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ARTICLE TYPE

Hopeachinols E-K, novel Oligostilbenoids from the Stem Bark of *Hopea chinensis*

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Five new stilbenolignans, hopeachinols E-I (**1-5**), two new stilbenoids, hopeachinols J and K (**6** and **7**), together with the known resveratrol trimer, vaticanol A (**8**), were isolated from the stem bark of *Hopea chinensis*. The structures and relative configurations of these natural products were elucidated by using spectroscopic and spectrometric methods. The (8*R*) absolute configuration of **1** was assigned by using the modified Mosher ester method. Hopeachinols E-I (**1-5**) possess unprecedented stilbenolignan skeletons, in which a stilbene trimer is linked with a phenylpropanoid unit through a pyran bridge. Hopeachinols J and K (**6** and **7**) have new skeletons in which a stilbene trimer is connected by an additional two-carbon unit through a furan moiety. An evaluation of the cytotoxicities against HCT116, MDA-MB-231, SMMC-7721, and HepG2 cell lines of the new hopeachinols showed that **1**, **2**, **4,** and **6** have moderate activities with IC_{50} values in the 10.39-18.72 μM range.

Introduction

Plants belonging to the Dipterocarpaceae family, mostly distributed in Southeast Asia, are well documented sources of biologically active stilbenoids.[1-3] They include stilbene dimers, trimers, tetramers, hexamers, heptamers and octamers with various molecular frameworks resulting from different oxidative condensation of resveratrol monomer. Some of these compounds have broad ranges of bioactivities including antimicrobial,[4] antitumor,[5,6] anti-inflammatory,[7] and anti-HIV.[8] Our previous studies on *Hopea chinensis*, a unique dipterocarpaceae species growing only in South China, have led to the identification of a number of oligostilbenoids that possess immunosuppressive and acetylcholinesterase (AChE) inhibitory activities.[9,10] In continuing studies, we have isolated five new stilbenolignans **1-5** and two stilbenoids **6** and **7**, along with one known oligostilbene **8** (Fig. 1) from the stem bark of *H. chinensis*. Compounds **1-5** share an unprecedented stilbenolignan skeleton in which a stilbene trimer is linked with a phenylpropanoid unit through a pyran ring.[11] In contrast, **6** and **7** are composed of a stilbene trimer connected by an additional two-carbon unit through a dihydrofuran. Biological evaluation showed that **1**, **2**, **4**, and **6** are cytotoxic towards HCT116, MDA-MB-231, SMMC-7721, and HepG2 cancer cells. Herein we describe their isolation, structure elucidation, and cytotoxicity evaluation.

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Results and Discussion

The stilbenolignans, hopeachinols E-I (**1-5**), and two stilbenoids, hopeachinols J and K (**6** and **7**), together with the known resveratrol trimer vaticanol A (**8**) were isolated from an EtOAc extract of the stem bark of *Hopea chinensis* by using successive chromatographic procedures (silica gel, Sephadex LH-20, RP-18, and HPLC).

Hopeachinol E (**1**), obtained as a yellow amorphous powder, was determined to have the molecular formula $C_{53}H_{44}O_{13}$ (*m/z* 889.2850 $[M + H]^+$, calcd 889.2855) by using positive ion HRESIMS and ^{13}C NMR data (Table 2). The ^1H NMR data (Table 1) showed the presence of three 4-hydroxyphenyl groups [δ_H 7.29 (2H, d, J = 8.4 Hz, H-2a/H-6a), 6.83 (2H, d, J = 8.4 Hz, H-3a/H-5a); 7.07 (2H, d, J = 8.4 Hz, H-2b/H-6b), 6.60 (2H, d, J = 8.4 Hz, H-3b/H-5b); 6.60 (2H, d, J = 8.4 Hz, H-2c/H-6c), and 6.36 (2H, d, J = 8.4 Hz, H-3c/H-5c)]; three 1,2,3,5-tetrasubstituted phenyl rings [δ_H 6.07 (1H, d, J = 2.4 Hz, H-12a), 6.46 (1H, d, J = 2.4 Hz, H-14a); 6.23 (1H, d, J = 2.4 Hz, H-10c), 6.22 (1H, d, J = 2.4 Hz, H-12c); and 6.76 (2H, s, H-2)]; a penta-substituted phenyl ring [δ_H 6.24 (1H, s, H-12b)]; a set of mutually coupled aliphatic protons [δ_H 6.18 (1H, d, J = 4.2 Hz, H-7a), and 4.51 (1H, d, J = 4.2 Hz, H-8a)]; and a contiguous sequence of four aliphatic methine protons [δ_H 5.16 (1H, s, H-7b), 4.48 (1H, d, J = 7.2 Hz, H-8b); 3.58 (1H, d, J = 7.2 Hz, H-7c), and 4.40 (1H, s, H-8c)]. Additionally, resonances were observed at δ_H 4.66 (1H, d, J = 7.8 Hz, H-7), 4.01 (1H, m, H-8), 2.80 (1H, dd, J = 15.6, 9.0 Hz, H-9α), 2.98 (1H, dd, J = 15.6, 4.8 Hz, H-9β), and 3.83 (6H, s, OCH_3). Comparison of the ^{13}C NMR data of **1** with those of vaticanol A (**8**) suggested that it possesses a substructure sharing the same skeleton with **8**.[12] HMBC correlations (Fig. 2) between H-7/C-2, C-8 and H-9/C-7, C-8, and ^1H-^1H COSY correlations between H-9/H-8 and H-8/H-7 showed that the remaining motif

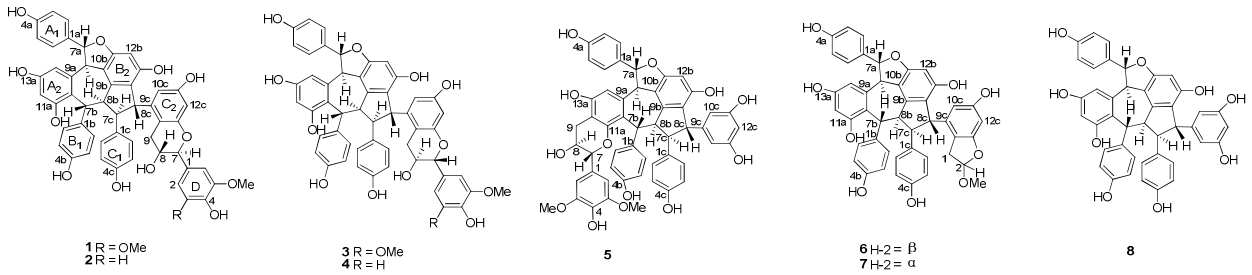


Fig. 1 Structures of compounds **1**-**8**.

