Benzylphenethylamine Alkaloids from *Hosta plantaginea* with Inhibitory Activity against Tobacco Mosaic Virus and Acetylcholinesterase

Yue-Hu Wang,[†,‡] Zhong-Kai Zhang,[§] Fu-Mei Yang,[⊥] Qian-Yun Sun,[⊥] Hong-Ping He,[†] Ying-Tong Di,[†,‡] Shu-Zhen Mu,[⊥,‡] Yang Lu,[∥] Ying Chang,[∥] Qi-Tai Zheng,[∥] Ming Ding,[§] Jia-Hong Dong,[§] and Xiao-Jiang Hao*[,†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China, Yunnan Key Laboratory of Agricultural Biotechnology, Yunnan Academy of Agricultural Sciences, Kunming 650223, People's Republic of China, Key Laboratory of Chemistry for Natural Products, Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, People's Republic of China, and Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, People's Republic of China

Five new benzylphenethylamine alkaloids, hostasine (**1**), 8-demethoxyhostasine, 8-demethoxy-10-*O*-methylhostasine, 10-*O*-methylhostasine, and 9-*O*-demethyl-7-*O*-methyllycorenine, along with 12 known compounds, were isolated from *Hosta plantaginea* by bioassay-guided fractionation. The structures of the new alkaloids were established by means of extensive spectroscopic methods, and the relative configuration of **1** was further confirmed by single-crystal X-ray diffraction. 7-Deoxy-*trans*-dihydronarciclasine ($IC_{50} = 1.80$ μM), a known alkaloid, showed strong activity against tobacco mosaic virus by the half-leaf method. Some of these alkaloids were also evaluated for their inhibitory activity against acetylcholinesterase. 8-Demethoxy-10-*O*-methylhostasine was found to possess significant activity, with an IC_{50} of 2.32 μM.

In our continuing investigation on active substances against tobacco mosaic virus (TMV) from plants,[1] a perennial herb, *Hosta plantaginea* (Lam.) Aschers (Liliaceae), was selected for study. This plant is widely distributed in the south of China. The whole plant is used to treat mastitis, tympanitis, ulcers, and carbuncles as a folk medicine in China.[2] The inhibitory effect of the crude extract from *H. plantaginea* against TMV has been previously reported,[3] but the chemical basis for this activity is unclear. The genus *Hosta* mainly contains glycosides of flavonoids and steroids, and some of the steroidal saponins show cytotoxicity against leukaemia HL-60 cells and human cervical carcinoma HeLa cells.[4]

In the present study, five new benzylphenethylamine alkaloids (hostasine, **1**; 8-demethoxyhostasine, **2**; 8-demethoxy-10-*O*-methylhostasine, **3**; 10-*O*-methylhostasine, **4**; and 9-*O*-demethyl-7-*O*-methyllycorenine, **5**), together with 12 known ones, were isolated from the whole plant of *H. plantaginea* by bioassay-guided fractionation. One of the known compounds, 7-deoxy-*trans*-dihydronarciclasine (**6**),[5] exhibited strong activity against TMV, with an IC_{50} value of 1.80 μM. In view of some benzylphenethylamine alkaloids such as galantamine and ungeremine being acetylcholinesterase (AChE) inhibitors,[6] 10 alkaloids from this plant were selected to test for inhibitory activity against AChE.

The results of bioactive screening and the structural elucidation of five new alkaloids are reported.

Results and Discussion

Isolation of Compounds and Antiviral Activity. The whole plant of *H. plantaginea*, collected from Guangzhou, P. R. China, was exhaustively extracted with 70% EtOH, and the extract (1000 g, 20.8%) was suspended in H_2O and partitioned to three fractions, petroleum (A, 80 g, 1.7%), EtOAc (B, 60 g, 1.2%), and H_2O (C, 800 g, 16.7%). Subsequently, fraction C was partitioned to three further fractions, H_2O (C_1, 600 g, 12.5%), MeOH (C_2, 110 g, 2.3%), and Me_2CO (C_3, 6 g, 0.1%), by D_{101} resin column chromatography.
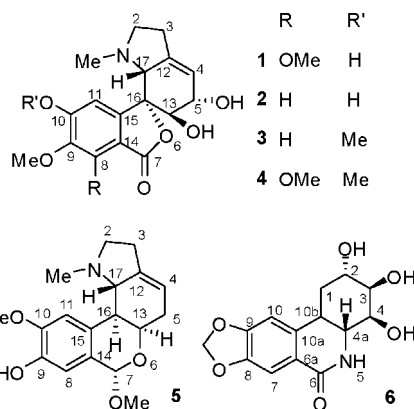
After a primary screening, fractions B and C_2 were found to be active against TMV, with inhibitory ratios of 91.4% and 100%, respectively, at 50 μg/mL. These fractions afforded 17 benzylphenethylamine alkaloids comprising five skeletal types. These included five new alkaloids, **1**–**5**, along with 12 known compounds, 7-deoxy-*trans*-dihydronarciclasine (**6**),[5] *O*-methyllycorenine,[7] albomaculine,[8] (+)-haemanthamine,[9] *O*-demethylhaemanthamine,[10] 8-*O*-demethylmaritidine,[10] haemanthidine,[11] yemenine C,[12] lycorine,[13] pseudolycorine,[14] ungeremine,[15] and norsanguinine.[16]

