

Antibacterial stilbenoids from the roots of *Stemona tuberosa*

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Abstract

Twelve dihydrostilbenes, stilbestemins N–Y (**1**–**12**), and a phenanthraquinone, stemanthraquinone (**13**), were isolated and identified from roots of *Stemona tuberosa*, along with five known dihydrostilbenes. Their structures were established on the basis of 1D and 2D NMR and other spectroscopic analyses. Dihydrostilbene **8** exhibited strong activity against *Bacillus pumilus* (MIT 12.5–25 µg/mL). Many tested compounds exhibited moderate antibacterial activities.

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1. Introduction

The roots of *Stemona* genus (Stemonaceae) have long been prescribed in traditional Chinese medicine as insecticidal and antitussive agents (Jiangsu New Medical College, 1986; Tsi and Duyfjes, 2000). Up to now extracts from roots of these plants are used for respiratory disorders, including pulmonary tuberculosis and bronchitis, and externally used against different insect pests (Philli and Ferreira de Oliveira, 2000; Xu, 2000). Pharmacological studies showed that the EtOH extract of *Stemona tuberosa* inhibited growth of many kinds of bacteria and fungi (Zhao et al., 1995). In our latest paper, we disclosed that several stilbenoids from roots of *Stemona sessilifolia* showed antibacterial activities against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Yang et al., 2006). These previous reports established that antimicrobial activities could be attributed to stilbenoids. To further identify stilbenoids related to the medicinal application, the CH₂Cl₂ fraction from roots of *S. tuberosa* collected in Yunnan province was investigated. Thirteen new derivatives, designed

as stilbestemins N–Y (**1**–**12**) and stemanthraquinone (**13**), were isolated and identified, along with a series of known dihydrostilbenes (**14**–**18**) (Pacher et al., 2002; Yang et al., 2006). Herein we report the isolation and structural elucidation of the new stilbenoids, and their antibacterial activities against some hospital pathogenic bacteria.

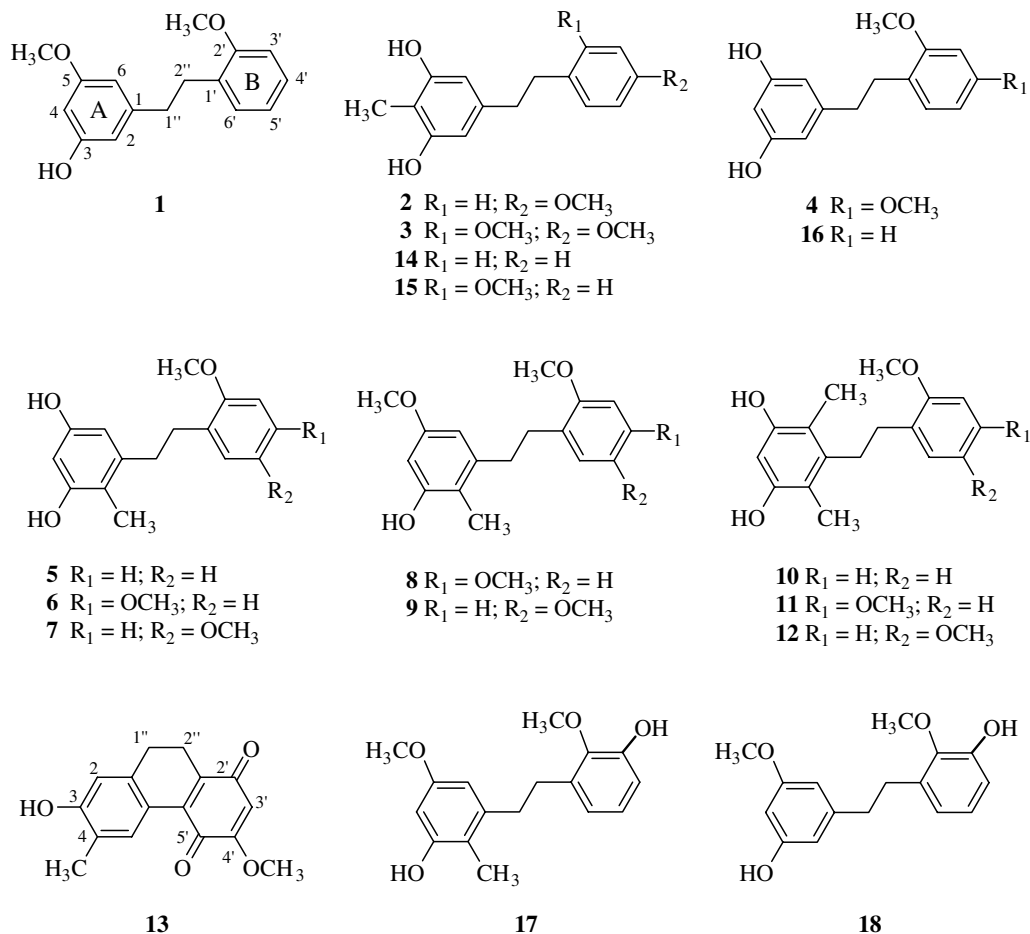
2. Results and discussion

The CH₂Cl₂ fraction from the roots of *S. tuberosa* was obtained as described in the experimental section. The HPLC analysis of this fraction with UV detection revealed the occurrence of two groups of stilbenoids. The majority with typical UV absorptions at 197–220 and 270–286 nm suggested the presence of dihydrostilbenes and the minority appeared to be phenanthraquinones with characteristic UV absorptions at 210–220, 280–286 and 295–310 nm. Preparative HPLC was applied for isolation of 18 stilbenoids. Among them, 13 compounds were identified to be hitherto unknown derivatives (see Fig. 1).