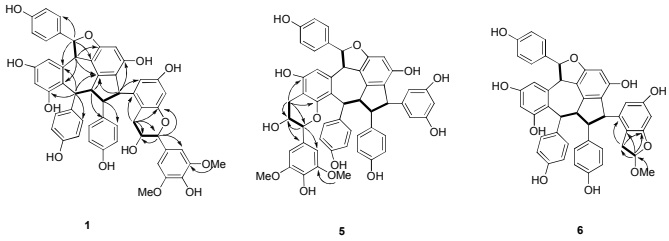


Fig. 2 Key HMBC correlations (H → C) for **1**, **5**, and **6**.

present in **1** is a C_6-C_3 moiety. The two partial structures of **1** are connected by an ether linkage between C-7/C-13c and a C–C bond between C-9/C-14c based on HMBC correlations between H-9/C-9c, C-13c and H-7/C-13c. Finally, long-range HMBC correlations showed that the methoxy groups are located at C-3 and C-5.

NOESY experiments were conducted to determine the relative configurations at the stereocenters in **1** (Fig. 3). The presence of NOE correlations between H-7a/H-14a and H-8a/H-2a(6a) suggested that the methine protons H-7a, H-8a are *trans* disposed, and that between H-8a/H-2b(6b) indicated that ring B_1 is α-oriented (H-7b: β-oriented). The NOE correlations between H-7b/H-2c(H-6c), H-8b/H-2b(H-6b), H-8b/H-10c and H-8c/H-2c(H-6c) showed that the relative orientations of the methine protons at C-7b, C-8b, C-7c and C-8c are β, α, α, and β oriented, respectively. Importantly, the similar NOE associations showed that the relative configurations of the stereocenters in the stilbene trimer unit in **1** are the same as those in vaticanol A (**8**).[12] In addition, H-7 and H-8 in **1** were *trans* oriented based on the NOE correlations between H-8/H-2(H-6). Finally, clear cross-peaks between H-7/H-9α and H-9β/H-8c were also observed.

Hopeachinol F (**2**), also obtained as a yellow amorphous powder, was shown to have the molecular formula $C_{52}H_{42}O_{12}$ (*m/z* 859.2745 $[M + H]^+$, calcd to be 859.2749) by analysis of its HRESIMS spectrometric and ^{13}C NMR data. The ^1H and ^{13}C NMR spectra of **2** (Table 1, 2) were closely similar to those of **1** except that the C-3 methoxy proton and carbon resonances at C-3 in the spectra of **1** are replaced by an aromatic proton and carbon in **2**. Moreover, the NOESY spectra of **1** and **2** are almost the same, indicating that both substances have the same relative configuration at their corresponding stereocenters.

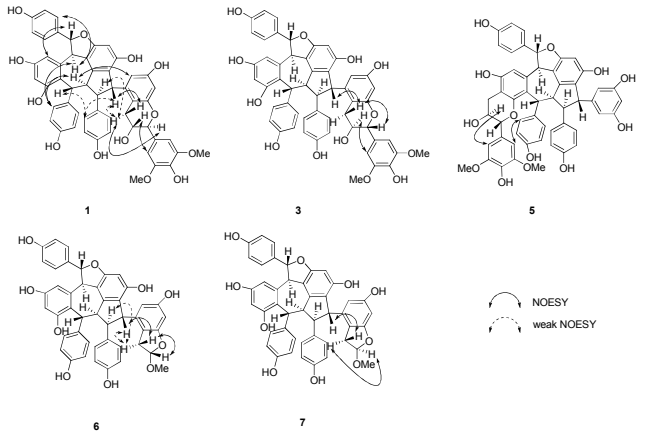


Fig. 3 Key NOESY correlations for **1**, **3**, **5**, **6**, and **7**.

Hopeachinol G (**3**), having the molecular formula $C_{53}H_{44}O_{13}$ was isolated as a yellow amorphous powder. The ^1H and ^{13}C NMR (Table 1, 2), HSQC, HMBC, ^1H-^1H COSY (see Supporting Information S15-S17), and NOESY data (Fig. 3) showed that **3** has the same planar structure and similar relative configurations as **1**. Structural difference between **1** and **3** was deduced using NOE correlations between H-7/H-9β, H-9α/H-7c and H-9β/H-8c, which showed that H-7 and H-8 are β- and α-oriented, respectively, in **3**.

Hopeachinol H (**4**), isolated as a yellow amorphous powder and having a molecular formula $C_{52}H_{42}O_{12}$, has a 30 mass units lower ($-OCH_2$) molecular weight than **3**. The spectroscopic data of **4** showed that it possesses a structure that is similar to **3**, except for the absence of a methoxy group. The D ring proton resonances in the ^1H NMR spectrum of **3**, which appear as an A_2 system [δ_H 6.76 (2H, s, H-2)], are replaced by an ABC system [δ_H 6.91 (1H, dd, J = 7.8, 1.8 Hz, H-2), 6.82 (1H, d, J = 7.8 Hz, H-3), 7.05 (1H, d, J = 1.8 Hz, H-6)] in **4** (Table 1). ^1H-^1H COSY, HMBC, and NOESY data provide support for the proposal that **4** is a demethoxy derivative of **3**.

