Alkaloids from Stems and Leaves of *Stemona japonica* and Their Insecticidal Activities

Chun-Ping Tang,[†] Tong Chen,[†] Robert Velten,[‡] Peter Jeschke,[‡] Ulrich Ebbinghaus-Kintscher,[§] Sven Geibel,[§] and Yang Ye*,[†]

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Zhangjiang High-Teck Park, Shanghai 201203, People's Republic of China, Bayer CropScience AG, Research Insecticides, Chemistry Insecticides, Alfred-Nobel-Strasse 50, Building 6240, D-40789 Monheim am Rhein, Germany, and Bayer CropScience AG, Research Insecticides, Biology Insecticides, Alfred-Nobel-Strasse 50, Building 6220, D-40789 Monheim am Rhein, Germany

Received August 15, 2007

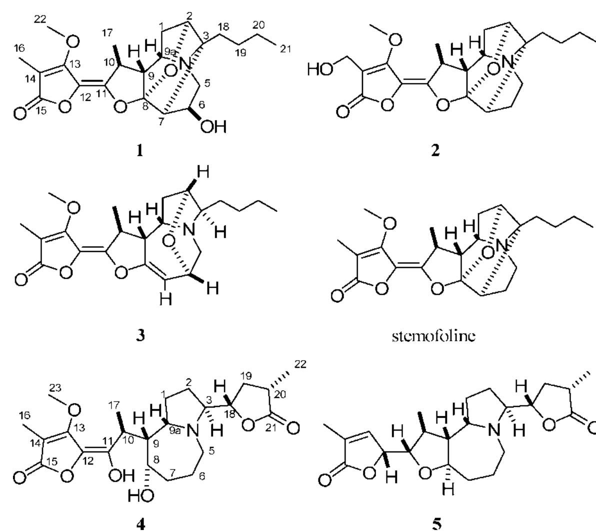
Five new alkaloids, 6*β*-hydroxystemofoline (**1**), 16-hydroxystemofoline (**2**), neostemofoline (**3**), protostemodiol (**4**), and 13-demethoxy-11(*S**),12(*R**)-dihydroprotostemonine (**5**), along with 10 known alkaloids, were isolated from stems and leaves of *Stemona japonica*. Their structures were elucidated by 1D and 2D NMR and other spectroscopic studies. The insecticidal activity of the agonist 16-hydroxystemofoline (**2**) and antagonist 13-demethoxy-11(*S**),12(*R**)-dihydroprotostemonine (**5**) was demonstrated by electrophysiological *in vitro* tests on the insect nicotinic acetylcholine receptor and by *in vivo* screenings against relevant agricultural insect pests.

Stemona japonica (Bl.) Miq. (Stemonaceae) is one of the original plants whose roots are prescribed in the Chinese Pharmacopoeia as "baibu". As a traditional Chinese medicine, baibu has been used as an antitussive and as an insecticidal agent.[1,2] Comprehensive investigations have been carried out to determine the phytochemical constituents related to these bioactivities. More than 80 alkaloids[2] have been isolated from the *Stemona* plants, and biological activities of some major components were described. Tuberostemonine-type alkaloids were reported to exhibit significant antitussive activity in the guinea pig cough model induced by citric acid aerosol stimulation.[3] Bisdehydrostemoninine, one of the stemonine-type alkaloids from *S. tuberosa*, also shows significant antitussive activity in the same model.[4] Insecticidal activity was demonstrated by stemofoline and its analogues, as well as by some extracts of *Stemona* plants.[5,6]

To further study the insecticidal constituents of baibu, we examined stems and leaves of *S. japonica*. Stemofoline and 15 other alkaloids have been reported previously from this plant.[7–12] In this paper, five new alkaloids (**1**–**5**), together with 10 known compounds, stemonamine,[8] isostemonamine,[8] maistemonine,[14] isomaistemonine,[12] stemofoline,[13] isostemofoline,[13] protostemonine,[7] isoprotostemonine,[9] croomine,[15] and stemocochinin,[16] were isolated. Stemofoline and the new isolates were tested for their *in vitro* and *in vivo* insecticidal activities.

