Antioxidant Lignans from the Fruits of *Broussonetia papyrifera*

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Nine new lignans, chushizisins A−I (**1**−**9**), and three known lignans, *threo*-1-(4-hydroxy-3-methoxyphenyl)-2-{4-[(*E*)-3-hydroxy-1-propenyl]-2-methoxyphenoxy}-1,3-propanediol (**10**), *erythro*-1-(4-hydroxy-3-methoxyphenyl)-2-{4-[(*E*)-3-hydroxy-1-propenyl]-2-methoxyphenoxy}-1,3-propanediol (**11**), and 3-[2-(4- hydroxyphenyl)-3-hydroxymethyl-2,3-dihydro-1-benzofuran-5-yl]propan-1-ol (**12**), were isolated from the fruits of *Broussonetia papyrifera*. Their structures were elucidated using spectroscopic methods. Compounds **1**, **5**, **6**, **8**, **9**, and **11** exhibited antioxidant activities against H_2O_2-induced impairment in PC12 cells, while compounds **1**, **2**, **4**, **7**, and **11** showed DPPH radical-scavenging activities with IC_{50} values of 236.8, 156.3, 273.9, 281.1, and 60.9 μM, respectively.

A variety of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis have been linked casually to oxidative injury. Reactive oxygen species (ROS) are considered as the major factors mediating oxidative damage.[1] ROS can be generated from cell lysis, oxidative burst, or the presence of an excess of free transition metals.[2,3] H_2O_2 is one type of ROS and is often used as a toxicant to establish *in vitro* models of oxidative stress, which can be used to evaluate the potential neuroprotective effects of substances.[4]

The fruits of *Broussonetia papyrifera* (Moraceae) have been used in traditional Chinese medicine for the treatment of age-related disorders, such as AD.[5] Previous reports indicated that its crude extract could improve the learning and memory abilities of mice.[6] Our biological test also showed that its EtOH extract could protect PC12 cells from H_2O_2-induced impairment. Therefore, it is reasonable to hypothesize that the fruits of *B. papyrifera* may contain neuroprotective substances. Diterpenoids, flavonoids, and alkaloids have been isolated from the leaves, twigs, and roots of this plant.[7−16] The compounds in the fruits that are responsible for the antioxidant effects have not been specifically identified. Thus, we conducted an investigation and isolated 12 lignans, including nine new ones (**1**−**9**). Herein, we describe the isolation, structural determination, and antioxidant properties of these lignans.

Results and Discussion

Chushizisins A (**1**) and B (**2**), with the same formula of $C_{19}H_{22}O_6$ derived from their positive HRESIMS, exhibited similar NMR signals (Tables 1 and 2), which implied that they are likely to be isomers. When compared with compounds **10** and **11**, isomers found in the wood of *Larix leptolepis*, the main difference was that compounds **1** and **2** both have an ABX and an AA′BB′ spin pattern, rather than two ABX systems in **10** and **11**.[17] Considering the difference in the molecular compositions, this indicated that an *O*-methyl group was missing in **1** and **2**. The HMBC correlation of an *O*-methyl with C-3′ (δ 151.9) (Figure 1, Supporting Information) indicated that this *O*-methyl group was linked to C-3′. The $J_{7',8'}$ value (16.0 Hz) indicated the *E* geometry of the double bond. The signal of H-7 was overlapped in the ^{1}H NMR spectrum when measured in methanol-d_4; thus, the proton spectra of **1** and **2** were remeasured in acetone-d_6. The $J_{7,8}$ values of 6.5 Hz for **1** and 5.3 Hz for **2** when measured in acetone-d_6 indicated a *threo*-configuration for **1** and an *erythro*-configuration for **2**.[18] The relative configurations of **1** and **2** at C-7 and C-8 were evident from their chemical shift difference at C-8, which was downfield shifted for the *threo* and upfield shifted for the *erythro* isomer (δ 87.6 in **1** and 86.5 in **2**).[19] Both **1** and **2** showed no optical activity, suggesting that both are racemates. Consequently, the structures of **1** and **2** were determined as *threo*-1-(4-hydroxyphenyl)-2-{4-[(*E*)-3-hydroxy-1-propenyl]-2-methoxyphenoxy}-1,3-propanediol (**1**) and *erythro*-1-(4-hydroxyphenyl)-2-{4-[(*E*)-3-hydroxy-1-propenyl]-2-methoxyphenoxy}-1,3-propanediol (**2**), respectively.