^1H and ^{13}C NMR (Table 1, 2), and HMBC (Fig. 2) data of hopeachinol I (**5**), obtained as a yellow amorphous powder and having the molecular formula of $C_{53}H_{44}O_{13}$, showed that this substance contains the same substructure as vaticanol A (**8**) and the same phenylpropanoid motif as **1**. The difference between **1** and **5** is the position of the C6-C3 unit. HMBC cross-peaks for H-9/C-11a, C-13a indicate that the two partial structures in **5** are connected by an ether linkage between C-7/C-11a and a C–C bond between C-9/C-12a. NOESY correlations (Fig. 3) show that the relative configurations at the stereogenic carbons in the stilbene trimer part of **5** are the same as those in vaticanol A (**8**). Also, the NOE correlations between H-2/H-8 and H-2/H-2b show that the methine protons at C-7 and C-8 are β- and α-oriented, respectively.

The ^1H NMR spectrum of hopeachinols J (**6**) (Table 1), which has the molecular formula $C_{45}H_{36}O_{10}$ and was isolated as a yellow amorphous powder, contains resonances for three sets of *ortho*-coupled protons on *p*-substituted phenyl moieties as an A_2B_2 system [δ_H 7.27 (2H, d, J = 9.0 Hz, H-2a/H-6a), 6.82 (2H, d, J = 9.0 Hz, H-3a/H-5a); 7.05 (2H, d, J = 8.4 Hz, H-2b/H-6b), 6.59 (2H, d, J = 8.4 Hz, H-3b/H-5b); 6.54 (2H, d, J = 8.4 Hz, H-2c/H-

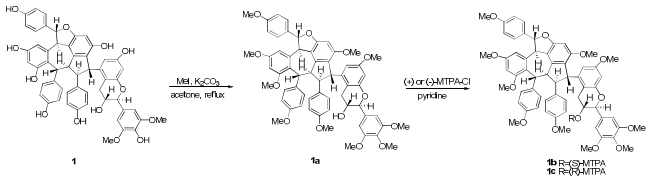
Table 1 ^{1}H NMR data for compounds **1**-**8**.

	1[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[b]
2a,6a	7.29 (d, 8.4)	7.28 (d, 8.4)	7.27 (d, 8.5)	7.28 (d, 8.4)	7.26 (d, 8.4)	7.27 (d, 9.0)	7.27 (d, 8.4)	7.28 (d, 8.5)
3a,5a	6.83 (d, 8.4)	6.83 (d, 8.4)	6.83 (d, 8.5)	6.83 (d, 8.4)	6.82 (d, 8.4)	6.82 (d, 9.0)	6.82 (d, 8.4)	6.84 (d, 8.5)
7a	6.18 (d, 4.2)	6.18 (d, 4.2)	6.18 (d, 3.5)	6.17 (d, 4.2)	6.14 (d, 3.6)	6.17 (d, 3.6)	6.16 (d, 3.6)	6.18 (d, 3.5)
8a	4.51 (d, 4.2)	4.51 (d, 4.2)	4.50 (d, 3.5)	4.50 (d, 4.2)	4.53 (d, 3.6)	4.49 (d, 3.6)	4.50 (d, 3.6)	4.50 (d, 3.5)
12a	6.07 (d, 2.4)	6.06 (d, 2.4)	6.05 (d, 2.0)	6.05 (d, 2.4)		6.06 (d, 2.4)	6.06 (d, 2.4)	6.08 (d, 2.0)
14a	6.46 (d, 2.4)	6.46 (d, 2.4)	6.46 (d, 2.0)	6.45 (d, 2.4)	6.61 (s)	6.47 (d, 2.4)	6.46 (d, 2.4)	6.49 (d, 2.0)
2b,6b	7.07 (d, 8.4)	7.07 (d, 8.4)	7.06 (d, 8.5)	7.06 (d, 8.4)	7.14 (d, 8.4)	7.05 (d, 8.4)	7.04 (d, 8.4)	7.08 (d, 8.5)
3b,5b	6.60 (d, 8.4)	6.60 (d, 8.4)	6.60 (d, 8.5)	6.60 (d, 8.4)	6.60 (d, 8.4)	6.59 (d, 8.4)	6.58 (d, 8.4)	6.62 (d, 8.5)
7b	5.16 (s)	5.16 (s)	5.13 (s)	5.13 (s)	5.17 (s)	5.14 (s)	5.12 (s)	5.16 (s)
8b	4.48 (d, 7.2)	4.47 (d, 6.6)	4.51 (d, 6.5)	4.51 (d, 7.2)	4.43 (d, 7.2)	4.49 (d, 7.2)	4.43 (d, 7.2)	4.53 (d, 7.0)
12b	6.24 (s)	6.24 (s)	6.24 (s)	6.24 (s)	6.23 (s)	6.22 (s)	6.22 (s)	6.25 (s)
2c,6c	6.60 (d, 8.4)	6.60 (d, 8.4)	6.56 (d, 8.5)	6.55 (d, 8.4)	6.34 (d, 8.4)	6.54 (d, 8.4)	6.56 (d, 8.4)	6.55 (d, 8.5)
3c,5c	6.36 (d, 8.4)	6.36 (d, 8.4)	6.35 (d, 8.5)	6.35 (d, 8.4)	6.39 (d, 8.4)	6.38 (d, 8.4)	6.37 (d, 8.4)	6.39 (d, 8.5)
7c	3.58 (d, 7.2)	3.58 (d, 6.6)	3.46 (d, 6.5)	3.46 (d, 7.2)	3.50 (d, 7.2)	3.58 (d, 7.2)	3.56 (d, 7.2)	3.65 (d, 7.0)
8c	4.40 (s)	4.40 (s)	4.38 (s)	4.37 (s)	4.11 (s)	4.19 (s)	4.22 (s)	4.21 (s)
10c	6.23 (d, 2.4)	6.23 (d, 2.4)	6.22 (d, 2.5)	6.22 (d, 2.4)	6.24 (d, 2.4)	6.11 (d, 2.4)	6.11 (d, 2.4)	6.30 (d, 2.0)
12c	6.22 (d, 2.4)	6.21 (d, 2.4)	6.21 (d, 2.5)	6.21 (d, 2.4)	6.16 (t, 2.4)	6.19 (d, 2.4)	6.20 (d, 2.4)	6.23 (t, 2.0)
14c					6.24 (d, 2.4)			6.30 (d, 2.0)
1						3.16 (dd, 15.6, 6.6) β 2.90 (dd, 15.6, 2.4) α	3.35 (dd, 15.6, 6.6) α 2.73 (dd, 15.6, 1.8) β	
2	6.76 (s)	6.92 (dd, 8.4, 1.8)	6.76 (s)	6.91 (dd, 7.8, 1.8)	6.67 (s)	5.67 (dd, 6.6, 2.4)	5.69 (dd, 6.6, 1.8)	
3		6.81 (d, 8.4)		6.82 (d, 7,8)				
6	6.76 (s)	7.05 (d, 1.8)	6.76 (s)	7.05 (d, 1.8)	6.67 (s)			
7	4.66 (d, 7.8)	4.68 (d, 8.4)	4.57 (d, 8.0)	4.59 (d, 7.8)	3.80 (d, 9.0)			
8	4.01 (m)	4.01 (m)	4.16 (m)	4.16 (m)	3.68 (m)			
9	2.98 (dd, 15.6, 4.8) β 2.80 (dd, 15.6, 9.0) α	2.97 (dd, 15.6, 4.8) β 2.81 (dd, 15.6, 9.0) α	3.11 (dd, 16.0, 5.5) α 2.63 (dd, 16.0, 9.0) β	3.09 (dd, 15.6, 5.4) α 2.64 (dd, 15.6, 9.0) β	3.04 (dd, 15.6, 5.4) 2.37 (dd, 15.6, 10.8)			
OCH_3	3.83 (6H, s)	3.85 (3H, s)	3.82 (6H, s)	3.84 (3H, s)	3.82 (6H, s)	3.48 (3H, s)	3.44 (3H, s)	