Seven compounds, representing all five skeletal types of alkaloids from this plant (7-deoxy-*trans*-dihydronarciclasine (**6**) of the isocarbostyril-type, 8-demethoxy-10-*O*-methylhostasine (**3**) and albomaculine of the lycorenine-type, *O*-demethylhaemanthamine and haemanthidine of the crinine-type, lycorine of the lycorine-type, and norsanguinine of the galanthamine-type), were tested for anti-TMV activity by the half-leaf method. 7-Deoxy-*trans*-dihydronarciclasine, which was isolated from both fractions B and C_2, showed inhibitory activity against TMV, with an IC_{50} value of 1.80 μM. The other six alkaloids tested were inactive.

The anti-TMV activity of 7-deoxy-*trans*-dihydronarciclasine was stronger than that of the positive control, ribavirin, with an IC_{50} value of 2989.60 μM under the conditions described in this assay. Effective TMV antagonists from plants are rare. An example is 7-demethoxytylophorine (antofine), a phenanthroindolizidine alkaloid from *Cynanchum komarovii*, which showed 65% inhibition against TMV at a concentration of 1.0 μg/mL.[17]

* To whom correspondence should be addressed. Tel: +86 0871 522 3263. Fax: +86 0871 521 9684. E-mail: haoxj@mail.kib.ac.cn.
† Kunming Institute of Botany.
‡ Graduate University of the Chinese Academy of Sciences.
§ Yunnan Academy of Agricultural Sciences.
⊥ Institute of Materia Medica.
∥ Key Laboratory of Chemistry for Natural Products.

Table 1. AChE Inhibitory Activity of Alkaloids from *H. plantaginea*

| compound	IC$_{50}$ (μM)
tacrine (positive control)	0.20
ungeremine	3.85
norsanguinine	1.43
8-demethoxy-10-*O*-methylhostasine (**3**)	2.32
10-*O*-methylhostasine (**4**)	113
lycorine	155
(+)-haemanthamine	452
albomaculine	inactive
7-deoxy-*trans*-dihydronarciclasine	inactive
haemanthidine	inactive
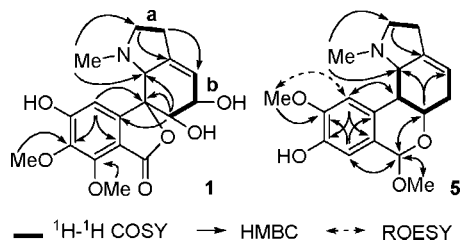
Figure 1. Key 2D NMR correlations of **1** and **5**.

AChE Inhibitory Activity. Some benzylphenethylamine alkaloids, such as galantamine (IC$_{50}$ = 1.07 μM), 11-hydroxygalantamine (IC$_{50}$ = 1.61 μM), epinorglantamine (IC$_{50}$ = 9.01 μM), sanguinine (IC$_{50}$ = 0.10 μM), assoanine (IC$_{50}$ = 3.87 μM), 1-*O*-acetyllycorine (IC$_{50}$ = 0.96 μM),[6b] and ungeremine (IC$_{50}$ = 0.35 μM),[6a] are effective AChE inhibitors. Thus, 10 of the alkaloids from *H. plantaginea* were selected to test for AChE inhibitory activity (Table 1). Ungeremine (IC$_{50}$ = 3.85 μM), norsanguinine (IC$_{50}$ = 1.43 μM), and 8-demethoxy-10-*O*-methylhostasine (**3**, IC$_{50}$ = 2.32 μM) showed strong activity.

AChE inhibitors are used in a major treatment for symptoms of the early stages of Alzheimer's disease.[6a] 8-Demethoxy-10-*O*-methylhostasine (**3**) was the first example of an effective AChE inhibitor among the lycorenine-type alkaloids. Noticeably, the activity of **3** was about 50 times stronger than that of 10-*O*-methylhostasine (**4**, IC$_{50}$ = 113 μM, Table 1), although the only difference between **3** and **4** is that **4** has one more methoxy group than **3**. On this basis, further studies of the AChE inhibitory activity of 8-demethoxy-10-*O*-methylhostasine and potential structure–activity relationships with related compounds are required.

