JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Phytotoxicity of Lignanamides Isolated from the Seeds of *Hyoscyamus niger*

Wen-Na Zhang, Jian-Guang Luo, and Ling-Yi Kong*

State Key Laboratory of Natural Medicines, Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, People's Republic of China

Supporting Information

ABSTRACT: Bioassay-guided fractionation of phytotoxic extracts prepared from the seeds of *Hyoscyamus niger* led to the isolation of three new lignanamides (**1**–**3**), along with six known lignanamides (**4**–**9**). The structures of the new compounds were determined by spectroscopic methods, including 1D and 2D nuclear magnetic resonance techniques, and high-resolution electrospray ionization mass spectrometry. The bioactivity analysis of the isolated compounds showed that compound **3** exhibited significant inhibition on the germination and radical elongation of *Allium fistulosum* at 10^{-4} M concentration.

KEYWORDS: *Hyoscyamus niger, lignanamides, phytotoxicity, Allium fistulosum, germination inhibition*

INTRODUCTION

Utilization of synthetic herbicides can provide an effective and economical means of weed control compared to cultivation, hoeing, and hand pulling.[1] However, these molecules not only have a specific target organism but also generate toxic effects on the environment and human health.[2] In addition to herbicidal agents of synthetic chemical origin, there are also a few so-called "biopesticides", which are natural or seminatural agents or organisms that can control specific weeds.[3,4]

Allelopathy, as secondary metabolite-mediated "chemical warfare" among plants, has been gaining application in present-day agricultural science.[5] Application of natural phytochemicals as weed management provides an alternative to chemical herbicides.[6] Many allelopathic natural compounds such as withanolides,[7,8] glycoalkaloids,[9] terpenoids, and isoprenoids have been reported in plants of the Solanaceae family.[10,11]

Hyoscyamus niger (henbane), a member of Solanaceae, are known as Tian-Xian-Zi in China.[12] Phytochemical studies on *H. niger* revealed the presence of tropane alkaloids, withanolides, steroidal saponins, glycosides, lignans, and lignanamides.[13]

In our research, the phytotoxic constituents of *H. niger* were investigated by bioassay-guided fractionation, and their germination and growth were tested on *Allium fistulosum* (monocotyledon) at the range of 10^{-4} to 5×10^{-6} M concentration.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter (Jasco, Tokyo, Japan). Ultraviolet (UV) spectra were recorded on a UV-2450 UV/vis spectrophotometer (Shimadzu, Tokyo, Japan). IR spectra (KBr disks) were recorded on a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV-500 NMR (^{1}H, 500 MHz; ^{13}C, 125 MHz) instrument (Bruker, Karlsruhe, Germany), with tetramethylsilane (TMS) as the internal standard. Electrospray ionization (ESI) and high-resolution (HR) ESI mass spectral data were acquired on an Agilent 1100 series LC/MSD ion trap mass spectrometer and a G1969A TOF-MS instrument (Agilent, Santa Clara, CA), respectively. All solvents used were of analytical grade (Jiangsu Hanbang Science and Technology Co., Ltd., Nanjing, China). Silica gel (Qingdao Marine Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (CHP20P, 75–150 μm) (Pharmacia, Uppsala, Sweden), MCI gel (Mitsubishi Chemical Industries Ltd., Japan), and ODS-C18 (40–63 μm) (Fuji, Japan) were used for column chromatography. Preparative high-performance liquid chromatography (HPLC) was carried out using an Agilent 1200 series (Agilent) instrument with a Shim-Pak RP-C18 column (20 mm i.d. × 200 mm) (Shimadzu, Japan) and a 1200 series multiple-wavelength detector at 210 and 280 nm. Thin-layer chromatography was performed on precoated silica gel GF_{254} plates (Qingdao Marine Chemical Co., Ltd., Qingdao, China) and detected by spraying with 10% H_2SO_4 in EtOH (v/v).

Plant Materials. The seeds of *H. niger* were purchased from a local market in Bozhou, Anhui province, China, in September 2010. The botanical identification was made by Prof. Min-jian Qin, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (no. 100705) is deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. Air-dried seeds of *H. niger* (15 kg) were ground and extracted with 95% EtOH under reflux (3 × 3 h). After concentration of the solution, the obtained crude extract (1 kg) was suspended in water and then partitioned with petroleum ether and $CHCl_3$ to afford a petroleum ether fraction and $CHCl_3$ fraction. From a preliminary test, we found that the $CHCl_3$ fraction exhibited inhibitory activity on the seed germination, shoot length, and radical elongation of *A. fistulosum*. Subsequently, the active $CHCl_3$ fraction (40 g) was submitted to an MCI gel column (75–150 μm, 120 g, Mitsubishi) eluted with a gradient solvent system of MeOH–H_2O (3:7 to 10:0, v/v) to yield four fractions (CA–CD). Fraction CC (MeOH–H_2O, 7:3) was chromatographed on a silica gel column (200–300 mesh, 100 g) using the mixture of $CHCl_3$–MeOH (9:1 to 1:1, gradient system) as the eluent to yield nine fractions (CC1–CC9). According to UV detection at 210 and 280 nm, subfraction CC5 was separated by preparative HPLC (20 mm i.d. × 200 mm, 40–63 μm, Shimadzu) using MeOH–H_2O (5:5, 10 mL/min) as the

