New Phenanthrenes and Stilbenes from *Dendrobium loddigesii*

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New phenanthrenes, loddigesiinols A (1) and B (7), and stilbenes, loddigesiinols C (8) and D (9), were isolated from 80% ethanol extract of *Dendrobium loddigesii* Rolfe (Orcharedceae) along with known compounds, including five phenanthrenes, three stilbenes, two lignans, and three sterols. Their structures were determined by spectroscopic studies. These compounds were evaluated for their inhibitory activities against nitric oxide (NO) production and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. Phenanthrenes (1—3, 7) and dihydrophenanthrenes (4—6) showed significant inhibitory activity against NO production. In particular, 2 showed stronger inhibitory activity against NO production than 5 and 6. This suggested that this phenanthrene is a stronger inhibitor of NO production than dihydrophenanthrene. The new phenanthrenes, loddigesiinols A (1) and B (7), significantly inhibited NO production with IC_{50} values of 2.6 and 10.9 μM, respectively. One new dihydrostilbene, loddigesiinol D (9), showed weak inhibitory activity against NO production with an IC_{50} value of 69.7 μM, but the other new dihydrostilbene, loddigesiinol C (8), did not show this activity at 100 μM.

Key words *Dendrobium loddigesii*; phenanthrene; stilbene; loddigesiinol; nitric oxide; 2,2-diphenyl-1-picrylhydrazyl radical scavenging

A Chinese herbal medicine, "Xiao Huang Cao Shi Hu" which is the dried stem of genus *Dendrobium*, has been used to treat fever, inflammation, and similar conditions.[1,2] Within genus *Dendrobium*, *Dendrobium nobile* Lindl. and *D. officinale* K. Kimura *et* Migo were originally used as "Xiao Huang Cao Shi Hu." However, these plants are very rare, and therefore, other plants of genus *Dendrobium*, including *D. loddigesii* Rolfe, *D. moniliforme* (L.) Sw., and *D. lohohense* Tang *et* Wang, have also been distributed as "Xiao Huang Cao Shi Hu" in medicinal herb markets in China. *D. loddigesii* parasitic on the trees and the rocks in Laos to South China.

During our screening of herbal medicines for active compounds with inhibitory activities against nitric oxide (NO) production, some plants of the genus *Dendrobium* were found to be as active as the herbal medicines.[3] Thus, we investigated 80% MeOH extracts of *D. loddigesii* in order to isolate inhibitors of NO production. New phenanthrenes, loddigesiinols A (**1**) and B (**7**), and stilbenes, loddigesiinols C (**8**) and D (**9**), were isolated from the active portions, *i.e.*, the *n*-hexane and EtOAc extracts, along with known compounds, including five phenanthrenes, three stilbenes, two lignans, and three sterols. Here, we describe the isolation and structure determinations of the new compounds, loddigesiinols A (**1**) and B (**7**) and loddigesiinols C (**8**) and D (**9**), and evaluate their effects against NO production and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging.

The 80% MeOH extract of *D. loddigesii* was partitioned in succession with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc extract, which showed strong inhibitory activity against NO production (inhibition rate: 43.4% at 30 μg/ml), was chromatographed on a silica gel column, and then purified using normal and reverse phase HPLC to obtain compounds **9**—**12** and **16**—**18**. Another active fraction, *n*-hexane extract (inhibition rate: 43.6% at 30 μg/ml), was also separated by normal and reverse phase column chromatography, and then purified by HPLC to obtain compounds **1**—**8** and **13**—**15**. Compounds **2**—**6** and **10**—**18** were found to be identical to moscatin[4] (plicatol B[5]), 5-hydroxy-2,4-dimethoxyphenanthrene,[6] lusianthridin,[7] rotundatin[8] (plicatol C[5]), hircinol,[9] moscatilin[10] (aloifol II[11]), gigantol,[12] batatasin III,[11] stigmasterol, β-sitosterol, sitostenone, dehydrovomifoliol,[13] (−)-pinoresinol,[14] and (−)-medioresinol,[15] respectively, by comparisons with spectroscopic data from the existing literature.

Loddigesiinol A (**1**) was obtained as a pale brownish amorphous powder. Its molecular formula was determined to be $C_{16}H_{14}O_3$ by high resolution (HR)-electron ionization (EI)-mass spectra (MS). The UV spectrum showed absorption peaks at 211, 259, 282, 315, and 362 nm, indicating the presence of a phenanthrene skeleton.[16] In the IR spectrum, absorption at 3286 cm^{-1} indicated hydroxy groups. ^{1}H-NMR revealed *meta*-coupled signals at δ_H 6.81 (d, J=2.4 Hz) and 6.99 (d, J=2.4 Hz), indicating the presence of a tetrasubstituted phenyl group; a spin system at δ_H 7.13 (dd, J=7.5, 0.9 Hz), 7.45 (t, J=7.5 Hz), and 7.84 (dd, J=7.5, 0.9 Hz), indicating the presence of a 1,2,3-trisubstituted phenyl group; an aromatic proton at δ_H 6.91 (1H, s), indicating the presence of a pentasubstituted benzene ring; and two methoxy signals at δ_H 4.03 and 4.11. ^{1}H–^{1}H correlation spectroscopy (COSY) also supported the above spin systems. In the ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra, six protonated olefin signals, four quaternary carbons, four oxygenated quaternary carbons, and two methoxy signals were observed.