The molecular formulas of chushizisins C (**3**) and D (**4**) were both established as $C_{19}H_{24}O_6$ by their positive HRESIMS. Furthermore, their NMR signals (Tables 1 and 2) were also similar. Comparing their NMR data with those of **1** and **2** revealed that the only difference was that the C-7′−C-8′ double bond in **1** and **2** was replaced by two methylenes in **3** and **4**. The $J_{7,8}$ values of 6.9 Hz for **3** and 5.3 Hz for **4** in acetone-d_6 indicated a *threo*- and an *erythro*-configuration for **3** and **4**, respectively.[18] Compounds **3** and **4** were optically inactive, suggesting that both are racemic. Thus, the structures of **3** and **4** were determined as *threo*-1-(4-hydroxyphenyl)-2-[4-(3-hydroxy-1-propyl)-2-methoxyphenoxy]-1,3-propanediol (**3**) and *erythro*-1-(4-hydroxyphenyl)-2-[4-(3-hydroxy-1-propyl)-2-methoxyphenoxy]-1,3-propanediol (**4**), respectively.

Chushizisin E (**5**) was isolated as an optically active powder and had the formula $C_{19}H_{22}O_5$ derived from its positive HRESIMS. Comparison of NMR data (Tables 1 and 2) with those of 3-[2-(4-hydroxyphenyl)-3-hydroxymethyl-2,3-dihydro-1-benzofuran-5-yl]-propan-1-ol (**12**) showed their close structural relationship. The only difference was that C-3 of **5** was substituted by an *O*-methyl group, instead of a proton as in **12**. This assumption was supported by the observed HMBC correlation from a proton signal at δ 3.90 to C-3. The $J_{7',8'}$ value (6.1 Hz) established a 7′,8′-*trans* configuration for **5**.[20] The absolute configuration of **5** was assigned as 7′*R*, 8′*S* by the negative Cotton effects at 288 nm ($\Delta\epsilon$ 0.6) and 238 nm ($\Delta\epsilon$ 1.3) and the positive Cotton effect at 224 nm ($\Delta\epsilon$ 1.0), in accordance with previously reported CD data.[20] Therefore, the structure of **5** was defined as (7′*R*,8′*S*)-3-methoxy-4′,9,9″-trihydroxy-4,7′-epoxy-5,8′-neolignan.

The molecular formula of chushizisin F (**6**) was determined to be $C_{19}H_{20}O_5$ by its positive HRESIMS. The IR spectrum of **6** showed the presence of a carbonyl group (1725 cm^{-1}). Comparing its ^{13}C NMR data with those of **5** revealed that compounds **5** and

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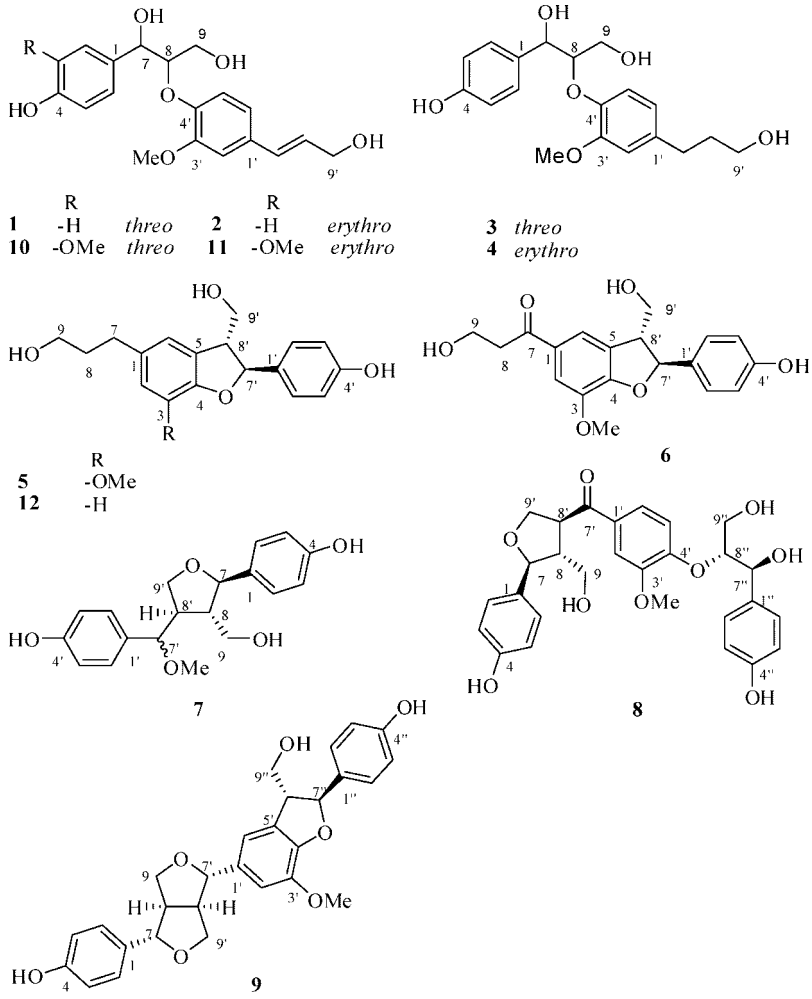

