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Bioactive Neolignans and Lignans from the Bark of *Machilus robusta*

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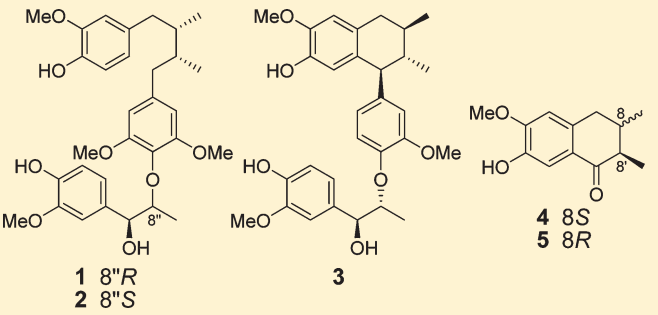
Supporting Information

ABSTRACT: Sixteen new neolignans and lignans (**1**−**16**), together with 12 known analogues, have been isolated from an ethanol extract of the bark of *Machilus robusta*. Compounds **1** and **2** showed activity against HIV-1 replication in vitro, with IC_{50} values of 2.52 and 2.01 μM, respectively. At 10 μM, **6**, **8**, and **9** reduced DL-galactosamine-induced hepatocyte (WB-F344 cells) damage, and **9** could additionally attenuate rotenone-induced PC12 cell damage. The known compounds (−)-pinoresinol (**17**) and (+)-lyoniresinol (**18**) were active against serum deprivation induced PC12 cell damage.



Machilus robusta W. W. Sm. (Lauraceae) is a plant that is widely distributed in southern China; however, no chemical or biological studies of this plant have been reported. As part of a continuing effort to assess the chemical diversity and biological activities of species of the genus *Machilus*,[1] an ethanol extract of the bark of *M. robusta* was investigated. We describe herein the isolation, structural elucidation, and biological assays of 16 neolignans and lignans (**1**−**16**), together with 12 known analogues. On the basis of IUPAC recommendations for the nomenclature of lignans and neolignans,[2] compounds **1** and **2** are categorized as uncommon 4′,8″-oxy-8,8′-sesquineolignans, **3** is a 4′,8″-oxy-2,7′-cyclo-8,8′-sesquineolignan, and **4** and **5** are abnormal 1′,2′,3′,4′,5′,6′-hexanor-2,7′-cyclolignans.

RESULTS AND DISCUSSION

Compound **1** had the molecular formula $C_{31}H_{40}O_8$, as indicated by HRESIMS combined with the NMR data. Its IR spectrum showed the presence of hydroxy (3490 cm^{-1}) and aromatic (1603, 1589, 1515, and 1463 cm^{-1}) functionalities. The NMR spectra of **1** displayed resonances (Table 1) attributable to two trisubstituted and one symmetric tetrasubstituted aromatic rings, four methoxy groups, an oxymethine, two methylenes, two methyls, respectively, attached to an aliphatic methine, and a methyl attached to a second oxymethine group. This suggested that compound **1** was a 4′,8″-oxy-8,8′-sesquineolignan with four *O*-methyl and three hydroxy substituents,[3] which was refined by 2D NMR data analysis (Supporting Information, Figures S8−S10). In the HMBC spectrum of **1**, correlations of H_2-7/C-1, C-2, C-6, C-8, C-8′, and C-9; H_2-7′/C-1′, C-2′, C-6′, C-8, C-8′, and C-9′; H-7″/C-1″, C-2″, C-6″, C-8″, and C-9″; and H-8″/C-4′, in combination with their chemical shifts, verified the presence of the three phenylpropyl units, the connection of C-8 and C-8′, and the oxygen bridge between C-4′ and C-8″. In addition, HMBC correlations from H-5 and O*Me*-3 to C-3, from H-2′/6′ and O*Me*-3′/5′ to C-3′/5′, and from H-5″ and O*Me*-3″ to C-3″ located the methoxy groups at C-3, C-3′, C-5′, and C-3″, respectively. This combined with the coupling constants of the aromatic protons (Table 1) assigned the hydroxy groups at C-4, C-4′, and C-4″, respectively. In the ^{1}H NMR spectrum, the small coupling constant between H-7″ and H-8″ (2.4 Hz) indicated the 7″,8″-*erythro* configuration.[4] In the CD spectrum, a negative Cotton effect at 242 nm ($\Delta\varepsilon$ −1.38) demonstrated that **1** had the 8″*R* configuration.[5] In addition, based on the bulkiness rule for the secondary alcohol,[6] a positive Cotton effect around 350 nm (the E band) in the $Rh_2(OCOCF_3)_4$-induced CD spectrum (Supporting Information Figure S29) supported the 7″*S* configuration, which was consistent with that defined by the 7″,8″-*erythro* and 8″*R* configuration. Oxidation of **1** by $RuO_2 \cdot 2H_2O$ in trifluoroacetic acid and trifluoroacetic anhydride[7] yielded the product **1a**, which had spectroscopic data (Supporting Information, Scheme S1 and Figures S1 and S13−S15) identical to those of the co-occurring **8** (see below). The 7′*S*,8*S*,8′*S* configuration of **1a** was proved by the $[\alpha]_D$ and CD data (Experimental Section). Accordingly, compound **1** was determined as (+)-(7″*S*,8*S*,8′*R*,-8″*R*)-4,4″-dihydroxy-3,3′,3″,5′-tetramethoxy-4′,8″-oxy-8,8′-sesquineolignan-7″-ol.

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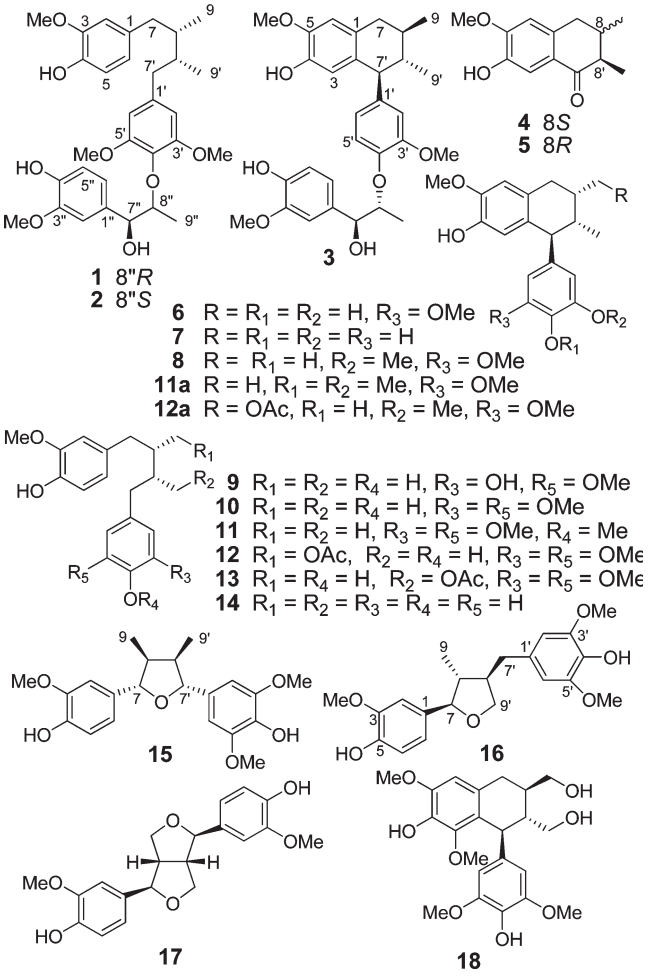

The spectroscopic data of compound **2** (Table 1 and Experimental Section) indicated that it was an isomer of **1**. Comparison of the NMR data of **2** and **1** demonstrated that H-6″, H-7″, and H-8″ and C-4′, C-6″, C-7″, C-8″, and C-9″ in **2** were shifted by $\Delta\delta_H$ +0.19, −0.18, and −0.42 and $\Delta\delta_C$ +2.3, +1.9, +6.3, +6.4, and +4.8 ppm, respectively. In addition, the coupling constant between H-7″ and H-8″ was changed from 2.4 Hz for **1** to 8.5 Hz for **2**. This suggested that **2** was the 7″,8″-*threo* isomer of **1**, which was proved by the 2D NMR data of **2** (Supporting Information, Figures S21−S23). The CD spectrum of **2** displayed a positive Cotton effect at 233 nm ($\Delta\varepsilon$ +2.07), demonstrating the 8″*S* configuration.[5] This was supported by a positive Cotton effect around 350 nm (the E band) in the $Rh_2(OCOCF_3)_4$-induced CD spectrum that suggested the 7″*S* configuration according to the bulkiness rule for the secondary alcohol[6] (Supporting Information, Figure S29). The $RuO_2 \cdot 2H_2O$ oxidation of **2** yielded the product **2a**, identical to **1a** (Supporting Information, Scheme S1 and Figures S1 and S26−S28), which confirmed the 8*S*,8′*R* configuration for **2**.