The results of NMR experiments are summarized in Table 1. The structure of **1** was determined by an heteronuclear multiple bond correlation (HMBC) experiment (Fig. 2). Two proton signals at δ_H 6.99 (H-1) and 6.81 (H-3) correlated with δ_C 157.1 (C-2), 109.6 (C-4a), and 102.5 (C-10). A methoxy proton signal at δ_H 4.11 correlated with δ_C 155.7

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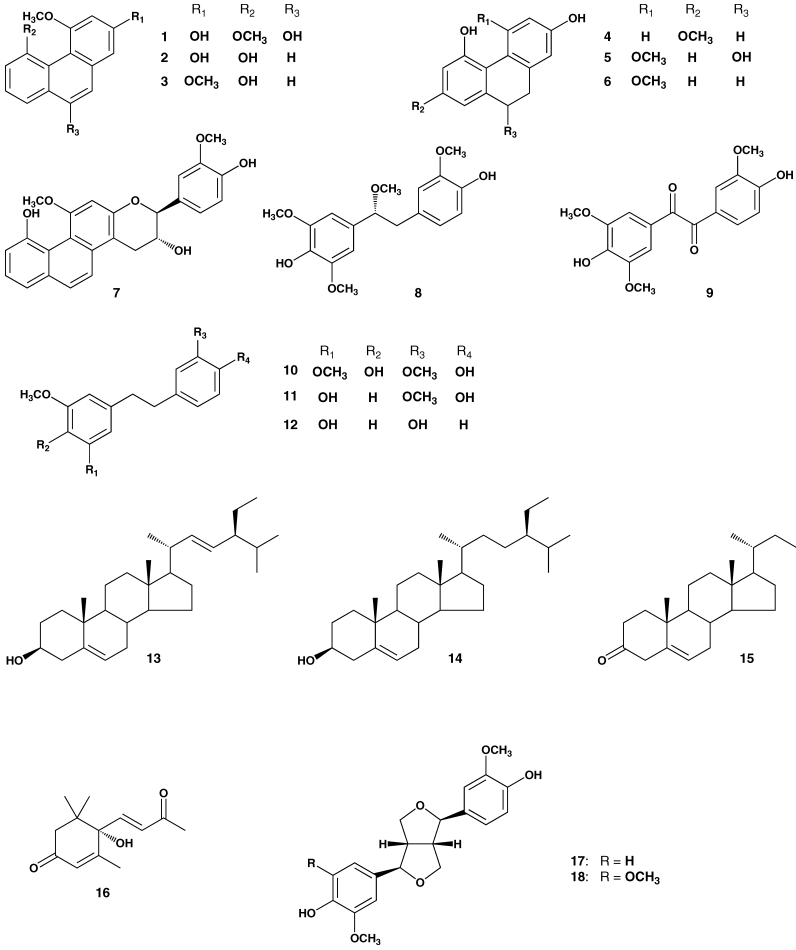

Fig. 1. Structures of Isolated Compounds from *Xiao Huang Cao Shi Hu*

Table 1. ^{13}C- and ^{1}H-NMR Data for Loddigesiinol A (**1**) (300 MHz in Acetone-d_6)

Position	δ_C	δ_H
1	106.6 d	6.99 1H, d, J=2.4 Hz
2	157.1 s	
3	100.0 d	6.81 1H, d, J=2.4 Hz
4	155.7 s	
4a	109.6 s	
4b	120.5 s	
5	154.6 s	
6	117.2 d	7.13 1H, dd, J=7.5, 0.9 Hz
7	126.6 d	7.45 1H, t, J=7.5 Hz
8	114.0 d	7.84 1H, dd, J=7.5, 0.9 Hz
8a	128.7 s	
9	154.4 s	
10	102.5 d	6.91 1H, s
10a	137.4 s	
4-OCH_3	58.3 q	4.11 3H, s
5-OCH_3	55.7 q	4.03 3H, s

(C-4), indicating the presence of a 2-hydroxy-4-methoxy-5,6-disubstituted benzene ring. In addition, an olefin proton signal at δ_H 7.45 (H-7) correlated with δ_C 154.6 (C-5) and 128.7 (C-8a), and two olefin proton signals at δ_H 7.13 (H-6) and 7.84 (H-8) also correlated with δ_C 120.5 (C-4b), indicating the presence of a 1-hydroxy-2,3-disubstituted benzene ring. The remaining olefin proton signal at δ_H 6.91 (H-10)