Results and Discussion

Compound **1** was obtained as a yellow, amorphous powder. The molecular formula was determined by HREIMS to be $C_{22}H_{29}NO_6$, one more oxygen atom than that of stemofoline. The strong absorption band at 3435 cm^{-1} in the IR spectrum suggested the presence of a hydroxyl group. The ^1H NMR spectrum displayed a methyl triplet at δ_H 0.89, a methyl doublet at δ_H 1.38, a methyl singlet at δ_H 2.05, and a *O*-methyl singlet at δ_H 4.13, which were all characteristic of stemofoline-type structures. Oxymethine resonances (δ_H 4.80/δ_C 74.7) in **1** instead of the methylene resonances in stemofoline suggested that **1** was an OH derivative of stemofoline. The OH group was placed at C-6 by the key HMBC correlation between C-6 (δ_C 74.7) and H-5 (δ_H 2.94), as well as by cross-peaks of H-6/H-5 and H-6/H-7 in the $^1H-^1H$ COSY spectrum. The relative configuration of **1** was determined by the ROESY experiment. ROESY correlations of H-9/H-9a, H-9/H-5*β*, H-9/H_3-17, H-9a/H-5*β*, H-9a/H_3-17, and H-5α/H_2-18 reflected the same cage conformation in the molecule as that in stemofoline. The α-orientation of H-6 was supported by correlations of H-6/H_2-18, together with the absence of correlations between H-6 and H-9*β*. Thus, the OH at C-6 was *β*-oriented. ^1H NMR and ^{13}C NMR resonances were assigned using COSY, ROESY, HMQC, and HMBC experiments. Accordingly, compound **1**was designated as 6*β*-hydroxystemofoline.



Compound **2** was obtained as a yellow, amorphous powder. The molecular formula $C_{22}H_{29}NO_6$ was established by HREIMS and ^{13}C NMR data, the same as that of **1**. The IR absorption band at 3427 cm^{-1} suggested the presence of an OH group. Analysis of the ^1H and ^{13}C NMR data indicated its stemofoline-type structural skeleton (Table 1). In comparison with stemofoline, the characteristic C-16 methyl resonance was absent and an additional oxymethylene resonance appeared at δ 4.50 (2H, s) in the ^1H NMR spectrum. Therefore, compound **2** was another oxidative product of stemofoline, where the OH group was attached to C-16. This assignment was supported by HMBC correlations between H_2-16 and C-13, C-14, and C-15. The relative stereochemistry of **2** was confirmed by the ROESY experiment.

Neostemofoline (**3**) was obtained as a colorless oil ($C_{22}H_{29}NO_5$ by HRESIMS, the same molecular formula as stemofoline). Compound **3** is also a stemofoline-type alkaloid, as indicated by characteristic ^1H NMR resonances [δ 0.93 (3H, t, $J = 7.0$ Hz, Me-

* To whom correspondence should be addressed. Tel: +86-21-50806726. Fax: +86-21-50807088. E-mail: yye@mail.shcnc.ac.cn.
† Shanghai Institute of Materia Medica, Chinese Academy of Sciences.
‡ Bayer CropScience AG, Research Insecticides, Chemistry Insecticides.
§ Bayer CropScience AG, Research Insecticides, Biology Insecticides.

10.1021/np070427k CCC: $40.75 © 2008 American Chemical Society and American Society of Pharmacognosy
Published on Web 12/29/2007

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1−3** in $CDCl_3$ (δ in ppm and J in Hz)