Received: November 15, 2011
Revised: January 24, 2012
Accepted: January 26, 2012
Published: January 26, 2012

mobile phase to yield **6** (12.5 mg, 0.03%) and **7** (33.0 mg, 0.08%). Subfraction CC6 was eluted for Sephadex LH-20 (CHP20P, 75−150 μm) with MeOH and further isolated by preparative HPLC using the solvent system of MeOH−H_2O (5:5, 10 mL/min) as the mobile phase to obtain **3** (4.0 mg, 0.01%). Subfraction CC7 was separated by repeat preparative HPLC (MeOH−H_2O, 4.5:5.5, 10 mL/min) to give pure **1** (1.5 mg, 0.003%) and **5** (37 mg, 0.09%). The fraction CC8 was further isolated by passage over a Sephadex LH-20 column (MeOH as the eluent) and an ODS column (40−63 μm, 40 g, Fuji) eluted with a gradient solvent system of MeOH−H_2O (5:5 to 10:0) and finally purified by preparative HPLC (MeOH−H_2O, 4.5:5.5, 10 mL/min) to afford **2** (2.2 mg, 0.005%) and **9** (45 mg, 0.1%). Using medium-pressure preparation liquid chromatography and preparative HPLC (MeOH−H_2O, 5:5, 10 mL/min), **4** (8.0 mg, 0.02%) and **8** (16.5 mg, 0.04%) were obtained from fraction CC9.

Data for Compound ***1****:* white amorphous powder; $[\alpha]_D^{25}$ +2.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.6), 253 (4.4), 278 (4.0), 310 (3.8) nm; IR (KBr) ν_{max} 3353, 1655, 1614, 1514, 1251 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 677 $[M + Cl]^-$; HRESIMS (negative mode) *m/z* 677.2262 $[M + Cl]^-$ (calcd for $C_{36}H_{38}N_2O_9Cl$, 677.2271).

Data for Compound ***2****:* white amorphous powder; $[\alpha]_D^{25}$ +7.3 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.6), 253 (4.4), 278 (4.0), 310 (3.8) nm; IR (KBr) ν_{max} 3353, 1655, 1614, 1514, 1251 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 643 $[M + H]^+$; HRESIMS (positive mode) *m/z* 665.2490 $[M + Na]^+$ (calcd for $C_{36}H_{38}N_2O_9Na$, 665.2470).

Data for Compound ***3****:* yellow amorphous powder; $[\alpha]_D^{25}$ +13.5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.2), 224 (4.0), 280 (3.9), 311 (3.8) nm; IR (KBr) ν_{max} 3396, 1693, 1642, 1590, 1515, 1276, 1241 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 653 $[M - H]^-$; HRESIMS (negative mode) *m/z* 653.2136 $[M - H]^-$ (calcd for $C_{36}H_{33}N_2O_{10}$, 653.2140).

Bioassay Activity. The phytotoxic activity of the extract and all isolated compounds on the seeds of *A. fistulosum* were assessed by quantitating the mean values for these parameters (germination average, shoot and radical elongation). Seeds of *A. fistulosum* collected during 2011 were obtained from Fuyang (Anhui, China). All undersized or damaged seeds were discarded, and the assay seeds were selected for uniformity. For the bioassays we used Petri dishes with 90 mm diameter with one sheet of Whatman no. 1 filter paper as the support. In three replicate experiments, germination and growth were conducted in aqueous solutions at controlled pH. Compounds to be assayed were first dissolved in DMSO, and then these solutions were diluted with MES (2-(*N*-morpholino)ethanesulfonic acid; 10 mM, pH 6) to obtain the final concentrations (10^{-4}, 5×10^{-5}, 10^{-5}, and 5×10^{-6} M) for each compound. Parallel controls (buffered aqueous solutions with DMSO and without test compounds) were also performed.[14] After addition of 25 seeds and 5 mL of test solution for 90 mm dishes, the Petri dishes were sealed with Parafilm to ensure closed-system models. The seeds were placed in a growth chamber, KBW Binder 240, at 25 °C in the dark. The germination percentage was determined daily for 5 days (no more germination occurred after this time). After that, the plants were frozen at −20 °C to avoid subsequent growth until the measurement process.[15,16]

Data are reported as percentage differences from the control in the graphics. Thus, zero represents the control; positive values represent stimulation of the parameter studied, and negative values represent inhibition.