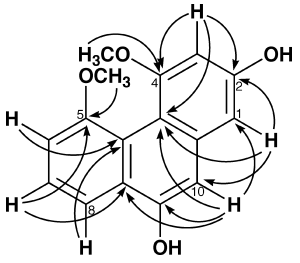

Fig. 2. Significant Correlation (^{1}H→^{13}C) Observed in HMBC of Loddigesiinol A (**1**)

correlated with δ_C 106.6 (C-1), 114.0 (C-8) and C-4a, and a methoxy proton signal at δ_H 4.03 correlated with C-5. Thus, the structure of **1** was determined to be 2,9-dihydroxy-4,5-dimethoxy phenanthrene.

Loddigesiinol B (**7**) was obtained as a colorless amorphous powder, and showed a molecular ion peak at *m*/*z* 418 in the EI-MS. The molecular formula was determined to be $C_{25}H_{22}O_6$ and agreed with 15 degree unsaturation from its HR-EI-MS (*m*/*z*: 418.14153, Calcd for $C_{25}H_{22}O_6$ 418.14161) and ^{1}H- and ^{13}C-NMR data. In the IR spectrum, absorption peaks at 3862, 1519, and 1428 cm^{-1} indicated the presence of hydroxy groups and aromatic rings. The ^{1}H-NMR, methylene proton signals at δ_H 3.03 (1H, dd, J=15.8, 8.3 Hz) and 3.35 (1H, dd, J=15.8, 5.5 Hz), two oxymethine proton signals at δ_H 4.24 (1H, ddd, J=8.3, 8.2, 5.5 Hz) and 4.79 (1H, d,

Table 2. ^{13}C- and ^{1}H-NMR Data for Loddigesiinol B (**7**) (600 MHz in CD_3OD)

Position	δ_C	δ_H
1	110.3 s	
2	154.5 s	
3	102.8 d	6.96 1H, s
4	155.4 s	
4a	115.7 s	
4b	120.4 s	
5	155.1 s	
6	117.3 d	7.13 1H, dd, J=7.6, 2.1 Hz
7	127.9 d	7.44 1H, t, J=7.6 Hz
8	121.7 d	7.42 1H, dd, J=7.6, 2.1 Hz
8a	135.2 s[a)]	
9	130.4 d	7.69 1H, d, J=8.9 Hz
10	121.9 d	7.67 1H, d, J=8.9 Hz
10a	135.1 s[a)]	
11	32.1 t	3.03 1H, dd, J=15.8, 8.3 Hz
		3.35 1H, dd, J=15.8, 5.5 Hz
12	68.9 d	4.24 1H, ddd, J=8.3, 8.2, 5.5 Hz
13	83.3 d	4.79 1H, d, J=8.2 Hz
1′	131.5 s	
2′	112.0 d	7.04 1H, d, J=1.4 Hz
3′	149.0 s	
4′	147.8 s	
5′	116.1 d	6.83 1H, d, J=8.2 Hz
6′	121.5 d	6.92 1H, dd, J=8.2, 1.4 Hz
4-OCH_3	58.8 q	4.03 3H, s
3′-OCH_3	56.5 q	3.83 3H, s

a) Assignments may be interchanged.

J=8.2 Hz), and two methoxy signals at δ_H 3.83 (3H, s) and 4.03 (3H, s) were observed. In the aromatic region, two spin systems were observed at δ_H 6.83 (1H, d, J=8.2 Hz), 6.92 (1H, dd, J=8.2, 1.4 Hz) and 7.04 (1H, d, J=1.4 Hz), and at δ_H 7.13 (1H, dd, J=7.6, 2.1 Hz), 7.44 (1H, t, J=7.6 Hz) and 7.42 (1H, dd, J=7.6, 2.1 Hz), and a singlet olefin signal was observed at δ_H 6.96. These data suggested the presence of a 1,3,4-trisubstituted benzene ring, a 1,2,6-trisubstituted benzene ring, and a pentasubstituted benzene ring, respectively.

In the ^{13}C-NMR and DEPT spectra, twenty-five signals indicating a methylene, two oxymethine, two methoxy, nine olefin methine signals, six quaternary olefin signals and five oxyquaternary olefin signals were observed. In consideration of the above spectral data, **7** had five ring systems. From the COSY and heteronuclear multiple quantum correlation (HMQC) spectra, five partial structures, A—E, and two methoxy groups were revealed (Fig. 3). Interrelationships of these partial structures were determined by an HMBC experiment. An olefin proton at δ_H 7.13 (H-6) correlated with δ_C 121.7 (C-8) and 155.1 (C-5); δ_H 7.42 (H-8) correlated with δ_C 117.3 (C-6), 120.4 (C-4b), and 130.4 (C-9); and δ_H 7.44 (H-7) correlated with δ_C 135.2 (C-8a) and C-5. In the partial structure B, δ_H 7.69 (H-9) correlated with C-4b, C-8a, and 135.1 (C-10a), and δ_H 7.67 (H-10) correlated with δ_C 110.3 (C-1), 115.7 (C-4a), C-8a, and C-10a. In addition, δ_H 6.96 (H-3) correlated with C-1, C-4a, δ_C 154.5 (C-2) and 155.4 (C-4). Furthermore, a methoxy signal (δ_H 4.03) correlated with C-4, and nuclear Overhauser effect (NOE) was observed between the methoxy signal and H-3. These results indicated the presence of a 5-hydroxy-4-methoxy-phenanthrene skeleton.