Compound **3**, $C_{30}H_{36}O_7$ (HRESIMS and the NMR data), exhibited spectroscopic data (Table 1 and Experimental Section) similar to **1**. Comparison of the NMR data of **3** with those of **1** indicated that the major difference was replacement of the C-7′ methylene group (H_2-7′ and C-7′) in **1** by a methine group in **3**. In addition, the 4-hydroxy-3-methoxyphenyl and 4′-oxy-3′,5′-dimethoxyphenyl moieties in **1** were substituted, respectively, by a 2-substituted 4-hydroxy-5-methoxyphenyl and a 4′-oxy-3′-methoxyphenyl moiety in **3**. This suggested that **3** was 4,4″-dihydroxy-3′,3″,5-trimethoxy-4′,8″-oxy-2,7′-cyclo-8,8′-sesquineolignan-7″-ol. The suggestion was confirmed by 2D NMR data analysis (Supporting Information, Figures S34−S36), which refined the assignments of the 1D NMR data of **3** (Table 1). Especially, HMBC correlations from H-7′ to C-1, C-1′, C-2′, C-3, C-6′, C-8, and C-9′, together with their chemical shifts, proved 2,7′-cyclization in **3**. The coupling constants ($J_{7b,8}$ = 12.0 Hz and $J_{7',8'}$ = 10.2 Hz) and NOE enhancements of H-8 and H_3-9′ upon irradiation of H-7′ indicated that H-8′ was *trans*-oriented to both H-7′ and H-8, and the 7″,8″-*erythro* configuration was indicated by the coupling constant (2.4 Hz)[3] between H-7″ and H-8″ (Table 1 and Supporting Information, Figure S32). In the CD spectrum of **3**, Cotton effects [positive at 274 ($\Delta\varepsilon$ +1.81) and negative at 291 nm ($\Delta\varepsilon$ −3.12)] indicated the 7′*S* configuration,[8] with a negative Cotton effect at 230 nm ($\Delta\varepsilon$ −1.56) suggesting the 8″*R* configuration.[5] In addition, the 7″*S* configuration defined by the 7″,8″-*erythro* orientation was supported by the positive E band (around 350 nm)[6] in the $Rh_2(OCOCF_3)_4$-induced CD spectrum of **3** (Supporting Information, Figure S40). Therefore, compound **3** was defined as (−)-(7′*S*,7″*S*,8*R*,8′*S*,8″*R*)-4,4″-dihydroxy-3′,3″,5-trimethoxy-4′,8″-oxy-2,7′-cyclo-8,8′-sesquineolignan-7″-ol.

Compound **4**, $C_{13}H_{16}O_3$ (HRESIMS and the NMR data), displayed absorption bands for hydroxy (3439 cm^{-1}), conjugated carbonyl (1665 cm^{-1}), and aromatic (1613 and 1509 cm^{-1}) functionalities in the IR spectrum. The NMR data (Table 1) indicated that it contained a 1,2,4,5-tetrasubstituted aromatic ring, an aromatic methoxy, a hydroxy, and a carbonyl group, as well as two methyls, a methylene, and two methines. On the basis of the splitting patterns and coupling constants for the resonances of the methyl, methylene, and methine protons, in combination with chemical shifts of the proton and carbon resonances of these units (Table 1), compound **4** was constructed to be 4-hydroxy-5-methoxy-1′,2′,3′,4′,5′,6′-hexanor-2,7′-cyclolignan-7′-one or its 5-hydroxy-4-methoxy isomer. In the HMBC spectrum of **4** (Supporting Information, Figure S48), correlations from H-3 to C-1, C-4, C-5, and C-7′, from O*H* to C-3, C-4, and C-5, and from O*Me* to C-5, together with their shifts, located the hydroxy and methoxy groups at C-4 and C-5, respectively. The coupling constants ($J_{7a,8}$ = 4.0 and $J_{7b,8}$ = 11.0 Hz) indicated that H-7b and H-8 occupied *trans* pseudo-diaxial positions. The NOE enhancements of H-7b and H_3-9 upon irradiation of H-8′ demonstrated that these protons were cofacial. On the basis of the octant rule for cyclohexenones,[9] a positive Cotton effect at 332 nm for the n → π* transition and a negative Cotton effect at 301 nm for the π → π* transition in the CD spectrum suggested that **4** had the 8′*R* configuration (Supporting Information, Figure S51), which was supported by calculation of CDs and specific rotations of the enantiomers (Supporting Information, Figure S177 and Table S2). Therefore, compound **4** was determined as (+)-(8*S*,8′*R*)-4-hydroxy-5-methoxy-1′,2′,3′,4′,5′,6′-hexanor-2,7′-cyclolignan-7′-one.

The spectroscopic data of compound **5** (Table 1 and Experimental Section) indicated that it was an isomer of **4**. However, the NMR resonances for the cyclohexenone moiety in **5** were significantly shifted as compared to **4** (Table 1). The 2D NMR data of **5** (Supporting Information, Figures S57−S59) revealed that it had the same planar structure as **4**. In the NOE difference spectrum of **5**, H-7b was enhanced upon irradiation of either H_3-9 or H_3-9′. This indicated that H_3-9 and H_3-9′ were cofacial and that H-7b and Me-9′ occupied the pseudo-diaxial positions,

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Table 1. NMR Spectroscopic Data (δ) of Compounds 1−5[a]