Structures of Alkaloids 1–5. Compound **1** was assigned molecular formula C$_{18}$H$_{21}$NO$_7$ by HRESI-MS. 1D and 2D NMR spectra of **1** show the presence of a pentasubstituted phenyl ring [δ_H 6.94 (1H, s, H-11); δ_C 108.8 (CH, C-11), 108.8 (qC), 143.2 (qC), 150.2 (qC), 153.9 (qC), and 162.0 (qC)], a trisubstituted double bond [δ_H 5.66 (1H, br s, H-4); δ_C 120.2 (CH, C-4) and 141.9 (qC)], an ester carbonyl [δ_C 169.4 (qC, C-7)], an *N*-methyl [δ_H 1.75 (3H, s); δ_C 43.5 (CH$_3$)], and two *O*-methyls [δ_H 3.84 (3H, s) and 4.05 (3H, s); δ_C 61.6 (CH$_3$) and 62.6 (CH$_3$)]. Except for the three carbons of an *N*-methyl and two *O*-methyls, there are 15 carbon atoms in the molecular skeleton of **1**. These characteristics indicate that **1** belongs to the group of lycorenine-type alkaloids.[18] Considering the *O*-bearing quaternary carbon resonance of δ_C 84.7 (s, C-16), **1** has characteristics of an alkaloid possessing a spirocyclic framework.[18]

The planar structure of **1** was deduced by 1D and 2D NMR spectra. The ^{1}H–^{1}H COSY spectrum exhibits two partial structures, **a** (C-2–C-3) and **b** (C-4–C-5–C-13) (Figure 1). The HMBC spectrum (Figure 1) displays correlations of NMe to C-2 and C-17, and H-2 to C-12, which reveals the presence of an *N*-methylpyrrolidine moiety. Based on the HMBC correlations of H-13 to C-16 and C-17, and H-3 to C-4, fragments **a** and **b** are connected to C-16 to form a six-membered ring. The correlations of OMe-8 to
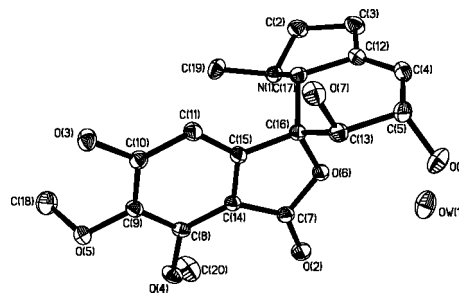
Figure 2. Perspective view (ORTEP) of **1**.

C-8 [δ_C 153.9 (qC)] and OMe-9 to C-9 [δ_C 143.2 (qC)] indicate that the two *O*-methyl groups are located on a benzene ring. The following assignments were elucidated by the HMBC correlations: C-11 by H-11 to C-16, C-15 by H-13 to C-15, C-9 by H-11 to C-9, and C-14 by H-11 to C-14. However, no correlations of H to C-7 were found in the HMBC spectrum of **1**.

The integrated structure and relative configuration of **1** were determined by single-crystal X-ray diffraction (Figure 2), and the compound was named hostasine. The absolute configuration has not been confirmed.

Compound **2** was assigned the molecular formula C$_{17}$H$_{19}$NO$_6$ by HRESI-MS. 1D NMR and HSQC spectra of **2** show the presence of a 1,2,4,5-tetrasubstituted phenyl ring. Comparing the 1D NMR data and MS spectrum of **2** with those of **1** indicates that **2** has one fewer *O*-methyl group than **1**. Based on the HMBC spectrum of **2**, which shows the correlations of H-8 to C-7, the difference between **2** and **1** is that the C-8 of **2** is not substituted by an *O*-methyl group.

The relative configuration of **2** was deduced to be similar to that of **1**, the latter unequivocally determined by single-crystal X-ray diffraction. Because the chemical shifts and coupling constants (see Table 2, below) in the ^{1}H NMR spectra and the specific rotations of the two compounds are very close, the relative configuration of **2** must be similar to that of **1**. Thus, **2** was elucidated as 8-demethoxyhostasine.

The molecular formula of **3** was determined as C$_{18}$H$_{21}$NO$_6$ by HRESI-MS. Comparison of 1D NMR spectroscopic data for **3** and **2** reveals that the only structural difference between the two compounds is that **3** has one more *O*-bearing methyl than **2**, namely, **3** possesses two *O*-methyl groups. The two *O*-methyl groups are located at C-9 and C-10, respectively, on the basis of the ROESY correlations of H-8/OMe-9 and H-11/OMe-10. Accordingly, **3** was determined as 8-demethoxy-10-*O*-methylhostasine.