HMBC correlations of methylene signals at δ_H 3.03 (H-11a) and 3.35 (H-11b) with C-1, C-2, and C-10a, of δ_H 4.24 (H-12) with C-1, and of δ_H 4.79 (H-13) with C-2 were observed. These data suggested the presence of a phenanthrenepyran skeleton in **7**. Furthermore, correlation of δ_H 6.83 (H-2′) with δ_C 83.3 (C-13), 147.8 (C-4′) and 121.5 (C-6′), of δ_H 6.83 (H-5′) with δ_C 131.5 (C-1′) and 149.0 (C-3′), and of δ_H 6.92 (H-6′) with δ_C 112.0 (C-2′), C-4′, and C-13 were observed. The long range correlation was observed between a methyl proton signal (δ_H 3.83), which correlated with H-2′ in a difference NOE experiment, and C-3′, indicating the presence of a 4-hydroxy-3-methoxyphenyl group. In the partial structure D, the following correlations were observed: H-12 with C-1′, H-13 with C-1′, C-2′ and C-6′, indicating that the 4-hydroxy-3-methoxyphenyl moiety was attached at C-13 of phenanthropyran. The coupling constants of H-11a (δ_H 3.03, dd, J=15.8, 8.3 Hz), H-12 (δ_H 4.24, ddd, J=8.3, 8.2, 5.5 Hz), and H-13 (δ_H 4.79, d, J=8.2 Hz) indicated that H-11a and H-12, and H-12 and H-13 were both oriented *trans* diaxially. Furthermore, NOE were observed from H-11a to H-13 and H-10; from H-11b to H-12 and H-10; from H-12 to H-11b, H-13, H-6′, and H-10; and from H-13 to H-11a, H-6′, H-2′. The results of NOE experiments supported the above stereo structure. The absolute stereochemistry of **7** was confirmed by circular dichroism (CD) spectrum. The stereochemistry of C-2 and 3 of 3′,4′-substituted flavanols were discussed by measurement of CD spectra. The CD spectrum of (−) robinetinidol (2*R*,3*S* configuration) shows negative cotton effect at 285 nm, and that of (+) fisetinidol (2*S*,3*R* configuration) shows positive cotton effect at 290 nm.[17)] The CD spectrum of **7** showed positive cotton effect at 286 nm ($\Delta\varepsilon$ 5.61) indicated 12*R*,13*S* configurations. From these data, the structure of **7** was determined to be 12*R*,13*S*-4,12-dihydroxy-3-methoxy-13-(4′-dihydroxy-3′-methoxyphenyl)-phenanthropyran.

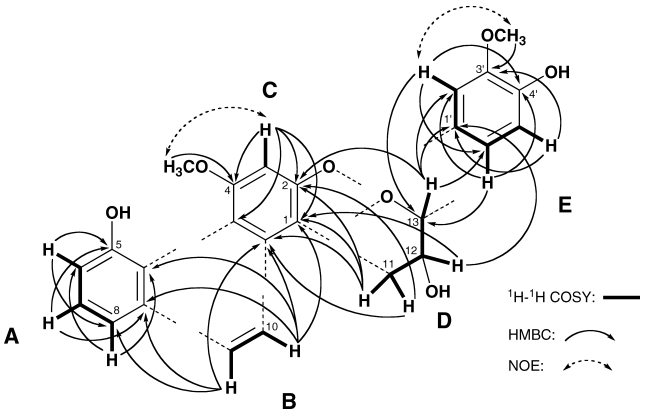

Fig. 3. ^{1}H–^{1}H COSY, HMBC and NOE Correlations of Loddigesiinol B (**7**)

Loddigesiinol C (**8**) was obtained as a colorless amorphous powder; $[\alpha]_D^{27}$ −0.6°. The molecular formula was determined to be $C_{18}H_{22}O_6$ by HR-EI-MS. The UV spectrum showed absorption peaks at 207 and 280 nm, and the IR spectrum showed absorption peaks at 3473 (hydroxy), 1517, and 1459 (aromatic ring) cm^{-1}. These data suggested that **8** was a phenol compound. The ^{1}H-NMR spectrum of **8**, two methylene signals at δ_H 2.74 (1H, dd, J=13.5, 6.0 Hz) and 2.95 (1H, dd, J=13.5, 7.0 Hz); an oxymethine proton at δ_H 4.22 (1H, dd, J=7.0, 6.0 Hz); and four methoxy protons at δ_H