	1		2		3		4		5	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		133.5		133.6		131.9		136.8		136.2
2	6.64 br s	111.4	6.64 br s	111.4		133.5		125.9		125.7
3		146.4		146.3	6.39 s	121.0	7.54 s	112.4	7.55 s	112.3
4		143.6		143.6		143.9		144.3		144.3
5	6.83 d (7.8)	113.8	6.83 d (8.0)	114.0		149.2		150.9		151.0
6	6.67 br s d (7.8)	121.7	6.67 br s d (8.0)	121.7	6.62 s	111.2	6.62 s	109.4	6.63 s	109.9
7a	2.73 dd (13.5, 5.1)	39.2	2.72 dd (13.5, 5.5)	39.2	2.83 dd (16.2, 4.8)	39.2	2.89 dd (16.5, 4.0)	37.4	2.99 dd (16.5, 4.5)	35.2
7b	2.35 dd (13.5, 9.3)		2.35 dd (13.5, 9.5)		2.67 dd (16.2, 12.0)		2.67 dd (16.5, 11.0)		2.73 dd (16.5, 7.0)	
8	1.79 m	39.3	1.78 m	39.3	1.72 m	35.4	1.97 m	36.6	2.41 m	34.1
9	0.88 d (6.6)	16.0	0.87 d (7.0)	16.1	1.14 d (6.0)	20.0	1.14 d (6.5)	20.3	0.97 d (7.0)	15.5
1′		138.1		137.8		138.6				
2′	6.38 br s	105.9	6.36 br s	105.9	6.56 d (1.8)	111.3				
3′		153.2		152.5		146.4				
4′		132.6		134.9		144.0				
5′		153.2		152.5	6.86 d (7.8)	114.0				
6′	6.38 br s	105.9	6.36 br s	105.9	6.67 dd (7.8, 1.8)	122.6				
7′a	2.78 dd (13.5, 4.5)	39.4	2.77 dd (13.5, 4.5)	39.3	3.44 d (10.2)	54.1		199.3		200.2
7′b	2.26 dd (13.5, 9.6)		2.26 dd (13.5, 10.0)							
8′	1.79 m	38.8	1.78 m	38.8	1.56 m	43.8	2.18 m	48.9	2.66 m	46.4
9′	0.86 d (4.8)	16.5	0.86 d (7.0)	16.4	0.93 d (6.0)	17.1	1.26 d (6.5)	12.8	1.14 d (7.0)	11.3
1″		132.0		132.7		131.7				
2″	6.98 br s	108.6	6.88 br s	109.5	6.83 d (1.8)	108.3				
3″		146.4		146.4		146.5				
4″		144.4		145.2		144.6				
5″	6.83 d (7.8)	114.0	6.86 d (8.0)	114.0	6.81 d (7.8)	113.9				
6″	6.67 br s d (7.8)	118.8	6.86 br s d (8.0)	120.7	6.37 dd (7.8, 1.8)	118.7				
7″	4.78 d (2.4)	72.8	4.60 d (8.5)	79.1	4.65 d (2.4)	72.5				
8″	4.34 m	82.2	3.92 m	86.6	4.05 m	81.8				
9″	1.12 d (6.3)	12.8	1.17 d (6.0)	17.6	1.06 d (6.6)	12.9				
3/5-OMe	3.89 s/	55.8/	3.87 s/	55.9/	/3.89 s	/55.8	/3.94 s	/56.0	/3.95 s	/56.0
3′/5′-OMe	3.85 s/3.85 s	56.1/56.1	3.84 s/3.84 s	55.9/55.9	3.89 s/	55.8/				
3″-OMe	3.86 s	56.0	3.86 s	55.9	3.82 s	55.9				
4/4′/4″-OH			4.95				5.48/		5.47/	

[a] Data (δ) were measured in $CDCl_3$ for ^1H at 300 MHz for **1**, 500 MHz for **2**, **4**, and **5**, and 600 MHz for **3**; for ^{13}C at 100 MHz for **1** and **2**, 125 MHz for **4** and **5**, and 150 MHz for **3**. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, $^1H-^1H$ COSY, gHSQC, and HMBC experiments.

whereas Me-9 was pseudoequatorial in **5** (Supporting Information, Figure S60). The CD spectrum of **5** gave a negative Cotton effect at 326 nm for the n → π^* transition and a positive Cotton effect at 302 nm for the $\pi \rightarrow \pi^*$ transition, indicating the 8′*R* configuration based on the octant rule for cyclohexenones[9] (Supporting Information, Figure S62). The 8*R*,8′*R* configuration of **5** was supported by calculation of CDs and specific rotations of the enantiomers (Supporting Information, Figure S180 and Table S4). Thus, compound **5** was the 8-epimer of **4**.

Compound **6**, $C_{20}H_{24}O_5$ (HRESIMS and the NMR data), exhibited spectroscopic data (Tables 2 and 3 and Experimental Section) diagnostic for 2,7′-cyclolignane analogues with two methoxy and three hydroxy groups substituted at the aromatic rings.[10] HMBC correlations from H-3, H-6, and O*Me*-5 to C-5, from H-6 to C-2, C-4, and C-7, from H-6′ and O*Me*-5′ to C-5′, and from H-7′ to C-1, C-2, C-3, C-1′, C-2′, C-6′, C-8′, and C-9′ (Supporting Information, Figure S70), together with the shifts of these proton and carbon resonances, indicated that compound **6** was 3′,4,4′-trihydroxy-5,5′-dimethoxy-2,7′-cyclolignan. In the NOE difference spectrum of **6**, H-2′, H-6′, and H-7a were enhanced by irradiation of either H-8 or H-8′, while H_3-9 and H_3-9′ were enhanced when H-7′ was irradiated. These enhancements combined with the coupling constant ($J_{7',8'}$ = 6.5 Hz) revealed that H-8 and H-8′ were *cis*-oriented, whereas H-7′ and H-8′ were *trans*-oriented.[11] The CD spectrum of **6** showed a negative Cotton effect at 287 nm ($\Delta\varepsilon$ −2.46) and a positive Cotton effect at 273 nm ($\Delta\varepsilon$ +2.99), suggesting the 7′*S* configuration[7] (Supporting Information, Figure S2). Thus,

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Table 2. ^{1}H NMR Spectroscopic Data (δ) of Compounds 6−16[a]

no.	6	7	8	9	10	11	11a	12	13	14	15	16
2				6.63 br s	6.62 br s	6.63 d (1.5)		6.58 d (1.8)	6.61 br s	6.61 br s	7.09 d (1.5)	6.88 d (1.8)
3	6.42 s	6.40 s	6.41 s				6.42 s					
5				6.83 d (8.0)	6.83 d (8.0)	6.82 d (8.0)		6.81 d (7.8)	6.83 d (7.8)	6.82 d (8.0)	6.81 d (8.0)	6.88 d (8.4)
6	6.56 s	6.56 s	6.57 s	6.66 br d (8.0)	6.67 br s d (8.0)	6.67 dd (8.0, 1.5)	6.57 s	6.66 dd (7.8, 1.8)	6.67 d br s (7.8)	6.64 d br s (8.0)	6.92 dd (8.0, 1.5)	6.82 dd (8.4, 1.8)
7a	2.87 dd (16.5, 5.0)	2.85 dd (16.0, 5.0)	2.92 dd (16.2, 5.4)	2.73 dd (14.0, 5.0)	2.72 dd (13.0, 4.5)	2.73 dd (13.0, 5.5)	2.92 dd (16.2, 5.4)	2.76 dd (13.8, 5.4)	2.70 dd (13.8, 6.0)	2.73 dd (13.5, 5.0)	4.42 d (5.0)	4.28 d (9.0)
7b	2.45 dd (16.5, 7.5)	2.44 dd (16.0, 7.5)	2.47 dd (16.2, 6.6)	2.27 dd (14.0, 9.5)	2.31 dd (13.0, 9.5)	2.33 dd (13.0, 9.0)	2.48 dd (16.2, 7.2)	2.49 dd (13.8, 9.0)	2.44 dd (13.8, 8.4)	2.30 dd (13.5, 9.5)		
8	2.04 m	2.02 m	2.05 m	1.75 m	1.77 m	1.78 m	2.05 m	2.06 m	2.02 m	1.74 m	2.26 m	1.71 m
9a	0.90 d (3.0)	0.89 d (6.6)	0.91 d (7.2)	0.85 d (6.5)	0.86 d (6.5)	0.86 d (7.0)	0.91 d (7.2)	4.08 dd (10.8, 6.6)	0.91 d (6.6)	0.83 d (6.5)	1.02 d (6.5)	1.00 d (6.6)
9b								4.00 dd (10.8, 5.4)				
2′	6.22 br s	6.48 br s	6.25 s	6.44 br s	6.35 s	6.34 br s	6.24 s	6.34 br s	6.31 br s	7.02 d (8.5)	6.76 s	6.39 br s
3′										6.75 d (8.5)		
5′		6.74 d (8.0)								6.75 d (8.5)		
6′	6.20 br s	6.50 d br s (8.0)	6.25 s	6.24 br s	6.35 s	6.34 br s	6.24 s	6.34 br s	6.31 br s	7.02 d (8.5)	6.76 s	6.39 br s
7′a	3.55 d (6.5)	3.57 d (6.5)	3.55 d (6.6)	2.69 dd (14.0, 5.5)	2.74 dd (13.0, 4.0)	2.75 dd (13.0, 5.0)	3.57 d (6.6)	2.74 dd (13.8, 6.6)	2.73 dd (13.8, 5.4)	2.73 dd (13.5, 5.0)	4.42 d (5.0)	2.86 dd (13.8, 5.4)
7′b				2.25 dd (14.0, 10.0)	2.27 dd (13.0, 9.0)	2.27 dd (13.0,9.5)		2.43 dd (13.8, 9.0)	2.40 dd (13.8, 9.0)	2.30 dd (13.5, 9.0)		2.53 dd (13.8, 9.6)
8′	1.93 m	1.91 m	1.95 m	1.75 m	1.77 m	1.78 m	1.97 m	2.06 m	2.02 m	1.74 m	2.26 m	2.23 m
9′a	0.88 d (3.0)	0.88 d (6.6)	0.88 d (6.6)	0.84 d (6.5)	0.85 d (6.5)	0.86 d (6.5)	0.89 d (6.6)	0.96 d (6.6)	4.05 dd (11.4, 7.2)	0.83 d (6.5)	0.99 d (6.5)	3.80 dd (8.4, 8.4)
9′b									3.97 dd (11.4, 5.4)			4.00 dd (8.4, 8.4)
3/5-OMe	3.86 s	/3.86 s	/3.87 s	3.86 s	3.85 s	3.85 s	3.87 s	3.88 s	3.85 s	3.86 s	3.85 s	3.91 s
3′/5′-OMe	/3.81 s		3.81 s/3.81 s	/3.84 s	3.85 s/3.85 s	3.83 s/ 3.83 s/	3.77 s/3.77 s	3.86 s/3.86 s	3.84 s/3.84 s		3.82 s/3.82 s	3.88 s/3.88 s
4′-OMe						3.83 s	3.83 s					
4/4′-OH	5.33 s/5.23 s	5.32 s/4.93 s	5.35 s/5.30 s	5.50 s/5.28 s/5.28 s			5.32 s/	5.48 s/5.38 s	5.46 s/5.36 s	/5.43 s		5.58 s/5.40 s
9/9′-OAc								2.04 s/	/2.00s			