Stilbestemin N (**1**) was obtained as colorless needles. The molecular formula, C₁₆H₁₈O₃, was inferred from its HREIMS (*m/z* 258.1252 [M]⁺). The IR absorptions at 3388 and 1597 cm^{−1} showed the existence of hydroxyl

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Fig. 1. Stilbenoids from *S. tuberosa*.

groups and benzenoids. The ^1H NMR spectrum of **1** (Table 1) showed signals for two methoxyl groups at δ_{H} 3.82 (3H, *s*) and 3.77 (3H, *s*), seven aromatic protons at δ_{H} 6.26–7.20, and four benzylic protons at δ_{H} 2.77–2.90 (4H, *m*) of a bibenzyl derivative (Kostechi et al., 2004; Zhao et al., 1995). The EIMS spectrum indicated fragments for a methoxybenzyl (m/z 121, $\text{C}_8\text{H}_9\text{O}^+$) and a hydroxy-methoxybenzyl (m/z 137, $\text{C}_8\text{H}_9\text{O}_2^+$). The positions of the functional groups in the two phenyl rings were ascertained from the chemical shifts and the splitting patterns of the aromatic protons of **1**. As a matter of fact, the chemical shifts and the splitting patterns of the four aromatic protons [δ_{H} 7.20 (1H, *ddd*, $J = 1.8, 7.9, 7.9$ Hz, H-4'), 7.10 (1H, *dd*, $J = 1.8, 7.9$ Hz, H-6'), 6.88 (1H, *m*, H-5') and 6.86 (1H, *d*, $J = 7.9$ Hz, H-3')] were strikingly similar to those of stilbostemin D (Zhao et al., 1995), which indicated the presence of a methoxyl group at C-2' in ring B of **1**. The remaining three aromatic proton resonances at δ_{H} 6.38 (1H, *dd*, $J = 1.3, 1.7$ Hz), 6.31 (1H, *dd*, $J = 1.3, 2.0$ Hz) and 6.26 (1H, *dd*, $J = 1.7, 2.0$ Hz) indicated the presence of a *meta*-trisubstituted benzyl fragment. The structure of **1** was confirmed by ROESY and HMBC experiments, with the detailed assignments of the ^1H and ^{13}C NMR resonances shown in Tables 1 and 2.

Stilbostemin O (**2**) and stilbostemin P (**3**) were both obtained as yellow amorphous powder. Their molecular formulae were determined to be $\text{C}_{16}\text{H}_{18}\text{O}_3$ (**2**: m/z 258.1247 $[\text{M}]^+$) and $\text{C}_{17}\text{H}_{20}\text{O}_4$ (**3**: m/z 288.1362 $[\text{M}]^+$) by HREIMS, respectively. The same fragment (m/z 137, $\text{C}_8\text{H}_9\text{O}_2^+$) was clearly observed in the EIMS spectra of **2** and **3**. This fragment was deduced to be a deoxygenated ring A, the same as that of stilbostemin B (**14**) (Zhao et al., 1995), by the presence of a singlet of two equivalent protons and a methyl in their ^1H NMR spectra [**2**: 6.22 (2H, *s*, H-2, H-6), 2.12 (3H, *s*, Me-4); **3**: 6.24 (2H, *s*, H-2, H-6), 2.11 (3H, *s*, Me-4)]. Such a structural moiety was confirmed by the NOE enhancement of the methylene (H-1'') by the irradiation of the two-proton singlet. Besides the deoxygenated ring A in both compounds, ring B of **2** was indicated to be a methoxybenzyl fragment by the EIMS peak (m/z 121, $\text{C}_8\text{H}_9\text{O}^+$). The *para*-substituted pattern of ring B was suggested further by a pair of double doublet at δ_{H} 6.82 (2H, *dd*, $J = 2.1, 6.5$ Hz, H-2', H-6') and 7.08 (2H, *dd*, $J = 2.1, 6.5$ Hz, H-3', H-5') in its ^1H NMR spectrum. Thus **2** was established as 3,5-dihydroxy-4'-methoxy-4-methyl bibenzyl. Similarly, ring B of **3** was suggested to be a dimethoxybenzyl by its EIMS fragment ($\text{C}_9\text{H}_{11}\text{O}_2^+$) at m/z 151. An obvious ABX aromatic-proton

Table 1

¹H NMR spectroscopic data of dihydrostilbenes **1–12** (**1–9**, **11**, **12** in CDCl₃, **10** in *d*-acetone, 400 MHz)