position	δ_H [c]			δ_C		
	1 [a]	**2** [a]	**3** [b]	**1** [a]	**2** [a]	**3** [a]
1	1.71 (ddd, 11.7, 3.0, 3.2)	1.71 (ddd, 12.2, 3.3, 3.3)	1.85 (2H, m)	33.8	33.3	25.8
	2.05 (m)	1.94 (d, 12.1)				
2	4.17 (s)	4.25 (br s)	2.50 (m)	78.8	78.6	65.3
3			3.76 (m)	84.0	82.9	73.6
5	2.94 (dd, 14.5, 3.6)	2.99 (ddd, 13.8, 8.4, 4.6)	2.82 (dd, 15.0, 0)	54.7	47.6	45.4
	3.50 (dd, 14.4, 8.8)	3.12 (m)	3.48 (dd, 15.0, 6.8)			
6	4.80 (ddd, 8.8, 5.9, 3.6)	1.82 (m)	4.20 (dd, 7.4, 6.8)	74.7	26.7	64.8
		1.90 (m)				
7	2.76 (d, 5.9)	2.70 (d, 6.0)	5.55 (dd, 7.4, 2.4)	53.7	50.0	104.5
8				112.0	113.0	159.5
9	2.80 (dd, 10.1, 3.6)	1.81 (dd, 6.5, 3.5)	3.06 (m)	47.9	47.6	49.1
9a	3.55 (m)	3.48 (m)	3.76 (m)	60.8	61.0	61.2
10	3.10 (dq, 10.0, 6.6)	3.11 (dq, 6.5, 3.5)	3.00 (dq, 6.6, 0)	34.9	35.0	38.3
11				148.5	151.0	145.2
12				127.7	127.5	125.6
13				162.9	165.5	162.7
14				98.5	101.6	98.3
15				169.8	169.3	169.7
16	2.05 (3H, s)	4.50 (2H, s)	2.09 (3H, s)	9.2	54.0	9.2
17	1.38 (3H, d, 6.6)	1.37 (3H, d, 6.5)	1.51 (3H, d, 6.5)	18.2	18.2	19.7
18	1.56 (2H, m)	1.56 (2H, m)	1.85 (m)	31.6	31.6	35.2
			2.13 (m)			
19	1.19 (m)	1.24 (m)	1.38 (2H, m)	27.4	27.4	29.5
	1.32 (m)	1.48 (m)				
20	1.34 (2H, m)	1.34 (2H, m)	1.38 (2H, m)	23.0	23.2	22.9
21	0.89 (3H, t, 7.0)	0.91 (3H, t, 7.1)	0.93 (3H, t, 7.0)	14.0	14.0	14.0
22	4.13 (3H, s)	4.28 (3H, s)	4.16 (3H, s)	58.8	59.5	58.9

[a] Recorded on a Bruker AM-400 NMR spectrometer. [b] Recorded on a Bruker AC-300 NMR spectrometer. [c] All integrals not indicated in the table are 1H.

21); 1.51 (3H, d, J = 6.5 Hz, Me-17); 2.09 (3H, s, Me-16); 4.16 (3H, s, OMe-22)] (Table 1). The 1D and 2D NMR data of **3** and stemofoline revealed that the side chain and rings C and D were similar. The olefinic proton resonance at δ 5.55 (1H, dd, J = 7.4, 2.4 Hz) and the carbon resonances at δ 104.5 and 159.5 indicated the presence of a double bond in the structure. The double bond was placed between C-7 and C-8 by the HMBC correlations between the olefinic carbon at δ 104.5 and the protons at δ 2.82 and 3.48 (featured for H-5 of stemofolines) and the proton at δ 3.06 (featured for H-9). The assignment was supported also by the correlation between the other olefinic carbon at δ 159.5 and the proton at δ 3.76 (featured for H-9a). Moreover, three structural segments, H-5 to H-6 [δ 4.20 (1H, dd, J = 7.4, 6.8 Hz)] to H-7, H-9a to H-1 to H-2 [δ 2.50 (1H, m)], and Me-21 to H-20 to H-19 to H-18 to H-3 [δ 3.76 (1H, m)], were deduced from correlations in the $^{1}H-^{1}H$ COSY spectrum. The low-field chemical shifts of H-2 and H-6 indicated that C-2 and C-6 bore oxygen functional groups. Thus an oxygen bridge was constructed between C-2 and C-6. Such assignment suggested a stable half-cage conformation in this molecule. The ROESY correlation of H-9aβ/H-2 indicated that H-2 was β-orientated and that the oxygen bridge was on the other face. The other correlations observed in the ROESY spectrum, such as H-9aβ/H$_2$-18, H-9β/H-5β, H-1α/H-6, and H-1α/H-7, supported the half-cage conformation and the α-orientation of H-3 (Figure 1). Therefore, the structure of **3** was fully established.

Protostemodiol (**4**) was obtained as a yellow, amorphous powder, $C_{23}H_{33}NO_7$ by HREIMS. EIMS showed a fragment peak at m/z 336, ascribable to the loss of the α-methyl γ-lactone ring ($C_5H_7O_2$). The IR spectrum showed OH absorption (3435 cm^{-1}) and absorptions indicating both α-methyl γ-lactone and α,β-unsaturated γ-lactone moieties (1766, 1732, 1684, and 1612 cm^{-1}). In the ^{13}C NMR spectrum, 23 carbon resonances resembled those of protostemonine. The ^{1}H NMR spectrum also featured the proton resonances for protostemonines [δ 1.28 (3H, d, J = 7.1 Hz, Me-22); 1.34 (3H, d, J = 7.2 Hz, Me-17); 2.07 (3H, s, Me-16) and 4.17 (3H, s, OMe-23)] (Table 2). Analyses of 1D and 2D NMR data resulted in the conclusion that **4** contained the protostemonine skeleton. The molecular weight of **4** was 18 amu more than that of