[a] Data (δ) were measured in $CDCl_3$ for ^{1}H at 500 MHz for **6**, **7**, **9**−**11**, and **14** and 600 MHz for **8**, **11a**, **12**−**14**, and **16** and in acetone-d_6 at 500 MHz for **15**. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, gHSQC, and HMBC experiments.

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Table 3. ^{13}C NMR Spectroscopic Data (δ) of Compounds 6−16[a]

no.	6	7	8	9	10	11	11a	12	13	14	15	16
1	127.6	127.7	127.8	133.8	132.7	133.6	127.8	132.3	132.8	134.1	135.0	133.6
2	130.4	130.6	131.0	111.4	111.4	111.4	130.8	111.2	111.3	111.4	110.7	108.5
3	116.0	116.1	116.2	146.3	146.3	146.3	116.2	146.5	146.4	146.2	148.2	146.6
4	143.3	143.0	143.7	143.5	143.6	143.6	143.7	143.9	143.8	143.5	146.8	145.2
5	145.0	143.5	145.2	113.9	113.9	114.0	145.2	114.1	114.0	114.0	115.4	114.0
6	110.5	110.5	110.8	121.7	121.7	121.7	110.8	121.6	121.6	121.7	119.9	119.4
7	35.2	35.1	35.7	38.8	39.1	39.2	35.7	33.2	39.8	39.3	88.2	88.7
8	29.1	28.9	29.9	39.1	38.9	38.8	29.9	42.7	34.9	38.9	45.2	48.6
9	15.7	15.6	15.8	16.2	16.2	16.4	15.9	65.3	15.6	16.2	13.0	14.9
1′	139.1	140.4	138.3	133.8	133.7	137.6	142.9	132.1	131.5	133.8	134.2	131.5
2′	109.6	114.9	106.2	109.3	105.6	105.9	106.5	105.5	105.4	130.1	104.6	105.2
3′	143.4	145.0	146.9	143.5	146.8	152.9	153.0	146.9	146.9	115.0	148.5	147.0
4′	130.6	141.4	133.1	130.2	132.9	136.0	136.4	133.0	132.9	153.4	136.1	133.1
5′	146.5	116.1	146.9	146.7	146.8	152.9	153.0	146.9	146.9	114.9	148.5	147.0
6′	104.0	121.8	106.2	103.8	105.6	105.9	106.5	105.5	105.4	130.1	104.6	105.2
7′	50.7	50.2	51.0	39.1	39.3	39.4	51.2	40.1	33.5	44.8	87.9	38.8
8′	40.5	40.6	40.8	39.2	39.0	39.0	40.7	35.0	42.6	38.4	45.6	49.1
9′	15.9	16.1	16.3	16.2	16.1	16.1	16.3	15.8	65.2	16.1	13.2	73.2
3/5-OMe	/55.8	/55.8	/56.0	55.8/	55.8/	55.8	/56.0	55.8	55.8	55.8	56.1	55.9
3′/5′-OMe	/56.1		56.6/56.6	/56.1	56.2/56.2	/56.0	56.3/56.3	56.2/56.2	56.2/56.2		56.6/56.6	56.3/56.3
4′-OMe						60.8	61.1					
9/9′-OAc								171.1, 21.0	171.1, 21.0			

[a] Data (δ) were measured in $CDCl_3$ for ^{13}C at 125 MHz for **6**−**11**, **11a**, and **14**, 150 MHz for **12**, **13**, and **16**, and 125 MHz in acetone-d_6 for **15**. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, gHSQC, and HMBC experiments.

compound **6** was (+)-(7′*S*,8*S*,8′*S*)-3′,4,4′-trihydroxy-5,5′-dimethoxy-2,7′-cyclolignan.

Compound **7** was the 5′-demethoxy analogue of **6**, as indicated by the spectroscopic data (Tables 2 and 3 and Experimental Section) and confirmed by comparison of the CD data of **7** and **6** and the reported analogues.[8c,12]

The spectroscopic data of compound **8** (Tables 2 and 3 and Experimental Section) demonstrated that it was the 3′-methoxy derivative of **6**. This was verified by 2D NMR data analysis of **8** (Supporting Information, Figures S85−S86), which supported the 1D NMR data assignments (Tables 2 and 3). In the NOE difference spectrum (Supporting Information, Figure S87), enhancements of both H_3-9 and H_3-9′ upon irradiation of H-7′ proved the relative configuration of **8**, while a negative Cotton effect at 287 nm ($\Delta\varepsilon$ −3.38) and a positive Cotton effect at 272 nm ($\Delta\varepsilon$ +2.57) in the CD spectrum confirmed the 7′*S* configuration.[8,10] Thus, compound **8** was identified as (+)-(7′*S*,8*S*,8′*S*)-4,4′-dihydroxy-3′,5,5′-trimethoxy-2,7′-cyclolignan.

Compound **9**, $C_{20}H_{26}O_5$ (HRESIMS and the NMR data), showed spectroscopic data (Tables 2 and 3 and Experimental Section) similar to those of **6**. However, comparison of the NMR data of **6** and **9** indicated substitution of the 1,2-disubstituted 4-hydroxy-5-methoxyphenyl unit in **6** by a 4-hydroxy-3-methoxyphenyl moiety in **9**. In addition, the methine group (CH-7′) in **6** was replaced by a methylene moiety (CH_2-7′) in **9**. This indicated that **9** was 3′,4,4′-trihydroxy-5,5′-dimethoxylignan, which was confirmed by 2D NMR data of **9** (Supporting Information, Figure S93−S95). Oxidative ring closure of **9** by using Ag_2O in benzene and acetone (2:1)[13] yielded a product (**9a**) that possessed spectroscopic data including the $[\alpha]_D$ and CD data identical to those of **6** (Supporting Information, Scheme S2 and Figures S2 and S98−S100). Therefore, the 8*S*,8′*R* configuration was assigned for **9**.

