Antitussive Stemoninine Alkaloids from the Roots of *Stemona tuberosa*

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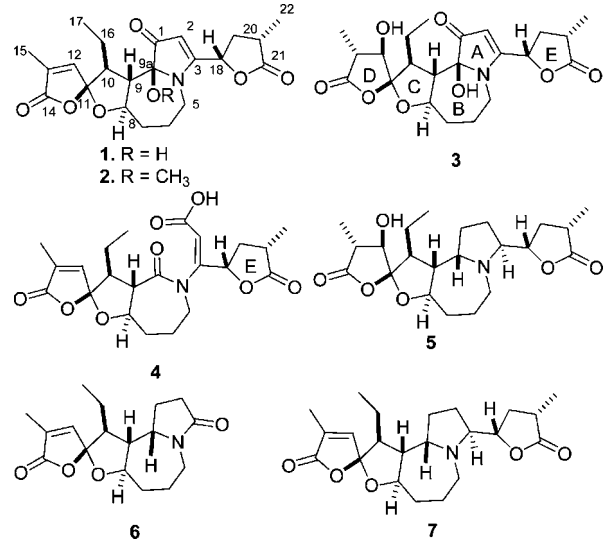
Investigation of the roots of *Stemona tuberosa* afforded five minor constituents, stemoenonine (**1**), 9a-*O*-methylstemoenonine (**2**), oxystemoenonine (**3**), 1,9a-*seco*-stemoenonine (**4**), and oxystemoninine (**5**), along with the known compound stemoninoamide (**6**). Their structures were elucidated by 1D and 2D NMR spectra and other spectroscopic studies. Alkaloids **1**, **2**, and **6**, as well as the representative stemoninine-type alkaloid, stemoninine (**7**), were screened for antitussive activity in the citric acid-induced guinea pig cough model. Compounds **6** and **7** exhibited strong antitussive activity after oral and intraperitoneal administrations.

We recently described the strong antitussive activity of bisdehydrostemoninine in the citric acid-induced guinea pig cough model.[1] This is the first demonstration of the *in vivo* antitussive effect of a pure stemoninine-type alkaloid, though the crude alkaloids of *Stemona tuberosa*, containing stemoninine as the main component, were found to show such an effect.[2] The major alkaloids of *S. tuberosa* were believed to be responsible for its bioactivities, but these constituents varied greatly with localities according to the previous reports.[2,3] So far three types of *Stemona* alkaloids, tuberostemonine, stemoninine, and croomine types, were identified from this species.[4] However, we found that *S. tuberosa* growing in Hainan produced specifically stemoninine-type alkaloids. This motivated us to conduct a further investigation on stemoninine analogues and their antitussive activities, especially stemoninine, the known representative of this structural type.

We performed a more extensive and thorough investigation on the residue extract of *S. tuberosa* collected in Hainan Province. Preparative HPLC was applied to search for minor compounds with the characteristic UV absorptions of stemoninine-type alkaloids. Five new minor constituents, stemoenonine (**1**), 9a-*O*-methylstemoenonine (**2**), oxystemoenonine (**3**), 1,9a-*seco*-stemoenonine (**4**), and oxystemoninine (**5**), and the known stemoninoamide (**6**) were isolated and identified. Alkaloids **1**, **2**, and **6**, as well as stemoninine (**7**), were screened in the *in vivo* citric acid-induced guinea pig cough model. Compounds **6** and **7**exhibited dose-dependent inhibition of citric acid-induced coughing.

