JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Gymnothelignans A–O: Conformation and Absolute Configuration Analyses of Lignans Bearing Tetrahydrofuran from *Gymnotheca chinensis*

Dahai He,[†] Lisheng Ding,[†] Hongxi Xu,*[,‡] Xinxiang Lei,[§] Hongping Xiao,[§] and Yan Zhou*[,†]

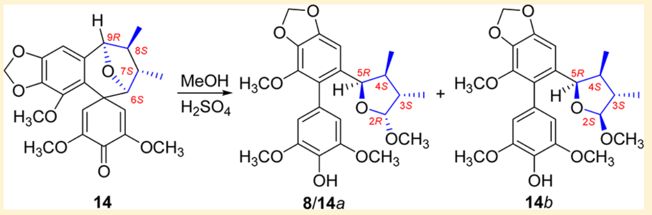
[†]Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, People's Republic of China

[‡]Shanghai University of Traditional Chinese Medicine, Shanghai 201203, People's Republic of China

[§]Wenzhou University, Wenzhou 325035, People's Republic of China

Supporting Information

ABSTRACT: Fifteen new lignans, gymnothelignans A–O (**1–15**), bearing tetrahydrofuran with variable conformations belonging to three potentially related skeletons were isolated from *Gymnotheca chinensis* Decne. The structures were elucidated by means of detailed spectroscopic analysis. Absolute configurations were assigned using X-ray single-crystal diffraction and chemical transformations. Moreover, by the homology, compounds **1–11** and eupomatilones were confirmed to have uniform *R*-configuration at C-5. However, a synthesized congener has long been mistaken as 5-epimer of eupomatilone-6. This work provides guidance for the absolute configuration establishment of the subeupomatilone family with *trans*-H-4—H-5 configuration.



INTRODUCTION

Gymnotheca chinensis Decne, as one of the endemic genera of seed plants in China, is a perennial herb of Saururaceae. The whole plants of *G. chinensis* have long been used as traditional herbal medicine to treat contusions and strains. Little phytochemical information about this genus is available except for *G. involucrata* Pei.[1] Our recent investigation on the constituents of *G. chinensis* led to the isolation of 15 new lignans (**1–15**), together with five known compounds kaempferol-4′,7-dimethyl-3-*O*-glucoside (**16**),[1] kaempferol-7-methyl-3-*O*-glucoside (**17**),[2,3] blumenol A (**18**),[4] 1-bisabolon (**19**),[5] and *β*-sitosterol (**20**).

The 15 new lignans, gymnothelignans A–O (**1–15**), bearing tetrahydrofuran (THF) with variable conformations belonged to three unusual lignan skeletons, namely, dibenzocyclooctene, eupomatilone, and eupodienone. The latter two rare types were only previously isolated from the Australian shrub *Eupomatia bennettii*[6,7] and *E. laurina*,[8,9] respectively. Owing to their attractive structures, there have been total syntheses of all of the members of eupomatilones[10–20] except eupomatilone-1. The absolute configuration of eupomatilone-6 has only just been recently proposed.[16] Moreover, it was reported that eupodienones could be rearranged to form dibenzocyclooctene derivatives.[9,21] Herein, we report the isolation, structural elucidation, and relative and absolute configuration of these compounds. We also explored the relationship between eupomatilones and eupodienones.