6 are analogues. The difference was that a methylene at δ 35.8 in **5** was replaced by a ketone at δ 199.5 in **6**. This was confirmed by HMBC correlations of H-2 (δ 7.55), H-6 (δ 7.64), and H-9 (δ 3.94) with C-7 (δ 199.5) in **6**. The $J_{7',8'}$ value (6.2 Hz) established the 7′,8′-*trans* configuration for **6**.[20] The structure of **6** was therefore deduced as (7′*R**,8′*S**)-3-methoxy-7-oxo-4′,9,9″-trihydroxy-4,7′-epoxy-5,8′-neolignan.

The HRESIMS established the formula of chushizisin G (**7**) as $C_{19}H_{22}O_5$. The NMR data of **7** (Table 3) were similar to those of the aglycone of 9-(β-D-glucopyranosyloxy)-3′-methoxy-3,4-(dimethylenedioxy)-7,9′-epoxylignan-4′-ol.[21] The HMBC spectrum of **7** exhibited H-8 (δ 1.75) correlating with C-1 (δ 133.6), H-7 (δ 4.58) with C-9 (δ 61.2) and C-8′ (δ 49.0), H-8′ (δ 2.50) and H-7′ (δ 3.98) with C-1′ (δ 131.7), and an *O*-methyl with C-7′ (δ 87.0) (Figure 1, Supporting Information). Therefore, the planar structure of **7** was established as shown. The $J_{7,8}$ value (7.8 Hz) indicated the 7,8-*trans* configuration. Furthermore, NOESY experiments revealed a correlation between H-7 and H-8′, which indicated that these hydrogens were cofacial. Thus, the structure of **7** was assigned as (7*R**,8*S**,8′*R**)-7′ξ-methoxy-4,4′,9,-trihydroxy-7,9′-epoxy-8,8′-lignan.

The molecular formula of chushizisin H (**8**) was defined as $C_{28}H_{30}O_9$ by its positive HRESIMS. The IR spectrum showed the presence of a ketone group (1725 cm^{-1}). The ^{1}H NMR spectrum displayed two AA′BB′ systems and an ABX system in the aromatic proton region (Table 3). In addition to an obvious *O*-methyl group, 27 carbon signals were observed in the ^{13}C NMR spectrum. Inspection of the NMR data (Table 3) disclosed that two partial structures in **8** were similar to chushizisins A (**1**) and G (**7**), respectively. All of these data suggested that **8** is a sesquilignan. The ^{1}H−^{1}H COSY spectrum showed two spin systems in the aliphatic region, representing H-7/H-8/H-9, H-8/H-8′/H-9′, and H-7″/H-8″/H-9″. The HMBC spectrum showed correlations (Figure 1) of H-7 with C-2 and C-9; H-8 with C-9′; H-8, H-8′, H-9′, H-2′, and H-6′ with a ketone group; H-8″ with C-4′; and an *O*-methyl with C-3′. These data established the planar structure of **8** as shown. ROESY experiments revealed correlations of H_2-9 with H-7 and H-8′, and H-7 with H-8′, which assigned the relative configuration of the furan ring. The $J_{7'',8''}$ value was 8.6 Hz, indicating a *threo*-configuration. Thus, the structure of **8** was assigned as (7*R**,8*S**,8′*R**)-7″,8″-*threo*-3′-methoxy-7′-oxo-4,4″,7″,9,9″-pentahydroxy-4′,8″:7,9′-bis-epoxy-8,8′-sesquineolignan.