Stemoenonine (**1**) was obtained as colorless needles by crystallization from benzene. Its molecular formula was determined to be $C_{22}H_{27}NO_7$ by elemental analysis (*anal.* C 63.38%, H 6.53%, N 3.36%). The IR absorption at 1770 cm^{-1} and the characteristic EIMS cleavage fragment at 318 $[M - C_5H_7O_2]^+$ indicated the presence of an α-methyl-γ-lactone moiety located at C-3. The ^1H NMR spectrum (Table 1) displayed three methyl resonances at δ_H 1.08 (3H, d, $J = 6.7$), 1.10 (3H, t, $J = 7.5$), and 1.85 (3H, d, $J = 1.4$) and three downfield protons at δ_H 6.86 (1H, q, $J = 1.4$), 5.50 (1H, dd, $J = 6.1, 9.5$), and 5.47 (1H, s), which were characteristic of a stemoninine-type skeleton.[1,5] The IR absorption at 3442 cm^{-1} suggested the presence of a hydroxy group. The D_2O exchangeable signal at δ_H 3.87 was assigned as a hydroxy proton. The ^{13}C NMR and DEPT spectra (Table 2) indicated 22 carbons including three CH_3, five CH_2, seven CH, and seven quaternary carbons. Analysis of 1D and 2D NMR data of **1** and bisdehydrostemoninine[1] revealed that rings B, C, D, and E were similar. Three segments were



revealed by $^1H-^1H$ COSY (bold lines in Figure 1) and further connected by the HMBC experiment (broken arrows in Figure 1). The HMBC correlations from the carbonyl carbon (δ_C199.5) to H-2 and H-9 suggested that the carbonyl was located at C-1, and the HMBC correlations from the quaternary carbon (δ_C 88.5) to H-2, H-8, and H-9 indicated that this carbon was C-9a and the hydroxy group was attached to this position. Thus, an enone moiety was present in ring A. Such a conclusion was further supported by the maximum UV absorption at 345 nm.

The relative configuation of **1** was determined by the ROESY experiment (Figure 2). The correlations of H-9/H-5b and H-9/H-7b indicated that the seven-membered ring was in a stable chair-type conformation and the 9a-hydroxy group was β-oriented. The correlations between H-10/H-8 and H-10/H-12 suggested an 11*R**-configuration. Detailed assignments of the ^1H and ^{13}C NMR signals were performed by HMQC, HMBC, and $^1H-^1H$ COSY experiments (Tables 1 and 2).

9a-*O*-Methylstemoenonine (**2**) was obtained as a yellow, amorphous powder. The molecular formula $C_{23}H_{29}NO_7$ was inferred from HREIMS (*m/z* 431.1940 $[M]^+$). The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1**, except for an additional *O*-methyl signal (δ_H 3.46/δ_C 50.8). The HMBC correlation from the *O*-methyl to C-9a suggested that this methoxy group was located at C-9a. The relative configuration of **2** was determined to be the same as that of **1** by the ROESY experiment. The ROESY correlation between the *O*-methyl and H-9 and H_3-17 indicated that this methoxy group was β-oriented.

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Table 1. ^{1}H NMR Data of Alkaloids **1**−**5** (400 MHz)