Statistical Treatment. The statistical significance of the differences between groups was determined by a Student's *t* test, calculating the mean values for every parameter (germination average, radical and shoot elongation) and their population variance within a Petri dish. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Isolation and Structure Elucidation. The 95% EtOH extract of dried seeds of *H. niger* was suspended in distilled water and then partitioned with petroleum ether and $CHCl_3$ successively. In a preliminary test for phytotoxic activities, the petroleum ether and H_2O fractions showed no effect, while the $CHCl_3$ fraction exhibited 28%, 35%, and 20% inhibitory activities on seed germination, radical length, and shoot elongation of *A. fistulosum* at 10.0 mg/mL concentration, respectively. Further chemical investigation of the bioactive $CHCl_3$ fraction using successive column chromatography over silica gel and Sephadex LH-20 and preparative HPLC resulted in the isolation of nine lignanamides (**1**−**9**), including three new lignanamides (**1**−**3**) (Figure 1).

Table 1. ^1H NMR (MeOD, 500 MHz) and ^{13}C NMR (MeOD, 125 MHz) Data of 1−3

	1		2		3	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		133.5		132.3		129.7
2	7.11 (d, 1.5)	111.9	7.15 (d, 2.0)	112.7	6.99 (d, 1.5)	110.3
3		149.0		148.7		149.6
4		147.4		147.6		148.9
5	6.79 (d, 8.5)	116.0	6.74 (d, 8.0)	115.5	6.86 (d, 8.5)	116.1
6	6.89 (dd, 1.5, 8.5)	120.7	6.81 (dd, 2.0, 8.0)	121.7	6.97 (dd, 1.5, 8.5)	120.0
7	5.14 (d, 3.5)	75.2	5.07 (d, 4)	75.3		91.8
8	4.56 (d, 3.5)	87.3	4.73 (d, 4)	86.6		140.7
9		171.9		171.5		161.7
1′		131.9		131.9		128.1
2′	7.29 (d, 1.5)	114.5	7.28 (s)	114.6	7.57 (d, 1.5)	112.0
3′		150.7		150.9		149.6
4′		149.2	6.93 (s)	117.7		155.2
5′	6.33 (d, 8.5)	117.3		149.4	6.88 (d, 8.5)	116.8
6′	6.83 (dd, 1.5, 8.5)	124.1	6.93 (s)	124.2	7.45 (dd, 1.5, 8.5)	127.0
7′	6.63 (d, 12.5)	137.5	6.65 (d, 12.5)	137.5		191.2
8′	5.92 (d, 12.5)	123.7	5.90 (d, 12.5)	123.6		150.9
9′		170.1		170.0		167.3
1″		131.1		130.9		130.9
2″, 6″	6.92 (d, 8.5)	130.8	6.71 (d, 8.5)	130.7	6.77 (d, 8.5)	130.6
3″, 5″	6.90 (d, 8.5)	116.4	6.59 (d, 8.5)	116.3	6.64 (d, 8.5)	116.4
4″		157.0		157.0		157.0
7″	2.62 (m)	35.6	2.37 (m)	35.5	2.45 (m)	35.2
			2.53 (m)			
8″	3.32 (m)	42.1	3.14 (m)	41.7	3.24 (m)	41.9
	3.46 (m)		3.34 (m)		3.28 (m)	
1‴		131.3		131.2		130.6
4‴		157.0		157.0		157.0
2‴, 6‴	6.98 (d, 8.5)	130.8	6.96 (d, 8.5)	130.7	6.84 (d, 8.5)	130.5
3‴, 5‴	6.69 (d, 8.5)	116.4	6.66 (d, 8.5)	116.3	6.65 (d, 8.5)	116.3
7‴	2.68 (m)	35.6	2.68 (m)	35.5	2.36 (m)	34.7
					2.67 (m)	
8‴	3.40 (m)	42.4	3.37 (m)	42.3	3.18 (m)	42.5
					3.37 (m)	
3-OMe	3.82 (s)	56.5	3.84 (s)	56.4	3.82 (s)	56.6
3′-OMe	3.74 (s)	56.5	3.61 (s)	56.5	3.92 (s)	56.6