Position	1	2	3	4	5	6	7	8	9	10	11	12
2	6.38 <i>dd</i>	6.22 <i>s</i>	6.24 <i>s</i>	6.27 <i>dd</i>	2.12 <i>s</i> (Me)	2.09 <i>s</i> (Me)	2.10 <i>s</i> (Me)	2.14 <i>s</i> (Me)	2.14 <i>s</i> (Me)	2.18 <i>s</i> (Me)	2.15 <i>s</i> (Me)	2.17 <i>s</i> (Me)
4	6.26 <i>dd</i>	2.12 <i>s</i> (Me)	2.11 <i>s</i> (Me)	6.20 <i>dd</i>	6.23 <i>d</i>	6.22 <i>d</i>	6.20 <i>d</i>	6.27 <i>d</i>	6.27 <i>d</i>	6.26 <i>s</i>	6.34 <i>s</i>	6.23 <i>s</i>
5	3.77 <i>s</i> (OMe)							3.74 <i>s</i> (OMe)	3.74 <i>s</i> (OMe)			
6	6.31 <i>dd</i>	6.22 <i>s</i>	6.24 <i>s</i>	6.27 <i>dd</i>	6.24 <i>d</i>	6.24 <i>d</i>	6.24 <i>d</i>	6.36 <i>d</i>	6.36 <i>d</i>	2.18 <i>s</i> (Me)	2.15 <i>s</i> (Me)	2.17 <i>s</i> (Me)
2'	3.82 <i>s</i> (OMe)	6.82 <i>dd</i>	3.80 <i>s</i> (OMe)	3.79 <i>s</i> (OMe)	3.83 <i>s</i> (OMe)	3.82 <i>s</i> (OMe)	3.82 <i>s</i> (OMe)	3.82 <i>s</i> (OMe)	3.75 <i>s</i> (OMe)	3.85 <i>s</i> (OMe)	3.87 <i>s</i> (OMe)	3.77 <i>s</i> (OMe)
3'	6.86 <i>d</i>	7.08 <i>dd</i>	6.45 <i>d</i>	6.45 <i>d</i>	6.97 <i>d</i>	6.55 <i>d</i>	6.80 <i>d</i>	6.46 <i>d</i>	6.79 <i>d</i>	6.87 <i>m</i>	6.56 <i>d</i>	6.80 <i>d</i>
4'	7.20 <i>ddd</i>	3.80 <i>s</i> (OMe)	3.80 <i>s</i> (OMe)	3.78 <i>s</i> (OMe)	7.20 <i>m</i>	3.78 <i>s</i> (OMe)	6.71 <i>dd</i>	3.80 <i>s</i> (OMe)	6.71 <i>dd</i>	7.21 <i>m</i>	3.79 <i>s</i> (OMe)	6.73 <i>dd</i>
5'	6.88 <i>m</i>	7.08 <i>dd</i>	6.40 <i>dd</i>	6.40 <i>dd</i>	6.85 <i>m</i>	6.42 <i>dd</i>	3.78 <i>s</i> (OMe)	6.41 <i>dd</i>	3.79 <i>s</i> (OMe)	6.89 <i>m</i>	6.46 <i>dd</i>	3.82 <i>s</i> (OMe)
6'	7.10 <i>dd</i>	6.82 <i>dd</i>	6.99 <i>d</i>	6.96 <i>d</i>	7.18 <i>m</i>	7.02 <i>d</i>	6.68 <i>d</i>	7.01 <i>d</i>	6.72 <i>d</i>	7.17 <i>m</i>	7.08 <i>d</i>	6.75 <i>d</i>
1''	2.79 <i>m</i>	2.80 <i>m</i>	2.79 <i>m</i>	2.78 <i>m</i>	2.78 <i>m</i>	2.66 <i>s</i>	2.79 <i>s</i>	2.75 <i>m</i>	2.80 <i>s</i>	2.85 <i>m</i>	2.77 <i>m</i>	2.87 <i>m</i>
2''	2.89 <i>m</i>	2.78 <i>m</i>	2.68 <i>m</i>	2.69 <i>m</i>	2.77 <i>m</i>	2.66 <i>s</i>	2.77 <i>s</i>	2.78 <i>m</i>	2.80 <i>s</i>	2.72 <i>m</i>	2.61 <i>m</i>	2.68 <i>m</i>

J (Hz): **1**: 2,4 = 1.7; 2,6 = 1.3; 4,6 = 2.0; 3',4' = 7.9; 4',5' = 7.9; 4',6' = 1.8; 5',6' = 7.9; **2**: 2',3' = 5',6' = 6.5; 2',6' = 3',5' = 2.1; **3**: 3',5' = 2.5; 5',6' = 8.2; **4**: 2,4 = 4,6 = 2.2; 2,6 = 2.0; 3',5' = 2.5; 5',6' = 7.9; **5**: 4,6 = 2.3; 3',4' = 8.4; **6**: 4,6 = 2.4; 3',5' = 2.3; 5',6' = 8.2; **7**: 4,6 = 2.5; 3',4' = 8.6; 4',6' = 2.8; **8**: 4,6 = 2.1; 3',5' = 2.3; 5',6' = 8.1; **9**: 4,6 = 2.1; 3',4' = 8.5; 4',6' = 2.3; **11**: 3',5' = 2.5; 5',6' = 8.2; **12**: 3',4' = 8.4; 4',6' = 3.1.

Table 2

¹³C NMR spectroscopic data of dihydrostilbenes **1–12** (**1–9**, **11**, **12** in CDCl₃, **10** in *d*-acetone, 100 MHz)