Compound **4** was assigned the molecular formula C$_{19}$H$_{23}$NO$_7$ by HRESI-MS. Compound **4** has one more *O*-methyl group than **3**, based on comparison of the 1D NMR data and MS spectrum of **4** with those of **3**. It is determined that the *O*-methyl group is attached to C-8 because the phenyl ring of **4** is pentasubstituted and the HMBC spectrum shows correlation of H-11 [δ_H 7.17 (1H, s)] to C-16 [δ_C 84.3 (qC)]. Hence, **4** was defined as 10-*O*-methylhostasine.

The molecular formula of **5** was elucidated as C$_{18}$H$_{23}$NO$_4$ by HRESI-MS. Detailed examination of 1D NMR spectroscopic data and MS spectra of **5** and the known *O*-methyllycorenine showed that **5** was very similar to *O*-methyllycorenine, except that **5** has two *O*-methyl groups and one hydroxy rather than the three *O*-methyl groups as in *O*-methyllycorenine. The two *O*-methyl groups of **5** are located at C-7, based on the HMBC correlation (Figure 1) of OMe-7 [δ_H 3.51 (3H, s)] to C-7 [δ_C 98.4 (CH)], and C-10, based on the ROESY correlation (Figure 1) of H-11 [δ_H 7.01 (1H, s)] to OMe-10 [δ_H 3.88 (3H, s)], respectively. Thus, **5** was identified as 9-*O*-demethyl-7-*O*-methyllycorenine.

Benzylphenethylamine alkaloids are usually found in the plants of the Amaryllidaceae family and are called Amaryllidaceae alkaloids. Harborne and Williams in 1995 considered this class of isoquinoline alkaloids, based on a common 15-carbon aromatic

Table 2. ¹H NMR Data for Compounds **1–5** (δ in ppm, J in Hz)

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
2	3.04 (1H, m)	3.02 (1H, m)	3.05 (1H, m)	3.06 (1H, m)	3.29 (1H, m)
	2.30 (1H, dd, 18.4, 9.2)	2.28 (1H, dd, 19.0, 9.0)	2.36 (1H, dd, 18.4, 9.2)	2.36 (1H, dd, 18.5, 9.2)	2.34 (1H, m)
3	2.53 (1H, m)	2.54 (1H, m)	2.58 (1H, m)	2.59 (1H, m)	2.52 (2H, m)
	2.48 (1H, m)	2.47 (1H, m)	2.48 (1H, m)	2.48 (1H, m)	
4	5.66 (1H, br s)	5.67 (1H, br s)	5.70 (1H, br s)	5.69 (1H, br s)	5.52 (1H, br d, 2.0)
5	4.05 (1H, br s)	4.06 (1H, s)	4.10 (1H, s)	4.09 (1H, br s)	2.65 (1H, m)
					2.34 (1H, m)
7					5.47 (1H, s)
8		7.26 (1H, s)	7.34 (1H, s)		6.83 (1H, s)
11	6.94 (1H, s)	7.10 (1H, s)	7.35 (1H, s)	7.17 (1H, s)	7.01 (1H, s)
13	3.58 (1H, s)	3.57 (1H, s)	3.60 (1H, s)	3.61 (1H, s)	4.29 (1H, br d, 5.5)
16					2.59 (1H, m)
17	3.36 (1H, s)	3.38 (1H, br s)	3.49 (1H, s)	3.49 (1H, s)	2.90 (1H, m)
NMe	1.75 (3H, s)	1.68 (3H, s)	1.66 (3H, s)	1.72 (3H, s)	2.17 (3H, s)
OMe-7					3.51 (3H, s)
OMe-8	4.05 (3H, s)			4.07 (3H, s)	
OMe-9	3.84 (3H, s)	3.91 (3H, s)	3.92 (3H, s)	3.84 (3H, s)	
OMe-10			3.96 (3H, s)	3.96 (3H, s)	3.88 (3H, s)

[a] Measured in CD₃OD at 400 MHz. [b] Measured in CDCl₃ at 500 MHz.