dx.doi.org/10.1021/jf2046784 | J. Agric. Food Chem. 2012, 60, 1682−1687

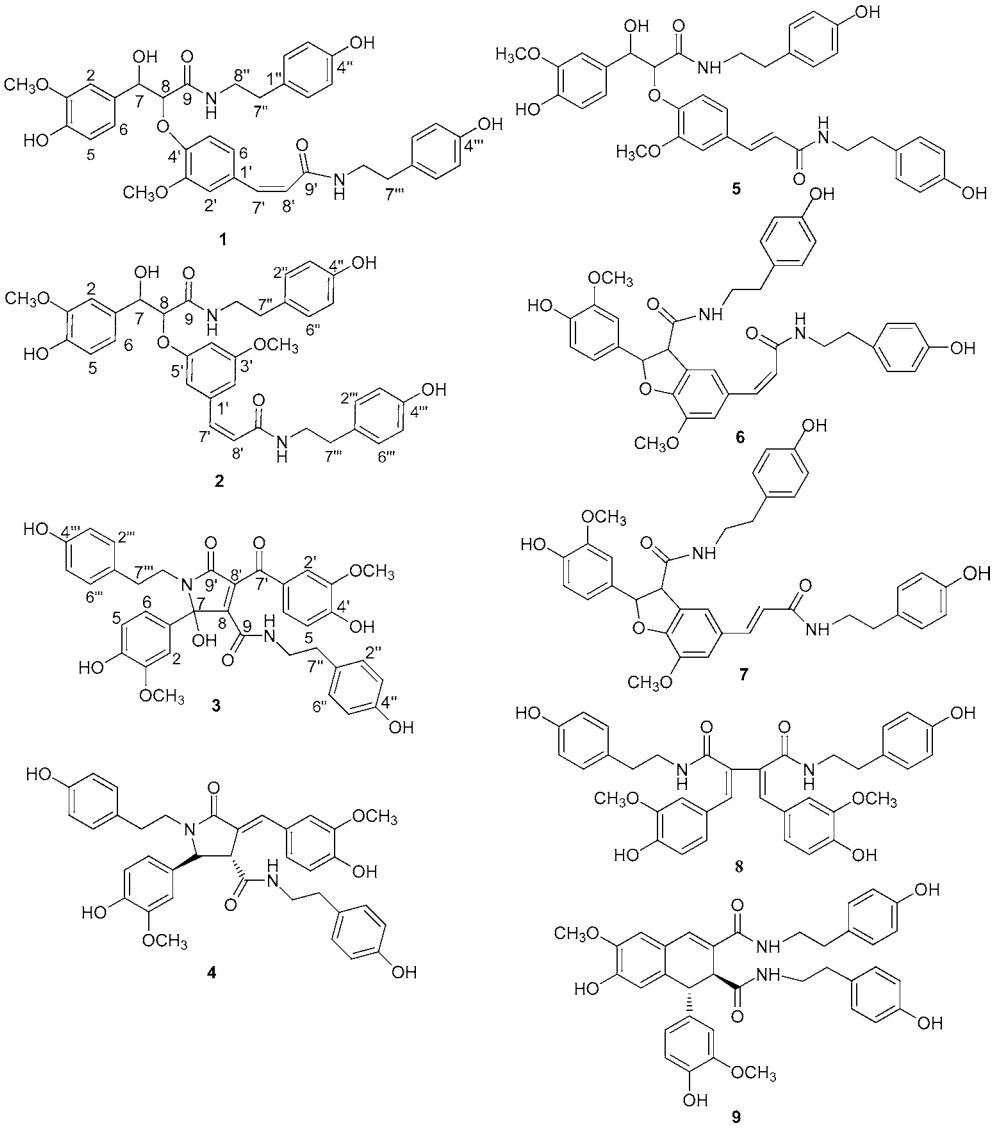

Figure 1. Structures of **1**–**9**.

Elucidation of 1. Compound **1** was obtained as a white amorphous powder ($[\alpha]_D^{25}$ +2.2), whose molecular formula was determined to be $C_{36}H_{38}N_2O_9$, as the HRESIMS spectrum had $[M + Cl]^-$ at *m*/*z* 677.2262 (calcd for $C_{36}H_{38}ClN_2O_9$, 677.2271). The UV maxima (203, 253, 278, and 310 nm) and IR bands (3353, 1655, 1614, 1514, and 1251 cm^{-1}) suggested that **1** contained aromatic rings, in addition to hydroxyl functions and a C–N bond. In the ^1H NMR spectrum, a pair of doublet protons at δ 6.63 (J = 12.5 Hz) and δ 5.92 (J = 12.5 Hz) indicated the presence of a *cis*-substituted double bond.[21] Six aromatic protons at δ 7.11 (d, J = 1.5 Hz), 6.79 (d, J = 8.5 Hz), and 6.89 (dd, J = 1.5, 8.5 Hz) and δ 7.29 (d, J = 1.5 Hz), 6.33 (d, J = 8.5 Hz), and 6.83 (dd, J = 1.5, 8.5 Hz) suggested the presence of two 1,3,5-trisubstituted aromatic rings. Furthermore, eight *ortho*-coupled protons of two disubstituted aromatic rings were present as doublets at δ 6.92 (2H, d, J = 8.5 Hz), 6.90 (2H, d, J = 8.5 Hz), 6.98 (2H, d, J = 8.5 Hz), and 6.69 (2H, d, J = 8.5 Hz). The ^1H and ^{13}C resonances of compound **1** were assigned by combination of rotating frame Overhauser effect spectroscopy (ROESY), heteronuclear multiple-bond correlation (HMBC), and heteronuclear single-quantum coherence (HSQC) experiments (Table 1, Figure 2). In the HSQC spectrum, the protons at δ 3.46 and 3.32 (2H, m, H-8″), 2.62 (2H, m, H-7″), 3.40 (2H, m, H-8‴), and 2.68 (2H, m, H-7‴), multiply correlated to the carbons at δ 42.1 (C-8″), 35.6 (C-7″), 42.4 (C-8‴), and 35.6 (C-7‴), revealed the presence of two 2-aminoethyl chains of a tyramine group. The presence of 8-*O*-linked 7-hydroxyferuloyl was determined by long-range correlations between the oxygenated methine proton signal at δ 5.14 (H-7) and the carbonyl carbon at δ 171.9 (C-9) and the aromatic carbon signals at δ 111.9 and 120.7 (C-2 and C-6) and between the proton signals at δ 4.56 (H-8) and 171.9 (C-9) and the oxygenated quaternary carbons at δ 149.2 (C-4′) in the HMBC spectrum. In addition, the correlations from the olefinic proton δ 6.63 (H-7′) to the carbonyl carbon δ 170.1 (C-9′) and methine carbons δ 114.5 (C-2′) and 124.1 (C-6′) and from aromatic proton signals at δ 7.29 and 6.83 (H-2′ and H-6′) to δ 149.2 (C-4′) indicated the presence of a 4′-*O*-linked feruloyl group (Figure 2). These were consistent with an 8-*O*-4′-lignan structure.[19] Finally, the HMBC evidence from H-8″ protons to C-9 and from the H-8‴ protons to C-9′ suggested two tyramine groups linked to C-9 and C-9′ carbons, respectively. The analysis of the ROESY spectrum (Figure 2) showed nuclear