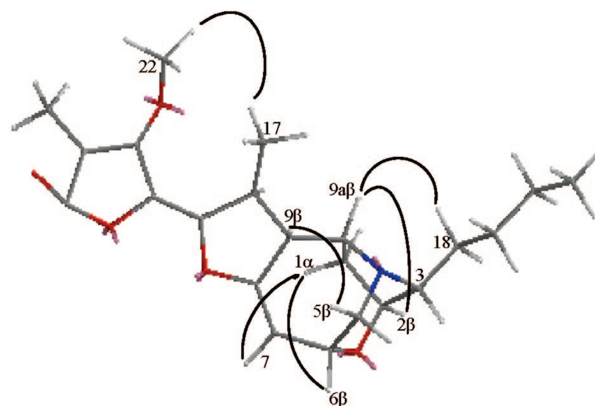


Figure 1. Key ROESY correlations and the possible conformation of **3** generated by computer.

protostemonine, consistent with an additional molecule of H_2O in **4**. Thus **4** was assumed to be a cleavage product (so-called hydrolysate) of protostemonine and the hydrolyzation might take place in the ring D, the same as in the case of stemodiol.[17] ROESY correlations of H-8/H-9aβ, H-8/H-9β, and H-8/H$_3$-17 indicated that H-8 was β-orientated and that the OH at this position was α-orientated. The configuration at C-10 was assumed to be the same, S*, as in protostemonine-type alkaloids.

Compound **5** was isolated as a colorless oil by preparative TLC, along with a known compound, stemocochinin.[16] They behaved similarly on TLC, and neither of them showed UV absorptions at 254 and 365 nm. The molecular formula of **5** ($C_{22}H_{31}NO_5$) was the same as that of stemocochinin. The ^{1}H and ^{13}C NMR spectra of **5** and stemocochinin were nearly superimposable, except for tiny differences in some proton resonances such as H-10, H-11, and H-13 (Table 2). The two compounds appeared to differ in configuration at the 11- and 12-positions. The relative configuration of **5** was established by the key ROESY correlations and further confirmed by the HGS molecular model. Once H-11 was assigned

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **4** and **5** in $CDCl_3$ (δ in ppm and J in Hz)

position	δ_H[d]		δ_C	
	4[a]	**5**[b]	**4**[a]	**5**[c]
1	1.44 (m)	1.81 (m)	26.9	26.8
	1.98 (m)	1.58 (m)		
2	1.94 (m)	1.92 (m)	29.1	26.7
	1.40 (m)	1.39 (m)		
3	3.25 (m)	3.24 (ddd, 5.6, 7.4, 9.6)	61.9	64.5
5	3.71 (2H, m)	2.89 (dd, 16.2, 15.6)	62.1	47.4
		3.45 (dd, 16.2, 5.2)		
6	1.70 (m)	1.39 (2H, m)	29.2	19.9
	1.72 (m)			
7	1.76 (2H, m)	1.28 (m)	33.6	35.1
		2.11 (m)		
8	4.32 (m)	3.91 (ddd, 10.2, 10.2, 4.0)	86.7	79.9
9	1.87 (m)	1.99 (m)	55.7	55.1
9a	3.21 (m)	3.63 (ddd, 5.0, 5.6, 10.5)	60.0	59.0
10	3.13 (dq, 7.2, 1.4)	1.90 (m)	39.6	40.6
11		3.52 (dd, 6.4, 7.6)	149.8	85.0
12		4.84 (m)	123.0	83.1
13		7.16 (m)	163.6	146.9
14			95.8	130.6
15			170.5	174.1
16	2.07 (3H, s)	1.93 (3H, s)	9.1	10.7
17	1.34 (3H, d, 7.2)	1.06 (3H, d, 6.2)	22.7	16.7
18	4.16 (m)	4.14 (ddd, 5.3, 7.5, 11.1)	81.0	83.1
19	1.52 (m)	1.53 (m)	34.1	34.4
	2.43 (ddd, 12.6, 8.7, 5.6)	2.36 (m)		
20	2.70 (m)	2.60 (m)	35.7	34.9
21			179.3	179.5
22	1.28 (3H, d, 7.1)	1.26 (3H, d, 7.0)	15.1	14.8
23	4.17 (3H, s)		59.0	