The spectroscopic data of compound **10** (Tables 2 and 3 and Experimental Section) indicated that it was the 3′-methoxy analogue of **9**, which was proved by the $RuO_2 \cdot 2H_2O$ oxidation[7] of **10** that generated **8** (Supporting Information, Scheme S1 and Figures S1 and S105−S106).

Compound **11** was the 4′-methoxy derivative of **9**, as demonstrated by its spectroscopic data (Tables 2 and 3 and Experimental Section) and confirmed by the $RuO_2 \cdot 2H_2O$ oxidative ring closure of **11** that produced **11a**, having CD spectroscopic features similar to those of **6**−**8** (Supporting Information, Schemes S1 and S2 and Figures S1, S2, and S114−S115).

The HRESIMS combined with the NMR data indicated that compound **12** had the molecular formula $C_{23}H_{30}O_7$. The NMR data of **12** resembled those of **10** (Tables 2 and 3) except for replacement of the resonances for Me-9 in **10** by those assignable to a CH_2OAc unit in **12**. The presence of OAc was supported by an absorption band at 1731 cm^{-1} in the IR spectrum. The 2D NMR data analysis verified that compound **12** was the 9-OAc derivative of **10**. Especially, HMBC correlations from H_2-9 to C-7, C-8, C-8′, and the carbonyl carbon of OAc, from H_3-9′ to C-7′, C-8, and C-8′, from H-2′/6′ to C-3′/5′, C-4′, and C-7′, and from O*Me*-3′/5′ to C-3′/5′, in combination with shifts of these proton and carbon resonances, proved the location of the substituents in **12**. Oxidation of **12** using Ag_2O yielded **12a**, of which the ^{1}H NMR spectrum showed a coupling constant of 8.4 Hz for H-7b and H-8, indicating that the two protons were *trans* pseudo-diaxially oriented. In addition, in the NOE difference spectrum of **12a**, H_3-9′ was enhanced by irradiation of either H-7b or H-7′ (Supporting Information, Figure S133). This

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revealed a *cis*-relationship between H-8 and H-8′. The CD spectrum of **12a** gave a negative Cotton effect at 289 nm ($\Delta\varepsilon$ −1.79) and a positive Cotton effect at 272 nm ($\Delta\varepsilon$ +2.49), suggesting the 7′*S* configuration. Accordingly, compound **12** was elucidated as (+)-(8*S*,8′*R*)-9-acetoxy-4,4′-dihydroxy-3,3′,5′-trimethoxylignan.

The spectroscopic data of compound **13** (Tables 2 and 3 and Experimental Section) demonstrated that it was the 9′-OAc isomer of **12**. This was confirmed by 2D NMR data, particularly, by HMBC correlations from H_2-7′ to C-1′, C-2′/6′, and C-9′, from O*H*-4 to C-3, C-4, and C-5, and from O*H*-4′ to C-3′/5′ and C-4′, in combination with shifts of these proton and carbon resonances. In addition, the similarity of the $[\alpha]_D$ and CD data of **13** and **12** supported that the two compounds had the same 8*S*,8′*R* configuration. Therefore, compound **13** was (+)-(8*S*,8′*R*)-9′-acetoxy-4,4′-dihydroxy-3,3′,5′-trimethoxylignan.

Compound **14** was (+)-(8*S*,8′*R*)-4,4′-dihydroxy-3-methoxylignan, as indicated by its spectroscopic data (Tables 2 and 3 and Experimental Section) and confirmed by 2D NMR data analysis (Supporting Information, Figures S148−S150), as well as by comparison of the $[\alpha]_D$ and CD data of **14** and **9**−**13**. This was also supported by comparison of the spectroscopic data of **14** and *threo*-4,4′-dihydroxy-3-methoxylignan (pycnantolol) with the opposite $[\alpha]_D$ value and undetermined absolute configuration.[14]

Compound **15** exhibited spectroscopic data (Tables 2 and 3 and Experimental Section) identical to those of fragransin C_1, but with opposite specific rotation.[15] 2D NMR data and NOESY data analysis of **15** (Supporting Information, Figures S193−S196) proved that it had the same planar structure and relative configuration as fragransin C_1. Although fragransin C_1 was reported to display no Cotton effect in its CD spectrum,[16] the CD spectrum of **15** showed a coupled CD curve, positive at 251 nm ($\Delta\varepsilon$ +0.37) and negative at 227 nm ($\Delta\varepsilon$ −1.09) (Supporting Information, Figure S162). On the basis of the CD exciton chirality rule,[17] compound **15** was assigned to have the 7*S*,7′*R*,8*S*,8′*R* configuration. Thus, compound **15** was defined as (−)-(7*S*,7′*R*,8*S*,8′*R*)-4,4′-dihydroxy-3,3′,5′-trimethoxy-7,7′-epoxylignan.

The spectroscopic data of compound **16** (Tables 2 and 3 and Experimental Section) indicated that it was an isomer of **15**. Comparison of the NMR data of **16** and **15** indicated that resonances assignable to the two oxymethylenes in **16** substituted those for O*CH*-7′ and *Me*-9′ in **15**, respectively. This indicated that **16** was 4,4′-dihydroxy-3,3′,5′-trimethoxy-7,9′-epoxylignan, which was confirmed by the 2D NMR data (Supporting Information, Figures S167−S169). In the NOE difference spectrum of **16**, irradiation of H-7 enhanced H-8′ and H_3-9 and irradiation of H-7′b enhanced H-8. The enhancements revealed that H-8 was *trans*-oriented to both H-7 and H-8′ on the tetrahydrofuran ring. The CD spectrum of **16** showed a coupled Cotton effect, positive at 235 nm and negative at 219 nm, indicating exciton coupling between the $\pi \rightarrow \pi^*$ transitions of the phenyl chromophores (Supporting Information, Figure S174). The positive chirality revealed the 7*R*,8*R*,8′*R* configuration for **16** on the basis of the CD exciton chirality rule.[18] Thus, compound **16** was assigned as (−)-(7*R*,8*R*,8′*R*)-4,4′-dihydroxy-3,3′,5′-trimethoxy-7,9′-epoxylignan.

The known compounds were identified by comparing the spectroscopic data with reported data as *meso*-dihydroguaiaretic acid,[19] (−)-(8*R*,8′*S*)-3,3′,4-trimethoxy-4′-hydroxylignan,[19] (+)-(8*R*,8′*R*)-3′,4,4′-trihydroxy-3-methoxylignan,[20] henricine B,[21] (+)-guaiacin,[22] (−)-isoguaiacin,[11] (−)-pinoresinol (**17**),[23] (−)-syringaresinol,[23] (+)-lyoniresinol (**18**),[24] (−)-(7′*R*,8*R*,8′*R*)-4,4′-dihydroxy-3,3′,5-trimethoxy-2,7′-cyclolignan,[1b] (−)-(7*S*,7′*S*,8*R*,8′*R*)-4,4′-dihydroxy-3,3′,5,5′-tetramethoxy-7,7′-epoxylignan-9,9′-diol,[17a] and (+)-(7*R*,8*R*,7′*E*)-4-hydroxy-3-methoxy-7,4′-epoxy-8,3′-neolignan-7′-ene.[25]