C	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
1					a 1.94, m; b 1.58, m
2	5.47, s	5.18, s	5.11, s	6.13, s	a 1.51, m; b 1.51, m
3					3.30, m
5a	3.62, m	3.53, m	3.51, m	3.95, m	3.50, d (14.0)
5b	3.69, m	3.63, m	3.51, m	3.28, m	2.93, dd (14.0, 15.7)
6a	1.62, m	1.62, m	1.82, m	1.98, m	1.92, m
6b	1.62, m	1.62, m	1.63, m	1.62, m	1.55, m
7a	2.14, bd (12.4)	2.13, m	2.18, m	2.32, m	2.12, m
7b	1.80, m	1.81, m	1.94, m	1.83, m	1.49, m
8	3.77, dd (10.1, 10.6)	3.50, m	3.53, m	4.00, m	3.96, dt (3.2, 10.2)
9	2.97, dd (10.1, 9.9)	2.81, m	2.51, t (10.3)	3.20, t (10.8)	2.38, m
9a					3.78, m
10	3.40, m	3.40, m	2.81, m	2.71, m	2.06, t (6.0)
12	6.86, q (1.4)	6.62, q (1.3)	3.98, d (9.9)	6.73, d (1.6)	3.81, d (10.0)
13			2.57, dq (9.9, 6.9)		2.58, m
15	1.85, d (1.4)	1.85, d (1.4)	1.29, d (7.0)	1.96, d (1.6)	1.33, d (7.1)
16	2.66, m	2.65, m	2.03, m	1.50, m	2.01, m
16	1.79, m	1.79, m	1.62, m	1.40, m	1.64, m
17	1.10, t (7.5)	1.10, t (7.5)	1.01, t (7.4)	0.82, t (7.3)	0.98, t (7.5)
18	5.50, dd (6.1, 9.5)	5.50, dd (6.1, 9.5)	5.20, m	4.98, m	4.29, m
19a	2.80, m	2.76, m	2.83, m	2.72, m	2.38, m
19b	1.37, m	1.35, m	1.94, m	2.60, m	1.52, m
20	2.87, m	2.85, m	2.79, m	1.94, m	2.61, m
22	1.08, d (6.7)	1.10, d (6.7)	1.32, d (6.7)	1.31, d (6.9)	1.26, d (6.6)
OMe		3.46, s			

[a] In C_5D_5N. [b] In $CDCl_3$.

Table 2. ^{13}C NMR Data of Alkaloids **1**−**5** (100 MHz)

C	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
1	199.5 C	198.9 C	198.8 C	166.8 C	26.6 CH_2
2	94.3 CH	94.6 CH	94.1 CH	117.9 CH	20.8 CH_2
3	177.5 C	177.4 C	178.0 C	155.3 C	63.6 CH
5	41.5 CH_2	41.8 CH_2	41.8 CH_2	51.0 CH_2	45.7 CH_2
6	26.6 CH_2	26.3 CH_2	26.2 CH_2	25.7 CH_2	26.4 CH_2
7	36.0 CH_2	35.1 CH_2	35.6 CH_2	35.9 CH_2	35.6 CH_2
8	80.0 CH	79.1 CH	79.4 CH	79.7 CH	81.7 CH
9	55.7 CH	54.4 CH	54.7 CH	53.8 CH	52.4 CH
9a	88.5 C	88.3 C	88.5 C	172.4 C	58.5 CH
10	47.8 CH	47.2 CH	42.9 CH	49.5 CH	47.1 CH
11	114.5 C	113.8 C	113.5 C	113.0 C	112.6 C
12	146.4 CH	144.9 CH	75.8 CH	144.5 CH	75.8 CH
13	133.2 C	133.4 C	41.5 CH	133.8 C	41.9 CH
14	172.0 C	171.8 C	175.8 C	171.4 C	175.2 C
15	10.4 CH_3	10.5 CH_3	12.4 CH_3	10.5 CH_3	12.6 CH_3
16	20.8 CH_2	20.0 CH_2	20.6 CH_2	20.3 CH_2	20.1 CH_2
17	13.1 CH_3	12.7 CH_3	12.1 CH_3	12.6 CH_3	12.3 CH_3
18	71.9 CH	71.1 CH	71.2 CH	78.0 CH	82.5 CH
19	36.5 CH_2	36.4 CH_2	36.3 CH_2	35.6 CH_2	34.3 CH_2
20	34.9 CH	34.9 CH	34.9 CH	35.1 CH	34.8 CH
21	178.1 C	177.9 C	177.5 C	178.1 C	179.3 C
22	15.4 CH_3	15.3 CH_3	15.2 CH_3	15.0 CH_3	14.8 CH_3
OMe		50.8 CH_3			

[a] In C_5D_5N. [b] In $CDCl_3$.

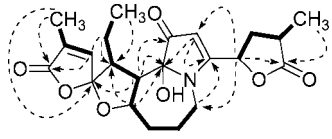


Figure 1. Important ^{1}H−^{1}H COSY (bold lines) and key HMBC correlations (broken arrows) of compound **1**.