[a] Recorded on a Bruker DRM-400 NMR spectrometer. [b] Recorded on a Bruker AC-300 NMR spectrometer. [c] Recorded on a Bruker AM-400 NMR spectrometer. [d] All integrals not indicated in the table are 1H.

to the β-orientation by the ROESY correlations from H-11 to H-9β and H_3-17, the key correlations of H-12/H-10, H-13/H-11, and H-13/H-10 allowed only the R*-configuration for C-12 in the HGS molecular model. In comparison with the corresponding J value of 11(S),12(S)-dihydrostemofoline, the $J_{11,12}$ value (ca 7.0 Hz) further supported the above conclusion.[18] Accordingly, compound **5** was established as 13-demethoxy-11(S*),12(R*)-dihydroprotostemonine.

The configuration at C-11 and C-12 in stemocochinin was not determined completely in the previous publication.[16] In fact, stemocochinin was found to be very unstable in organic solvents, especially in chloroform. The relative configuration of stemocochinin could still be assumed by comparison with the literature data. Wang et al. reported a pair of C-12 isomers, saxorumamide and isosaxorumamide, whose structures were very similar to **5** and stemocochinin except for the absence of ring E and the presence of a ketone at C-3.[19] The corresponding NMR resonances of stemocochinin and **5** agreed well with those of saxorumamide and isosaxorumamide, respectively.[19] In the ^{1}H NMR spectrum of stemocochinin, the $J_{11,12}$ value (ca. 3.0 Hz) was clearly identified, which was consistent with the corresponding J value in 11(S),12(R)-dihydrostemofoline. Therefore, stemocochinin was deduced to be 13-demethoxy-11(S*),12(S*)-dihydroprotostemonine.

Similar to commercialized neonicotinoids,[20] the mode of action (MoA) of stemofoline was determined to be agonist at insect nicotinic acetylcholine receptors (nAChRs) by using biochemical and electrophysiological approaches.[21] The pure alkaloid had fast acting insecticidal, antifeedant, and repellent activities,[16,18] but was significantly lower in activity than commercial products. Therefore, screening of the stemofoline-type compounds was carried out in order to identify novel derivatives having higher potency than stemofoline.

It was found that all stemofolines tested *in vitro* are active on isolated neurons from the pest insect *Heliothis virescens*. Stemofoline, **1**, **2**, and **3** act as agonists, whereas **4** and **5** are antagonists at the insect nAChR. For compound **1**, an EC_{50} of 157 nM indicated lower potency than stemofoline (EC_{50}: 50 nM), whereas **2** showed a stronger effect (EC_{50}: 12 nM) than stemofoline. The position of the OH group (6β-position/cage structure vs 16-position/side chain) in the molecule is significant for the electrophysiological *in vitro* effect. Compound **4** was only a weak antagonist (IC_{50} around 10 μM) at the *Heliothis* nAChR. Compound **5** at 10 μM blocked more than 90% of the response induced by 10 μM acetylcholine and was slightly more active than **4**.

As shown by *in vivo* screening results, stemofoline was the most active against sucking and chewing insects. Lower activity was observed for **2**, whereas **1** was inactive at 500 g/ha. The lower *in vivo* activity of **2**, in comparison to stemofoline, could be due to reduced uptake and/or rapid metabolism. This could also explain why **1** was completely inactive *in vivo* at the application rate of 500 g/ha.

In conclusion, *Stemona* alkaloids of four types, stemofoline-type, protostemonine-type, maistemonine-type, and croomine-type, have been isolated from stems and leaves of *S. japonica.* Stemofoline-type alkaloids, especially stemofoline, are the main constituents of the crude alkaloidal extract. We believe that the significant insecticidal activity of the stemofolines is closely related to the plant defense system. The insecticidal stemofolines can efficiently prevent insects from biting the aerial parts of the plant, and stemofolines are present in significant amounts in stems and leaves but are nearly undetectable in roots.