dx.doi.org/10.1021/jf2046784 | J. Agric. Food Chem. 2012, 60, 1682–1687

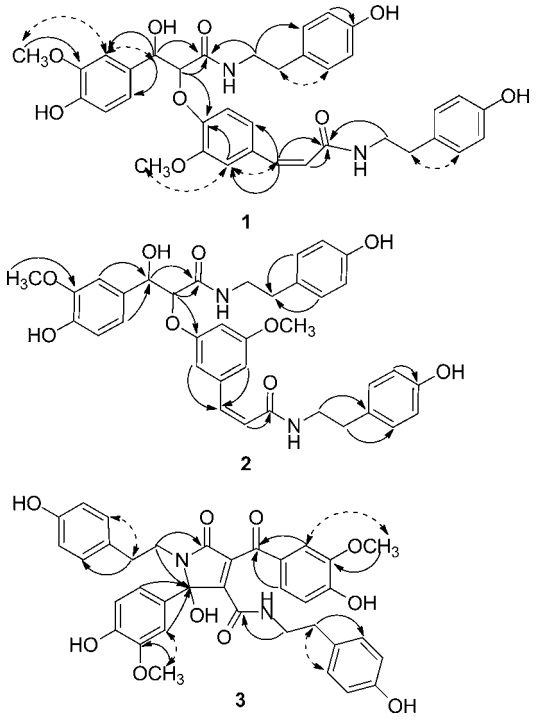

Figure 2. Key HMBC (H → C) and ROESY (dashed arrows) correlations of **1**, **2**, and **3**.

Overhauser effects (NOEs) between the H-7 proton and H-2 and H-6, the H-2 proton and 3-OMe, H-7′ and H-2′ and H-6′, H-2′ and 3′-OMe, H-7″ and H-2″ and H-6″, and H-7‴ and H-2‴ and H-6‴. These data confirmed the structure of **1** as depicted, named *cis*-cannabisin E. Its *trans*-isomer has been isolated from *Cannabis sativa*.[22] Due to the presence of two chiral carbons (C-7 and C-8) in **1**, the small coupling constant between H-7 and H-8 (3.5 Hz) in the ^{1}H NMR spectrum indicated the 7,8-*erythro* configuration.[17,18]

Elucidation of ***2***. Compound **2** has the same molecular formula as **1**, which was deduced to be $C_{36}H_{38}N_2O_9$ as the HRESIMS spectrum showed a pseudomolecular ion at 665.2490 $[M + Na]^+$ (calcd for $C_{36}H_{38}N_2O_9Na$, 665.2470). The ^{1}H NMR spectrum of **2** was also similar to that of **1**, with the exception of trisubstituted aromatic proton signals at δ 7.28 (s) and 6.93 (2H, s) instead of ABX-type coupled aromatic proton signals at δ 7.29 (1H, d, J = 1.5 Hz), 6.33 (1H, d, J = 8.5 Hz), and 6.83 (1H, dd, J = 1.5 Hz, 8.5 Hz). The ^{1}H and ^{13}C data of **2** were assigned on the basis of HMBC and HSQC experiments (Table 1, Figure 2). An oxygenated proton signal at δ 4.73 (H-8) showing HMBC correlations with the quaternary carbon at δ 149.4 (C-5′) and the carbonyl carbon at δ 171.5 (C-9) indicated the presence of an ether function connecting between C-5′ and C-8. In addition, the small $^{3}J_{H\text{-}7,H\text{-}8}$ value (4 Hz) of **2** was also indicative of an *erythro* derivative.[17,18] On the basis of these findings, the structure of **2** was defined as shown in Figure 1 and named cannabisin K.