The molecular formula of **3** was determined by HRESIMS to be $C_{22}H_{29}NO_8$ (*m*/*z* 436.1986 $[M + 1]^+$), with nine unsaturation degrees. The IR spectrum indicated the presence of a hydroxy group (3425 cm^{-1}) and a lactone moiety (1774 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **3** (Tables 1 and 2) revealed a stemoninine-type basic skeleton. The enone moiety of ring A was retained in **3**, but the signals of the allylic moiety in ring D of **1** and **2** were replaced by signals of a methyl doublet (δ_H 1.29/δ_C 12.4), a methine double-quartet (δ_H 2.57/δ_C 41.5), and an oxymethine doublet (δ_H 3.98/δ_C 75.8) in **3**. These data suggested that the double bond in ring D might be hydrated. The assumption was supported by HMBC correlations of C-14/H-12, C-14/H-15, C-12/H-15, and C-11/H-13. The relative configuration of **3** was fixed by its ROESY spectrum (Figure 2). The correlations of H-9/H-5b and H-9/H-7b indicated a stable chair-type conformation of the seven-membered ring and a β-orientation of the C-9a hydroxy group. The cross-peaks between H-10/H-12 and H-12/H_3-15 indicated that the 12-hydroxy group was β-orientated and the methyl was α-orientated.

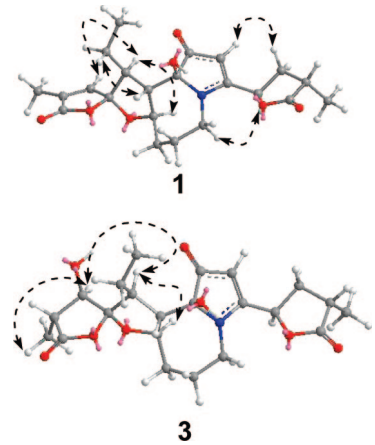


Figure 2. Key NOE correlations (broken arrow) and possible conformation of **1** and **3**.

Compound **4** was obtained as a yellow, amorphous powder. Its molecular formula was established by HRESIMS as $C_{22}H_{27}NO_8$ with 10 unsaturation degrees. The IR absorption band at 1724 cm^{-1} indicated the presence of a carboxylic carbonyl, which was supported by the characteristic ion peak at 388 $[M - COOH]^+$ in the EIMS. The IR absorptions at 1770 and 1655 cm^{-1} suggested the existence of lactone and lactam moieties, which were in accordance with the carbonyl signals at δ_C 171.4, 172.4, and 178.1 in the ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR data of rings B, C, D, and E of **4** were very similar to those of **1** (Tables 1 and 2). In ring A, a carboxylic carbonyl at δ_C 166.8 and a lactam carbonyl at δ_C 172.4 instead of the carbonyl (C-1) and the quaternary carbon (C-9a) in **1** were observed in **4**. These changes suggested that the

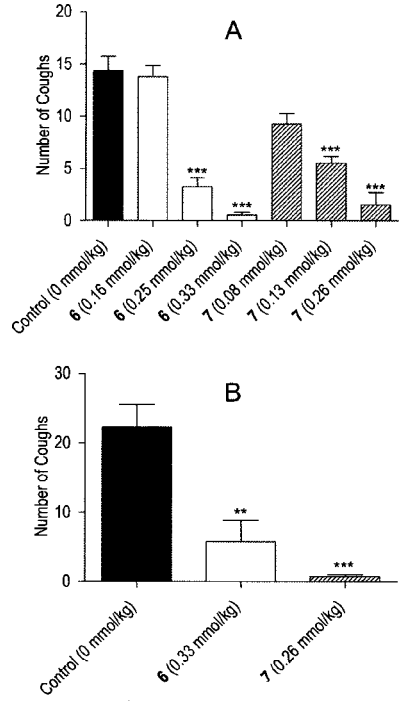


Figure 3. Antitussive activity of alkaloids **6** and **7** in guinea pigs treated with a single ip (A) and oral (B) dose. Data are expressed as mean ± SEM ($n = 4-8$). $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the corresponding vehicle control.

linkage between C-1 and C-9a was broken and ring A was opened, which was supported by the HMBC correlations from H-9 to C-9a and from H-2 to C-1. The relative configuration of **4** was also shown to be the same as that of **1** by its ROESY experiment. Thus, **4** was established as an unprecedented 1,9a-*seco*-stemoenonine.