C	1	2	3	4	5	6	7	8	9	10	11	12
1	145.2 <i>s</i>	140.9 <i>s</i>	141.6 <i>s</i>	145.6 <i>s</i>	143.5 <i>s</i>	143.0 <i>s</i>	143.5 <i>s</i>	143.2 <i>s</i>	142.9 <i>s</i>	141.5 <i>s</i>	141.9 <i>s</i>	141.4 <i>s</i>
2	106.7 <i>d</i>	107.8 <i>d</i>	107.8 <i>d</i>	108.1 <i>d</i>	114.1 <i>s</i>	113.5 <i>s</i>	114.8 <i>s</i>	114.1 <i>s</i>	114.2 <i>s</i>	114.4 <i>s</i>	114.4 <i>s</i>	114.4 <i>s</i>
3	160.8 <i>s</i>	154.5 <i>s</i>	154.5 <i>s</i>	156.5 <i>s</i>	154.8 <i>s</i>	156.5 <i>s</i>	152.3 <i>s</i>	154.5 <i>s</i>	154.6 <i>s</i>	152.1 <i>s</i>	154.4 <i>s</i>	152.1 <i>s</i>
4	98.9 <i>d</i>	107.6 <i>s</i>	107.4 <i>s</i>	100.2 <i>d</i>	100.4 <i>d</i>	100.6 <i>d</i>	100.8 <i>d</i>	99.1 <i>d</i>	99.2 <i>d</i>	100.6 <i>d</i>	101.4 <i>d</i>	100.5 <i>d</i>
5	156.5 <i>s</i>	154.5 <i>s</i>	154.5 <i>s</i>	156.5 <i>s</i>	154.0 <i>s</i>	156.0 <i>s</i>	153.7 <i>s</i>	158.1 <i>s</i>	158.1 <i>s</i>	152.1 <i>s</i>	154.4 <i>s</i>	152.1 <i>s</i>
6	108.0 <i>d</i>	107.8 <i>d</i>	107.8 <i>d</i>	108.1 <i>d</i>	108.6 <i>d</i>	107.9 <i>d</i>	108.9 <i>d</i>	107.4 <i>d</i>	107.3 <i>d</i>	114.4 <i>s</i>	114.4 <i>s</i>	114.4 <i>s</i>
1'	130.1 <i>s</i>	133.8 <i>s</i>	122.6 <i>s</i>	122.5 <i>s</i>	130.0 <i>s</i>	123.1 <i>s</i>	131.9 <i>s</i>	122.8 <i>s</i>	131.6 <i>s</i>	130.5 <i>s</i>	124.0 <i>s</i>	131.7 <i>s</i>
2'	157.5 <i>s</i>	113.7 <i>d</i>	159.2 <i>s</i>	159.0 <i>s</i>	157.7 <i>s</i>	160.1 <i>s</i>	155.1 <i>s</i>	158.4 <i>s</i>	151.9 <i>s</i>	157.6 <i>s</i>	159.7 <i>s</i>	151.9 <i>s</i>
3'	120.4 <i>d</i>	129.3 <i>d</i>	98.5 <i>d</i>	98.5 <i>d</i>	110.3 <i>d</i>	98.8 <i>d</i>	111.7 <i>d</i>	98.6 <i>d</i>	111.3 <i>d</i>	110.3 <i>d</i>	99.6 <i>d</i>	111.3 <i>d</i>
4'	127.2 <i>d</i>	157.8 <i>s</i>	158.3 <i>s</i>	158.2 <i>s</i>	127.4 <i>d</i>	159.0 <i>s</i>	111.5 <i>d</i>	159.3 <i>s</i>	111.2 <i>d</i>	127.3 <i>d</i>	160.8 <i>s</i>	111.1 <i>d</i>
5'	110.3 <i>d</i>	129.3 <i>d</i>	103.7 <i>d</i>	103.8 <i>d</i>	120.5 <i>d</i>	104.8 <i>d</i>	154.1 <i>s</i>	103.9 <i>d</i>	153.5 <i>s</i>	120.5 <i>d</i>	105.6 <i>d</i>	153.6 <i>s</i>
6'	129.8 <i>d</i>	113.7 <i>d</i>	129.9 <i>d</i>	130.0 <i>d</i>	130.3 <i>d</i>	130.4 <i>d</i>	116.7 <i>d</i>	130.0 <i>d</i>	116.4 <i>d</i>	129.8 <i>d</i>	131.1 <i>d</i>	116.5 <i>d</i>
1''	36.2 <i>t</i>	37.7 <i>t</i>	35.9 <i>t</i>	36.1 <i>t</i>	34.0 <i>t</i>	35.1 <i>t</i>	34.2 <i>t</i>	34.7 <i>t</i>	34.5 <i>t</i>	30.8 <i>t</i>	32.2 <i>t</i>	30.8 <i>t</i>
2''	32.1 <i>t</i>	36.7 <i>t</i>	31.6 <i>t</i>	31.3 <i>t</i>	31.5 <i>t</i>	31.6 <i>t</i>	31.7 <i>t</i>	31.1 <i>t</i>	31.9 <i>t</i>	30.6 <i>t</i>	30.7 <i>t</i>	30.7 <i>t</i>
2-Me					10.1 <i>q</i>	10.4 <i>q</i>	10.4 <i>q</i>	10.3 <i>q</i>	10.4 <i>q</i>	10.9 <i>q</i>	11.6 <i>q</i>	10.9 <i>q</i>
4(6)-Me		7.7 <i>q</i>	7.7 <i>q</i>							10.9 <i>q</i>	11.6 <i>q</i>	10.9 <i>q</i>
3-OMe	55.2 <i>q</i>							55.2 <i>q</i>	55.3 <i>q</i>			
5-OMe								55.4 <i>q</i>	55.9 <i>q</i>	55.2 <i>q</i>	56.1 <i>q</i>	55.8 <i>q</i>
2'-OMe	55.3 <i>q</i>		55.3 <i>q</i>	55.3 <i>q</i>	55.2 <i>q</i>	55.4 <i>q</i>	56.1 <i>q</i>	55.4 <i>q</i>	55.9 <i>q</i>	55.2 <i>q</i>	56.1 <i>q</i>	55.8 <i>q</i>
4'-OMe		55.3 <i>q</i>	55.3 <i>q</i>	55.3 <i>q</i>		55.2 <i>q</i>		55.4 <i>q</i>			55.9 <i>q</i>	
5'-OMe							56.0 <i>q</i>		55.7 <i>q</i>			55.9 <i>q</i>

substitution pattern [δ_{H} 6.40 (1H, *dd*, *J* = 2.5, 8.2 Hz, H-5'), 6.45 (1H, *d*, *J* = 2.5 Hz, H-3'), 6.99 (1H, *d*, *J* = 8.2 Hz, H-6')] of ring B reflected a 2,4 or 2,5-dimethoxybenzyl moiety. The locations of methoxyl groups were established as a 2,4-substitution by the ROESY experiment. Therefore **3** was designated as 3,5-dihydroxy-2',4'-dimethoxy-4-methyl bibenzyl. The detailed assignments of the ¹H and ¹³C NMR signals are shown in Tables 1 and 2.