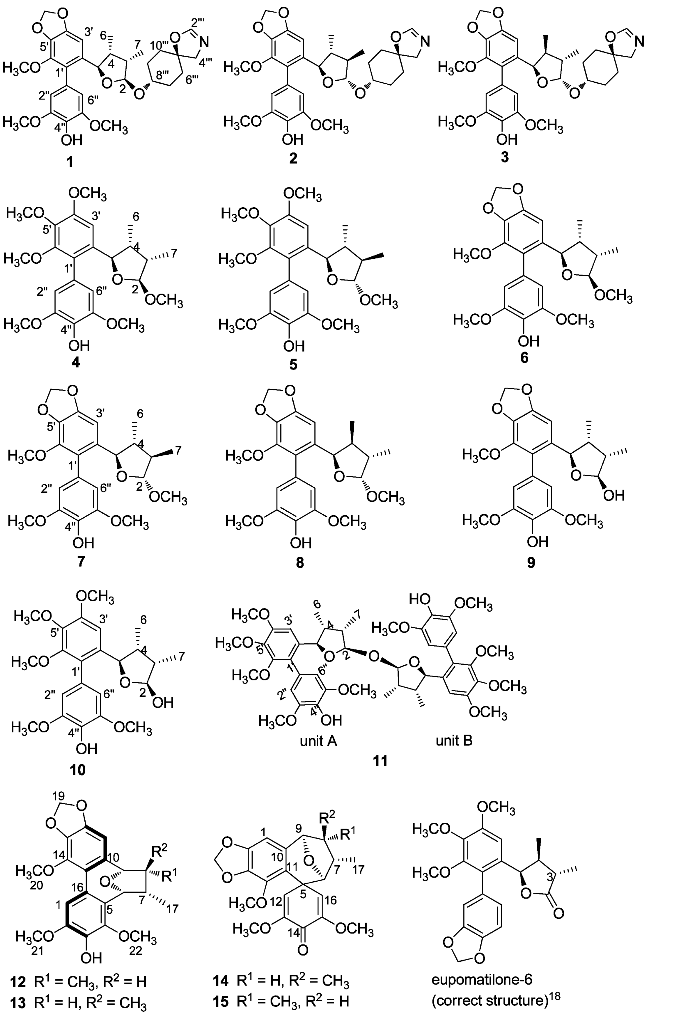
RESULTS AND DISCUSSION

Dried and powdered whole plants of *G. chinensis* were extracted with ethanol at room temperature to give an extract, which was suspended in H_2O and extracted with petroleum ether and ethyl acetate successively. The ethyl acetate extracts were subjected to silica gel column chromatography followed by reversed-phase HPLC to yield 15 new lignans and five known compounds. Structures of these compounds were elucidated by a combination of detailed spectroscopic analysis. The absolute configurations were determined by X-ray single-crystal diffraction and chemical conversions.

Gymnothelignan A (**1**) was obtained as a colorless crystal. Its molecular formula, $C_{30}H_{37}NO_9$, was established by HRESIMS, which requires 13 degrees of unsaturation. The IR (KBr) spectrum showed absorption bands due to hydroxyl (3437 cm^{-1}) and aromatic groups (1619, 1474 cm^{-1}). The ^1H and ^{13}C NMR (Table 1) spectra displayed signals of three methoxy groups, two secondary methyl groups, one methylenedioxy group, five methylene groups, five methine groups, four sp^2 carbons, and 10 non-hydrogenated carbons (assigned by DEPT, Table 1).

The analyses of $^1H–^1H$ COSY and HMBC spectra (Figure 1) suggested that the gross structure has three subunits (units A–C), as shown in Figure 2. The connection of C-1′ to C-1″ was deduced from the HMBC correlations of H-2″/6″ to C-1′ (it possesses an unusual doubly attached ring system which

Received: June 15, 2012
Published: September 4, 2012



exhibits hindered rotation about the biaryl bond[6]). The correlations of 3″-OCH_3 to C-3″ and 5″-OCH_3 to C-5″ positioned the two methoxy groups to C-3″ and C-5″, respectively. In addition, a hydroxyl group was connected to C-4″ by analysis of the chemical shift of C-4″, which was shifted ($\Delta\delta$ = −12.4 and −12.3, respectively) in comparison with those of C-3″ and C-5″. The presence of a methylenedioxy group was assigned by the HMBC correlations of OCH_2O to C-4′ and C-5′, as well as H-3′ to C-4′ and C-5′. Similarly, a methoxy group at C-6′ was also assigned. Thus, the gross structure of unit A was proposed as shown in Figure 1. In unit B, the $^1H-^1H$ COSY cross-peaks revealed the sequential connections of C-2−C-7. This was also supported by the HMBC correlations of H-2 to C-7 and H-5 to C-6. The downfield chemical shifts revealed that C-2 and C-5 were both oxygenated. HMBC correlations of H-2/C-5 and H-5/C-2 displayed the connection of C-2 to C-5 via an ether bond. The ^{13}C NMR chemical shift of C-2 (δ_C 108.6) was indicative of a ketal or hemiketal. In unit C, the sequential connections of C-6‴−C-10‴ were confirmed by the $^1H-^1H$ COSY spectrum. HMBC correlations of H-4‴ at δ_H 2.80 to C-2‴/6‴/10‴ as well as those of H-2‴ at δ_H 7.12 to C-4‴/C-5‴ are in association with the relatively downfield ^{13}C NMR chemical shifts[22] of C-2‴ and C-4‴, and HRMS confirmed that an oxazoline ring fused with a cyclohexanol at C-5‴.

The connection of C-2′ (unit A) to C-5 (unit B) was established by the analyses of HMBC correlations of H-5 to C-1′ and C-3′, and H-3′ to C-5. The connection between units B and C was achieved by HMBC correlation of H-2 to C-8‴, giving rise to the connection of C-2 to C-8‴ through an ether bond. NOESY cross-peaks of H-2 to H-8‴ also supported the connection of units B and C (Figure 2).

The stereochemistry of different substituent groups on the THF (unit B) was designated using a NOESY experiment. The cross-peaks of H-2/H_3-7, H-5/H_3-6, and H-4/3′ indicated that H-2, H-5, H_3-6, and H_3-7 were cofacial. Likewise, H-3, H-4, and units A and C were on the opposite face. The assignments were also buttressed by vicinal coupling constant values of H-2−H-3 (2.1 Hz) and H-4−H-5 (7.7 Hz) when the dihedral angle of H-2−H-3 took the value close to 90° and that of H-4−H-5 was close to 180°. In the molecule, the bulky biphenyl group unit A was in the equatorial position, whereas unit C was in the axial position (Figure 3). On the basis of single-crystal Cu Kα X-ray diffraction (Figure S84, Supporting Information), C-7 was revealed to have α-orientation of the THF moiety. The absolute stereochemistry of **1** was also determined (Flack parameter, 0.2(3), calculated using 1217 Friedel pairs).[23] Gymnothelignan A (**1**) was then assigned as (2*S*,3*S*,4*R*,5*R*)-3,4-dimethyl-2-hydroxyl-5-(4″-hydroxyl-4′,5′-methylenedioxy-6′,3″,5″-trimethoxylbiphenyl-2′-yl)tetrahydrofuranyl-8‴-oxo-1‴-oxa-3‴-azaspiro[4.5]dec-2‴-enyl-2,8‴-ether .