[a] Measured at 600 MHz for ^{1}H NMR in acetone-d_6. [b] Measured at 500 MHz for ^{1}H NMR in acetone-d_6.

6c), and 6.38 (2H, d, J = 8.4 Hz, H-3c/H-5c)]; two sets of *meta*-coupled protons as an AB system corresponding to 1,2,3,5-tetrasubstituted phenyl moieties [δ_H 6.06 (1H, d, J = 2.4 Hz, H-12a), 6.47 (1H, d, J = 2.4 Hz, H-14a); 6.11 (1H, d, J = 2.4 Hz, H-10c), and 6.19 (1H, d, J = 2.4 Hz, H-12c)]; a proton of a penta-substituted phenyl group [δ_H 6.22 (1H, s, H-12b)]; a sequence of four aliphatic methane protons [δ_H 5.14 (1H, s, H-7b), 4.49 (1H, d, J = 7.2 Hz, H-8b); 3.58 (1H, d, J = 7.2 Hz, H-7c), and 4.19 (1H, s, H-8c)]; and a set of mutually coupled aliphatic protons [δ_H 6.17 (1H, d, J = 3.6 Hz, H-7a), and 4.49 (1H, d, J = 3.6 Hz, H-8a)]. In addition, other resonances occured at δ_H 3.16 (1H, dd, J = 15.6, 6.6 Hz, H-1β), 2.90 (1H, dd, J = 15.6, 2.4 Hz, H-1α), 5.67 (1H, dd, J = 6.6, 2.4 Hz, H-2), and 3.48 (3H, s, OCH_3). ^{13}C and 2D NMR (^{1}H-^{1}H COSY, HMBC, and NOESY) data showed that **6** is also structurally similar to vaticanol A (**8**).[12] HMBC correlations (Fig. 2) between H-1/C-9c, C-13c and H-2/C-1, C-13c, C-14c revealed the existence of a C-14c/C-1/C-2 sequence, and that a methoxy group is located at C-2. Finally, cross-peaks in NOE correlations (Fig. 3) between H-1β/H-2, H-1β/H-8c, H-1α/H-7c and H-1α/H-8c showed that H-2 is β-oriented.

The final substance isolated as an amorphous yellow powder from the stem bark of *H. chinensis* is hopeachinol K (**7**, $C_{53}H_{44}O_{13}$). ^{1}H and ^{13}C NMR (Table 1, 2), HSQC, HMBC, and



Scheme 1 Formation of the *R*- and *S*-MTPA esters from **1**.

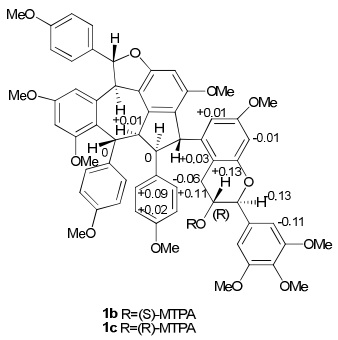


Fig. 4 $\Delta\delta$ (δ_S - δ_R) values (ppm) for the MTPA-esters **1b** and **1c**.

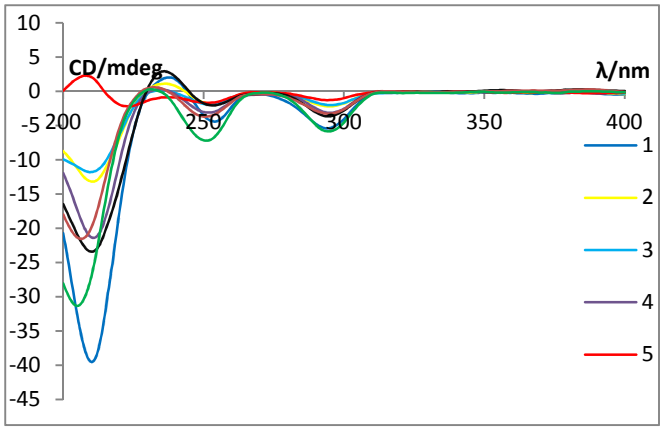


Fig. 5 CD spectra of compounds **1**-**8**.