Table 3. ¹³C NMR Data for Compounds **1–5** (δ in ppm)

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
2	56.8 (CH₂)	56.8 (CH₂)	56.8 (CH₂)	56.7 (CH₂)	56.6 (CH₂)
3	28.7 (CH₂)	28.7 (CH₂)	28.7 (CH₂)	28.7 (CH₂)	27.9 (CH₂)
4	120.2 (CH)	120.1 (CH)	120.3 (CH)	120.3 (CH)	116.7 (CH)
5	72.5 (CH)	72.5 (CH)	72.4 (CH)	72.4 (CH)	31.6 (CH₂)
7	169.4 (qC)	172.2 (qC)	171.4 (qC)	168.7 (qC)	98.4 (CH)
8	153.9 (qC)	106.8 (CH)	107.0 (CH)	153.5 (qC)	113.5 (CH)
9	143.2 (qC)	152.3 (qC)	152.7 (qC)	143.7 (qC)	145.2 (qC)
10	162.0 (qC)	156.7 (qC)	156.3 (qC)	161.0 (qC)	146.5 (qC)
11	108.8 (CH)	111.9 (CH)	107.5 (CH)	103.9 (CH)	112.0 (CH)
12	141.9 (qC)	141.9 (qC)	141.6 (qC)	141.6 (qC)	139.3 (qC)
13	77.7 (CH)	78.0 (CH)	78.1 (CH)	77.8 (CH)	66.6 (CH)
14	108.8 (qC)	116.3 (qC)	118.8 (qC)	111.3 (qC)	126.4 (qC)
15	150.2 (qC)	148.2 (qC)	147.1 (qC)	150.1 (qC)	129.2 (qC)
16	84.0 (qC)	84.7 (qC)	85.0 (qC)	84.3 (qC)	43.0 (CH)
17	67.3 (CH)	67.2 (CH)	67.1 (CH)	67.3 (CH)	67.9 (CH)
NMe	43.5 (CH₃)	43.4 (CH₃)	43.4 (CH₃)	43.4 (CH₃)	44.0 (CH₃)
OMe-7					55.4 (CH₃)
OMe-8	62.6 (CH₃)			62.8 (CH₃)	
OMe-9	61.6 (CH₃)	56.5 (CH₃)	56.7 (CH₃)	61.8 (CH₃)	
OMe-10			56.9 (CH₃)	57.1 (CH₃)	56.1 (CH₃)

[a] Measured in CD₃OD at 100 MHz. [b] Measured in CDCl₃ at 125 MHz.

nucleus, to be unique to the Amaryllidaceae.[19] However, there were two exceptions: *Urginea altissima* (L.f.) Baker (Hyacinthaceae), from which lycorine and acetylcaranine were isolated, and *Dioscorea dregeana* (Kunth) Dur. & Schinz (Dioscoreaceae), from which crinamine was isolated.[19]

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Horiba SEPA-300 polarimeter. Column chromatography (CC) was performed over silica gel G (200–300 and 300–400 mesh), silica gel H (10–40 μm), D₁₀₁ resin (Qingdao Marine Chemical Ltd., Qingdao, P. R. China), and Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden). TLC was conducted on precoated silica gel plates GF₂₅₄ (Qingdao). HPLC separations were performed using an Agilent 1100 series pump equipped with a diode array detector and an NH₂ analytical column (5 μm, φ 4.6 × 250 mm, Waters). 1D and 2D NMR spectra were recorded on BRUKER AM-400 and DRX-500 spectrometers with TMS as internal standard. MS were measured on a VG Auto Spec-3000 mass spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 infrared spectrophotometer. Melting points were determined using an X-4 melting point apparatus (Yingyu Yuhua Apparatus Factory, Gongyi, P. R. China) and were not corrected. UV spectra were recorded on a Shimadzu double-beam 210A spectrometer.

Plant Material. *H. plantaginea* was collected from South China Botanical Garden, the Chinese Academy of Sciences (CAS), Guangzhou, P. R. China, in September 2005. The plant was identified by Assistant Professor Qi-Fei Yi (South China Botanical Garden, CAS), and a voucher specimen (No. GD0509) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS. Seeds of *Nicotiana tabacum* cv. K326 and *Nicotiana glutinosa* were provided by Yunnan Key Laboratory of Agricultural Biotechnology, Yunnan Academy of Agricultural Sciences, P. R. China.

Extraction and Isolation. The whole plant of *H. plantaginea* (4.8 kg) was exhaustively extracted with EtOH (70%). The solvent was evaporated under reduced pressure to give a residue (1000 g, 20.8%), which was suspended in H₂O and partitioned to three fractions, petroleum ether (A, 80 g, 1.7%), EtOAc (B, 60 g, 1.2%), and H₂O (C, 800 g, 16.7%). Subsequently, fraction C was partitioned to three further fractions, H₂O (C₁, 600 g, 12.5%), MeOH (C₂, 110 g, 2.3%), and Me₂CO (C₃, 6 g, 0.1%), by D₁₀₁ resin CC.

Fraction B was fractionated by silica gel CC (CHCl₃–MeOH, 10:1 and 4:1) to afford two major fractions (B₁ and B₂). Fraction B₁ was submitted to chromatography on a silica gel column (CHCl₃–MeOH–NH₄OH, 200:10:1; petroleum ether–Et₂NH, 4:1) to afford albomaculine (500 mg). Fraction B₂ was recrystallized from CHCl₃–MeOH (1:1) to afford **6** (100 mg).