Gymnothelignan B (**2**) had the same molecular formula of $C_{30}H_{37}NO_9$ of **1** by HRESIMS. The ^1H and ^{13}C NMR (Table 1) spectra indicated that it was a diastereoisomer of **1**. By comparing the ^1H NMR spectra with those of **1**, the signals of H-6‴−H-10‴ were shifted ($\Delta\delta$ = −0.10 to −0.20), which indicated that unit C at C-2 (unit B) of **2** should have α-orientation because the bulk effects from unit A could be substantially relieved (the alternative explanation that epimerization occurred at C-5 was less likely due to the chemical shift of C-5 and bulk biphenyl group[6]). Moreover, the ^{13}C NMR chemical shift of C-2 was shifted ($\Delta\delta$ = −2.6), and the chemical shift of C-5 was shifted ($\Delta\delta$ = +0.6) instead (it was probably a result of epimerization occurring at C-2). Similarly, the signals of C-3 (δ_C 40.8) and C-7 (δ_C 9.4) were shifted ($\Delta\delta$ = −3.4 and −1.8, respectively). This indicated that epimerization(s) occurred in the molecule.[24] Correlation observed in the NOESY (Figure 2) spectrum of H-2/H_3-7 suggested that H-2 and H_3-7 were cofacial. The similar correlation of H-4/H-3′, which was also observed in the molecule of **1**, was indicative of H-4 being in the β-orientation. Similarly, H-5 and H_3-6 were on the opposite face. Obviously, epimerizations occurred at C-2 and C-3 in comparison to **1**. Since the vicinal coupling constants of H-2−H-3 and H-4−H-5 took moderate value (4−5 Hz), the conformation of the THF moiety should be a result of interconversion of two conformational isomers (conformers 1 and 2, Figure 3). In summary, the structure of **2** was determined to be the 2,3-bisepimer of **1**.

Gymnothelignans C (**3**) and H (**8**) only differed from unit C by a methyl group at C-2 based on the HRESIMS and NMR data analyses (Table 1 for **3**, Tables 2 and 3 for **8**). The structure of unit C of **3** was established by comparing its ^{13}C NMR with that of **2**. The main seco-lignan part was assigned as follows (take **8** for example). Correlations of H-2/H-3, H-3/H-3′, and H-5/H-4 were observed in the NOESY spectrum (Figure S36, Supporting Information). This showed that H-2, H-3, H_3-6, and biphenyl were cofacial, whereas H-4, H-5, H_3-7, and 2-OCH_3 were on the opposite face. The method aforementioned was applied for the determination of THF (Figure 3). Except for 2-OCH_3, all of the substituents were in the equatorial position. The configuration of **8** was finally

Table 1. NMR Data for Compounds 1–3 (CD_3COCD_3)[a]

no.	1		2		3	
	δ_C, mult	δ_H	δ_C	δ_H	δ_C	δ_H
2	108.6, CH	4.86 d (2.1)	106.0	5.21 d (4.7)	104.1	5.17 d (3.9)
3	44.2, CH	2.15 m[b]	40.8	2.38 m[b]	42.7	1.82 m*[,b]
4	44.1, CH	2.35 m[b]	44.1	2.01 m[b]	47.2	1.79 m*[,b]
5	84.3, CH	4.51 d (7.7)	84.9	4.56 d (4.3)	79.4	5.07 d (8.1)
6	12.2, CH_3	0.71 d (7.0)	15.5	0.74 d (7.2)	16.5	0.69 d (6.5)
7	11.2, CH_3	0.79 d (7.3)	9.4	0.90 d (7.2)	12.8	0.94 d (6.4)
2′	137.1, C		137.7		134.4	
3′	103.0, CH	7.03 s	101.6	6.69 s	103.1	6.63 s
4′	149.3, C		149.4		149.0	
5′	136.6, C		137.0		136.9	
6′	141.5, C		141.8		141.8	
1′	129.7, C		128.8		128.8	
1″	127.4, C		127.6		127.8	
2″	110.0, CH	6.42 d (1.7)	110.0	6.44 br s	109.4	6.40 br s
3″	148.3, C		148.5		148.5	
4″	135.9, C		135.9		135.9	
5″	148.2, C		148.3		148.3	
6″	108.4, CH	6.45 d (1.7)	108.3	6.45 br s	108.1	6.43 br s
OCH_2O	102.1, CH_2	6.03, 6.01, each 1H, d (1.0)	102.0	6.01, 6.00, each 1H, br s	102.0	6.01, 2H, br s
6′-OCH_3	60.1, CH_3	3.74 s	60.1	3.74 s	60.1	3.75 s
3″-OCH_3	56.8, CH_3	3.82 s	56.8	3.82 s	56.7	3.81 s
5″-OCH_3	56.7, CH_3	3.81 s	56.7	3.82 s	56.7	3.81 s
2‴	146.5, CH	7.12 t (1.7)	146.5	7.09 br s	146.5	7.09 br s
4‴	46.2, CH_2	2.80 d (1.7)	46.5	2.69 d (1.4)	46.4	2.69 dd (1.7)
5‴	84.1, C		83.8		83.9	
6‴	32.9, CH_2	1.62–2.02 m*	32.6	1.52–1.82 m*	32.7	1.51–1.82 m*
7‴	28.3, CH_2	1.62–2.02 m*	28.3	1.52–1.82 m*	28.6	1.51–1.82 m*
8‴	73.0, CH	3.81 m*[,b]	72.6	3.70 m[b]	72.5	3.71 m[b]
9‴	30.3, CH_2	1.62–2.02 m*	30.3	1.52–1.82 m*	30.3	1.51–1.82 m*
10‴	33.0, CH_2	1.62–2.02 m*	32.7	1.52–1.82 m*	32.8	1.51–1.82 m*

