemofoline (asparagamine A), with a (*Z*)-configured lactone ring and an (*E*)-configured double bond in the *n*-butenyl side chain, was isolated from the roots of *S. collinsae* together with stemofoline and 2'-hydroxystemofoline. All compounds were characterized by ¹H and ¹³C NMR data, including those of two-dimensional methods (H/H COSY and HSQC for C/H correlation). The chemical shift data of the cage-type pentacyclic cores of the three stemofoline derivatives were very similar; especially the ¹³C shifts indicated that only the C₄ side chain differs in this series of compounds (compare Table 1 and ref 15). The 2'-hydroxy side chain of the new 2'-hydroxystemofoline could be clearly derived from the H/H COSY spectrum, showing the connectivities CH₂—CH(OH)—CH₂—CH₃. The chemical shift values for C-17 and 17-H ($\delta_C = 71.2$ and $\delta_H = 3.62$) and the coupling pattern of 16-H₂ (only one geminal and one vicinal coupling each) were also conclusive for the position of the hydroxy group.

Regarding the high insecticidal potential of the leaf extract of *S. collinsae*, which is in the activity range of the preferentially used perennial tuberous roots, it provides an even better economic approach for insect control than the root extract, since it represents a more productive source of replenishing raw material. However, in-depth toxicological studies would be required to prove that the extracts have no adverse effects on mammals. Stemofoline was already reported to be highly toxic against silkworm larvae, *Bombyx mori*, but proved to be completely inactive against larvae of the cabbage armyworm *Mamestra brassicae*, even at concentrations as high as 100 ppm (7). In light of these results, *Stemona* species might also provide selective agricultural pesticides without harmful side effects toward nontarget organisms. The high contact toxicity as well as the rapid toxication of pest insects make their alkaloids particularly suited for application in agriculture. Moreover, because of its pronounced repellent activity, tuberostemonine could also be used as a new nontoxic pest control agent. More detailed analyses concerning the mode-of-action of various *Stemona* alkaloids are already in progress in our laboratory.

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