Stilbostemin Q (**4**) was obtained as a yellow amorphous powder. Its molecular formula was established as C₁₆H₁₈O₄ by HREIMS (*m/z* 274.1204 [M]⁺). Its IR spec-

trum showed the existence of hydroxyl groups (3454 cm⁻¹) and benzenoid moieties (1601 cm⁻¹). The EIMS fragmentation pattern of **4** indicated fragments of a dimethoxybenzyl (*m/z* 151, C₉H₁₁O₂⁺) and a dihydroxybenzyl (*m/z* 123, C₇H₇O₂⁺). Three aromatic proton signals [6.27 (2H, *dd*, *J* = 2.0, 2.2 Hz, H-2, H-6), 6.20 (1H, *dd*, *J* = 2.2, 2.2 Hz, H-4)] were identical to those of ring A in stilbostemin A (Pacher et al., 2002). Moreover, **4** was elucidated to share the same ring B of **3** by the ABX signals [6.40 (1H, *dd*, *J* = 2.5, 7.9 Hz, H-5'), 6.45 (1H, *d*, *J* = 2.5 Hz, H-3'), 6.96 (1H, *d*, *J* = 7.9 Hz, H-6')] in the ¹H

NMR spectrum. The structure of **4** was thus identified as 3,5-dihydroxy-2',4'-dimethoxyl bibenzyl.

Stilbostemin R (**5**), a yellow amorphous powder, exhibited the molecular formula $C_{16}H_{18}O_3$ from HREIMS (m/z 258.1262 $[M]^+$). The 1H NMR spectrum of **5** (Table 1) showed signals of an aromatic methoxyl group at δ_H 3.83 (3H, s), an aromatic methyl group at δ_H 2.12 (3H, s), six aromatic protons at δ_H 6.23–7.20 and a four-proton multiplet at δ_H 2.75–2.80. The EIMS fragmentation pattern of **5** suggested the presence of fragments of a dihydroxymethylbenzyl (m/z 137, $C_8H_9O_2^+$) and a methoxybenzyl (m/z 121, $C_8H_9O^+$). Two aromatic protons at δ_H 6.23 (1H, d, $J = 2.3$ Hz) and δ_H 6.24 (1H, d, $J = 2.3$ Hz) suggested a *meta*-substitution of ring A. The methyl group was assigned at 2-position by ROESY cross-peaks between the methyl group and the methylene (δ_H 2.78). The rest 1H and ^{13}C NMR signals in **5** were closely similar to those of **1**, indicating that both compounds shared the same ring B. Thus the structure of **5** was determined as 3,5-dihydroxy-2'-methoxy-2-methyl bibenzyl.

Stilbostemins S (**6**) and T (**7**) were both obtained as yellow amorphous powder. Their molecular formulae $C_{17}H_{20}O_4$ were established by HREIMS (**6**: m/z 288.1359 $[M]^+$, **7**: m/z 288.1348 $[M]^+$). The IR spectra showed the existence of a hydroxyl group (**6**: 3348 cm^{-1} , **7**: 3411 cm^{-1}) and a benzene moiety (**6**: 1593 cm^{-1} , **7**: 1610 cm^{-1}). The EIMS fragmentation patterns of **6** and **7** indicated fragments of a dihydroxy-methylbenzyl (m/z 137, $C_8H_9O_2^+$) and a dimethoxybenzyl (m/z 151, $C_9H_{11}O_2^+$). Comparison of the 1H and ^{13}C NMR spectroscopic data of **6** and **7** with those of **5** indicated that three compounds shared the same dihydroxymethylbenzyl ring A. The substitution pattern of the dimethoxybenzyl fragment of **6** was the same to that of **3**, while that of **7** was similar to the ring B in stilbostemin K (Yang et al., 2006). The structures of **6** and **7** were further confirmed by ROESY experiments. The detail assignments of the 1H and ^{13}C NMR signals are shown in Tables 1 and 2.

Stilbostemins U (**8**) and V (**9**) were obtained as colorless oil. Their molecular formulae were deduced as $C_{18}H_{22}O_4$ by HREIMS spectra. The EIMS spectral fragmentation patterns of **8** and **9** indicated fragments of a dimethoxybenzyl (m/z 151, $C_9H_{11}O_2^+$) and a hydroxy-methyl-methoxybenzyl (m/z 151, $C_9H_{11}O_2^+$). The 1H and ^{13}C NMR spectra of **8** were similar to those of **6**, except that one more methoxyl was present in **8**, which was also indicated by its molecular formula. The substitution pattern of **8** was established by the ROESY experiment. A ROESY cross-peak between the methoxyl group at δ_H 3.74 and H-4 and H-6 suggested the methoxyl group was attached to C-5 in ring A. The substitution patterns of rings A and B were confirmed by the HMBC correlations. Thus, **8** was identified as 3-hydroxy-5,2',4'-trimethoxy-2-methyl bibenzyl. The substitution pattern of ring A in **9** was same to that in **8**, while its ring B was same to that of **7** according to the 1H and ^{13}C NMR spectroscopic data comparison. The structure of **9** was further confirmed by ROESY and HMBC experiments.

The molecular formula of stilbostemin W (**10**) was established to be $C_{17}H_{20}O_3$ by HREIMS. The 1H NMR spectrum (Table 1) disclosed signals for a 1,2-disubstituted benzene, an aromatic proton, a methoxyl group, two methyl groups, as well as two methylenes, indicating the presence of a pentasubstituted bibenzyl. Considering the special ion fragments at m/z 151 ($C_9H_{11}O_2^+$), 121 ($C_8H_9O^+$) and aromatic protons in rings A and B, it was concluded that the methoxyl group was located in ring B, while the methyl and hydroxyl groups were in ring A. Meanwhile, a symmetrically 3,5-dihydroxy-2,6-dimethyl substituted ring A was suggested by four signals in aromatic region (δ_C 141.5, 114.4, 152.1, 100.6) in the ^{13}C NMR spectrum and the NOE correlations from two methyl groups at δ_H 2.18 to H-1''. The substitution of ring B resembled to that of **5** on the basis of 1H and ^{13}C NMR spectra and NOE correlations. Furthermore, HMBC data confirmed this substitution pattern and also allowed the assignment of the 1H and ^{13}C NMR signals (Tables 1 and 2).