Table 2 ^{13}C NMR data for compounds **1**-**7**.

	1[a]	2[a]	3[b]	4[a]	5[a]	6[a]	7[a]
1a	134.5	134.5	134.5	134.4	134.4	134.3	134.3
2a,6a	128.2	128.2	128.1	128.1	128.1	128.1	128.1
3a,5a	116.2	116.2	116.2	116.2	116.2	116.1	116.1
4a	158.1	158.1	158.1	158.2	158.1	158.0	158.1
7a	86.8	86.8	86.9	86.8	86.7	86.7	86.9
8a	50.3	50.3	50.4	50.3	50.0	50.3	50.3
9a	144.9	144.9	144.9	144.9	141.8	144.8	144.8
10a	119.5	119.5	119.4	119.3	120.3	119.1	119.4
11a	157.9	157.9	157.9	157.9	154.3	157.9	157.8
12a	101.4	101.4	101.5	101.4	107.2	101.2	101.3
13a	156.4	156.4	156.5	156.5	153.9	156.4	156.4
14a	103.5	103.4	103.5	103.4	103.3	103.1	103.2
1b	138.7	138.7	138.7	138.7	138.7	138.6	138.7
2b,6b	129.3	129.4	129.3	129.3	129.4	129.2	129.3
3b,5b	115.5	115.5	115.5	115.5	115.5	115.4	115.4
4b	155.9	155.9	155.9	155.9	155.9	155.8	155.8
7b	36.0	36.0	36.1	36.0	36.1	36.1	36.1
8b	48.5	48.5	48.6	48.5	48.3	48.8	48.6
9b	145.4	145.4	145.4	145.4	145.0	145.1	145.2
10b	119.0	119.0	119.1	119.0	118.8	118.8	118.8
11b	160.2	160.2	160.2	160.2	160.0	160.1	160.1
12b	95.5	95.5	95.6	95.5	95.5	95.3	95.3
13b	155.4	155.4	155.3	155.3	155.8	155.4	155.6
14b	122.5	122.4	122.6	122.6	122.4	121.5	121.4
1c	135.4	135.4	135.5	135.5	136.0	135.6	135.5
2c,6c	130.0	130.0	129.9	129.9	129.6	129.6	129.7
3c,5c	115.0	115.0	115.2	115.1	115.2	115.1	115.0
4c	156.6	156.6	156.7	156.7	156.6	156.5	156.5
7c	62.2	62.2	62.9	62.8	64.6	63.1	62.5
8c	54.0	54.0	53.9	53.8	57.4	55.2	55.4
9c	145.1	145.1	145.4	145.4	147.3	142.3	142.1
10c	107.4	107.4	107.3	107.3	106.8	106.1	106.3
11c	157.2	157.2	157.3	157.3	159.4	158.7	158.7
12c	101.9	101.9	102.0	102.0	101.4	96.2	96.3
13c	156.7	156.8	156.8	156.8	159.4	160.2	160.2
14c	111.6	111.6	111.6	111.5	106.8	115.4	115.5
1	130.9	132.0	131.0	132.0	131.1	36.0	36.1
2	106.1	121.4	106.3	121.6	105.9	108.8	108.7
3	148.5	115.4	148.6	115.5	148.3		
4	136.7	147.3	136.7	147.4	136.3		
5	148.5	148.1	148.6	148.2	148.3		
6	106.1	111.9	106.3	111.8	105.9		
7	83.2	82.9	83.2	82.9	82.8		
8	68.9	68.9	68.7	68.6	68.5		
9	32.9	33.0	32.5	32.5	30.3		
OCH_3	56.6	56.3	56.7	56.3	56.6	55.6	55.6

[a] Measured at 150 MHz for ^{13}C NMR in acetone-d_6. [b] Measured at 125 MHz for ^{13}C NMR in acetone-d_6.

^{1}H-^{1}H COSY data (see Supporting Information S39-S41) demonstrated that **7** had the same molecular structure as **6**. However, H-2 in **7** is α-oriented based on the NOE correlations of H-2/H-1α and H-1β/H-8c (Fig. 3).

To further assign the absolute configurations of these compounds, the C-8-OH Mosher ester of **1** was prepared after protection of the phenol groups (Scheme 1).[13,14] The $\Delta\delta$ (δ_S - δ_R) value distribution pattern clearly indicated an 8*R* configuration (Fig. 4). Thus, the absolute configuration of **1** was 7a*R*, 8a*R*, 7b*R*, 8b*S*, 7c*S*, 8c*S*, 7*S*, 8*R*. Moreover, the CD spectrum of **1** was similar to those of **2**-**8**, indicating that these compounds have the same absolute configurations at the stereogenic carbons in their common substructure, vaticanol A (**8**) (Fig. 5). Therefore, their structures were determined as shown in Fig. 1.

Table 3 In vitro cytotoxicity of **1**-**7**.

compound	HCT116	MDA-MB-231	SMMC-7721	HepG2
1	17.89 ± 1.27	14.34 ± 0.65	12.18 ± 0.36	NA
2	14.74 ± 0.93	18.72 ± 0.71	10.39 ± 0.87	NA
3	NA	NA	NA	NA
4	NA	NA	NA	15.76 ± 1.05
5	NA	NA	NA	NA
6	NA	NA	NA	12.35 ± 0.43
7	NA	NA	NA	NA
Doxorubicin[a]	1.79 ± 0.21	3.54 ± 0.34	2.98 ± 0.53	1.27 ± 0.28

[a] Positive control; NA not active (IC_{50} > 40 μM); ± results are expressed as mean ± SEM, n=3.