The cage structural moiety of stemofolines is pivotal to their insecticidal activity. Any variations in this moiety weaken the activity. Compound **1**, with an OH group at C-6, exhibited lower activity than its nonhydroxy counterpart stemofoline. The half-cage derivative (**3**) is a partial agonist due to the significant changes in the cage moiety. Electrophysiological investigations demonstrated that the agonist (**2**), which has the cage structure, has an effect at the insect nAChR similar to that of stemofoline.

Experimental Section

General Experimental Procedures. Optical rotations were taken on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a

Nicolet Magna FT-IR 750 spectrophotometer with KBr disks. 1D and 2D NMR spectra were recorded on Bruker AM-400, Bruker DRM-400, Bruker AC-300, and INVOR-600 NMR spectrometers. The chemical shift (δ) values are given in ppm with residual $CHCl_3$ (δ_H 7.26, δ_C 77.0) as internal standard, and coupling constants (J) are in Hz. EIMS and HREIMS spectra were recorded on a Finnigan MAT-95 mass spectrometer. ESIMS and HRESIMS spectra were recorded on a Micromass LC-MS-MS mass spectrometer. CC and TLC were carried out using silica gel obtained from Qingdao Ocean Chemical Co.

Plant Material. The stems and leaves of *Stemona japonica* were collected in 1998 from Anji County, Zhejiang Province, China, and identified by Professor Jin-Gui Shen. A voucher specimen (No. 1999301) has been deposited at the Herbarium in Shanghai Institute of Materia Medica.

Extraction and Isolation. The dried and ground stems and leaves of *S. japonica* (ca. 20 kg) were macerated three times during a 3-day period with 25 L of 95% EtOH, and the combined EtOH extracts were pooled and evaporated under reduced pressure. The residue was treated with 2% HCl to pH 1–2 and extracted three times with Et_2O. Then, the acidic solution was basified with concentrated ammonia solution to pH 9–10 and extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were evaporated under reduced pressure, yielding 40.7 g of crude alkaloid. The crude extract (40.0 g) was dissolved in 250 mL of acetone. Stemofoline was then crystallized from the filtered solution as white needles. Recrystallization from acetone yielded 31.5 g of stemofoline. The mother liquid was concentrated, and the residue (6.4 g) was subjected to column chromatography (CC) over silica gel and eluted with petroleum ether−acetone (3:1, 2:1, 1:1) and then acetone, giving fractions A−S. These fractions were further purified by repeated CC over silica gel and then Sephadex LH-20. Stemonamine (20 mg) and isostemonamine (25 mg) were isolated from fraction A, maistemonine (120 mg) and isomaistemonine (40 mg) were obtained from fraction B, and isostemofoline (360 mg) was obtained from fraction C. Fractions D−F were combined and dissolved in hot acetone, and 1.4 g of stemofoline crystallized as small white needles. Protostemonine (250 mg) was obtained from fraction I using a RP-18 column eluted with MeOH−H_2O (6:4). Similarly, isoprotostemonine (120 mg) was purified from fraction J. From faction K compound **1** (60 mg) was separated by CC over silica gel eluted with $CHCl_3$−MeOH (15:1). Fractions M and N were combined and subjected to a RP-18 column eluted with MeOH−H_2O in a step gradient (30%−70%), yielding compounds **2** (60 mg) and **3** (8 mg). Croomine (8 mg), accompanied by a mixture, was isolated from fraction R. This mixture was further purified by preparative TLC to yield stemocochinin (20 mg) and **5** (40 mg). Compound **4** (120 mg) was isolated from fraction S by a RP-18 column eluted with MeOH−H_2O (30%–50%).

6*β*-Hydroxystemofoline (1): yellow, amorphous powder; $[\alpha]^{20}_D$ +490.0 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 295.5 (4.70) nm; IR (KBr) ν_{max} 3435, 2956, 1747, 1687, 1620, 1460, 1396, 1365, 1219, 1149, 1051, 1012, 991, 756, 673 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS m/z 403 $[M]^+$, 386, 220 (100), 136; HREIMS m/z 403.1999 (calcd for $C_{22}H_{29}NO_6$, 403.1995).

16-Hydroxystemofoline (2): yellow, amorphous powder; $[\alpha]^{20}_D$ +286.7 (c 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 296 (4.39) nm; IR (KBr) ν_{max} 3427, 1743, 1684, 1608, 1462, 1254, 1180, 1140, 997, 777, 685 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS m/z 403 $[M]^+$ (100), 386, 136; HREIMS m/z 403.1984 (calcd for $C_{22}H_{29}NO_6$, 403.1995).