Elucidation of ***3***. Compound **3** was obtained as a yellow amorphous powder, whose molecular formula was determined to be $C_{36}H_{34}N_2O_{10}$, deduced by the HRESIMS experiment. The IR spectrum of **3** also indicated the presence of a hydroxyl group (3396 cm^{-1}), a carbonyl group (1693 cm^{-1}), an aromatic ring (1590 and 1515 cm^{-1}), and a C−N bond (1276 and 1241 cm^{-1}). The ^{1}H NMR spectrum exhibited two tyramine moieties and two ABX-type coupled aromatic proton signals, indicating

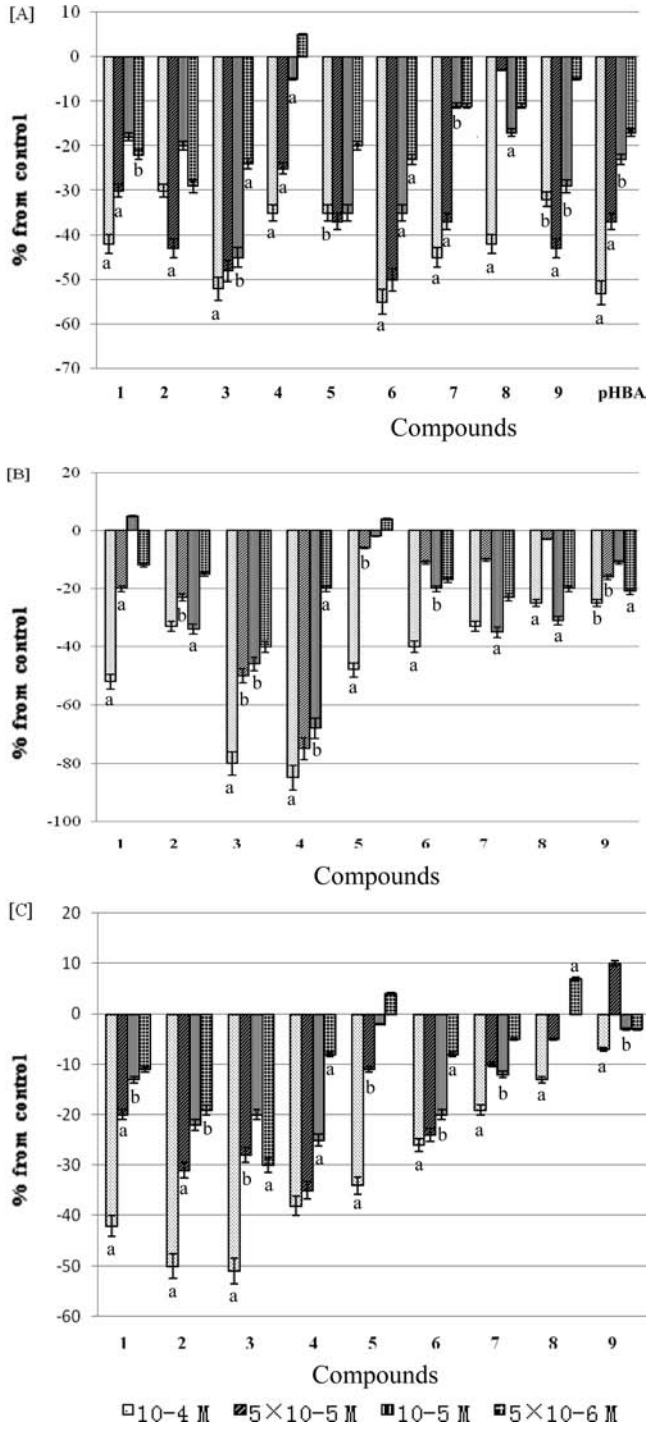

Figure 3. (A) Effect of **1**−**9** and pHBA (4-hydroxybenzoic acid) on germination of *A. fistulosum*. (B) Effect of **1**−**9** on the radical length of *A. fistulosum*. (C) Effect of **1**−**9** on the shoot length of *A. fistulosum*. Values are presented as percentage differences from the control and are not significantly different with $p > 0.05$ for Student's t test: a, $p < 0.01$; b, $0.01 < p < 0.05$.

that **3** was also a lignanamide compound. The ^{1}H and ^{13}C resonances of **3** were assigned by combination of ROESY, HMBC, and HSQC experiments (Table 1, Figure 2). In the HMBC spectrum, the cross-peaks of H-8″ and H-8‴ with C-9 and C-9′ indicated two tyramine groups linked to C-9 and C-9′ carbons, respectively. Furthermore, the cross-peaks between H-8‴ (δ 3.37 and 3.18) and an oxygenated carbon, C-7 (δ 91.8), in

dx.doi.org/10.1021/jf2046784 | J. Agric. Food Chem. 2012, 60, 1682−1687

the HMBC spectrum led to the conclusion that a carboxamide central moiety was present. C-8 was connected to C-8′ to form a five-membered α,β-unsaturated lactam ring on the basis of a pair of remaining quaternary olefinic carbon signals at δ 140.7 (C-8) and 150.9 (C-8′). In the ROESY experiment, the protons of the methoxyl group at δ 3.82 (3-OMe) had a relation with the proton at δ 6.99 (H-2), and the proton of the methoxyl group at δ 3.92 (3′-OMe) had a relation with the proton at δ 7.57 (H-2′). From the above observations, the structure of **3** was defined as shown in Figure 1 and named cannabisin L.