Stilbostemins X (**11**) and Y (**12**) were isolated as light yellow oil and a yellow amorphous powder, respectively. From their HREIMS data, the same molecular formula, $C_{18}H_{22}O_4$, was established for both compounds. Their 1H NMR spectra (Table 1) exhibited similar signals for one 1,2,4-trisubstituted benzene, one aromatic proton, two methoxy groups, two methyl groups, as well as two methylenes, indicating the presence of a hexasubstituted bibenzyl in their structures (Table 1). The EIMS fragments of a dimethoxybenzyl (m/z 151, $C_9H_{11}O_2^+$) and a dihydroxy-dimethylbenzyl (m/z 151, $C_9H_{11}O_2^+$) for compounds **11** and **12** further indicated the similarity of their structures. The ROESY and HMBC experiments suggested that both **11** and **12** shared the same 3,5-dihydroxy-2,6-dimethyl benzyl ring A as that of **10**. As the ring B substitution pattern of **11** was the same as that of **6**, the structure of **11** was determined as 3,5-dihydroxy-2',4'-dimethoxy-2,6-dimethyl bibenzyl. While the substitution pattern of ring B in **12** was the same as that of **7**, the structure of **12** was constructed as 3,5-dihydroxy-2',5'-dimethoxy-2,6-dimethyl bibenzyl.

Stemanthraquinone (**13**) was obtained as an orange amorphous solid and its molecular formula was established as $C_{16}H_{14}O_4$ by HREIMS. The typical UV absorption maxima at 275, 339 and 496 nm and the strong IR bands at 1647, 1625, 1608, 1564 and 1413 cm^{-1} indicated a phenanthraquinone moiety (Tezuka et al., 1990). Its 1H NMR spectrum (Table 3) showed a broad singlet at δ_H 2.69, assigned to H₂-1'' and H₂-2'' of phenanthraquinones, three isolated olefinic protons at δ_H 5.91 (1H, s), 6.66 (1H, s) and 7.89 (1H, s), a methoxyl group (δ_H 3.85) and a methyl group (δ_H 2.27). Two carbonyl signals (δ_C 181.4 and 187.5) were suggested to be a *para*-quinone compared with those of *para*- and *ortho*-quinone. By comparison with the above spectroscopic data with those of ephemanthraquinone (Tezuka et al., 1991), **13** should be a derivative of 1'',2''-dihydrophenanthrene-2',5'-quinone. The NOE

Table 3
¹H and ¹³C NMR spectroscopic data of stemanthraquinone (**13**) (in CDCl₃, 400 and 100 MHz)

Position	δ _H	δ _C
1		139.2 <i>s</i>
2	6.66 <i>s</i>	114.5 <i>d</i>
3		155.6 <i>s</i>
4	7.89 <i>s</i>	121.9 <i>d</i>
5		133.3 <i>s</i>
6		136.7 <i>s</i>
1'		139.2 <i>s</i>
2'		181.4 <i>s</i>
3'	5.91 <i>s</i>	107.5 <i>d</i>
4'		158.6 <i>s</i>
5'		187.5 <i>s</i>
6'		136.9 <i>s</i>
1''	2.69 <i>br s</i>	27.1 <i>t</i>
2''	2.69 <i>br s</i>	20.3 <i>t</i>
4-Me	2.27 <i>s</i>	15.6 <i>q</i>
4'-OMe	3.85 <i>s</i>	56.2 <i>q</i>

correlation between the methoxyl group and the olefinic proton at δ_H 5.91 suggested methoxyl group was adjacent to the olefinic proton. The almost identical chemical shift values of the olefinic proton (δ_H 5.91) and the methoxyl group (δ_H 3.85) with those of ephemeranthoquinone suggested they were located at positions 3' and 4' in ring B, respectively. The NOE correlation between the proton at δ_H 6.66 and H-1'' indicated that it was located at the 2-position. Additionally, the NOE correlation between the methyl group at δ_H 2.27 and H-5 confirmed the methyl functionality at the 4-position. Thus the remaining position 3 should be substituted by a hydroxyl group. The HMBC correlations provided more evidence to confirm the substituted pattern in rings A and B. Therefore, structure **13** was established as 3-hydroxy-4'-methoxy-4-methyl-1'',2''-dihydrophenanthrene-2',5'-quinone.

Stilbenoids were the main non-alkaloid constituents from *S. tuberosa*. So far stilbenoids were isolated from many other *Stemona* species as their characteristic components. It was reported that the aromatic C-methylation of stilbenoids was a typical chemical feature of the *Stemona* genus (Kostechi et al., 2004; Pacher et al., 2002). Thus feature was also observed in stilbenoids from the roots of *S. tuberosa*. All of the above derivatives, except for compounds **1** and **4**, were characterized by the C-methylation of ring A. Moreover, the naturally occurring 2,6-dimethyl substituted dihydrostilbenes were rarely found in the plant kingdom, and so far from only the phytochemical investigation of *S. tuberosa*.

All new compounds were assayed for the antibacterial activities against six hospital pathogenic bacteria including four Gram-positive bacteria *S. aureus* ATCC 25923, *Bacillus pumilus* ATCC 21356, *Bacillus subtilis*, *Cryptococcus neoformans*, and a Gram-negative bacterium *Klebsiella pneumoniae* ATCC 13883, and a fungus *Candida albicans* *in vitro*. Two antibacterial agents, gentamycin and amphotericin B, were used as positive controls in these tests.

Table 4
 Antibacterial activities of stilbenoids **1–13**

Compounds and Control	Minimum inhibitory concentration (μg/mL)		
	<i>B. pumilus</i>	<i>C. neoformans</i>	<i>K. pneumoniae</i>
1	200	200	>100
2	200	>200	>50
3	200	200	100
4	200	200	>50
5	200	200	>200
6	200	>200	100
7	200	100	>50
8	12.5–25	50	100
9	200	>200	>200
10	200	>200	>200
11	50	50	200
12	200	50	200
13	>200	>200	>200
Gentamycin	3.125	>200	3.125
Amphotericin B	>200	1.562	>200

Among the test compounds, compound **8** displayed good antimicrobial activity against *B. pumilus* at the level of MIC 12.5–25 μg/mL (Table 4). Meanwhile, compounds **8**, **11** and **12** showed moderate antibacterial activities against *C. neoformans*, with MICs at 50 μg/mL. Compounds **2**, **4** and **7** exhibited moderate antibacterial activities against *K. pneumoniae* at the level of MICs 50 μg/mL.