Fraction C₂ was fractionated by silica gel CC (CHCl₃–MeOH, 10:1, 4:1, 1:1, and 0:1) to afford seven fractions (C₂₁–C₂₇). Fraction C₂₁ was rechromatographed on a Sephadex LH-20 column (MeOH), on a silica gel column (petroleum ether–Et₂NH, 4:1), and by HPLC using an NH₂ analytical column with MeCN–H₂O (10:90) elution to yield **3** (8 mg) and **4** (4 mg). Fraction C₂₂ was recrystallized from MeOH to yield haemanthidine (1000 mg) and the mother liquor. The latter was evaporated in vacuo to dryness and separated by silica gel CC, eluted with petroleum ether–Et₂NH (4:1) to afford (+)-haemanthamine (500 mg). Fraction C₂₃ was recrystallized from CHCl₃–MeOH (1:1) to afford lycorine (1500 mg) and the mother liquor. The latter was evaporated in vacuo to dryness and

separated by silica gel CC, eluted with CHCl₃–Me₂CO–NH₄OH (90:30:0.6 and 70:35:1) to give *O*-methyllycorenine (9 mg) and **5** (10 mg), and then by HPLC using an NH₂ analytical column with MeOH–H₂O (60:40) elution to afford **1** (6 mg) and **2** (5 mg). Fraction C₂₄ was recrystallized from CHCl₃–MeOH (1:1) to yield pseudolycorine (500 mg). Fraction C₂₅ was recrystallized from CHCl₃–MeOH (1:1) to afford **6** (100 mg). Fraction C₂₆ was rechromatographed on a Sephadex LH-20 column (MeOH) and on silica gel columns (CHCl₃–Et₂NH, 4:1; CHCl₃–Me₂CO, 1:5; CHCl₃–Me₂CO–NH₄OH, 40:10:1) to afford yemenine C (50 mg), *O*-demethylhaemanthamine (100 mg), norsanguinine (35 mg), and ungeremine (5 mg). Fraction C₂₇ was rechromatographed on silica gel columns (CHCl₃–Me₂CO–NH₄OH, 40:10:1; EtOAc–MeOH–Et₂NH, 10:1:1) to afford 8-*O*-demethylmaritidine (50 mg).

Hostasine (1): colorless crystals (MeOH); mp 202–205 °C; [α]²⁶_D −34.0 (*c* 0.21, MeOH); UV (MeOH) λ_max (log ε) 397 (1.32), 360 (1.01), 344 (1.79), 263 (3.87), 223 (4.34) nm; IR (KBr) ν_max 3468, 2925, 1745, 1593, 1369, 1005 cm⁻¹; ¹H and ¹³C NMR, see Tables 2 and 3; ESI-MS *m/z* 364 [M + H]⁺; HRESI-MS *m/z* 386.1224 [M + Na]⁺ (calcd for C₁₈H₂₁NO₇Na, 386.1215).

8-Demethoxyhostasine (2): colorless solid; [α]²⁶_D −29.5 (*c* 0.36, MeOH); UV (MeOH) λ_max (log ε) 392 (2.02), 297 (3.62), 260 (3.71), 223 (4.28) nm; IR (KBr) ν_max 3424, 1743, 1631, 1596, 1317, 1087, 1016 cm⁻¹; ¹H and ¹³C NMR, see Tables 2 and 3; ESI-MS *m/z* 334 [M + H]⁺; HRESI-MS *m/z* 334.1295 [M + H]⁺ (calcd for C₁₇H₂₀NO₆, 334.1290).

8-Demethoxy-10-*O*-methylhostasine (3): colorless solid; [α]²⁶_D −76.4 (*c* 0.53, MeOH); UV (MeOH) λ_max (log ε) 380 (1.90), 343 (1.57), 295 (3.65), 258 (3.76), 224 (4.37) nm; IR (KBr) ν_max 3421, 2929, 1752, 1630, 1599, 1499, 1306, 1092, 1006 cm⁻¹; ¹H and ¹³C NMR, see Tables 2 and 3; ESI-MS *m/z* 348 [M + H]⁺; HRESI-MS *m/z* 348.1450 [M + H]⁺ (calcd for C₁₈H₂₂NO₆, 348.1447).

10-*O*-Methylhostasine (4): colorless solid; [α]²⁶_D −65.7 (*c* 0.38, MeOH); UV (MeOH) λ_max (log ε) 392 (2.13), 369 (1.98), 291 (3.30), 261 (3.88), 223 (4.38) nm; IR (KBr) ν_max 3418, 2928, 1757, 1599, 1480, 1260, 1107, 1017 cm⁻¹; ¹H and ¹³C NMR, see Tables 2 and 3; ESI-MS *m/z* 378 [M + H]⁺; HRESI-MS *m/z* 378.1554 [M + H]⁺ (calcd for C₁₉H₂₄NO₇, 378.1552).