The HRESIMS of oxystemoninine **5**, exhibiting the $[M + H]^+$ peak at *m/z* 408.2374, established the molecular formula as $C_{22}H_{33}NO_6$. The IR absorptions at 3438 and 1770 cm^{-1} indicated the presence of a hydroxy and a lactone group, respectively. The ^1H and ^{13}C NMR spectra of **5** were similar to those of stemoninine,[5] except for the signals of ring D, which closely resembled those of **3**. Thus, an α-methyl-β-hydroxylactone ring D was revealed in this molecule. The structure of **5** was confirmed by HMBC and $^1H-^1H$ COSY experiments. The relative configuration was determined by its ROESY spectrum.

The compounds that were available in sufficient quantities for the antitussive study using the guinea pig citric acid model were stemoenonine (**1**), 9a-*O*-methylstemoenonine (**2**), stemoninoamide (**6**), and stemoninine (**7**). The representative stemoninine-type alkaloid **7** and the major alkaloid **6**exhibited a similar dose-dependent inhibition of citric acid-induced coughing with similar ID_{50} values of 0.197 mmol/kg (CI: 0.101 to 0.305) and 0.130 mmol/kg (CI: 0.067 to 0.198), respectively. At the highest ip doses (0.33 mmol/kg for **6** and 0.26 mmol/kg for **7**) both alkaloids showed about a 90% inhibition of coughing ($P < 0.001$; Figure 3A). Both compounds were also administered orally and had a comparable potency to reduce coughing by over 70% ($P < 0.01$; Figure 3B). However, no significant differences in antitussive activity were observed between oral and ip routes of **6** at 0.33 mmol/kg and **7** at 0.26 mmol/kg ($P > 0.05$), suggesting that both alkaloids might have a good oral absorption. Alkaloids **1** and **2** were available only in small quantities and could be evaluated only in comparison with **6** and **7** for antitussive activity following icv administration. Compared with the vehicle control, all four alkaloids decreased coughing, but the inhibition was not significant (~10−40% inhibition, $P > 0.05$; Figure 4). The relatively lower antitussive activity via icv administration is probably due to the low dosage administered and/or the weak action of the alkaloids in the central nervous system. Further investigations are warranted to identify whether the antitussive activity of these alkaloids is mediated centrally and/or peripherally.

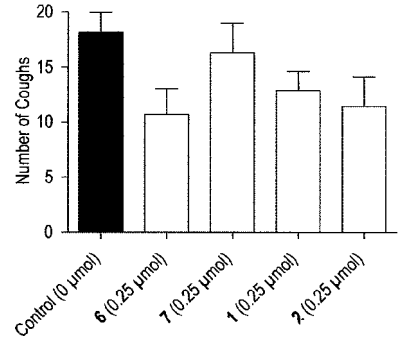
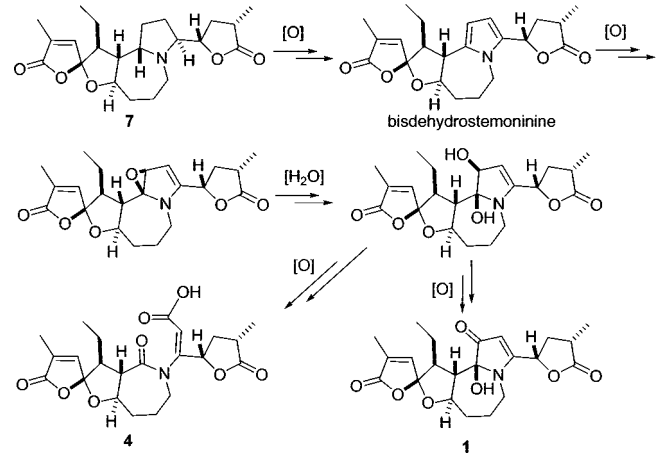