Table 3. ^{13}C- and ^{1}H-NMR Data for Loddigesiinols C (**8**) and D (**9**) (300 MHz)

Position	**8** (acetone-d_6)		**9** ($CDCl_3$)	
	δ_C	δ_H	δ_C	δ_H
1	132.8 s		124.6 s	
2, 6	104.8 d	6.51 1H, s	107.8 d	7.21 1H, s
3, 5	148.0 s		148.3 s	
4	135.6 s		143.3 s	
1′	130.6 s		126.2 s	
2′	113.6 d	6.65 1H, d, J=1.8 Hz	111.2 d	7.55 1H, d, J=2.1 Hz
3′	147.2 s		148.5 s	
4′	145.2 s		153.6 s	
5′	114.8 d	6.66 1H, d, J=8.1 Hz	115.5 d	6.94 1H, d, J=8.4 Hz
6′	122.4 d	6.55 1H, dd, J=8.1, 1.8 Hz	126.6 d	7.55 1H, dd, J=8.4, 2.1 Hz
α	85.8 d	4.22 1H, dd, J=7.0, 6.0 Hz	193.7 s	
α'	44.8 t	2.74 1H, dd, J=13.5, 6.0 Hz 2.95 1H, dd, J=13.5, 7.0 Hz	193.5 s	
3,5-OCH_3	56.4 q	3.77 3H, s	56.6 q	3.86 3H, s
3′-OCH_3	55.9 q	3.73 3H, s	56.2 q	3.93 3H, s
α-OCH_3	56.3 q	3.13 3H, s		
4-OH		7.08 1H, s		
4′-OH		7.25 1H, s		

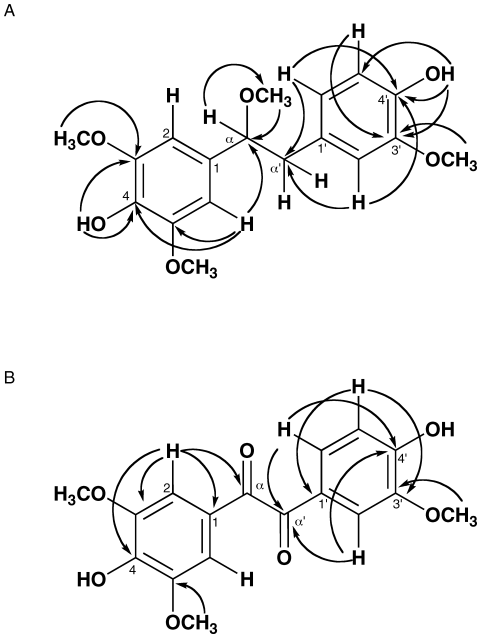

Fig. 4. HMBC Correlation (^{1}H→^{13}C) of Loddigesiinols C (**8**) and D (**9**)

3.13 (3H, s), 3.73 (3H, s) and 3.77 (6H, s) were observed. In the aromatic region, a singlet signal at δ_H 6.51 (2H, s), indicating the presence 1,3,4,5-tetrasubsutituted phenyl group, and at δ_H 6.55 (1H, dd, J=8.1, 1.8 Hz), 6.65 (1H, d, J=1.8 Hz) and 6.66 (1H, d, J=8.1 Hz), indicating the presence of 1,3,4-trisubstituted phenyl group, were observed. Additionally, two hydroxy proton signals at δ_H 7.08 (1H, s) and 7.25 (1H, s) were also observed. From the ^{13}C-NMR and DEPT spectra, eighteen carbon signals indicating four methoxyls, a methylene, an oxymethine, five olefin methines, two quaternary carbons and five oxygenated quaternary carbons were observed. In the HMBC experiment (Fig. 4A), four methoxy proton signals at δ_H 3.13, 3.73, and 3.77 (×2) correlated with δ_C 85.5 (C-α), 147.2 (C-3′) and 148.0 (C-3, 5), respectively. Also, two hydroxy proton signals at δ_H 7.08 and 7.25 correlated with δ_C 135.6 (C-4) and 145.2 (C-4′), respectively. All of the oxygenated carbons were assigned. An olefin proton signal at δ_H 6.51 (H-2, 6) correlated with C-α, 135.6 (C-4), C-3, and C-5. Olefin proton signals on another ring, δ_H 6.65 (H-2′) and 6.55 (H-6′), correlated with δ_C 44.8 (C-α') and C-4′. Additionally, H-2′ and H-5′ also correlated with C-3′. Thus, the structure of **8** was determined to be 4,4′-dihydroxy-3,3′,5,α'-tetramethoxy-dihydrostilbene. The planner structure of **8** was the same as nobilin B.[18] Because the optical rotation of **8** was different from that of nobilin B ($[\alpha]_D^{27}$ +1.4°), both compounds should be mutual enantiomers. The CD spectrum of **8** showed a negative cotton effect at 224 nm ($\Delta\varepsilon$ −0.70), the chiral center of **8** was determined to be *R*-configuration.[19—21]