9-*O*-Demethyl-7-*O*-methyllycorenine (5): white solid; [α]²⁶_D +122.6 (*c* 0.65, CHCl₃); UV (CHCl₃) λ_max (log ε) 284 (3.47), 240 (3.69) nm; IR (KBr) ν_max 3425, 1620, 1587, 1511, 1280, 1045 cm⁻¹; ¹H and ¹³C NMR, see Tables 2 and 3; ESI-MS *m/z* 318 [M + H]⁺; HRESI-MS *m/z* 318.1701 [M + H]⁺ (calcd for C₁₈H₂₄NO₄, 318.1705).

7-Deoxy-*trans*-dihydronarciclasine (6): colorless sharp needles (CHCl₃–MeOH); mp 303−304 °C; [α]²⁴_D +80.4 (*c* 0.45, DMSO); ¹³C NMR (DMSO-*d₆*, 100 MHz) δ 164.4 (qC, C-6), 150.7 (qC, C-9), 146.0 (qC, C-8), 138.1 (qC, C-10a), 123.3 (qC, C-6a), 107.0 (CH, C-7), 104.4 (CH, C-10), 101.7 (CH₂, –OCH₂O–), 71.7 (CH, C-3), 69.8 (CH, C-4), 68.7 (CH, C-2), 55.2 (CH, C-4a), 34.3 (CH, C-10b), 28.4 (CH₂, C-1); ESI-MS *m/z* 294 [M + H]⁺. The ¹³C NMR data were assigned for the first time; ¹H NMR same as reported in the literature.⁶

X-ray Single-Crystal Structure Determination of Hostasine (1). Crystal data: C₁₈H₂₁O₇N·H₂O; MW = 363.36 (no water of crystallization); monoclinic system, space group *P*2₁; crystal cell parameters *a* = 6.747(1) Å, *b* = 17.447(4) Å, *c* = 8.254(2) Å, β = 113.12(3)°, *V* = 893.6(4) Å³, *Z* = 2, *d* = 1.410 g/cm³. A crystal of dimensions 0.20 × 0.30 × 0.60 mm was used for X-ray measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω−2θ scans, 2θ_max = 50.0°), using Mo Kα radiation. The total number of independent reflections measured was 1994, of which 1988 were observed (|*F*|² ≥ 2σ|*F*|²). The crystal structure of **2** was solved by the direct method SHELXS-97²⁰ and expanded using difference Fourier techniques, refined by the program and method NOMCSDP²¹ and full-matrix least-squares calculations. H atoms were fixed at calculated positions. The final indices were *R₁* = 0.0430, w*R₂* = 0.1180, *S* = 1.123. Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Center as supplementary publication CCDC-623070 (available free of charge at http://www.ccdc.cam.ac.uk/deposit or from the CCDC, 12 Union Rd., Cambridge CB2 1EZ, UK).

Antiviral Biological Assay. TMV (U1 strain) was obtained from Yunnan Key Laboratory of Agricultural Biotechnology, Yunnan Academy of Agricultural Sciences, P. R. China, and multiplied in *Nicotiana tabacum* cv. K326. Activity against TMV was assessed by the conventional half-leaf method.¹⁷,²² Fresh leaves of *Nicotiana glutinosa* were inoculated by the juice-leaf rubbing method. After 2 h, each leaf was cut in half along the main vein. Half-leaves of these inoculated leaves were immersed into the solution of compounds or positive control, ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), a commercial antiviral agent, respectively, and other half-leaves into distilled H₂O as negative control. The solutions were then cultured at 20 °C for 72 h. The inhibition ratio was calculated by comparing the number of local lesions on the two half-leaves. Three repetitions were conducted for each sample tested.

Microplate Assay for AChE Inhibition. The AChE inhibitory activities of samples were measured quantitatively by Ellman's method.⁶ᵃ,²³ The absorbance was measured 10 times at 405 nm every 15 s. The reaction rate was calculated using Microplate Manager software, and percentage inhibition was determined by comparing the rate for the sample to that for the blank.

Supporting Information Available: ¹H and ¹³C NMR, COSY, HMQC, HMBC, and ROESY spectra for compounds **1** and **2**. This material is available free of charge via the Internet at http:// pubs.acs.org.