Figure 4. Antitussive activity of alkaloids **1**, **2**, **6**, and **7** in guinea pigs treated with a single icv dose. Data are expressed as mean ± SEM.

Scheme 1. Proposed Biosynthetic Pathway of Compounds **1** and **4**



In conclusion, five new minor constituents (**1**−**5**) were isolated from *S. tuberosa*. All these compounds contain a basic stemoninine-type skeleton. From a structural point of view, they can be considered as the oxidative products of stemoninine. Since these new compounds can be detected in the crude alkaloid fraction by HPLC methods, they are indeed naturally occurring constituents in this plant (HPLC profiles in the Supporting Information). These stemoninine-type alkaloids, together with those reported in our last paper, indicated that the stemoninines were the representative type of *Stemona* alkaloids in Hainan *S. tuberosa*.[1] The major alkaloids, stemoninine, bisdehydrostemoninine, and stemonamide, showed strong antitussive effects in the citric acid-induced guinea pig cough model, respectively, suggesting that Hainan *S. tuberosa* could be used as an anticough agent.

In addition, stemoninine derivatives showed diverse rings A and D, which is not often seen in reported *Stemona* alklaoids. Compounds **1**−**3** were found for the first time to possess an enone fragment in ring A among the isolated *Stemona* alkaloids. Alkaloid **4** is the first example of a 1,9a-*seco Stemona* alkaloid.[2−4,6] The proposed biosynthetic pathway is indicated in Scheme 1. The pyrrolidine ring in **7** is dehydrogenated to a pyrrole ring in bisdehydrostemoninine. Then a double bond is epoxidized and hydrolyzed to form the vicinal dihydroxy functionality. One hydroxy group is then oxidized to form an enone fragment. Alternative oxidations would form the carboxylic acid **4** and lactam **1**.

Experimental Section

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

Nicolet Magna FT-IR 750 spectrophotometer with KBr disks. NMR spectra were recorded on Bruker AM-400 and INVOR-600 NMR spectrometers. The chemical shift (δ) values are given in ppm with TMS 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. Analytical HPLC was performed on a Waters 2690 instrument equipped with a 996 PAD (photodiode array detector) and coupled with an Alltech ELSD 2000 detector. Chromatographic separation was carried out on a C18 column (125 × 4.0 mm, 5 μm, Merck), using a gradient solvent system comprised of H_2O (A) and CH_3CN (B), with a flow rate of 1.0 mL/min. The temperature for the ELSD drift tube was set at 105 °C, and the air flow was 3.2 L/min. Preparative HPLC was performed on a Varian SD1 instrument equipped with a 320 single wave detector. Chromatographic separation was carried out on C18 columns (220 × 25 mm, 10 μm, Merck), using a gradient solvent system comprised of H_2O (A) and CH_3CN (B), with a flow rate of 15 mL/min. Si was used for flash chromatography and was produced by Qingdao Marine Chemical Industrials. TLC was carried out on precoated Si GF254 plates (Yantai Chemical Industrials), and the TLC spots were viewed at 254 nm and visualized by spraying Dragendorff's reagent. The sample of stemoninine was obtained from another batch of Hainan *S. tuberosa* species in a previous investigation.