Chushizisin I (**9**) had the molecular formula $C_{28}H_{28}O_7$ derived from its positive HRESIMS. The ^{1}H NMR spectrum of **9** exhibited two AA′BB′systems and an ABX system in the aromatic proton region. The ^{13}C NMR spectrum exhibited 27 carbons and an additional *O*-methyl (Table 3), suggesting **9** to be a sesquilignan, with partial pinoresinol- and dehydrodiconiferyl alcohol-type constituent units. Comparison of its ^{13}C NMR data with those of hedyotol-A[22] showed that **9** possessed two *p*-hydroxyphenyl moieties instead of two 4-hydroxy-3-methoxyphenyl groups, as in hedyotol-A. HMBC correlation (Figure 1) of an *O*-methyl with C-3′ assigned the position of the *O*-methyl group attached at C-3′. H-7 and H-8 as well as H-7′ and H-8′ should be in a *trans* relationship in view of the coupling constants ($J_{7,8}$ = 5.2 and $J_{7',8'}$ = 4.8 Hz).[22] Furthermore, the chemical shifts of C-7 and C-7′ suggested that the aryl groups were *cis*-oriented relative to the bridge-head protons, H-8 and H-8′.[22] A *trans* relationship between H-7″ and H-8″ was inferred from the $J_{7'',8''}$ coupling of 6.1 Hz.[16] Considering these data, the structure of **9** was assigned as (7*S**,7′*S**,7″*R**,8*R**,8′*R**,8″*S**)-3′-methoxy-4,4″,9″-trihydroxy-4′,7″:7,9′:7′,9-triepoxy-5′,8″,8,8′-sesquineolignan.

Table 1. ^{1}H NMR Data for Compounds **1**−**6**[a] in Methanol-d_4

no.	1	2	3	4	5	6
2	7.24 d (8.3)	7.24 d (8.3)	7.24 d (8.3)	7.22 d (8.3)	6.71 br s	7.55 br s
3	6.74 d (8.3)	6.74 d (8.3)	6.74 d (8.3)	6.72 d (8.3)		
5	6.74 d (8.3)	6.74 d (8.3)	6.74 d (8.3)	6.72 d (8.3)		
6	7.24 d (8.3)	7.24 d (8.3)	7.24 d (8.3)	7.22 d (8.3)	6.71 br s	7.64 br s
7	4.87 d (6.6)[b]	4.88 d (5.4)[b]	4.85 d (6.9)[b]	4.87 d (5.3)[b]	2.62 t (8.0)	
8	4.25 m	4.25 m	4.15 m	4.21 m	1.81 m	3.18 t (6.2)
9	3.69 dd (11.5, 3.7)	3.84 m	3.67 dd (12.0, 3.7)	3.83 dd (11.7, 5.7)	3.56 t (6.5)	3.94 t (6.2)
	3.42 dd (11.5, 5.0)	3.78 m	3.40 dd (12.0, 5.0)	3.72 dd (11.7, 3.4)		
2′	7.05 s	7.05 s	6.85 d (1.4)	6.80 d (1.8)	7.18 d (7.2)	7.18 d (8.5)
3′					6.76 d (7.2)	6.76 d (8.5)
5′	7.01 d (8.1)	7.01 d (8.1)	6.98 d (8.1)	6.80 d (8.1)	6.76 d (7.2)	6.76 d (8.5)
6′	6.74 d (8.1)	6.74 d (8.1)	6.72 d (8.1)	6.66 d (8.1)	7.18 d (7.2)	7.18 d (8.5)
7′	6.53 d (16.0)	6.53 d (16.0)	2.62 t (7.1)	2.60 t (8.5)	5.48 d (6.1)	5.62 d (6.2)
8′	6.26 dt (16.0, 5.8)	6.26 dt (16.0, 5.8)	1.80 m	1.79 m	3.44 m	3.55 m
9′	4.20 d (5.8)	4.20 d (5.8)	3.55 t (6.5)	3.54 t (6.5)	3.80 dd (11.0, 6.0), 3.72 dd (11.0, 6.4)	3.84 m
OMe	3.87 s	3.87 s	3.85 s	3.79 s	3.90 s	3.90 s

[a] ^{1}H NMR data of **1**−**6** at 500 MHz. [b] Measured in acetone-d_6.