[a]Spectra recorded at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR. Chemical shifts (δ) are in ppm and coupling constants (J) in Hz. [b]Assigned by $^1H-^1H$ COSY. *Signals overcharged.

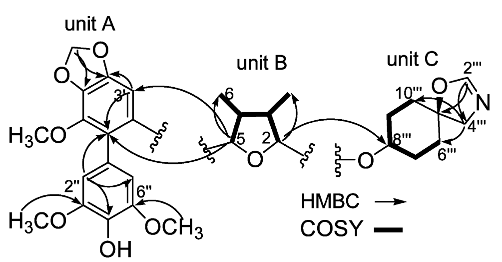


Figure 1. Key $^1H-^1H$ COSY and HMBC correlations supporting the structures of units A–C and their final assembly into gymnothelignan A.

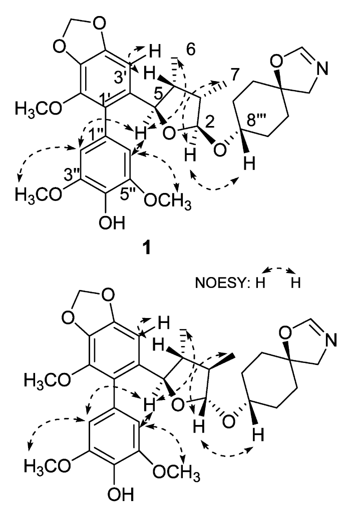


Figure 2. Key NOESY spectra of compounds **1** and **2**.

confirmed by single-crystal X-ray diffraction (Figure S85, Supporting Information). The relative stereochemistry was thus determined as 2*R**,3*S**,4*S**,5*R**.

Gymnothelignan D (**4**) was found to have the molecular formula $C_{24}H_{32}O_8$ by HRESIMS. The ^1H and ^{13}C NMR spectra (Table 4) were very similar to those of **1** except for the OCH_3 replacing unit C at C-2. Single-crystal[25] X-ray diffraction (Figure S86, Supporting Information) revealed that **4** had two conformers (conformers **4**a and **4**b, Figure 3) on the THF moiety, but only a single conformer in solution (acetone) was observed. The chemical shifts values of H-2 and H-5 in acetone-d_6 were distinguishable, and the vicinal coupling constants of H-4–H-5 (9.9 Hz) and H-2–H-3 (0 Hz) were in favor of the conformation of THF in **4** (conformer **4**b, Figure 3) when dihedral angle of H-4–H-5 was near 180° and that of H-2–H-3 was near 90°. Finally, the absolute configuration was established by single-crystal X-ray diffraction, as (2*S*,3*S*,4*R*,5*R*)-3,4-dimethyl-2-methoxyl-5-(4″-hydroxyl-4′,5′,6′,3″,5″-pentamethoxylbiphenyl-2′-yl)THF.

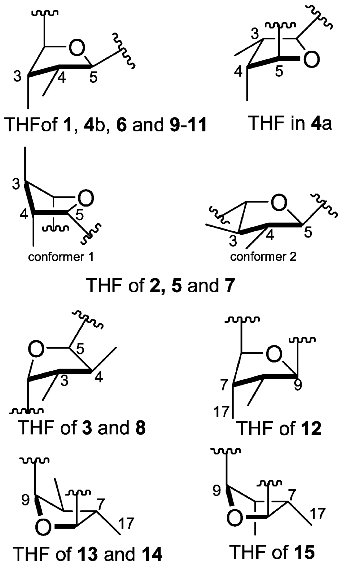


Figure 3. Conformations of THF in compounds **1**−**15**.