Additional isolated compounds were identified as heliotropamide (**4**),[20] cannabisin E (**5**),[19] *N-cis*-grossamide (**6**),[21] *N-trans*-grossamide (**7**),[22] hyoscyamide (**8**),[23] and cannabisin D (**9**)[24] by comparison of their spectroscopic data to previously reported values. **4** was isolated for the first time from Solanaceae.

Phytotoxicity Bioassays. Since the compounds were isolated from the phytotoxic fraction of *H. niger* ($CHCl_3$ extract), they were further analyzed individually for their phytotoxicity on the seeds of *A. fistulosum*. This species was selected as representative of the main monocotyledon commercial crops. It has been used extensively as a model organism because of its fast germination and high sensitivity and because it allows comparison of bioassay results for many different compounds. The assays were performed according to the procedures optimized by Macías et al.[25] Results are reported as percentage differences of germination, radical elongation, and shoot elongation from the control (Figure 3).

Compounds **3** and **6** caused about 55% inhibition on germination of *A. fistulosum* at the highest concentration of 10^{-4} M (Figure 3). The effect is near that of 4- hydroxybenzoic acid, which is known as a germination inhibitor.[26,27] Compounds **7** and **8** caused about 45% and 42% inhibition at 10^{-4} M concentration. All the compounds inhibited the radical length of *A. fistulosum* at the highest concentration (10^{-4} M). Among them, **3** and **4** reduced the radical elongation by 80% at a concentration of 10^{-4} M, while **1** and **5** showed approximately 50% inhibition. This suggests that the carboxamide moiety plays an important role in inhibition of radical elongation. For inhibitory assay on the shoot elongation of *A. fistulosum*, only **2** and **3** caused approximately 50% inhibition. Additionally, **1** was a more powerful inhibitor of the radical and shoot elongation than **5**, and **6** was also a more powerful inhibitor than **7**, which indicated that the *cis*-isomer was more effective on the phytotoxic activity than the *trans*-isomer. A previous report showed the lignanamides from *Aptenia cordifolia* exhibited phytotoxic activity on the seeds of *Lactuca sativa*.[16] Our present research further confirms their potential phytotoxic role and suggests the lignanamides could be considered as selective natural product herbicides.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Spectra (HRESIMS, ^1H, ^{13}C NMR, ROESY, HSQC, HMBC) of *cis*-cannabisin E, cannabisin K, and cannabisin L (**1**−**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone/Fax: +86-25-83271405. E-mail: cpu_lykong@126.com.

Funding

This research work was supported by the National Natural Science Foundation of China (Grant 81073009), the Cultivation Fund of the Key Scientific and Technical Innovation Project, Ministry of Education of China (Grant 707033), the Scaling Project for Innovation Scholars, Natural Science Foundation of Jiangsu Province, China (Grant BK2008039), and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge Dr. Ming Hua Yang for measuring the ROESY, HSQC, and HMBC NMR spectra.