Plant Material. The plant material (7.0 kg) was collected in Hainan Province, China, and was identified by Professor Yi Zhong. A voucher specimen (YYE-20020612) was deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried roots of *S. tuberosa* were ground into powder and extracted with 95% EtOH. After evaporation of the collected percolate, the crude extract was acidified with dilute HCl (4%) to pH = 1−2 and partitioned between Et_2O and H_2O. The aqueous part was basified with aqueous NH_3 to pH = 9−10 and extracted with Et_2O to afford 88 g of crude alkaloid. This residue was subjected to chromatography over Si and eluted with petroleum ether−acetone (2:1, 1:1, 1:2, 1:3) and acetone and MeOH (each 2 L) to yield six major fractions. Fractions 4 and 5 were rechromatographed over Si, preparative HPLC, and Sephadex LH-20 to obtain stemoenonine (**1**) (53 mg), 9a-*O*-methylstemoenonine (**2**) (48 mg), oxystemoenonine (**3**) (16 mg), 1,9a-*seco*-stemoenonine (**4**) (21 mg), oxystemoninine (**5**) (13 mg), and stemoninoamide (**6**) (1.0 g).

Stemoenonine (1): colorless needles (benzene); mp 192.5−195.0 °C; $[\alpha]^{20}_D$ +10.5 (*c* 1.6, MeOH); IR ν_{max} (KBr) 3442, 1799, 1770, 1650, 1500, 11509, 1030, 970 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; EIMS *m*/*z* 417 $[M]^+$, 344, 318, 232, 176, 175, 108, 83; *anal*. C 63.38%, H 6.53%, N 3.36%, calcd for $C_{22}H_{27}NO_7$ C 63.29%, H 6.51%, N 3.35%.

9a-*O*-Methylstemoenonine (2): yellow, amorphous powder; $[\alpha]^{20}_D$ −8 (*c* 0.17, $CHCl_3$); IR ν_{max} (KBr) 3415 (weak, water), 1767, 1670, 1497, 1450, 1128, 970, 758 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; EIMS *m*/*z* 431 $[M]^+$, 417, 399, 370, 344, 316, 232; HREIMS *m*/*z*431.1940 (calcd for $C_{23}H_{29}NO_7$, 431.1944).

Oxystemoenonine (3): yellow, amorphous solid; $[\alpha]^{20}_D$ −2 (*c* 0.10, $CHCl_3$); IR ν_{max} (KBr) 3425, 1774, 1651, 1456, 1198, 1030, 937 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; EIMS *m*/*z* 435 $[M]^+$, 417, 362, 334, 294, 232; ESIMS *m*/*z* 436.2 $[M + 1]^+$, 458.2 $[M + Na]^+$, 434.3 $[M - 1]^-$; HRESIMS *m*/*z*436.1986 (calcd for $C_{22}H_{30}NO_8$, 436.2001).

1,9a-*seco*-Stemoenonine (4): yellow, amorphous powder; $[\alpha]^{20}_D$ −47 (*c* 0.20, $CHCl_3$); IR ν_{max} (KBr) 3442, 1770, 1724, 1655, 1425, 1164, 1022, 974, 876, 762 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; EIMS *m*/*z* 433 $[M]^+$, 415, 388 $[M - COOH]^+$, 345, 331, 317, 303, 276, 194; ESIMS *m*/*z* 434.2 $[M + 1]^+$, 432.3 $[M - 1]^-$; HRESIMS *m*/*z* 434.1798 (calcd for $C_{22}H_{28}NO_8$, 434.1815).

Oxystemoninine (5): yellow, amorphous powder; $[\alpha]^{20}_D$ −36 (*c* 0.12, $CHCl_3$); IR ν_{max} (KBr) 3439, 2933, 1770, 1458, 1194, 939 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; ESIMS *m*/*z* 408.4 $[M + 1]^+$; HRESIMS *m*/*z* 408.2374 (calcd for $C_{22}H_{34}NO_6$, 408.2362).