Gymnothelignan E (**5**) was assigned as a diastereomer of **4** by comparing its HRESIMS and NMR (Table 4) with those of **4**. The ^{13}C NMR chemical shifts of C-2 (δ_C 108.3) and C-3 (δ_C 40.3) shifted ($\Delta\delta$ = −3.3 and −3.9, respectively) in comparison to that of **4**, indicating that epimerization(s) had occurred in the molecule. Characteristic NOESY (Figure S24, Supporting Information) cross-peaks of H-2/H_3-7, H_3-7/H-4, 2-OCH_3/H-5, and H-3/5 distinctly confirmed the proposed relative stereochemistry structure of **5**. The vicinal coupling constants (H-2−H-3 and H-4−H-5) along with the relative configuration of different substituents on the THF suggested that THF had two interconvertible conformers (Figure 3). Henceforth, the structure of **5** was elucidated as the 2,3-bisepimer of **4**.

Gymnothelignans F (**6**) and G (**7**) had the same molecular formulas as **8**, as shown by HRESIMS. The NMR spectra (Tables 2 and 3) revealed that **6**, **7**, and **8** were diastereomers. Both **6** and **7** demonstrated *trans*-H-4−H-5 configuration by comparing their ^{13}C NMR chemical shifts of C-5 with that of **4** (the alternative explanation that the γ-gauche interaction[26] had been substantially relieved by comparing the ^{13}C NMR chemical shifts of C-2′ with that of **8**, in which C-2′ was shielded by C-6 when it demonstrated *cis*-H-4−H-5). NOESY (Figures S28 and S32, diagnostic cross-peaks of H-2/H_3-7, H-4/H-3′, H-5/H_3-6, and 2-OCH_3/H-3′ for **6**, H-2/H_3-7/H-4, H-4/H-3′, and H-5/H_3-6 for **7**; Supporting Information) and vicinal coupling constants (H-2−H-3 and H-4−H-5) allowed the assignment of relative stereochemistry and conformation of THF (Figure 3). Compounds **6** and **7** were subsequently established to be the 2,4- and 3,4-bisepimer of **8**, respectively.

Gymnothelignans I (**9**) and J (**10**) were found to be demethyl analogues of **6** and **4**, respectively, based on the HRESIMS and NMR data (Tables 2, 3, and 4) analyses, with major differences in the ^{13}C NMR chemical shifts of C-2.

Table 3. ^{13}C NMR Data for Compounds **6**−**9** and **11** (Acetone-d_6)[a]

no.	6	7	8	9	11
2	111.5	108.2	106.2	104.3	105.9
3	44.0	40.4	42.6	44.1	44.0
4	44.4	44.3	46.6	45.1	44.6
5	84.1	84.9	79.5	83.8	84.3
6	11.8	15.2	16.2	12.2	11.8
7	11.3	9.1	12.5	11.2	11.4
2′	137.2	137.7	134.3	137.0	137.3
3′	102.8	101.4	102.9	103.4	102.8
4′	149.5	149.3	148.9	149.3	149.5
5′	136.3	136.9	136.9	137.1	136.1
6′	141.4	141.9	141.7	141.4	141.5
1′	130.1	128.5	128.6	129.5	130.2
1″	127.4	127.5	127.5	127.6	127.4
2″	110.1	110.0	109.3	110.2	110.0
3″	148.2	148.5	148.5	148.3	148.3[b]
4″	135.9	135.9	135.9	135.9	135.9
5″	148.1	148.4	148.2	148.2	148.1[b]
6″	108.5	108.3	108.2	108.5	108.5
2-OCH_3	55.0	54.9	54.5		
OCH_2O	102.1	102.0	102.0	102.0	102.1
6′-OCH_3	60.1	60.1	60.1	60.1	60.1
3″-OCH_3	56.8	56.7	56.7	56.8	56.7
5″-OCH_3	56.7	56.8	56.7	56.8	56.7

[a]Spectra recorded at 150 MHz. Chemical shifts (δ) are in ppm. [b]Signals may be exchangeable.

Table 2. ^{1}H NMR Data for Compounds **6**−**9** and **11** (Acetone-d_6)[a]

no.	6	7	8	9	11
2	4.55 s	4.97 d (4.9)	4.91 d (4.4)	5.04 d (1.3)	5.07 br s
3	2.15 m[b]	1.97 m[b]	1.86 m[b,*]	2.02 m[b,*]	2.36 m[c]
4	2.35 m[b]	2.36 m[b]	1.82 m[b,*]	2.34 m[b]	2.47 m[c]
5	4.53 d (8.9)	4.54 d (4.2)	5.01 d (8.6)	4.48 d (8.0)	4.55 d (8.9)
6	0.72 d (7.0)	0.64 d (7.2)	0.69 d (6.9)	0.69 d (7.0)	0.79 d (6.9)
7	0.76 d (7.3)	0.84 d (7.2)	0.91 d (6.6)	0.77 d (7.3)	0.85 d (7.3)
3′	6.86 s	6.68 s	6.64 s	7.16 s	6.91 s
2″	6.42 d (1.6)	6.42 d (1.7)	6.39 br s	6.42 d (1.6)	6.44 br s
6″	6.45 d (1.7)	6.45 d (1.7)	6.44 br s	6.44 d (1.4)	6.44 br s
2-OCH_3	3.38 s	3.22 s	3.21 s		
OCH_2O	6.02, 6.02 (each 1H, d, 0.9)	5.99, 5.98 (each 1H, d, 0.9)	6.01, 6.00 (each 1H, d, 1.0)	6.00, 6.00 (each 1H, br s)	6.02, 6.01 (each 1H, br s)
6′-OCH_3	3.74 s	3.73 s	3.74 s	3.73 s	3.74 s
3″-OCH_3	3.82 s	3.81 s	3.81 s	3.81 s	3.82 s
5″-OCH_3	3.80 s	3.79 s	3.80 s	3.81 s	3.81 s