Table 2. ^{13}C NMR Data for Compounds **1**−**6**[a] in Methanol-d_4

no.	1	2	3	4	5	6
1	133.3	133.5	132.9	133.5	136.8	133.3
2	129.3	129.2	129.3	129.2	113.9	113.6
3	116.0	115.8	116.0	115.8	145.2	146.5
4	158.2	157.9	158.1	157.9	147.5	154.4
5	116.0	115.8	116.0	115.8	129.0	130.5
6	129.3	129.2	129.3	129.2	117.9	120.1
7	74.1	73.9	74.1	73.9	35.8	199.5
8	87.6	86.5	88.1	86.5	32.9	41.8
9	61.8	62.0	61.7	62.0	62.2	58.9
1′	133.0	133.2	138.3	133.2	134.2	132.6
2′	111.3	111.5	113.9	111.5	128.3	128.4
3′	151.9	152.0	151.7	152.0	116.3	116.4
4′	149.3	149.0	147.6	149.0	158.4	158.8
5′	119.3	119.3	119.9	119.3	116.3	116.4
6′	120.8	120.7	122.0	120.7	128.3	128.4
7′	131.4	131.4	35.5	131.4	88.8	90.2
8′	128.7	128.6	32.7	128.6	55.4	54.5
9′	63.7	63.7	62.2	63.7	65.0	64.2
OMe	56.6	56.6	56.6	56.6	56.6	56.6

[a] ^{13}C NMR data of **1**−**6** at 125 MHz.

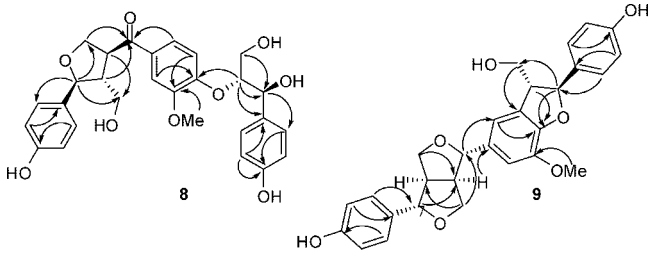

Figure 1. Key HMBC correlations of compounds **8** and **9**.

The known compounds were identified as *threo*-1-(4-hydroxy-3-methoxyphenyl)-2-{4-[(*E*)-3-hydroxy-1-propenyl]-2-methoxyphenoxy}-1,3-propanediol,[23] *erythro*-1-(4-hydroxy-3-methoxyphenyl)-2-{4-[(*E*)-3-hydroxy-1-propenyl]-2-methoxyphenoxy}-1,3-propanediol,[23] and 3-[2-(4-hydroxyphenyl)-3-hydroxymethyl-2,3-dihydro-1-benzofuran-5-yl]propan-1-ol,[24] respectively, by comparison of their spectroscopic data with literature data.

Oxidative stress is well recognized as an important risk factor for the occurrence of AD.[25] Therefore, compounds **1**−**9**, **11**, and **12** were evaluated for their antioxidant activities via MTT and DPPH assays (Table 4). Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a strong free radical scavenger used for the treatment of patients with acute brain infraction, was used as a positive control.[26] Compounds **1**, **5**, **6**, **8**, **9**, and **11** exhibited antioxidant activities against H_2O_2-induced impairment in PC12 cells, with concentrations ranging from 0.16 to 100 μM. Compounds **1**, **2**, **4**, **7**, and **11** showed DPPH radical scavenging activity with IC_{50} values of 236.8, 156.3, 273.9, 281.1, and 60.9 μM, respectively. Importantly, compound **11** is the only one that appears to show significant antioxidant behavior in both the MTT and DPPH assays.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were obtained on a Shimadzu double-beam 210A spectrometer. IR spectra were obtained on a Tensor 27 spectrometer with KBr pellets or film. NMR spectra were recorded on a Bruker AV-400 or a DRX-500 spectrometer with TMS as an internal standard. FABMS were recorded with a VG Autospec-3000 spectrometer. ESIMS and HRESIMS were recorded with an API QSTAR Pulsar 1 spectrometer. CD spectra were recorded on a JASCO J-810 spectropolarimeter. Silica gel (200−300 mesh, Qingdao Marine Chemical Inc., People's Republic of China), RP-18 (40−60 μm, Daiso Co., Japan), and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm × 25 cm, column. Fractions were monitored by TLC, and spots were visualized with heat, after spraying with 10% H_2SO_4 in EtOH.