Cytotoxicities of all of the new natural products characterized in this investigation were determined against four human cancer cell lines (HCT116, MDA-MB-231, SMMC-7721, and HepG2) using the MTT method[15] with doxorubicin as a positive control (Table 3). Hopeachinols H (**4**) and J (**6**) are cytotoxic against HepG2 with respective IC_{50} values of 15.76 and 12.35 μM. In addition, hopeachinols E (**1**) and F (**2**) exhibit moderate cytotoxic activities against HCT116, MDA-MB-231, and SMMC-7721 cell lines with IC_{50} values in the 10.39-18.72 μM range, whereas their stereoisomeric analogs **3** and **4** are inactive (IC_{50} > 40 μM) against these same cell lines, indicating that stereochemistry may play an important role in determining cytotoxic activity.

Conclusions

In summary, we isolated and characterized a number of new stilbenolignan natural products from the stem bark of dipterocarp *H. chinensis*, including hopeachinols E-I (**1**-**5**), and two stilbenoids, hopeachinols J and K (**6** and **7**). Dipterocarp plants typically use resveratrol as a building block to construct resveratrol oligomers having diverse carbon skeletons.[1-10] However, stilbenolignans are rare in nature, especially those in which the stilbenoid and lignan motifs are connected by C-C bonds. Examples of stilbenolignans are aiphanol from *Aiphanes aculeate*,[11] shanciol A and shanciol B from *Pleione bulbocodioides*,[16] and trigonopol B from *Dendrobium trigonopus*.[17] To the best of our knowledge, hopeachinols E-I (**1**-**5**) are the first examples of stilbenolignans in which a stilbene trimer and one lignan unit are linked through a pyran ring. It is possible to speculate that **1**-**7** are biosynthetically derived from the common intermediate vaticanol A (**8**) *via* a route involving sequential intramolecular free radical cyclization reactions with phenylpropanoid or two-carbon units.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Rudolph Autopol III automatic polarimeter. UV spectra were recorded on a Hitachi U-3000 spectrophotometer in MeOH. IR spectra were measured on a Nexus 870 FT-IR spectrometer. CD spectra were acquired on a JASCO J-810 spectrometer. HRESIMS spectra were recorded on an Agilent

6210 TOF LC/MS equipped with an electrospray ionization (ESI) probe operating in positive or negative ion mode with direct infusion. The semi-preparative HPLC was accomplished over a Hypersil ODS column (5 μm, 250 mm×10 mm, Thermo Fisher Scientific, USA) on a Hitachi HPLC system consisted of a L-7110 pump (Hitachi) and a L-7420 UV-VIS Detector (Hitachi). NMR spectroscopic data were acquired in acetone-d_6 or $CDCl_3$ on BRUKER DRX500, BRUKER AVANCE □ 600, or BRUKER AVANCE Ⅲ 400 NMR spectrometer with tetramethylsilane (TMS) and solvent signals as internal references. Acetone-d_6 or $CDCl_3$ for NMR measurements were purchased from Sigma-Aldrich Chemicals. Analytical TLC was performed on GF254 (Qingdao Marine Chemical Factory, Qingdao, China) plates with 0.2 mm layer thickness. Column chromatography was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Factory, Qingdao, China), reverse phase (ODS) silica gel (12 nm, S-50 μm, YMC, Japan) or Sephadex LH-20 (GE, USA). All chemicals used in the study were of analytical grade.

Plant material. The stems and twigs of *H. chinensis* were collected in August 2008 from the Botanical Garden at Jianfeng Town, Ledong County, Hainan Island (China). A voucher specimen (no. IFB-20080820) was preserved at the Institute of Functional Biomolecules, Nanjing University. The specimen was identified by Prof. X. J. Tian (Nanjing University, Nanjing, China).

Extraction and Isolation. All of the fractions left over from previous isolations[9,10] were combined and concentrated to a residue (63 g), which gave six fractions A-F upon column chromatography on silica gel (1 kg, 200–300 mesh) using a gradient of CH_2Cl_2-MeOH (100:10 to 0:100) based on TLC monitoring. All fractions were analyzed by LC-HRMS to search for the unusual molecular formulas, which may indicate the existence of new compounds. Fraction C and D were selected for further separation. Fraction C (9.8 g), given by elution with CH_2Cl_2/MeOH (100:20), was subjected to reversed phase ODS column with a step gradient of MeOH-H_2O (20:80 to 100:0) to give six subfractions C1-C7, respectively. Chromatography of C2 over Sephadex LH-20 (100% MeOH) followed by the semi-preparative HPLC (MeCN/H_2O, 31:69, 2 mL/min) gave **6** (1.2 mg, t_R = 37.1 min) and **7** (1.1 mg, t_R = 41.7 min). Compound **4** (1.0 mg, t_R = 53.2 min) and **1** (11.5 mg, t_R = 87.1 min) were obtained from HPLC (MeOH/H_2O, 41:59, 2mL/min) over C3. C4 was chromatographed over Sephadex LH-20 (100% MeOH) to give **8** (530 mg). Fraction D (4.2 g), given by elution with CH_2Cl_2/MeOH (100:30), was further purified by using Sephadex LH-20 (100% MeOH) and column chromatography (CH_2Cl_2/MeOH from 100:15 to 100:30) followed by HLPC (MeOH/H_2O, 40:60, 2 mL/min) gave **3** (8.0 mg, t_R = 23.0 min), **5** (5.0 mg, t_R = 29.2 min), and **2** (3.0 mg, t_R = 40.1 min).