[a]Spectra recorded at 600 MHz. Chemical shifts (δ) are in ppm and coupling constants (J) in Hz. [b]Assigned by NOESY. [c]Assigned by ^{1}H−^{1}H COSY. *Signals overcharged.

Table 4. NMR Data for Compounds 4, 5, and 10 (Acetone-d_6)[a]

	4		5		10	
no.	H	C	H	C	H	C
2	4.58 s	111.6	5.00 d (4.9)	108.3	5.07 d (2.0)	104.4
3	2.15 m[b]	44.2	2.01 m[b]	40.3	2.11 m[b]	44.3
4	2.40 m[b]	44.5	2.38 m[b,*]	44.3	2.40 m[b]	45.0
5	4.57 d (9.9)	84.2	4.62 d (4.0)	85.0	4.55 d (7.7)	83.9
6	0.71 d (7.0)	11.9	0.65 d (7.2)	15.3	0.70 d (7.0)	12.4
7	0.77 d (7.3)	11.3	0.85 d (7.2)	9.1	0.79 d (7.3)	11.3
2′		137.4		138.7		138.2
3′	7.08 s	107.5	6.89 s	106.1	7.41 s	110.1
4′		153.9		153.6		153.7
5′		142.2		142.1		142.0
6′		151.6		152.1		151.6
1′		130.3		129.0		129.7
1″		127.5		127.7		127.7
2″	6.44 br s	110.0	6.45 d (1.4)	109.9	6.45 br s	108.4
3″		148.2		148.4		148.2
4″		135.8		135.9		135.8
5″		148.1		148.4		148.2
6″	6.45 br s	108.4	6.48 br s	108.2	6.47 br s	108.2
2-OCH_3	3.44 s	55.1	3.25 s	54.9		
4′-OCH_3	3.87 s	61.2	3.87 s	61.3	3.86 s	61.2
5′-OCH_3	3.80 s	60.8	3.83 s	60.8	3.82 s	60.7
6′-OCH_3	3.58 s	56.1	3.58 s	56.3	3.58 s	56.1
3″-OCH_3	3.81 s	56.7	3.81 s	56.8	3.82 s	56.8
5″-OCH_3	3.81 s	56.7	3.81 s	56.8	3.82 s	56.8

[a]Spectra recorded at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR. Chemical shifts (δ) are in ppm and coupling constants (J) in Hz. [b]Assigned by NOESY. *Signals overcharged.

NOESY (Figures S40 and S44, Supporting Information) correlations (H-2/H_3-7, H-4/H-3′, and H-5/H_3-6 for **9**, H-2/H_3-7, H-4/H-3′, and H-5/H_3-6 for **10**) were similar to those of corresponding compounds **6** and **4**, suggesting the same relative stereochemistry of different substituents on THF in the two pairs of compounds. The conformations of THF of **9** and **10** are shown in Figure 3. Their structures were established as shown.

Gymnothelignan K (**11**) was shown to have the molecular formula $C_{44}H_{50}O_{15}$ by HRESIMS. Its ^1H and ^{13}C NMR spectra (Tables 2 and 3) were in good agreement with those of **9**. Hence, compound **11** was presumed to be a dimer of **9** with a C_2 symmetry axis. Key HMBC (Figure S48, Supporting Information) correlation from H-2b to C-2a (or H-2a to C-2b) confirmed the linkage via an oxygen bridge between C-2a of unit A and C-2b of unit B. NOESY (Figure S49, Supporting Information) cross-peaks of H-2/H_3-7, H-4/H-3′, and H-5/H_3-6 were in agreement with those of **9**. Therefore, it was concluded that the conformations of THF (Figure 3) of both **11** and **9** were identical despite different substituents on the THF moiety. Thus, the absolute configuration of **11** was established as 2*S*,3*S*,4*R*,5*R*.