In the preliminary in vitro assays, compounds **1** and **2** inhibited HIV-1 replication with IC_{50} values of 2.52 and 2.01 μM, respectively (the positive control efavirenz gave a 42.5 ± 13.1% inhibition at 0.001 μM). At 10 μM, compounds **6**, **8**, and **9** reduced DL-galactosamine (GalN)-induced hepatocyte (WB-F344 cells) damage with 77.2 ± 4.1%, 64.0 ± 5.4%, and 68.0 ± 3.7% inhibition, respectively, while the positive control bicyclol gave a 67.0 ± 4.6% inhibition.[26] At the same concentration, compound **9** attenuated rotenone-induced PC12 cell damage by increasing the cell viability from 73.0 ± 9.6% to 96.2 ± 15.6%, and (−)-syringaresinol (**17**) and (+)-lyoniresinol (**18**) showed activities against serum deprivation induced PC12 cell damage by increasing the cell viability from 66.6 ± 9.2% to 73.2 ± 8.8% and 79.4 ± 4.5%, respectively. Other compounds were inactive in these assays. In addition, the isolates were also assessed for cytotoxicity against several human cancer cell lines[27] and Fe^{2+}-cystine-induced rat liver microsomal lipid peroxidation,[28] but were inactive at a concentration of 1.0 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter, and UV spectra were obtained on a Cary 300 spectrometer. CD spectra were recorded on a JASCO J-815 CD spectrometer. IR spectra were recorded on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission). 1D and 2D NMR spectra were acquired at 300, 500, or 600 MHz for ^{1}H and 100, 125, or 150 MHz for ^{13}C, respectively, on Varian Mecury-300 MHz or INOVA 400, 500 MHz, or SYS 600 MHz spectrometers, in $CDCl_3$ or acetone-d_6, with solvent peaks used as references. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer. HRESIMS data were measured using an Agilent Technologies 6520 Accurate Mass Q-TOF LC/MS spectrometer. Column chromatography (CC) was performed with silica gel (200−300 mesh, Qingdao Marine Chemical Inc. Qingdao, People's Republic of China), Pharmadex LH-20 (Amersham Biosciences, Inc., Shanghai, China), and MCI gel (CHP20P). Preparative TLC separation was performed with high-performance silica gel TLC plates ($HSGF_{254}$, glass precoated, Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, China). HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector, with a Prevail (250 × 10 mm i.d.) preparative column packed with C18 (5 μM). TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating.

Plant Material. The bark of *M. robusta* was collected in August 2006 at Dayao Mountain, Guangxi Province, People's Republic of China. The plant was identified by Mr. Guang-Ri Long (Guangxi Forest Administration, Guangxi 545005, China). A voucher specimen (no. 041) was deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China.

Extraction and Isolation. The air-dried bark of *M. robusta* (5.3 kg) was powdered and extracted with 45 L of aqueous 95% EtOH at room temperature for 3 × 48 h. The EtOH extract was evaporated under reduced pressure to yield a dark brown residue (535 g). The residue was suspended in H_2O (2000 mL) and partitioned with EtOAc (8 × 2000 mL). After removal of solvent, the EtOAc extract (261.0 g) was subjected to CC over silica gel, eluting with a gradient of increasing

acetone (0−100%) in petroleum ether, to produce nine fractions (A−I) on the basis of TLC analysis. Fraction B (17.5 g) was further fractionated via silica gel CC, eluting with petroleum ether−EtOAc (100:5−100:20), to yield B_1−B_5. Fraction B_4 was subjected to CC over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1), to obtain **14** (0.5 mg). B_5 was separated by normal silica gel CC, eluting with a gradient of increasing Me_2CO in $CHCl_3$ (0−100%), to yield $B_{5\text{-}1}$−$B_{5\text{-}4}$. $B_{5\text{-}2}$ was subjected to Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (5:5:1), to give $B_{5\text{-}2\text{-}1}$−$B_{5\text{-}2\text{-}11}$, of which $B_{5\text{-}2\text{-}5}$ was purified by high-performance silica gel preparative TLC plates, using a mobile phase of petroleum ether−Me_2CO (1.3:1), to obtain **4** (1.5 mg) and **5** (1.0 mg). Fraction D (2.0 g) was chromatographed over MCI gel, eluting with a gradient of increasing MeOH (30−100%) in H_2O, to give D_1−D_6. D_3 (1.023 g) was chromatographed over Sephadex LH-20, eluting with a gradient of petroleum ether−$CHCl_3$−MeOH (2:2:1). Subsequent fractions were separated by RP-HPLC using a mobile phase of MeOH−H_2O (62:38 or 67:33) to afford **6** (12.6 mg), **7** (1.0 mg), **8** (6.2 mg), **10** (50.2 mg), and **15** (15.1 mg). Fraction E (3.0 g) was chromatographed over MCI gel, eluting with a gradient of increasing MeOH (30−100%) in H_2O, to give nine subfractions (E_1−E_9). E_6 was subjected to CC over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (2:2:1), to give a fraction that was resolved by RP-HPLC using a mobile phase of MeOH−H_2O (72:28) to afford **1** (25.0 mg), **2** (89.0 mg), and **3** (0.8 mg). E_3 was subjected to CC over Sephadex LH-20, eluting with petroleum ether−$CHCl_3$−MeOH (2:2:1), to give **11** (21.2 mg) and fractions $E_{3\text{-}1}$−$E_{3\text{-}8}$. Fraction $E_{3\text{-}4}$ was separated by RP-HPLC using a mobile phase of MeOH−H_2O (59:41) to afford compounds **9** (4.1 mg), **12** (2.5 mg), and **13** (1.6 mg) and subfractions $E_{3\text{-}4\text{-}1}$−$E_{3\text{-}4\text{-}3}$. Fraction $E_{3\text{-}4\text{-}2}$ was purified by RP-HPLC by using a mobile phase of MeOH−H_2O (55:45) to afford **16** (3.0 mg).

*(+)-(7′′S,8S,8′R,8′′R)- 4,4′′-Dihydroxy-3,3′,3′′,5′-tetramethoxy-4′,8′′-oxy-8,8′-sesquineolignan-7′′-ol (**1**):* white, amorphous solid; $[\alpha]^{20}_D$ +3.1 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (5.05), 232 (4.37, sh), 280 (3.75) nm; CD (MeOH) 242 ($\Delta\varepsilon$ −1.38) nm; IR ν_{max} 3490, 2958, 2935, 2873, 1603, 1589, 1515,1463, 1425, 1271, 1124, 1033 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; (+)-ESIMS *m/z* 563 $[M + Na]^+$, 579 $[M + K]^+$; (+)-HRESIMS *m/z* 563.2628 $[M + Na]^+$ (calcd. for $C_{31}H_{40}O_8Na$, 563.2615).

*(+)-(7′′S,8S,8′R,8′′S)-4,4′′-Dihydroxy-3,3′,3′′,5′-tetramethoxy-4′,8′′-oxy-8,8′-sesquineolignan-7′′-ol (**2**):* white, amorphous solid; $[\alpha]^{20}_D$ + 44.1 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.84), 232 (4.08, sh), 280 (3.40) nm; CD (MeOH) 214 ($\Delta\varepsilon$ +2.19), 233 ($\Delta\varepsilon$ + 2.07) nm; IR ν_{max} 3443, 2958, 2936, 2873, 1603, 1589, 1515, 1463, 1424, 1271, 1125, 1035, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; (+)-ESIMS *m/z* 563 $[M + Na]^+$, 579 $[M + K]^+$; (+)-HRESIMS *m/z* 563.2617 $[M + Na]^+$ (calcd for $C_{31}H_{40}O_8Na$, 563.2615).

*(−)-(7′′S,7′′S,8R,8′S,8′′R)-4,4′′-Dihydroxy-3′,3′′,5-trimethoxy-4′,8′′-oxy-2,7′-cyclo-8,8′-sesquineolignan-7′′-ol (**3**):* white, amorphous solid; $[\alpha]^{20}_D$ −40.5 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.43), 232 (3.52, sh), 280 (3.13) nm; CD (MeOH) 230 ($\Delta\varepsilon$ −1.56), 274 ($\Delta\varepsilon$ +1.81), 291 ($\Delta\varepsilon$ −3.12) nm; IR ν_{max} 3437, 2960, 2931, 2873, 1605, 1514, 1464, 1452, 1431, 1267, 1246, 1209, 1151, 1034 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data, see Table 1; (+)-ESIMS *m/z* 531 $[M + Na]^+$, 547 $[M + K]^+$; (+)-HRESIMS *m/z* 531.2362 $[M + Na]^+$ (calcd. for $C_{30}H_{36}O_7Na$, 531.2353).