3. Experimental

3.1. General

IR spectra were recorded on Nicolet Magna FT-IR 750 spectrophotometer whereas NMR spectra were acquired 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 Finnigan MAT-95 mass spectrometer whereas ESIMS and HRESIMS spectra were obtained on a Micromass LC-MS-MS mass spectrometer. Silica gel was used for flash chromatography and was produced by Qingdao Marine Chemical Industrials. TLC was carried out on precoated silica gel GF254 plates (Yantai Chemical Industrials).

3.2. HPLC

Analytical HPLC was performed on a Waters 2690 instrument with a 996 PAD (Photodiode Array Detector) and 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 H₂O (A) and CH₃CN (B) containing 0.1% HCO₂Cl, at a flow rate of 1.0 mL/min. 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 with a 320 single wave detector. Chromatographic separation was carried out on two C18 columns (220 × 25 mm, 10 μm, Merck; 220 × 50 mm, 10 μm, Merck), using a gradient solvent system comprised of H₂O (A) and CH₃CN (B) containing 0.1% HCO₂Cl, at a flow rate of 15 and 60 mL/min, respectively.

3.3. Plant Material

The plant material was collected in Wenshan County, Yunnan Province in May 2005, PR China, and identified by Prof. Jin-Gui Shen. A voucher (No. SIMM-YYE-050401) was deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.4. Extraction and Isolation

Air-dried roots of *S. tuberosa* (7 kg) were ground into powder and extracted with 10 L 95% ethanol in room temperature for three times (three days each). After evaporation of the collected percolate, the crude extract was acidified with dilute HCl (4%) to pH 1–2 and partitioned between CH₂Cl₂ and H₂O. The CH₂Cl₂ solubility fraction was evaporated to dryness in vacuo to yield a crude extract (158 g). An aliquot of a crude extract (10 g) was subjected to silica gel cc eluted with petroleum ether–acetone (18:1, 9:1, 5:1, 4:1, 2:1, 1:1, v/v), acetone and MeOH to yield seven fractions. Fraction 3 (520 mg) was applied to a silica gel column (petroleum ether–acetone 12:1) to afford **8** (5 mg), **14** (254 mg), **16** (12 mg) and a mixture (68 mg). The latter was further separated by preparative HPLC with a gradient elution MeCN–H₂O (50:50 → 80:20, v/v) to yield **1** (26 mg) and **9** (4 mg). Fraction 4 (180 mg) was purified by preparative HPLC (MeCN:H₂O: 45:55 → 60:40, v/v) to afford **2** (13 mg), **10** (3 mg) and **15** (102 mg). Fraction 6 (1.2 g) was separated by preparative HPLC using a gradient of MeCN:H₂O (40:60 → 68:32, v/v) for eluting to give **17** (8 mg), **18** (24 mg) and other four subfractions. Further purification of each subfraction by silica gel cc (petroleum ether–acetone 4:1) gave **3** (6 mg), **4** (14 mg), **5** (26 mg), **6** (12 mg), **7** (22 mg), **11** (3 mg) and **12** (6 mg). Fraction 7 (61 mg) was subjected to silica gel and Sephadex LH-20 cc to afford **13** (5 mg).

3.5. Physical data of new compounds

3.5.1. Stilbostemin N (**1**)

Colorless needles (hexane–acetone), mp 82–85 °C; UV (MeOH) λ_{max} 210.0, 278.3 nm; IR ν_{max} (KBr) 3388, 1597, 1495, 1464, 1242, 1147, 1061, 754 cm^{−1}; for ¹H NMR and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 259.1 [M+1]⁺, 257.1 [M-1][−]; EIMS *m/z* 258 [M]⁺, 227 [M − OCH₃]⁺, 137, 121, 91; HREIMS *m/z* 258.1252 (calcd for C₁₆H₁₈O₃, 258.1256).

3.5.2. Stilbostemin O (**2**)

Yellow amorphous powder; UV (MeOH) λ_{max} 210.5, 279.3 nm; IR ν_{max} (KBr) 3396, 1597, 1512, 1238, 1074,

831 cm^{−1}; for ¹H NMR and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 259.1 [M+1]⁺, 257.0 [M-1][−]; EIMS *m/z* 258 [M]⁺, 242, 228, 137, 121, 91; HREIMS *m/z* 258.1247 (calcd for C₁₆H₁₈O₃, 258.1256).

3.5.3. Stilbostemin P (**3**)

Yellow amorphous powder; UV (MeOH) λ_{max} 205.1, 278.3 nm; IR ν_{max} (KBr) 3419, 1763, 1614, 1508, 1456, 1209, 1155, 1040, 833 cm^{−1}; for ¹H NMR and ¹³C NMR data, see Tables 1 and 2; EIMS *m/z* 288 [M]⁺, 151, 149, 137, 121, 91; HREIMS *m/z* 288.1362 (calcd for C₁₇H₂₀O₄, 288.1362).

3.5.4. Stilbostemin Q (**4**)

Yellow amorphous powder; UV (MeOH) λ_{max} 214.5, 278.3 nm; IR ν_{max} (KBr) 3454, 3240, 1601, 1506, 1207, 1155, 1043, 827 cm^{−1}; for ¹H NMR and ¹³C NMR data, see Tables 1 and 2; EIMS *m/z* 274 [M]⁺, 152, 151, 123, 121, 91, 77; HREIMS *m/z* 274.1204 (calcd for C₁₆H₁₈O₄, 274.1205).