Gymnothelignan L (**12**) was found to have molecular formula $C_{22}H_{24}O_7$ by HRESIMS. The ^1H and ^{13}C NMR spectra (Tables 5 and 6) indicated that it had a dibenzocyclooctene skeleton. The HMBC (Figure 4) correlations of H-6/C-9 and H-9/C-6 presented the 6,9-epoxide. The correlations of H-1 to C-2, C-3, C-5, C-15, and C-16 corroborated the proton δ_H 7.24 at C-1, differing from that of a congener reported in the literature.[27] In addition, a strong correlation of H_3-22/H-6 was observed in the NOESY spectrum (Figure 4, excluding the possibility of δ_H 7.24 at C-4). The correlations of H-6 to H_3-17 and H-9 to H_3-18 suggested H-6/H_3-17 and H-9/H_3-18 to be cofacial. The vicinal coupling constants (H-6 and H-9), along with assigned relative stereochemistry of H_3-17 and H_3-18, allowed the conformation assignment of THF, as shown in Figure 3. Compound **12** was established as 6*S**,7*S**,8*R**,9*R** and *R**-biphenyl.[28]

Table 5. ^1H NMR Data for Compounds 12−15 (Acetone-d_6)[a]

no.	12	13	14	15
1	7.24 s	7.28 s	6.42s	6.50 s
6	5.09 d (1.9)	5.08 d (5.7)	3.43 d (2.4)	3.41 d (2.7)
7	2.22 m[b]	1.84 m[b]	2.03 m[b,*]	2.76 m[b]
8	2.33 m[b]	2.08 m[b,*]	1.97 m[b,*]	2.31 m[b]
9	4.48 d (5.5)	4.99 d (6.2)	4.85 d (5.6)	4.71 s
11	6.50 s	6.36 s		
12			6.20 d (2.3)	6.19 d (2.3)
16			6.08 d (2.3)	6.09 d (2.3)
17	1.03 d (7.1)	1.16 d (6.7)	1.11 d (6.9)	1.02 d (7.4)
18	1.01 d (7.1)	0.59 d (6.8)	0.85 d (6.8)	1.05 d (7.2)
19	6.02, 5.96 (each 1H, d, 1.0)	6.06, 5.99 (each 1H, d, 0.8)	5.97, 5.96 (each 1H, d, 0.9)	5.96, 5.94 (each 1H, d, 0.9)
20	3.68 s	3.68 s	3.65 s	3.65 s
21	3.81 s	3.81 s	3.56 s	3.56 s
22	3.85 s	3.83 s	3.67 s	3.69 s

[a]Spectra recorded at 600 MHz. Chemical shifts (δ) are in ppm and coupling constants (J) in Hz. [b]Assigned by NOESY. *Signals overcharged.

Table 6. ^{13}C NMR Data for Compounds 12−15 (Acetone-d_6)[a]

no.	12	13	14	15
1	113.4	114.7	102.1	101.1
2	146.6	146.7	148.7	149.2
3	138.8	138.5	137.7	137.5
4	144.5	144.9	144.3	144.0
5	130.6	132.6	49.5	46.7
6	85.2	82.2	92.3	92.4
7	42.6	53.5	42.6	36.7
8	49.4	46.1	46.8	45.8
9	90.6	87.9	83.1	85.7
10	140.6	133.7	131.0	134.5
11	103.7	103.9	120.6	120.1
12	147.6	147.6	118.8	118.6
13	139.4	139.3	150.3	149.7
14	143.7	143.4	175.6	175.6
15	124.6	125.7	154.0	154.0
16	123.7	124.0	122.0	121.7
17	13.7	17.5	19.4	15.5
18	13.9	13.8	14.0	15.1
19	102.2	102.3	102.4	102.1
20	56.4	56.7	59.7	59.7
21	60.2	60.4	55.2	55.2
22	60.4	61.5	55.5	55.5

[a]Spectra recorded at 150 MHz. Chemical shifts (δ) are in ppm.

dx.doi.org/10.1021/jo301225v | J. Org. Chem. 2012, 77, 8435−8443

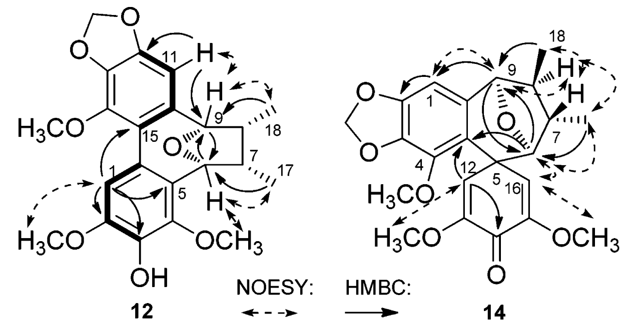


Figure 4. Selected NOESY and HMBC spectra of compounds **12** and **14**.

Gymnothelignan M (**13**) was assigned as a diastereomer of **12** by comparison of the HRMS and NMR (Tables 5 and 6) data with that of **12**. Diagnostic cross-peaks in the NOESY (Figure S57, Supporting Information) of H-11/H-9, H-11/H_3-18, H-7/H_3-18, H-7/H_3-17, and H-6/H_3-17 supported that H_3-17 and H_3-18 were in the α- and β-orientation, respectively. Consequently, compound **13** was proposed to be an epimer of **12** at C-8. The vicinal coupling constants of H-6 and H-9 along with substituents on the THF allowed the conformation assignment of THF as shown in Figure 3. The structure of **13** was therefore elucidated as the 8-epimer of **12**.