Plant Material. The fruits of *B. papyrifera* were purchased from Yunnan Corporation of Materia Medica, Yunnan Province, People's Republic of China, and identified by Mr. Hong-Yan Sun, at Yunnan Corporation of Materia Medica. A voucher specimen (CHYX0043) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. The dried and powdered fruits of *B. papyrifera* (30 kg) were extracted with 95% EtOH (3 × 50 L) under reflux. The extract was concentrated and suspended in H_2O, followed by successive partition with petroleum ether (3 × 5 L), EtOAc (3 × 5 L), and *n*-BuOH (3 × 5 L), respectively. The *n*-BuOH extract (50 g) was separated with a silica gel column (5 × 60 cm, 200−300 mesh, 800 g) using a gradient elution of $CHCl_3$/MeOH (9:1, 7:1, 5:1, 3:1, each 2 L) to afford fractions A−C. Fraction A (12 g) was subjected to gel filtration on Sephadex LH-20 (MeOH) to give three subfractions, A1−A3. Fractions A2 (3 g) and A3 (5 g) were subjected to repeated RP-18 (MeOH/H_2O, 30−50%) and semipreparative HPLC (MeOH/H_2O, 37:63) to yield **9** (10 mg) from A2 and **10** (2 mg) and **11** (5 mg) from A3, respectively. Fraction B (15 g) was submitted to chromatography on Sephadex LH-20 (MeOH) to yield four fractions, B1−B4. Fractions B2 (1 g), B3 (3 g), and B4 (2 g) were each subjected to repeated RP-18 (MeOH/H_2O, 20−70%) and semipreparative HPLC (MeOH/H_2O, 32:68) to afford compounds **1** (6 mg), **2** (5 mg), **3** (4 mg), and **4** (5 mg) from B2, **5** (7 mg) and **6** (4 mg) from B3, and **7** (6 mg), **8** (4 mg), and **12** (3 mg) from B4, respectively.

Chushizisin A (1): colorless oil; $[\alpha]^{27}_D$ 0.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 263 (4.05), 204 (4.63) nm; IR (film) ν_{max} 3423, 2924, 1614, 1511, 1265, 1225, 1134, 1029, 835 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS (positive) *m*/*z* 346 $[M]^+$; HRESIMS (positive) *m*/*z* 369.1304 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_6Na$, 369.1314).

Chushizisin B (2): colorless oil; $[\alpha]^{27}_D$ 0.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 266 (4.07), 204 (4.50) nm; IR (film) ν_{max} 3424,

Table 3. ^{1}H and ^{13}C NMR Data for Compounds **7**−**9**[a]

no.	**7**		**8**		**9**	
1		133.6		132.7		132.7
2	7.17 d (8.5)	128.9	7.24 d (8.5)	129.3	7.20 d (8.3)	128.0
3	6.74 d (8.5)	116.1	6.78 d (8.5)	116.1	6.77 d (8.3)	116.0
4		158.1		158.5		158.0
5	6.74 d (8.5)	116.1	6.78 d (8.5)	116.1	6.77 d (8.3)	116.0
6	7.17 d (8.5)	128.9	7.24 d (8.5)	129.3	7.20 d (8.3)	128.0
7	4.58 d (7.8)	84.9	4.63 d (8.8)	85.1	4.71 d (5.2)	87.0
8	1.75 m	53.5	2.88 m	54.4	3.13 m	55.3
9	3.16 dd (10.2, 4.0)	61.2	3.60 m	61.0	4.20 m	72.5
	3.00 dd (10.2, 5.7)				3.77 m	
1′		131.7		131.5		135.6
2′	7.09 d (8.5)	130.0	7.64 br s	112.8	6.90 br s	111.7
3′	6.75 d (8.5)	116.2		151.3		145.2
4′		158.5		154.8		148.8
5′	6.75 d (8.5)	116.2	7.14 d (9.0)	116.0		129.9
6′	7.09 d (8.5)	130.0	7.63 d (9.0)	124.4	6.90 br s	116.0
7′	3.98 d (9.3)	87.0		200.2	4.70 d (4.8)	87.4
8′	2.50 m	49.0	4.26 m	50.3	3.13 m	55.0
9′	4.17 dd (9.0, 4.2)	71.8	4.15 m	71.7	4.24 m	72.2
	3.90 dd (9.0, 9.0)				3.80 m	
1″				133.0		133.7
2″			7.24 d (8.3)	129.3	7.17 d (8.3)	128.5
3″			6.72 d (8.3)	116.0	6.74 d (8.3)	115.8
4″				158.2		158.2
5″			6.72 d (8.3)	116.0	6.74 d (8.3)	115.8
6″			7.24 d (8.3)	129.3	7.17 d (8.3)	128.5
7″			4.66 d (8.6)[b]	73.8	5.51 d (6.1)	88.8
8″			4.56 m	86.0	3.47 m	54.9
9″			3.74 m	62.0	3.79 m	64.6
			3.50 m			
Ome	3.14 s	56.5	3.93 s	56.6	3.86 s	56.5