Neostemofoline (3): colorless oil; $[\alpha]^{23}_D$ +230.0 (c 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 305.5 (4.32) nm; IR (KBr) ν_{max} 3433, 2956, 2929, 2872, 1749, 1672, 1622, 1460, 1209, 1165, 1055, 1018, 754 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS (positive) m/z 388.2111 $[M + H]^+$ (calcd for $C_{22}H_{30}NO_5$, 388.2124).

Protostemodiol (4): yellow, amorphous powder; $[\alpha]^{23}_D$ +124.7 (c 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 305.5 (4.25) nm; IR (KBr) ν_{max} 3435, 1766, 1732, 1684, 1612, 1460, 1400, 1277, 1215, 1159, 1126, 1061, 1016, 933, 756, 675 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; EIMS m/z 435 $[M]^+$, 336, 168; ESIMS m/z 436 $[M + H]^+$, 872 $[2M + 2H]^+$, 1307 $[3M + 2H]^+$; HREIMS m/z 435.2242 (calcd for $C_{23}H_{33}NO_7$, 435.2257).

13-Demethoxy-11(*S),12(*R**)-dihydroprotostemonine (5):** yellow oil; $[\alpha]^{23}_D$ +53.3 (c 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 294 (3.49) nm; IR (KBr) ν_{max} 3435, 2962, 2875, 1761, 1657, 1456, 1159, 1045, 729 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; EIMS m/z 389 $[M]^+$, 292, 290 (100), 136; HRESIMS (positive) m/z 390.2307 $[M + H]^+$ (calcd for $C_{22}H_{32}NO_5$, 390.2280).

***In Vitro* Assay.** Electrophysiological recordings were done on isolated neuronal cell bodies obtained from fifth instar *Heliothis virescens* larvae, as described elsewhere, with slight modification.[22] In short, the whole nervous systems of several larvae were dissected and placed into dissociation buffer (Sigma C-1419). The ganglia were incubated for 5 min at 37 °C with 4 mg/mL Dispase, centrifuged, and resuspended in culture buffer by gentle aspiration with a fire-polished pasteur pipet. Cell somata were plated onto glass coverslips previously coated with concanavalin-A (400 μg/mL) and laminin (4 μg/mL). The cells were kept at room temperature in a culture medium based on supplemented Leibowitz's L-15 medium and used for experiments on the following 4 days. Electrophysiological recordings were done with the whole-cell voltage clamp technique.[23] The microelectrodes were pulled from borosilicate glass capillaries. The resistance of the fire-polished pipettes was 4–7 MΩ using the internal and external solutions described below. All experiments were done at room temperature (22–25 °C). The cells were placed in a perfusion chamber of approximately 0.5 mL volume and superfused continuously (flow rate 3 mL/min) with external bath solution driven by gravity. The fluid in the chamber was thereby renewed every 10 s and completely washed out in less than 60 s. The external bath contained 150 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM Hepes (pH 7.4 adjusted with NaOH). The (internal) pipet solution contained 120 mM CsF, 30 mM CsCl, 10 mM Cs-EGTA, 1 mM $CaCl_2$, and 10 mM Hepes (pH 7.4 adjusted with CsOH). Currents were measured with an L/M-EPC 7 patch clamp amplifier (List, Darmstadt, Germany). The holding potential was −70 mV. Current records were low-pass Bessel filtered at 315 Hz and digitized at 1 kHz sample rate. Data storage and analysis were done with the pClamp V 6.03 software package (Axon Instruments, Foster City, CA). Stemofolines and acetylcholine were applied to the cells using the U-tube-reversed-flow technique with applications of 2−4 s duration at intervals of 1 min.

***In Vivo* Assay.** To determine the insecticidal activity of *Stemona* sp. alkaloids, a standard screening on the agriculturally relevant pest-insects such as caterpillars of the armyworm (*Spodoptera frugiperda*) and green peach aphid (*Myzus persicae*) with the test compounds administered by foliar spray application (500, 100, 20, and 4 g/ha) was used. Cabbage leaves (*Brassica oleracea*), heavily infested by one of the insect species, were treated with the *Stemona* sp. alkaloids of the desired concentration. After the desired period of time, the percent killed is determined; 100% means that all caterpillars or aphids have been killed.