*(+)-(8S,8′R)-4-Hydroxy-5-methoxy-1′,2′,3′,4′,5′,6′ -hexanor-2,7′-cyclolignan-7′-one (**4**):* white needles ($CHCl_3$); mp 159−161 °C; $[\alpha]^{20}_D$ +74.3 (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.28) nm, 233 (4.24) nm, 273 (4.05) nm, 317 (3.24) nm; CD (MeOH) 301 ($\Delta\varepsilon$ −0.24), 332 ($\Delta\varepsilon$ +0.30); IR ν_{max} 3439, 2969, 2929, 2874, 1665, 1613, 1509, 1452, 1315, 1270, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 3; (+)-ESIMS *m/z* 221 $[M + H]^+$, 243 $[M + Na]^+$; (+)-HRESIMS *m/z* 221.1174 $[M + H]^+$ (calcd for$C_{13}H_{17}O_3$, 221.1172), 243.0994 $[M + Na]^+$ (calcd for $C_{13}H_{16}O_3Na$, 243.0992).

*(+)-(8R,8′R)-4-Hydroxy-5-methoxy-1′,2′,3′,4′,5′,6′-hexanor-2,7′-cyclolignan-7′-one (**5**):* white, amorphous solid; $[\alpha]^{20}_D$ +90.0 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (4.72), 233 (4.62), 274 (4.55), 315 (4.17) nm; CD (MeOH) 302 ($\Delta\varepsilon$ +0.18), 326 ($\Delta\varepsilon$ −0.03); IR ν_{max} 3355, 2961, 2925, 2853, 1659, 1602, 1509, 1468, 1278, 1025 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 3; (+)-ESIMS *m/z* 221 $[M + H]^+$, 243 $[M + Na]^+$, 463 $[2 M + Na]^+$, 479 $[2 M + K]^+$; (+)-HRESIMS *m/z* 221.1169 $[M + H]^+$ (calcd for $C_{13}H_{17}O_3$, 221.1172), 243.0994 $[M + Na]^+$ (calcd for $C_{13}H_{16}O_3Na$, 243.0992).

*(+)-(7′S,8S,8′S)-3′,4,4′-Trihydroxy-5,5′-dimethoxy-2,7′-cyclolignan (**6**):* white, amorphous powder, $[\alpha]^{20}_D$ +66.6 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.86), 237 (4.22, sh), 284 (3.63) nm; CD (MeOH) 273 ($\Delta\varepsilon$ +2.99), 287 ($\Delta\varepsilon$ −2.46) nm; IR ν_{max} 3410, 2960, 2875, 2842, 1613, 1513, 1464, 1456, 1245, 1208, 1024 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 3; (+)-ESIMS *m/z* 345 $[M + H]^+$, 367 $[M + Na]^+$, 383 $[M + K]^+$ 711[2 M + $Na]^+$; (+)-HRESIMS *m/z* 345.1706 $[M + H]^+$ (calcd for $C_{20}H_{25}O_5$, 345.1697), 367.1521 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_5Na$, 367.1516).

*(+)-(7′S,8S,8′S)-3′,4,4′-Trihydroxy-5-methoxy-2,7′-cyclolignan (**7**):* white, amorphous solid, $[\alpha]^{20}_D$ +11.8 (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.32), 232 (3.80, sh), 286 (3.46) nm; CD (MeOH) 274 ($\Delta\varepsilon$ +1.55), 294 ($\Delta\varepsilon$ −2.26) nm; IR ν_{max} 3374, 2957, 2932, 2876, 1594, 1511, 1451, 1372, 1353, 1275, 1249, 1205 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 3; (+)-ESIMS *m/z* 315 $[M + H]^+$, 337 $[M + Na]^+$, 353 $[M + K]^+$; (+)-HRESIMS *m/z* 315.1590 $[M + H]^+$ (calcd for $C_{19}H_{23}O_4$, 315.1591), 337.1412 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_4Na$, 337.1410).

*(+)-(7′S,8S,8′S)-4,4′-Dihydroxy-3′,5,5′-trimethoxy-2,7′-cyclolignan (**8**):* white, amorphous powder; $[\alpha]^{20}_D$ +97 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.35), 283 (3.28) nm; CD (MeOH) 272 ($\Delta\varepsilon$ +2.57), 287 ($\Delta\varepsilon$ −3.38) nm; IR ν_{max} 3418, 2959, 2882, 2840, 1615, 1514, 1460, 1247, 1215, 1116 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 3; (+)-ESIMS *m/z* 359 $[M + H]^+$, 381 $[M + Na]^+$, 397 $[M + K]^+$, 739 $[2 M + Na]^+$.

*(+)-(8S,8′R)-3′,4,4′-Trihydroxy-5,5′-dimethoxylignan (**9**):* white, amorphous powder; $[\alpha]^{20}_D$ +22.5 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.94), 232 (4.14, sh), 281 (3.43) nm; CD (MeOH) 271 ($\Delta\varepsilon$ +0.27), 291 ($\Delta\varepsilon$ −0.88) nm; IR ν_{max} 3454, 2970, 2959, 2870, 2851, 1611, 1514, 1467, 1269, 1102, 1021, 932, 818, 796 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 3; (+)-ESIMS *m/z* 347 $[M + H]^+$, 369 $[M + Na]^+$, 385 $[M + K]^+$; (+)-HRESIMS *m/z* 345.1708 $[M - H]^-$ (calcd for $C_{20}H_{25}O_5$, 345.1707).

*(+)-(8S,8′R)-4,4′-Dihydroxy-3,3′,5′-trimethoxylignan (**10**):* white, amorphous powder, $[\alpha]^{20}_D$ +6.01 (*c* 0.98, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.94), 232 (3.71), 283 (3.23) nm; CD (MeOH) 264 ($\Delta\varepsilon$ +0.03), 286 ($\Delta\varepsilon$ −0.01) nm; IR ν_{max} 3383, 2962, 1611, 1513, 1456, 1429, 1271, 1231, 1119, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 3; ESIMS *m/z* 361 $[M + H]^+$, 383 $[M + Na]^+$, and 399 $[M + K]^+$.

*(+)-(8S,8′R)-4-Hydroxy-3,3′,4′,5′-tetramethoxylignan (**11**):* white, amorphous powder; $[\alpha]^{20}_D$ +4.4 (*c* 0.090 $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.68), 230 (3.99), 280 (3.35) nm; IR ν_{max} 3434, 2957, 2934, 2840, 1590, 1513, 1460, 1423, 1270, 1239, 1128, 1035, 1011 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 3; (+)-ESIMS *m/z* 375 $[M + H]^+$, 397 $[M + Na]^+$, and 413 $[M + K]^+$; (+)-HRESIMS *m/z* 397.2010 $[M + Na]^+$ (calcd for $C_{22}H_{30}O_5Na$, 397.1991).

Chemical Transformation of Compounds 1, 2, 10, and 11. While a suspension of $RuO_2 \cdot 2H_2O$ (15 mg) in a mixture of anhydrous CH_2Cl_2 (0.5 mL), TFA (0.75 mL), and TFAA (0.35 mL) was stirred at −10 °C, a solution of **1** (15 mg) in CH_2Cl_2 (0.5 mL) was added dropwise, followed immediately by adding BF_3-Et_2O (37.5 μL). After stirring at 18 °C (±2 °C) for 8 h, the reaction mixture was treated with a saturated $NaHCO_3$ solution (2 mL) at 0 °C and extracted with $CHCl_3$ (×3). The $CHCl_3$ extract was evaporated under reduced pressure, and the residue was subjected to RP-HPLC using a mobile phase of MeOH−H_2O (75:25) to afford **1a** (2.5 mg), of which the ^{1}H NMR and CD data were identical to those of the natural product **8** isolated from the plant material (Supporting Information, Figures S3). By following the same procedure, **2a**, **10a**, and **11a** were obtained from **2**, **10**, and **11**, respectively. **2a** and **10a** had spectroscopic data identical to those of **1a** and **8**. Compound **11a** was determined to be (+)-(7′*S*,8*S*,8′*S*)-4-hydroxy-3′,4′,5,5′-tetramethoxy-2,7′-cyclolignan on the basis of the following data: $[\alpha]^{20}_D$ +75 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.72), 284 (3.37) nm; CD (MeOH) 270 (Δε +2.58), 288 (Δε −2.29) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 3.