Antitussive Activity of Alkaloids. The well-established citric acid-induced guinea pig cough model[1,7] is generally recognized as the most relevant model for predicting the clinical efficacy of drugs treating cough in man.[8,9] Briefly, unrestrained conscious Dunkin-Hartley guinea pigs were randomly divided into groups for different treatments. A single dose of alkaloid **6** (50, 75, and 100 mg/kg) or **7** (30, 50, and 100 mg/kg) was given intraperitoneally or orally (100 mg/kg for both alkaloids), respectively. Furthermore, due to the limited amounts of alkaloids **1** and **2**, a single dose (0.25 μM in 10 μL) of these two compounds was given via intracerebroventricular (icv) injection. The same icv administration was also conducted for alkaloids **6** and **7** for comparison. A standard stereotaxic procedure[10] was used for the icv injection. Briefly, the anaesthetized individual animal was placed in a stereotaxic apparatus. A 22 gauge stainless steel guide cannula was inserted into the lateral ventricle and anchored to the skull by two 3.2 mm stainless steel mounting screws and dental acrylic. After 4 days recovery, the animal was subjected to icv injection of individual compound through the cannula with a maximum volume of 10 μL.

The treated animal was individually placed into a transparent Perspex airtight chamber. At 30 min after ip and oral treatment or 3 min after icv injection, each animal was exposed to 0.5 M citric acid aerosols for 8 min with a flow rate of 0.5 mL/min. During the aerosol exposure, the animal was continuously monitored, and cough sounds were recorded and analyzed by Cool Edit 2000 software (Syntrillium, Phoenix, AZ). Cough episodes were determined using our previously developed software CoughCount-CHHK (2003 copyright).[7,10] For ip administration, the alkaloids were dissolved in DMSO, while for icv and oral administration, the alkaloids were dissolved in Tween 80 in saline (5:95, v/v). All studies were conducted in parallel. Antitussive activity was evaluated and expressed as the percentage of cough inhibition based on the comparison of numbers of cough episodes recorded in the treated group with the corresponding vehicle control group. Statistical analysis between control and treated groups was calculated using a one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison tests. Statistical analysis of differences between ip and oral effect estimates (weighted mean differences) was calculated using *z*-tests. *P* values less than 0.05 were considered significant. ID_{50} values were calculated by linear regression analysis.

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Supporting Information Available: Spectroscopic data of compounds **1**−**5** and HPLC profiles for the extract and the crude alkaloid of *S. tuberosa* and the major alkaloids **1**, **2**, and **4**−**6** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) 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.
(2) Xu, Y.-T.; Hon, P.-M.; Jiang, R.-W.; Cheng, L.; Li, S.-H.; Chan, Y.-P.; Xu, H.-X.; Shaw, P.-C.; But, P. P.-H. *J. Ethnopharmacol.* **2006**, *108*, 46–53.
(3) Jiang, R.-W.; Hon, P.-M.; Zhou, Y.; Chan, Y.-M.; Xu, Y.-T.; Xu, H.-X.; Greger, H.; Shaw, P.-C.; But, P. P-H. *J. Nat. Prod.* **2006**, *69*, 749–754.
(4) Greger, H. *Planta Med.* **2006**, *72*, 99–113.
(5) Cheng, D.-L.; Guo, J. *J. Nat. Prod.* **1988**, *51*, 202–211.
(6) *Jiangsu New Medical College. Dictionary of Chinese Traditional Medicine*; Shanghai People's Publishing House: Shanghai, P. R. China, 1977; pp 858−861.
(7) Chung, H.-S.; Hon, P. M.; Lin, G.; But, P. P.-H.; Dong, H. *Planta Med.* **2003**, *69*, 914–920.
(8) Karlsson, J. A.; Fuller, R. W. *Pulm. Pharmacol. Ther.* **1999**, *12*, 215–228.
(9) Belvisi, M. G.; Bolser, D. C. *Pulm. Pharmacol. Ther.* **2002**, *15*, 249–250.
(10) McLeod, R. L.; Gertner, S. B.; Hey, J. A. *Eur. J. Pharmacol.* **1991**, *209*, 141–142.

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