Acknowledgment. Financial support from the Ministry of Science and Technology (2004CB518902) and from the Shanghai Commission of Science and Technology (06DZ22028) is gratefully acknowledged.

Supporting Information Available: ^{13}C and ^1H NMR and ROESY spectra for compounds **1**−**5** and ^{13}C and ^1H NMR spectra for stemocochinin are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Pilli, R. A.; Ferreira de Oliveira, M. C. *Nat. Prod. Rep.* **2000**, *17*, 117–127.
(2) Greger, H. *Planta Med.* **2006**, *72*, 99–113.
(3) Chung, H.-S.; Hon, P.-M.; Lin, G.; But, P. P.-H.; Dong, H. *Planta Med.* **2003**, *69*, 914–920.
(4) Lin, L.-G.; Zhong, Q.-X.; Cheng, T.-Y.; Tang, C.-P.; Ke, C.-Q.; Lin, G.; Ye, Y. *J. Nat. Prod.* **2006**, *69*, 1051–1054.
(5) Ujvary, I. In *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*; Yamamoto I., Casida J. E., Eds.; Springer Press: Tokyo, 1999; pp 29–69.
(6) Brem, B.; Seger, C.; Pacher, T.; Hofer, O.; Vajrodaya, S.; Greger, H. *J. Agric. Food Chem.* **2002**, *50*, 6383–6388.
(7) Irie, H.; Harada, H.; Ohno, K.; Mizutuni, T.; Uyeo, S. *J. Chem. Soc. D* **1970**, 268–269.
(8) Iizuka, H.; Irie, H.; Masaki, N.; Osaki, K.; Uyeo, S. *J. Chem. Soc., Chem. Commun.* **1973**, 125–126.
(9) Ye, Y.; Xu, R.-S. *Chin. Chem. Lett.* **1992**, *3*, 511–514.
(10) Ye, Y.; Qin, G.-W.; Xu, R.-S. *Phytochemistry* **1994**, *37*, 1205–1208.
(11) Ye, Y.; Qin, G.-W.; Xu, R.-S. *J. Nat. Prod.* **1994**, *57*, 665–669.
(12) Zou, C.-Y.; Fu, H.-Z.; Lei, H.-M.; Li, J.; Lin, W.-H. *J. Chin. Pharm. Sci.* **1999**, *8*, 185–190.
(13) Irie, H.; Harada, H.; Ohno, K.; Mizutani, T.; Uyeo, S. *Chem. Commun.* **1970**, 1066.

(14) Lin, W.-H.; Ye, Y.; Xu, R.-S. *Chin. Chem. Lett.* **1991**, *2*, 369–370.
(15) Xu, R.-S.; Lu, Y.-J.; Chu, J.-H.; Iwashita, T.; Naoki, H.; Naya, Y.; Nakanishi, K. *Tetrahedron* **1982**, *38*, 2667–2670.
(16) Kaltenegger, E.; Brem, B.; Mereiter, K.; Kalchhauser, H.; Kählig, H.; Hofer, O.; Vajrodaya, S.; Greger, H. *Phytochemistry* **2003**, *63*, 803–816.
(17) Ye, Y.; Xu, R.-S. *Chin. Chem. Lett.* **1992**, *3*, 511–514.
(18) Mungkornasawakul, P.; Pyne, S. G.; Jatisatienr, A.; Supyen, D.; Jatisatienr, Ch.; Lie, W.; Ung, A. T. *J. Nat. Prod.* **2004**, *67*, 675–677.
(19) Wang, Y.-Z.; Tang, C.-P.; Dien, P.-H.; Ye, Y. *J. Nat. Prod.* **2007**, *70*, 1356–1359.
(20) Jeschke, P.; Nauen, R. *Comprehensive Molecular Insect Science*; Gilbert, L. I., Latrou, K., Sargeet, S., Eds.; Elsevier Ltd.: Oxford, UK, 2005; Vol. *5*, p 53−105.
(21) Lind, R. J.; Greenhow, D. T.; Blythe, J.; Goodchild, J.; Hirst, E.; Dunbar, S. J.; Earley, F. G. P. *BCPC Conf. Pests Dis.* **2002**, *1*, 145–152.
(22) Oland, L. A.; Müller, T.; Kettenmann, H.; Hayashi, J. J. *Neurosci. Methods* **1996**, *69*, 103–112.
(23) Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. *Pflügers Arch.* **1981**, *391*, 85–100.

NP070427K