Loddigesiinol D (**9**) was obtained as a pale yellow amorphous powder. A molecular ion peak was found at *m*/*z* 332 in its EI-MS. The molecular formula was determined to be $C_{17}H_{16}O_7$ from HR-EI-MS (*m*/*z*: 332.08900, Calcd for $C_{17}H_{16}O_7$ 332.08960). In the IR spectrum, absorption bands at 3413 cm^{-1} (hydroxy), 1646 cm^{-1} (conjugated carbonyl), and 1513 cm^{-1} and 1459 cm^{-1} (aromatic ring) were observed. The ^{1}H- and ^{13}C-NMR spectra of **9** were very similar to those of **8**. However, a methine, two methylenes, and a methoxy signals that were observed in the NMR data of **8** were absent in the spectrum of **9**. The ^{13}C-NMR spectrum of **9**, two carbonyl carbon signals (δ_C 193.5, 193.7) were observed, and a methoxy signal (δ_C 56.3 in **8**) was absent in the spectrum of **9**. Two phenyl groups, 5-hydroxy-3,4-dimethoxyphenyl and 4-hydroxy-3-methoxyphenyl, were revealed by 2D NMR experiments. In the HMBC experiment, an olefin proton signal at δ_H 7.21 (H-2, 6) correlated with δ_C 193.7 (C-α). Two olefin proton signals at δ_H 7.55 (H-2′) and 7.35 (H-6′) correlated with another carbonyl carbon signal at δ_C 193.5 (C-α'). From these results, the structure of **9** was determined to be 4,4′-dihydroxy-3,3′,5-trimethoxy-α,α'-dioxostilbene. This compound was previously identified from a tissue culture source by GC/MS.[22] However, our work is the first to isolate and determine the structure of this compound from a natural source.

Table 4. Inhibitory Activities against NO Production and DPPH Radical Scavenging of Compounds **1**—**18**

Compound	IC_{50} (μM)		Compound	IC_{50} (μM)	
	NO	DPPH		NO	DPPH
1	2.6	26.1	**10**	—	—
2	6.4	59.8	**11**	—	—
3	5.3	—	**12**	—	—
4	4.6	62.2	**13**	—	—
5	29.1	—	**14**	—	—
6	29.2	—	**15**	—	—
7	10.9	—	**16**	—	—
8	—	23.7	**17**	89.5	—
9	69.7	—	**18**	5.0	—
Aminoguanidine	17.5	21.7	Resveratrol	22.0	28.7

—: IC_{50} >100 μM.

Compounds **1**—**18** were evaluated for their inhibitory activity against NO production in a murine macrophage-like cell line, RAW 264.7, and for DPPH radical-scavenging activity (Table 4). Phenanthrenes (**1**—**3**, **7**) and dihydrophenanthrenes (**4**—**6**) showed significant inhibitory activity against NO production with IC_{50} values of 2.6, 6.4, 5.3, 10.9, 29.1, 29.2 and 4.6 μM, respectively. In particular, **2** showed stronger inhibitory activity against NO production than **5** and **6**. This suggested that this phenanthrene is a stronger inhibitor of NO production than dihydrophenanthrene. In contrast, α,α'-diketo-dihydrostilbene (**9**) inhibited NO production with an IC_{50} value of 69.7 μM, while the other dihydrostilbenes (**8**, **10**—**12**) did not show this inhibitory activity.

Phenanthrenes (**1**, **2**, **4**) and a stilbene (**8**) also showed DPPH radical-scavenging activity. These results indicated that phenanthrenes possess stronger DPPH radical-scavenging activity than stilbenes, similar to the inhibitory activity against NO production. Other compounds, however, did not show these activities. Furthermore, dihydrostilbene (**8**), which has a methoxy group at C-α, showed DPPH radical-scavenging activity as strong as aminoguanidine used as a positive control, but another dihydrostilbene (**10**) did not show this activity. These data suggested that for this biological activity, it is very important that any substituted group(s) appear at α or α' in dihydrostilbene.[23]

Experimental

General Experimental Procedures Optical rotation and CD spectra were measured using a JASCO P-1020 polarimeter and a JASCO J-720 spectropolarimeter. UV spectra were obtained using a Shimadzu UV-160 spectrophotometer and IR spectra were recorded using a JASCO FT/IR-30E spectrophotometer. MS data were obtained using a JEOL GC mate spectrometer. The ^{1}H- and ^{13}C-NMR spectra were obtained from a Varian Mercury-300Plus spectrometer (300 MHz) and a JEOL ECA 600 spectrometer (600 MHz), using tetramethylsilane (TMS) as an internal standard.