Compound (1): Yellow amorphous powder; $[\alpha]^{25}_D$ = -156.3 (*c* = 0.175, MeOH). UV (MeOH): λ_{max} (log ε) = 209 (5.91), 284 (4.91) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) =210 (-11.78), 234 (+0.17), 252 (-1.90), 269 (-0.30), 295 (-1.99) nm; IR (KBr): ν_{max} = 3357, 2965, 2930, 2850, 1614, 1513, 1456, 1330, 1261, 1220, 1174, 1138, 1109, 1039, 827 cm^{-1}. HRESIMS: *m/z* 889.2850 $[M + H]^+$ (calcd for $C_{53}H_{45}O_{13}$, 889.2855). For 1D and 2D NMR data, see Table 1, 2, and Supporting Information.

Compound (2): Yellow amorphous powder; $[\alpha]^{25}_D$ = -129.8 (*c* = 0.060, MeOH). UV (MeOH): λ_{max} (log ε) = 204 (5.23), 284 (4.22) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) =211 (-21.39), 234 (+0.35), 252 (-3.09), 274 (-0.45), 295 (-3.14) nm; IR (KBr): ν_{max} = 3358, 2963, 2925, 2852, 1614, 1513, 1455, 1384, 1262, 1174, 1137, 1097, 1035, 802 cm^{-1}. HRESIMS: *m/z* 859.2745 $[M + H]^+$ (calcd for $C_{52}H_{43}O_{12}$, 859.2749). For 1D and 2D NMR data, see Table 1, 2, and Supporting Information.

Compound (3): Yellow amorphous powder; $[\alpha]^{25}_D$ = -151.2 (*c* = 0.260, MeOH). UV (MeOH): λ_{max} (log ε) = 204 (5.98), 283 (4.88) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) =211 (-39.29), 238 (+2.03), 254 (-4.41), 268 (-0.41), 294 (-5.41) nm; IR (KBr): ν_{max} = 3362, 2967, 2933, 2849, 1614, 1513, 1456, 1330, 1220, 1174, 1138, 1112, 1039, 831 cm^{-1}. HRESIMS: *m/z* 889.2850 $[M + H]^+$ (calcd for $C_{53}H_{45}O_{13}$, 889.2855). For 1D and 2D NMR data, see Table 1, 2, and Supporting Information.

Compound (4): Yellow amorphous powder; $[\alpha]^{25}_D$ = -69.1 (*c* = 0.065, MeOH). UV (MeOH): λ_{max} (log ε) = 204 (4.82), 284 (3.61) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) =211 (-13.15), 237 (+1.12), 254 (-1.78), 275 (-0.20), 295 (-2.19) nm; IR (KBr): ν_{max} = 3355, 2925, 2853, 2062, 1662, 1614, 1513, 1456, 1384, 1261, 1224, 1174, 1138, 1108, 1036, 821, 803 cm^{-1}. HRESIMS: *m/z* 859.2745 $[M + H]^+$ (calcd for $C_{52}H_{43}O_{12}$, 859.2749). For 1D and 2D NMR data, see Table 1, 2, and Supporting Information.

Compound (5): Yellow amorphous powder; $[\alpha]^{25}_D$ = -53.7 (*c* = 0.125, MeOH). UV (MeOH): λ_{max} (log ε) = 204 (5.65), 222 (5.70), 287 (5.01) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) =208 (+2.30), 223 (-2.19), 239 (-0.86), 252 (-1.67), 272 (-0.12), 294 (-1.28) nm; IR (KBr): ν_{max} = 3366, 2962, 2926, 2853, 1613, 1513, 1463, 1384, 1336, 1261, 1221, 1174, 1108, 1040, 832, 806 cm^{-1}. HRESIMS: *m/z* 889.2857 $[M + H]^+$ (calcd for $C_{53}H_{45}O_{13}$, 889.2855). For 1D and 2D NMR data, see Table 1, 2, and Supporting Information.

Compound (6): Yellow amorphous powder; $[\alpha]^{25}_D$ = -217.3 (*c* = 0.040, MeOH). UV (MeOH): λ_{max} (log ε) = 204 (5.09), 284 (4.16) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) =206 (-21.51), 232 (+0.60), 251 (-3.70), 272 (-0.40), 294 (-3.26) nm; IR (KBr): ν_{max} = 3365, 2962, 2926, 2854, 1613, 1512, 1450, 1384, 1261, 1225, 1174, 1101, 1035, 802 cm^{-1}. HRESIMS: *m/z* 737.2379 $[M + H]^+$ (calcd for $C_{45}H_{37}O_{10}$, 737.2381). For 1D and 2D NMR data, see Table 1, 2, and Supporting Information.

Compound (7): Yellow amorphous powder; $[\alpha]^{25}_D$ = -291.4 (*c* = 0.035, MeOH). UV (MeOH): λ_{max} (log ε) = 204 (4.85), 284 (3.90) nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) =206 (-15.30), 234 (-0.07), 251 (-2.55), 272 (-0.31), 294 (-2.28) nm; IR (KBr): ν_{max} = 3343, 2957, 2926, 2854, 1705, 1614, 1513, 1455, 1384, 1261, 1228, 1175, 1105, 1036, 1004, 932, 833 cm^{-1}. HRESIMS: *m/z* 737.2366 $[M + H]^+$ (calcd for $C_{45}H_{37}O_{10}$, 737.2381). For 1D and 2D NMR data, see Table 1, 2, and Supporting Information.