3.5.5. Stilbostemin R (**5**)

Yellow amorphous powder; UV (MeOH) λ_{max} 211.0, 278.3 nm; IR ν_{max} (KBr) 3288, 1599, 1497, 1468, 1244, 1140, 1032, 748 cm^{−1}; for ¹H NMR and ¹³C NMR data, see Tables 1 and 2; EIMS *m/z* 258 [M]⁺, 227 [M − OCH₃]⁺, 149, 137, 121, 91; HREIMS *m/z* 258.1262 (calcd for C₁₆H₁₈O₃, 258.1256).

3.5.6. Stilbostemin S (**6**)

Yellow amorphous powder; UV (MeOH) λ_{max} 209.8, 279.5 nm; IR ν_{max} (KBr) 3348, 1612, 1593, 1508, 1290, 1211, 1153, 1140, 1041, 814 cm^{−1}; for ¹H NMR and ¹³C NMR data, see Tables 1 and 2; EIMS *m/z* 288 [M]⁺, 167, 151, 137, 121, 91; HREIMS *m/z* 288.1359 (calcd for C₁₇H₂₀O₄, 288.1361).

3.5.7. Stilbostemin T (**7**)

Yellow amorphous powder; UV (MeOH) λ_{max} 211.0, 286.6 nm; IR ν_{max} (KBr) 3411, 1610, 1500, 1227, 1138, 1051, 1028, 800 cm^{−1}; for ¹H NMR and ¹³C NMR data, see Tables 1 and 2; EIMS *m/z* 288 [M]⁺, 256, 167, 151, 149, 137, 121, 91; HREIMS *m/z* 288.1348 (calcd for C₁₇H₂₀O₄, 288.1361).

3.5.8. Stilbostemin U (**8**)

Colorless oil; UV (MeOH) λ_{max} 208, 233 (sh), 285 nm; IR ν_{max} (KBr) 3386, 2942, 2834, 1618, 1589, 1502, 1465, 1309, 1226, 1141, 1054, 804 cm^{−1}; for ¹H NMR and ¹³C NMR data, see Tables 1 and 2; EIMS *m/z* 302 [M]⁺, 151, 121, 91, 77, 65; HREIMS *m/z* 302.1523 (calcd for C₁₈H₂₂O₄, 302.1518).

3.5.9. Stilbostemin V (**9**)

Colorless oil; UV (MeOH) λ_{max} 210, 232 (sh), 280 nm; IR ν_{max} (KBr) 3401, 2945, 2835, 1615, 1557, 1501, 1287, 1113, 1024, 1002, 817 cm^{−1}; for ¹H NMR and ¹³C NMR

data, see Tables 1 and 2; EIMS m/z 302 $[M]^+$, 151, 121, 91, 77, 65; HREIMS m/z 302.1524 (calcd for $C_{18}H_{22}O_4$, 302.1518).

3.5.10. Stilbostemin W (10)

Light yellow amorphous powder; UV (MeOH) λ_{\max} 207, 230 (sh), 282 nm; IR ν_{\max} (KBr) 3457, 1618, 1498, 1214, 1125, 1024, 1014, 821 cm^{-1} ; for 1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 272 $[M]^+$, 151, 136, 121, 91; HREIMS m/z 272.1418 (calcd for $C_{17}H_{20}O_3$, 272.1412).

3.5.11. Stilbostemin X (11)

Light yellow oil; UV (MeOH) λ_{\max} 206, 233 (sh), 289 nm; IR ν_{\max} (KBr) 3501, 3423, 2919, 1598, 1502, 1465, 1224, 1076, 796, 711 cm^{-1} ; for 1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 302 $[M]^+$, 151, 135, 121, 91, 77, 65; HREIMS m/z 302.1518 (calcd for $C_{18}H_{22}O_4$, 302.1518).

3.5.12. Stilbostemin W (12)

Yellow amorphous powder; UV (MeOH) λ_{\max} 207, 235 (sh), 282 nm; IR ν_{\max} (KBr) 3418, 1605, 1497, 1303, 1141, 1047, 1023, 802, 723 cm^{-1} ; for 1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS m/z 302 $[M]^+$, 151, 121, 91, 77; HREIMS m/z 302.1521 (calcd for $C_{18}H_{22}O_4$, 302.1518).

3.5.13. Stemanthraquinone (13)

Orange amorphous powder; UV (MeOH) λ_{\max} 210, 275, 339, 496 nm; IR ν_{\max} (KBr) 3554, 3355, 2925, 2852, 1647, 1625, 1608, 1564, 1413, 1259, 1230, 1205, 1049, 1028 cm^{-1} ; for 1H NMR and ^{13}C NMR data, see Table 3; EIMS m/z 270 $[M]^+$, 255, 238, 207; HREIMS m/z 270.0894 (calcd for $C_{16}H_{14}O_4$, 270.0892).

3.6. Antibacterial activity

The anti-bacterial activity was determined using broth dilution techniques as previously described (Gatsing et al., 2006). The solutions (maximum concentration) of the compounds (i.e. compounds that induced zones of inhibition) were prepared in DMSO, serially (2-fold) diluted and 0.5 mL of each dilution was introduced into a test tube containing 4.4 mL of Selenite broth; then 0.1 mL of bacteria suspension (5×10^5 cfu/mL) was added and the mixture was homogenized. The total volume of the mixture was 5 mL, with the test-compound concentrations in the tube ranging from 200 to 6.25 $\mu g/mL$ and those of the standard

compounds, i.e. Gentamycin and Amphotericin B, ranging from 200 to 3.125 $\mu g/mL$ and 200 to 1.562 $\mu g/mL$, respectively. After 24 h of incubation at 37 °C, the MIC was reported as the lowest concentration of anti-microbial that prevented visible growth.

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