Plant Materials Whole plants of *D. loddigesii* were obtained from a market in Guangdong Province, People's Republic of China, in October 2004. It was identified by Dr. Tomoko Takamiya (Research Unit of Biofunctional Organic Chemistry, College of Pharmacy, Nihon University) using sequence analysis of internal transcribed spacer region between 18S and 5.8S rDNA and BLAST search of the NCBI database. Voucher specimens have been deposited at the Research Unit of Pharmacognosy, College of Pharmacy, Nihon University.

Extraction and Isolation The air-dried stems of *D. loddigesii* (5 kg) were extracted twice with 80% MeOH. The 80% MeOH solutions were combined and evaporated to dryness to give 327 g of a crude extract (45.0% of inhibitory activity of NO production at 100 μg/ml). The extract was suspended in H_2O (1 l) and partitioned with *n*-hexane (3×1 l), EtOAc (3×1 l), and *n*-BuOH (3×1 l) to give extracts of *n*-hexane (17.5 g, 43.6% of inhibitory activity of NO production at 30 μg/ml), EtOAc (33.3 g, 43.4% of inhibitory activity of NO production at 30 μg/ml), *n*-BuOH (51.6 g, 27.5% of inhibitory activity of NO production at 30 μg/ml), and H_2O (187 g, 4.6% of inhibitory activity of NO production at 30 μg/ml), respectively. The *n*-hexane extract (17 g) was chromatographed by silica gel column chromatography (Kiesel gel 60 N, 8×27 cm, E. Merck Darmstadt, Frankfurt, Germany), using a step gradient of a solvent mixture of *n*-hexane–EtOAc (100 : 0→0 : 100) to give 11 fractions. Fraction 8 (Hx-8, 1.07 g) was crystallized from $CHCl_3$–MeOH to give compound **13** (292 mg). Fraction 7 (Hx-7, 739 mg) was subjected to silica gel column chromatography (Wako gel C-300, 12×17 cm, Wako pure Chemical Industry Ltd., Tokyo, Japan) and eluted with a solvent mixture of *n*-hexane and EtOAc with increasing polarity. These fractions (200 ml each) were combined based on TLC monitoring into 9 portions (Hx-7-1 to -9). Hx-7-4 was applied to a Sephadex LH-20 column (2.5×25 cm, Pharmacia Fine Chemical Co., Ltd.), eluted with $CHCl_3$–MeOH (50 : 50), and were further purified by preparative HPLC [Senshu pak PEGASIL ODS, 10×250 mm, Senshu Science Co., Ltd., Tokyo Japan; mobile phase: MeCN–H_2O (70 : 30) and (55 : 45); flow rate: 4 ml/min; detection: UV at 254 nm] to obtained **3** (43 mg). Hx-6 (672 mg) and Hx-7 (112 mg) and the mother liquor of Hx-8 were combined, and then chromatographed on a silica gel column (silica gel 60 N, 3.5×35 cm), and eluted with $CHCl_3$–MeOH (100 : 0→0 : 100) to give 3 fractions (Hx-6-1, 2, 3). Hx-6-2 (109 mg) was re-crystallized from $CHCl_3$–MeOH to give **14** (27.2 mg). Hx-6-3 (382 mg) was purified by HPLC [YMC-Pak Pro C18, 10×150 mm; mobile phase: MeOH–H_2O (95 : 5), and PEGASIL Silica 60-5, 10×250 mm; mobile phase: *n*-hexane–$CHCl_3$ (55 : 45); column temperature: 40 °C] to give **15** (26.0 mg). The EtOAc fraction (33.2 g) was subjected to a silica gel column (Wako gel C-300, 8.0×20 cm) eluted with *n*-hexane–EtOAc (100 : 0→0 : 100) to give 7 fractions (E-1 to -7). E-5 (3.62 g) was chromatographed on a silica gel (Wako gel C-300, 3.5×34 cm) eluted with $CHCl_3$–MeOH (100 : 0→0 : 100) to give 7 portions (E-5-1 to -7). E-5-2 (1.09 g) was purified by MPLC [ULTRA PAK ODS-S-50B, 26×300 mm, Yamazen, Co., Ltd., Kyoto, Japan; mobile phase: MeOH–H_2O (60 : 40)] to give **1** (12.5 mg) and **10** (677 mg), respectively. E-5-3 was purified by HPLC [CAPCELL PAK C18, 10×250 mm (Shiseido, Tokyo, Japan); mobile phase: MeOH–H_2O (55 : 45)] to give **6** (8.0 mg), **11** (106 mg) and **2** (12.7 mg), respectively. Fractions 5 and 6 (E-5, -6, 512 mg) were purified by HPLC [Senshu Pak AQUASIL, 10×250 mm; mobile phase: $CHCl_3$–MeOH (98 : 2) and CAPCELL PAK C18, 10×250 mm; mobile phase: MeCN–H_2O (40 : 60)] to give **4** (9.8 mg) and **12** (161 mg). E-6 (4.44 g) was applied to Sephadex LH-20 column (6.0×19 cm) eluted with $CHCl_3$, MeOH, and acetone–H_2O (80 : 20). Following this, it was chromatographed on ODS (Chromatorex ODS, 2.5×19 cm, Fuji Silysia Chem. Ltd., Aichi, Japan), eluted with a stepwise solvent mixture [MeOH–H_2O (10 : 90→100 : 0)], and purified by HPLC [PEGASIL Silica 60-5, 10×250 mm; mobile phase: *n*-hexane–EtOAc (60 : 40), temperature; 40 °C] to give **5** (60.1 mg), **7** (4.9 mg), **8** (39.3 mg), **9** (5.5 mg), **16** (3.1 mg), **17** (12.2 mg), and **18** (2.6 mg), respectively.