[a] ^{1}H and ^{13}C NMR data of **7**−**9** at 500 and 125 MHz, respectively, measured in methanol-d_4. [b] Measured in acetone-d_6.

Table 4. Antioxidant Activities of Compounds **1**−**9**, **11**, and **12** by MTT and DPPH Assays

	MTT assay (viability, %)[a,b]					DPPH assays
	0.16 μM	0.8 μM	4 μM	20 μM	100 μM	(IC_{50}, μM)[c]
control	100***					
model[d]	33.71 ± 3.24					
1	35.68 ± 3.59	34.48 ± 7.58	40.34 ± 3.95***	43.94 ± 5.13***	29.46 ± 1.83*	236.8
2	31.11 ± 4.31	29.68 ± 3.98	31.67 ± 4.31	31.91 ± 3.98	30.67 ± 3.99	156.3
3	33.64 ± 3.06	31.64 ± 3.04	32.58 ± 4.17	37.76 ± 4.08	29.63 ± 5.73	>500
4	32.98 ± 3.70	36.05 ± 2.39	36.87 ± 1.59	30.78 ± 3.53	30.90 ± 3.93	273.9
5	39.41 ± 3.09*	35.78 ± 3.77	41.99 ± 2.74***	43.63 ± 3.17***	39.12 ± 3.78*	>300
6	38.39 ± 0.14	42.61 ± 1.69**	39.03 ± 3.50*	40.83 ± 1.39***	38.98 ± 5.23	>300
7	34.05 ± 7.74	31.10 ± 4.42	36.53 ± 2.98	32.94 ± 5.69	35.17 ± 4.58	281.1
8	35.01 ± 4.32	37.76 ± 1.11	37.47 ± 1.66	36.52 ± 2.15	40.08 ± 4.12*	>300
9	34.63 ± 1.65	32.52 ± 1.17	40.48 ± 2.73*	39.11 ± 4.29*	34.33 ± 2.01	>300
11	34.61 ± 4.81	38.38 ± 2.99*	39.55 ± 3.31***	37.82 ± 1.37*	37.48 ± 1.40*	60.9
12	38.18 ± 4.97	37.55 ± 1.48	38.55 ± 5.26	35.53 ± 2.40	35.15 ± 5.58	>300
edaravone[e]	40.17 ± 4.45	38.32 ± 0.70	39.08 ± 2.88	36.47 ± 2.36	35.42 ± 1.46	43.6

[a] Activities of the tested compounds against H_2O_2-induced impairment in PC12 cells. [b] $n = 6$, each value represents the mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs model. [c] DPPH radical scavenging activities of the tested compounds. [d] H_2O_2-induced cell viability without the addition of the compounds. [e] Positive control.

2933, 1614, 1511, 1461, 1451, 1266, 1226, 1134, 1031 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS (positive) *m/z* 346 $[M]^+$; HRESIMS (positive) *m/z* 369.1304 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_6Na$, 369.1314).

Chushizisin C (3): colorless oil; $[\alpha]^{27}_D$ +1.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 277 (3.70), 226 (4.31), 204 (4.68) nm; IR (film) ν_{max} 3424, 2935, 1616, 1512, 1460, 1451, 1266, 1224, 1028, 834 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS (positive) *m/z* 348 $[M]^+$; HRESIMS (positive) *m/z* 371.1480 $[M + Na]^+$ (calcd for $C_{19}H_{24}O_6Na$, 371.1470).

Chushizisin D (4): colorless oil; $[\alpha]^{27}_D$ 0.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 277 (3.57), 226 (4.20), 204 (4.60) nm; IR (film) ν_{max} 3428, 2928, 1615, 1512, 1460, 1452, 1267, 1223, 1031, 833 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS (positive) *m/z* 348 $[M]^+$; HRESIMS (positive) *m/z* 371.1480 $[M + Na]^+$ (calcd for $C_{19}H_{24}O_6Na$, 371.1470).