*(+)-(8S,8′R)-9-Acetoxy-4,4′-dihydroxy-3,3′,5′-trimethoxylignan (**12**):* white, amorphous powder; $[\alpha]^{20}_D$ +7.3 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (5.00), 232 (4.34, sh), 281 (3.90) nm; CD (MeOH) 221 (Δε +1.63), 268 (Δε +0.52), 290 (Δε −0.32) nm; IR ν_{max} 3432, 2955, 2935, 2852, 1732, 1611, 1515, 1460, 1369, 1240, 1116, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 150 MHz) data, see Table 3; (+)-ESIMS *m/z* 441 $[M + Na]^+$, 457 $[M + K]^+$; (+)-HRESIMS *m/z* 441.1891 $[M + Na]^+$ (calcd for $C_{23}H_{30}O_7Na$, 441.1884).

Chemical Transformations of Compounds 9 and 12. A solution of **9** (13.0 mg) in benzene (0.9 mL) and acetone (0.45 mL) was reacted over Ag_2O (11.6 mg) at room temperature for 3 h. The reaction mixture was filtered, the filtrate was evaporated under reduced pressure, and the residue was purified by RP-HPLC using a mobile phase of MeOH−H_2O (59:41), to give product **9a** (4.3 mg), of which the ^{1}H NMR and CD data were identical to those of the natural product **6** isolated from the plant material (Supporting Information, Figures S17). By following the same procedure, **12a** (0.6 mg) was obtained from **12** (2.5 mg). Compound **12a** was determined to be (+)-(7′*S*,8*S*,8′*S*)-9-acetoxy-4,4′-dihydroxy-3′,5,5′-trimethoxy-2,7′-cyclolignan on the basis of the following data: white, amorphous powder; $[\alpha]^{20}_D$ +73.6 (*c* 0.05, $CHCl_3$); CD (MeOH) 241 (Δε +4.04), 272 (Δε +2.49), 289 (Δε −1.79) nm; ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.22 (1H, s, OH), 6.98 (1H, s, OH), 6.69 (1H, s, H-5), 6.34 (3H, s, H-3, H-2′, and H-6′), 4.08 (1H, dd, *J* = 10.8 and 5.4 Hz, H-9a), 3.91 (1H, dd, *J* = 10.8 and 8.4 Hz, H-9b), 3.81 (3H, s, O*Me*-5), 3.72 (6H, s, O*Me*-3′/5′), 3.67 (1H, d, *J* = 6.0 Hz, H-7′), 2.87 (1H, dd, *J* = 16.2 and 5.4 Hz, H-7a), 2.59 (1H, dd, *J* = 16.2 and 8.4 Hz, H-7b), 2.25 (1H, m, H-8′), 2.17 (1H, m, H-8), 0.91 (3H, s, H_3-9′); (+)-ESIMS *m/z* 439 $[M + Na]^+$, 455 $[M + K]^+$; (−)-ESIMS *m/z* 415 $[M - H]^-$.

*(+)-(8S,8′R)-9′-Acetoxy-4,4′-dihydroxy-3,3′,5′-trimethoxylignan (**13**):* white, amorphous powder, $[\alpha]^{20}_D$ +13.5 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.86), 232 (4.05, sh), 281 (3.37) nm; CD (MeOH) 208.5 (Δε +2.28), 239 (Δε +1.58), 271 (Δε +0.96), 290 (Δε −0.79) nm; IR ν_{max} 3437, 2958, 2939, 2843, 1731, 1612, 1515, 1461, 1429, 1368, 1116, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 150 MHz) data, see Table 3; (+)-ESIMS *m/z* 419 $[M + H]^+$, 441 $[M + Na]^+$, 457 $[M + K]^+$; (+)-HRESIMS *m/z* 441.1888 $[M + Na]^+$ (calcd for $C_{23}H_{30}O_7Na$, 441.1884).

*(+)-(8S,8′R)-4,4′-Dihydroxy-3-methoxylignan (**14**):* white, amorphous powder; $[\alpha]^{20}_D$ +8.7 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.08), 224 (3.84, sh), 280 (3.19) nm; CD (MeOH) 269 (Δε +0.07), 302 (Δε −0.01); IR ν_{max} 3361, 3199, 2957, 2925, 2853, 1632, 1613, 1514, 1454, 1234 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) data, see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 3; (+)-ESIMS *m/z* 301 $[M + H]^+$; (+)-HRESIMS *m/z* 301.1794 $[M + H]^+$ (calcd for $C_{19}H_{25}O_3$, 301.1798); 323.1617 $[M + Na]^+$ (calcd for $C_{19}H_{24}O_3Na$, 323.1618).

*(−)-(7S,7′R,8S,8′R)-4,4′-Dihydroxy-3,3′,5′-trimethoxy-7,7′-epoxylignan (**15**):* white, amorphous powder, $[\alpha]^{20}_D$ −2.2 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.55), 232 (3.83, sh), 280 (3.30) nm; CD (MeOH) 227 (Δε −1.09), 251 (Δε +0.37); IR ν_{max} 3431, 2961, 2937, 2875, 2844, 1613, 1518, 1463, 1429, 1213, 1116 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 2 and Table 3; (+)-ESIMS *m/z* 375 $[M + H]^+$, 397 $[M + Na]^+$, 413 $[M + K]^+$, 771 $[2 M + Na]^+$; HRESIMS *m/z* 375.1796 $[M + H]^+$ (calcd for $C_{21}H_{27}O_6$, 375.1802), 397.1614 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_6Na$, 397.1622).

*(−)-(7R,8R,8′R)-4,4′-Dihydroxy-3,3′,5′-trimethoxy-7,9′-epoxylignan (**16**):* white, amorphous powder; $[\alpha]^{20}_D$ −25.6 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.06), 232 (3.26, sh), 280 (2.70) nm; CD (MeOH) 219 (Δε −1.13), 235 (Δε +1.82); IR ν_{max} 3418, 2956, 2936, 2872, 2843, 1612, 1517, 1462, 1274, 1215, 1116, 1034 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data, see Table 2 and Table 3; (+)-ESIMS *m/z* 375 $[M + H]^+$, 397 $[M + Na]^+$; HRESIMS *m/z* 375.1809 $[M + H]^+$ (calcd for $C_{21}H_{27}O_6$, 375.1802), 397.1632 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_6Na$, 397.1622).

Anti-HIV Activity Assay. See ref 29.

Cells, Culture Conditions, and Cell Proliferation Assay. See ref 29c.

Protective Effect of DL-Galactosamine-Induced WB-F344 Cell Damage. See ref 1b.

PC12 Cell Protection Assay. See ref 30. PC12 cells at a density of 5×10^3 cells per well in 96-well plates were cultured in DMEM media (Hyclone) supplemented with 5% fetal bovine serum (FBS, Hyclone), 5% horse serum (Hyclone), and L-glutamine (2 mM). Cultures were maintained at 37 °C in 5% CO_2 in a humidified incubator. After incubation for 48 h, compounds at concentrations of 10 and/or 4 μM rotenone were added to the cells. After incubation for another 48 h, 10 μL of the 5 mg/mL MTT (Sigma) was added and maintained for 4 h. Absorbance was measured at 570 nm using an Ultramark microplate reader. Cell viability was evaluated.

■ ASSOCIATED CONTENT

Supporting Information. Copies of spectra of compounds **1**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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