Inhibitory Activity of Activated Macrophages-Like Cell Line, RAW 264.7, against NO Production Cells were seeded at 1.2×10^6 cells/ml into a 96-well flat-bottomed plate (Sumitomo Bakelite, #8096R, Tokyo) and then incubated at 37 °C for 2 h. A test sample was added to the culture along with *Escherichia coli* lipopolysaccharide (LPS, 100 ng/ml) and recombinant mouse interferon-γ (0.33 ng/ml). The cells were then incubated at 37 °C for 16 h and subsequently chilled on ice. Culture supernatant (100 μl) was added in duplicate wells of the 96-well flat-bottomed plates. A standard solution of $NaNO_2$ was placed in different wells on the same plate. To quantify nitrite, 50 μl of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% *N*-1-naphthyletylenediamide-dihydrochloride) was added to each well. After 10 min, the reaction products were colorimetrically quantified at 550 nm using a microplate reader (Bio-Rad, Model 3550) and the background absorbance (630 nm) was subtracted. Cytotoxicity of test samples was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Two hundred microliters of cells suspension (1.0×10^5 cells/ml) was seeded into a 96-well flat-bottomed plate and a test sample was added, then incubated at 37 °C for 16 h. After incubation, MTT was added and incubated for 4 h. The color product was dissolved in DMSO (150 μl), and colorimetrically quantified at 520 nm using a microplate reader and the background absorbance (655 nm) was subtracted.

DPPH Radical-Scavenging Activity Radical-scavenging activity was determined according to Blois method.[24] The assay mixture contained 40 μl of 0.5 mM DPPH radical solution, 145 μl of ethanol, 40 μl of 0.5 M acetic acid buffer, and 0.4 μl of sample solution. The solution was rapidly mixed and the scavenging capacity was measured electrophotometrically by moni-

toring the decrease in absorbance at 520 nm determined after 30 min; scavenging activity was calculated as a percentage of radical reduction.

Loddigesiinol A (**1**): Pale brown amorphous powder, EI-MS *m/z* 270 $[M]^+$, HR-EI-MS *m/z*: 270.08920 (Calcd for $C_{16}H_{14}O_4$: 270.08919), UV λ_{max} (MeOH) nm (log ε): 211 (4.20), 259 (4.60), 282 (4.33), 315 (3.81), IR (KBr) cm^{-1}: 3286, 1548, 1464.

Loddigesiinol B (**7**): Colorless amorphous powder, $[\alpha]_D^{24}$ −2° (c=0.49, MeOH), EI-MS *m/z*: 418 $[M]^+$, HR-EI-MS *m/z*: 418.14153 (Calcd for $C_{25}H_{22}O_6$: 418.14161), UV λ_{max} (MeOH) nm (log ε): 205 (4.52), 257 (4.53), 287 (4.35), 322 (3.93), CD (MeOH): nm ($\Delta\varepsilon$): 216 (2.24), 227 (2.39), 252 (4.87), 272 (4.30), 286 (5.61), IR (KBr) cm^{-1}: 3862, 1519, 1428.

Loddigesiinol C (**8**): Colorless amorphous powder, $[\alpha]_D^{27}$ −0.6° (c=1.0, MeOH), EI-MS *m/z* 334 $[M]^+$, HR-EI-MS *m/z* 334.14115 (Calcd for $C_{18}H_{22}O_6$: 334.14161), UV λ_{max} (MeOH) nm (log ε): 207 (4.50), 280 (3.50), CD (MeOH): nm ($\Delta\varepsilon$): 224 (−0.70), IR (KBr) cm^{-1}: 3473, 1517, 1459.

Loddigesiinol D (**9**): Pale yellow amorphous powder, EI-MS *m/z*: 332 $[M]^+$, HR-EI-MS *m/z*: 332.08900 (Calcd for $C_{17}H_{16}O_7$: 332.08960), UV λ_{max} (MeOH) nm (log ε): 208 (4.26), 325 (4.03), IR (KBr) cm^{-1}: 3413, 1646, 1513, 1459.

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