Methylation of compound 1. Hopeachinol E (**1**, 10 mg) was allowed to react with K_2CO_3 (300 mg) and MeI (200 mg) in dry acetone under reflux for 12 h. Upon completion of the reaction, the resulting mixture was dried under a N_2 stream, dissolved in CH_2Cl_2 (10 mL), washed with water (15 mL) and brine (15 mL), dehydrated with $MgSO_4$, followed by *in vacuo* evaporation of the organic solvent, and then purified by Sephadex LH-20 column (CH_2Cl_2/MeOH 1:1) to give **1a** (11 mg) as a white amorphous powder. ^1H NMR (500 MHz, $CDCl_3$): 7.31 (2H, d, *J* = 8.0 Hz, H-2a/H-6a), 7.09 (2H, d, *J* = 8.0 Hz, H-2b/H-6b), 6.88 (2H, d, *J* = 8.0 Hz, H-3a/H-5a), 6.71 (2H, s, H-2/H-6), 6.64 (2H, d, *J* = 8.0 Hz, H-3b/H-5b), 6.52 (2H, br d, *J* = 8.0 Hz, H-2c/H-6c), 6.49 (1H, br s, H-14a), 6.43 (2H, br d, *J* = 8.0 Hz, H-3c/H-5c), 6.42 (1H, d, *J* = 2.0 Hz, H-12c), 6.36 (1H, d, *J* = 2.0 Hz, H-10c), 6.35 (1H, s, H-12b), 6.23 (1H, d, *J* = 3.0 Hz, H-7a), 5.94 (1H, br s, H-12a), 5.14 (1H, s, H-7b), 4.73 (1H, d, *J* = 9.0 Hz, H-7), 4.54 (1H,

d, J = 6.5 Hz, H-8b), 4.52 (1H, d, J = 3.0 Hz, H-8a), 4.38 (1H, s, H-8c), 4.07 (1H, m, H-8), 3.88 (6H, s, OMe), 3.86 (3H, s, OMe), 3.81 (3H, s, OMe), 3.74 (3H, s, OMe), 3.71 (3H, s, OMe), 3.69 (3H, s, OMe), 3.68 (3H, s, OMe), 3.66 (3H, s, OMe), 3.52 (1H, d, J = 6.5 Hz, H-7c), 3.23 (3H, s, OMe), 3.11 (1H, dd, J = 16.0, 5.5 Hz, H-9), 2.77 ppm (1H, dd, J = 16.0, 9.0 Hz, H-9). HRESIMS: *m/z*: found 1023.3974, calcd. for $C_{61}H_{60}O_{13}Na$, 1023.3926 $[M + Na]^+$.

Preparation of the (*S*)- and (*R*)-MTPA Esters (1b and 1c) of compound 1a. To two separate solutions of compound **1a** (2.0 mg) in pyridine (1 mL) with DMAP (4-*N,N*-dimethylaminopyridine, 1.0 mg), *R*- and *S*-MTPA chloride (10 μL) were respectively added. The mixture was allowed to react for 12 h at rt and then quenched by the addition of CH_3OH (100 μL). The acylation products (**1b** and **1c**) were purified by reversed-phase HPLC (MeOH/H_2O, 92:8). *S*-(**1b**) and *R*-MTPA esters (**1c**) were eluted at the retention times of 15.6 and 16.8 min, respectively. The ^1H chemical shifts around the stereocenters of **1b** and **1c** were assigned unequivocally by ^1H NMR and COSY analyses. *S*-MTPA ester (**1b**): ^1H NMR (selected signals, 400 MHz, $CDCl_3$): 6.58 (2H, s, H-2/H-6), 6.47 (2H, br d, J = 8.8 Hz, H-2c/H-6c), 6.42 (1H, d, J = 2.4 Hz, H-12c), 6.38 (2H, br d, J = 8.8 Hz, H-3c/H-5c), 6.36 (1H, d, J = 2.4 Hz, H-10c), 5.66 (1H, m, H-8), 5.11 (1H, s, H-7b), 4.93 (1H, d, J = 8.4 Hz, H-7), 4.50 (1H, d, J = 6.8 Hz, H-8b), 4.33 (1H, s, H-8c), 3.42 (1H, d, J = 6.8 Hz, H-7c), 3.18 (1H, dd, J = 16.0, 5.6 Hz, H-9), 2.97 ppm (1H, dd, J = 16.0, 9.2 Hz, H-9); ESIMS *m/z* 1239.4 $[M + Na]^+$. *R*-MTPA ester (**1c**): ^1H NMR (selected signals, 400 MHz, $CDCl_3$): 6.69 (2H, s, H-2/H-6), 6.43 (1H, d, J = 2.4 Hz, H-12c), 6.38 (2H, br d, J = 8.8 Hz, H-2c/H-6c), 6.36 (2H, br d, J = 8.8 Hz, H-3c/H-5c), 6.35 (1H, d, J = 2.4 Hz, H-10c), 5.53 (1H, m, H-8), 5.11 (1H, s, H-7b), 5.06 (1H, d, J = 8.4 Hz, H-7), 4.49 (1H, d, J = 6.8 Hz, H-8b), 4.30 (1H, s, H-8c), 3.42 (1H, d, J = 6.8 Hz, H-7c), 3.24 (1H, dd, J = 16.0, 5.6 Hz, H-9), 2.86 ppm (1H, dd, J = 16.0, 9.2 Hz, H-9); ESIMS *m/z* 1239.4 $[M + Na]^+$.

Cytotoxic activity assay. Compound **1-7** were evaluated for cytotoxicity against four cell lines, HCT116 (human colon cancer), MDA-MB-231 (human breast cancer), SMMC-7721 (human hepatic carcinoma), HepG2 (human hepatic carcinoma) (all from the Jiangsu Provincial Center for Disease Prevention and Control), using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[15] Doxorubicin HCl (Sigma-Aldrich) was used as a positive control, and the medium without compounds as a negative control in the bioassay.

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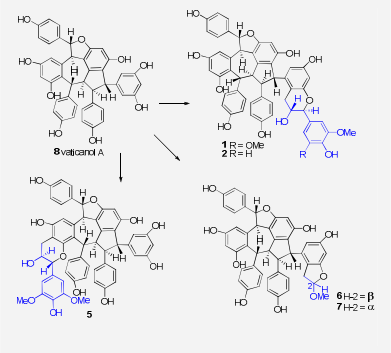
Seven novel oligostilbenoids, isolated from *Hopea chinensis*, may biosynthetically derived from the common intermediate vaticanol A.



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Hopeachinols E-K, novel Oligostilbenoids from the Stem Bark of *Hopea chinensis*

Keywords: Dipterocaceae / *Hopea chinensis* / resveratrol oligomers / oligostilbenoids / cytotoxicity activity