■ REFERENCES

(1) Dayan, F. E.; Cantrell, C. L.; Duke, S. O. Natural products in crop protection. *Bioorg. Med. Chem.* **2009**, *17*, 4022−4034.
(2) Huang, H. Z.; Morgan, C. M.; Asolkar, R. N.; Koivunen, M. E. Phytotoxicity of sarmentine isolated from long pepper (*Piper longum*) fruit. *J. Agric. Food Chem.* **2010**, *58*, 9994−10000.
(3) Vyvyan, J. R. Allelochemicals as leads for new herbicides and agrochemicals. *Tetrahedron* **2002**, *58*, 1631−1646.
(4) Dayan, F. E.; Romagni, J. G.; Duke, S. O. Investigating the mode of action of natural phytotoxins. *J. Chem. Ecol.* **2000**, *26*, 2079−2094.
(5) Tharayil, N.; Bhowmik, P. C.; Xing, B. S. Preferential sorption of phenolic phytotoxins to soil: Implications for altering the availability of allelochemicals. *J. Agric. Food Chem.* **2006**, *54*, 3033−3040.
(6) Batish, D. R.; Setia, N.; Singh, H. P.; Kohli, R. K. Phytotoxicity of lemon-scented eucalypt oil and its potential use as a bioherbicide. *Crop Prot.* **2004**, *23*, 1209−1214.
(7) Nicotro, V. E.; Gill, R. R.; Vaccarini, C; Burton, G. 15,21-Cyclowithanolides from *Jaborosa bergii*. *J. Nat. Prod.* **2003**, *66*, 1471−1475.
(8) Nicotro, V. E.; Ramacciotti, N. S.; Gill, R. R. Phytotoxic withanolides from *Jaborosa rotacea*. *J. Nat. Prod.* **2006**, *69*, 783−789.
(9) Sun, F.; Li, S. Y.; He, D. J.; Cao, G. Effects of glycoalkaloids from *Solanum* plant on cucumber radical growth. *Phytochemistry* **2010**, *71*, 1534−1538.
(10) Abrosca, B. D.; Greca, M. D.; Fiorentino, A. Structure elucidation and phytotoxicity of C_{13} *nor*-isoprenoids from *Cestrum parqui*. *Phytochemistry* **2004**, *65*, 497−505.
(11) Abrosca, B. D.; Greca, M. D.; Fiorentino, A. S. Structural characterization of phytotoxic terpenoids from *Cestrum parqui*. *Phytochemistry* **2005**, *66*, 2681−2688.
(12) The Editorial Committee of the Chinese Pharmacopoeia. *Chinese Pharmacopoeia*, Chinese Medicine Science and Technology Press: Beijing, China, 2010; Vol.*1*, p 51.
(13) Ji, J.; Shi, L.; Yu, X. W. Chemical and pharmacological researches on *Hyoscyamus niger*. *Chin. Herbal Med.* **2011**, *3*, 117−126.
(14) Macías, F. A.; Lacret, R.; Varela, R. M.; Nogueiras, C.; Molinillo, J. M. G. Isolation and phytotoxicity of terpenes from *Tectona grandis*. *J. Chem. Ecol.* **2010**, *36*, 396−404.
(15) Cutillo, F.; Abrosca, B. D.; Greca, M. D. Cinnamic acid amides from *Chenopodium album*: Effects on seeds germination and plant growth. *Phytochemistry* **2003**, *64*, 1381−1387.
(16) DellaGreca, M.; Previtera, L.; Purcaro, R.; Zarrelli, A. Cinnamic acid amides and lignanamides from *Aptenia cordifolia*. *Tetrahedron* **2006**, *62*, 2877−2882.
(17) Wuts, P. G. M.; Bigelow, S. S. γ-Alkoxyallylboronates as useful reagents for preparation of differentially protected diol derivatives. *J. Org. Chem.* **1982**, *47*, 2498−2500.
(18) Matsuda, N.; Kikuchi, M. Studies on the constituents of *Lonicera* species. X. Neolignan glycosides from the leaves of *Lonicera gracilipes* var. *glandulosa* maxim. *Chem. Pharm. Bull.* **1996**, *44*, 1676−1679.

(19) Sakakibara, I.; Ikeya, Y.; Hayashi, K.; Okada, M. Three acyclic bis-phenylpropane lignanamides from fruits of *Cannabis sativa*. *Phytochemistry* **1995**, *38*, 1003−1007.
(20) Guntern, A.; Ioset, J. R.; Queiroz, E. F. Heliotropamide, a novel oxopyrrolidine-3-carboxamide from *Heliotropium ovalifolium*. *J. Nat. Prod.* **2003**, *66*, 1550−1553.
(21) King, R. R.; Calhoun, L. A. Characterization of cross-linked hydroxycinnamic acid amides isolated from potato common scab lesions. *Phytochemistry* **2005**, *66*, 2468−2473.
(22) Seca, A. M. L.; Silva, A. M. S.; Silvestre, A. J. D.; Cavaleiro, J. A. S. Lignanamides and other phenolic constituents from the bark of kenaf (*Hibiscus cannabinus*). *Phytochemistry* **2001**, *58*, 1219−1223.
(23) Ma, C. Y.; Liu, W. K.; Che, C. T. Lignanamides and nonalkaloidal components of *Hyoscyamus niger* seeds. *J. Nat. Prod.* **2002**, *65*, 206−209.
(24) Sakakibara, I.; Ikeya, Y.; Hayashi, K.; Mitsuhashi, H. Three phenyldihydronaphthalene lignanamides from fruits of *Cannabis sativa*. *Phytochemistry* **1992**, *31*, 3219−3223.
(25) Macías, F. A.; Castellano, D.; Molinillo, J. M. G. Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. *J. Agric. Food Chem.* **2000**, *48*, 2512−2521.
(26) Mizutani, J. Select allelochemicals. *Crit. Rev. Plant Sci.* **1999**, *18*, 221−223.
(27) Sebeson, J. M.; Mitchell, E.; Snyder, F. W. Effect of phenolic acids on alpha-amylase *in vitro* and early growth of sugar beet. *J. Am. Soc. Sugar Beet Technol.* **1969**, *15*, 556−561.