References and Notes

(1) Li, Y. M.; Wang, L. H.; Li, S. L.; Chen, X. Y.; Shen, Y. M.; Zhang, Z. K.; He, H. P.; Xu, W. B.; Shu, Y. L.; Liang, G. D.; Fang, R. X.; Hao, X. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8083–8088.
(2) Wang, F. Z.; Tang, J. In *Flora Reipublicae Popularis Sinicae*; Wu, Z. Y., Eds; Science Press: Beijing, 1980; Vol. 14, pp 49–52.
(3) Lin, C. L.; Qiu, W. F. *Acta Phytophylacica Sin.* **1987**, *14*, 217–220.
(4) (a) Budzianowski, J. *Phytochemistry* **1990**, *29*, 643–3647. (b) Mimaki, Y.; Kanmoto, T.; Kuroda, M.; Sashida, Y.; Satomi, Y.; Nishino, A.; Nishin, H. *Phytochemistry* **1996**, *42*, 1065–1070. (c) Mimaki, Y.; Kameyama, A.; Kuroda, M.; Sashida, Y.; Hirano, T.; Oka, K.; Koike, K.; Nikaido, T. *Phytochemistry* **1997**, *44*, 305–310. (d) Mimaki, Y.; Kuroda, M.; Kameyama, A.; Yokosuka, A.; Sashida, Y. *Phytochemistry* **1998**, *48*, 1361–1369.
(5) Pettit, G. R.; Pettit, G. R., III; Backhaus, R. A. *J. Nat. Prod.* **1993**, *56*, 1682–1687.
(6) (a) Rhee, I. K.; Appels, N.; Hofte, B.; Karabatak, B.; Erkelens, C.; Stark, L. M.; Flippin, L. A.; Verpoorte, R. *Biol. Pharm. Bull.* **2004**, *27*, 1804–1809. (b) Houghton, P. J.; Ren, Y.; Howes, M. J. *Nat. Prod. Rep.* **2006**, *23*, 181–199.
(7) Codina, C.; Bastida, J.; Viladomat, F.; Fernández, J. M.; Bergoñón, S.; Rubiralta, M.; Quirion, J. C. *Phytochemistry* **1993**, *32*, 1354–1356.
(8) Briggs, C. K.; Highet, P. F.; Highet, R. J.; Wildman, W. C. *J. Am. Chem. Soc.* **1956**, *78*, 2899–2904.
(9) Frahm, A. W. *Magn. Reson. Chem.* **1985**, *23*, 804–808.
(10) Kihara, M.; Koike, T.; Imakura, Y.; Kida, K.; Shingu, T.; Kubayashi, S. *Chem. Pharm. Bull.* **1987**, *35*, 1070–1075.
(11) Hohmann, J.; Forgo, P.; Molnár, J.; Wolfard, K.; Molnár, A.; Thalhammer, T.; Máthé, I.; Sharples, D. *Planta Med.* **2002**, *68*, 454–457.
(12) Abdel-Halim, O. B.; Morikawa, T.; Ando, S.; Matsuda, H.; Yoshikawa, M. *J. Nat. Prod.* **2004**, *67*, 1119–1124.
(13) Likhitwitayawuid, K.; Angerhofer, C. K.; Chai, H.; Pezzuto, J. M.; Cordell, G. A.; Ruangrungsi, N. *J. Nat. Prod.* **1993**, *56*, 1331–1338.
(14) Seaforth, C. E.; Williams, G. S.; Tinto, W. F. *Fitoterapia* **1998**, *69*, 79.
(15) Stark, L. M.; Lin, X. F.; Flippin, L. A. *J. Org. Chem.* **2000**, *65*, 3227–3230.
(16) Abdallah, O. M. *Phytochemistry* **1995**, *39*, 477–478.
(17) An, T. Y.; Huang, R. Q.; Yang, Z.; Zhang, D. K.; Li, G. R.; Yao, Y. C.; Gao, J. *Phytochemistry* **2001**, *58*, 1267–1269.
(18) Latvala, A.; Önür, M. A.; Gözler, T.; Linden, A.; Kivçak, B.; Hesse, M. *Phytochemistry* **1995**, *39*, 1229–1240.
(19) Mulholland, D. A.; Crouch, N.; Decker, B.; Smith, M. T. *Biochem. Syst. Ecol.* **2002**, *30*, 183–185.
(20) Sheldrick, G. M. *SHELX-97, Program for Crystal Structure Refinement*; University of Gottingen: Gottingen, Germany, 1997.
(21) Lu, Y.; Wu, B. M. *Chin. Chem. Lett.* **1992**, *3*, 637–640.
(22) Yao, Y. C.; Yang, Z.; Gao, J.; An, T. Y.; Yu, X. S.; Li, G. R.; Huang, R. Q. *Neimenggu Gongye Daxue Xuebao* **2002**, *21*, 1–4.
(23) Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88–95.