Chushizisin E (5): colorless, amorphous powder; $[\alpha]^{27}_D$ −9.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 282 (3.63), 225 (4.32), 206 (4.61) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 288 (−0.6), 238 (−1.3), 224 (1.0); IR (KBr) ν_{max} 3419, 2936, 2879, 1614, 1516, 1499, 1451, 1330, 1214, 1140, 1058, 833 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS (positive) *m/z* 330 $[M]^+$; HRESIMS (positive) *m/z* 353.1366 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_5Na$, 353.1364).

Chushizisin F (6): colorless, amorphous powder; $[\alpha]^{27}_D$ −18.9 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 283 (3.96), 204 (4.45) nm; IR (KBr) ν_{max} 3424, 2960, 2932, 1725, 1615, 1515, 1274, 1154 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS (positive) *m/z* 345 $[M + H]^+$; HRESIMS (positive) *m/z* 367.1153 $[M + Na]^+$ (calcd for $C_{19}H_{20}O_6Na$, 367.1157).

Chushizisin G (7): colorless oil; $[\alpha]^{27}_D$ +17.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 277 (3.48), 226 (4.19), 202 (4.18) nm; IR (film) ν_{max} 3424, 2923, 1614, 1515, 1235, 1059, 833 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; FABMS (positive) *m/z* 330 $[M]^+$, 423 $[M + Gly + H]^+$; HRESIMS (positive) *m/z* 353.1362 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_5Na$, 353.1364).

Chushizisin H (8): colorless oil; $[\alpha]^{27}_{D}$ 0.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 306 (3.89), 277 (4.09), 226 (4.44), 202 (4.49) nm; IR (KBr) ν_{max} 3424, 2959, 2928, 2850, 1725, 1615, 1597, 1513, 1268, 1032, 834 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; FABMS (positive) *m/z* 511 $[M + H]^+$; HRESIMS (positive) *m/z* 533.1784 $[M + Na]^+$ (calcd for $C_{28}H_{30}O_9Na$, 533.1787).

Chushizisin I (9): colorless oil; $[\alpha]^{27}_{D}$ +20.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 278 (3.80), 225 (4.45), 208 (4.70) nm; IR (film) ν_{max} 3420, 2936, 2874, 1614, 1516, 1451, 1370, 1331, 1217, 1171, 1144, 1048, 831 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; FABMS (positive) *m/z* 477 $[M + H]^+$; HRESIMS (positive) *m/z* 499.1741 $[M + Na]^+$ (calcd for $C_{28}H_{28}O_7Na$, 499.1732).

Antioxidant Assay against H_2O_2-Induced Impairment in PC12 Cells. The antioxidant assay was performed using the modified method described by Wang.[27] PC12 cells were grown in RPMI 1640 supplement with 5% fetal calf serum, 10% horse serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 20 mM L-glutamine. The cell suspensions, which were adjusted to 1×10^6/mL, were seeded into 96-well culture plates at 100 μL/well and incubated at 37 °C, 5% CO_2 for 24 h, followed by an incubation with H_2O_2 (final concentration 200 μM) and different concentrations of the tested compounds (final concentrations of 0.16 to 100 μM) for 3 h. After treatment, cell viability was measured by the MTT method, as described by Mosmann.[28] Briefly, cells in 96-well plates were rinsed with phosphate-buffered saline (PBS), and then MTT (0.4 mg/mL) was added to each well. The plates were incubated for 4 h at 37 °C. After the medium with MTT was removed, cells and dye crystals were solubilized with 200 μL of DMSO, and optical density was measured at 570 nm on a microplate reader (Zenyth 200, Anthos Orig., Austria).

DPPH Radical Scavenging Activity Assay. The DPPH assay was carried out using the method described by Blois.[29] Briefly, 10 μL of various concentrations of the tested compounds (final concentrations ranging from 0.16 to 100 μM) was added to 190 μL of DPPH solution (0.1 mM in EtOH). The mixture was allowed to react for 30 min at room temperature. The absorbance of the solution was read at 517 nm with a spectrophotometer (Zenyth 200, Anthos Orig., Austria). The percentage of radical scavenging activity (RSA%) was calculated as follows: $RSA\% = [(A_c - A_t)/A_c] \times 100\%$, where A_c is the average absorbance of the control and A_t is the absorbance of the test compounds.

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Supporting Information Available: HMBC correlations of **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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