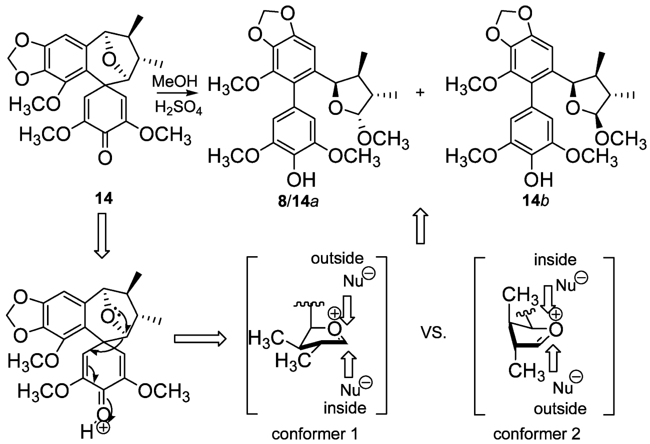
Gymnothelignan N (**14**) was assigned to have molecular formula $C_{22}H_{24}O_7$ on the basis of HRESIMS. The ^1H and ^{13}C NMR spectrum (Tables 5 and 6) showed that it probably belonged to the eupodienone[8] family. The HMBC (Figure 4) correlations of H-6/C-9 and H-9/C-6 confirmed an epoxide at C-6 and C-9. The stereochemistry of epoxide at C-6 and C-9 was proposed to be in α-orientation due to the α-orientation of a substituent at C-9 of eupodienone (it was favorable for cyclization occurring at C-6 and C-9 without epimerizing). Two *trans*-methyl groups (H_3-17 and H_3-18) were determined by NOESY (Figure 4, cross-peaks of H-9/H-8 and H-6/H_3-17). The conformation of THF (Figure 3) favored the proposed relative configuration. Finally, the absolute configuration was established by single-crystal X-ray diffraction (Figure S85, Supporting Information) as (6*S*,7*S*,8*S*,9*R*)-6,9-epoxy-7,8-dimethyl-2,3-methylenedioxy-4,13,15-trimethoxy-10,11-benzospiro[5.6]dodec-13,15-dien-14-one.

Gymnothelignan O (**15**) was found to have molecular formula $C_{22}H_{24}O_7$ by HRESIMS. Its ^1H and ^{13}C NMR spectra (Tables 5 and 6) were quite close to those of **14**. Detailed analysis of ^1H and ^{13}C NMR spectra suggested that compounds **14** and **15** were diastereomeric at C-8. The vicinal coupling constants (H-9/H-8) value was 5.6 Hz for **14**, whereas that of **15** was 0 Hz, indicating an epimerization at C-8. NOESY (Figure S65, Supporting Information) correlations of H-6/H_3-17 and H-9/H_3-18 supported that H-6, H-9, H_3-17, and H_3-18 existed in the same orientation. The conformation of THF is shown in Figure 3.

In order to determine the absolute configurations of compounds **3** and **8**, compound **14** underwent nucleophilic substitution reactions[29,30] to afford **8**/**14*a*** and **14*b***.[31] The possible mechanism of the conversion of **14** to **8** is shown in Scheme 1.[29] Therefore, a total of four chiral centers of **3**/**8** were undoubtedly determined to be 2*R*,3*S*,4*S*,5*R*.

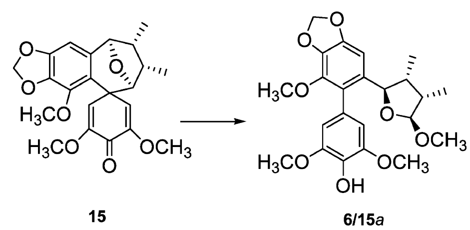
Similarly, treatment **15** afforded **15*a*** (Scheme 2; a possibly minor product could not be detected probably due to the steric hindrance). The structure of **15** was thus determined to be the 8-epimer of **14**.

Scheme 1. Possible Mechanism of 14 to 8[a]



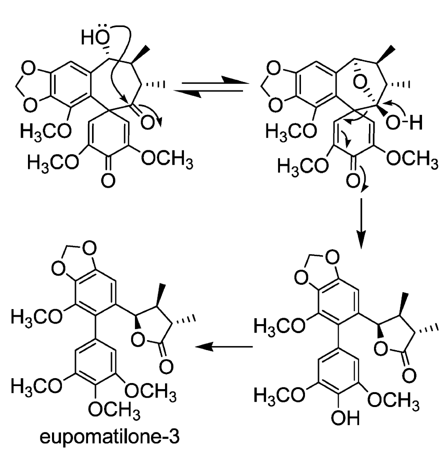
[a]The major product could arise from either inside attack on the diequatorial oxocarbenium ion **1** or outside attack on the diaxial cation **2**.[29] Actually, the minor product was also elucidated by comparison of the NMR spectra with those of **8**.

Scheme 2. Chemical Transformation of 15 to 15*a*

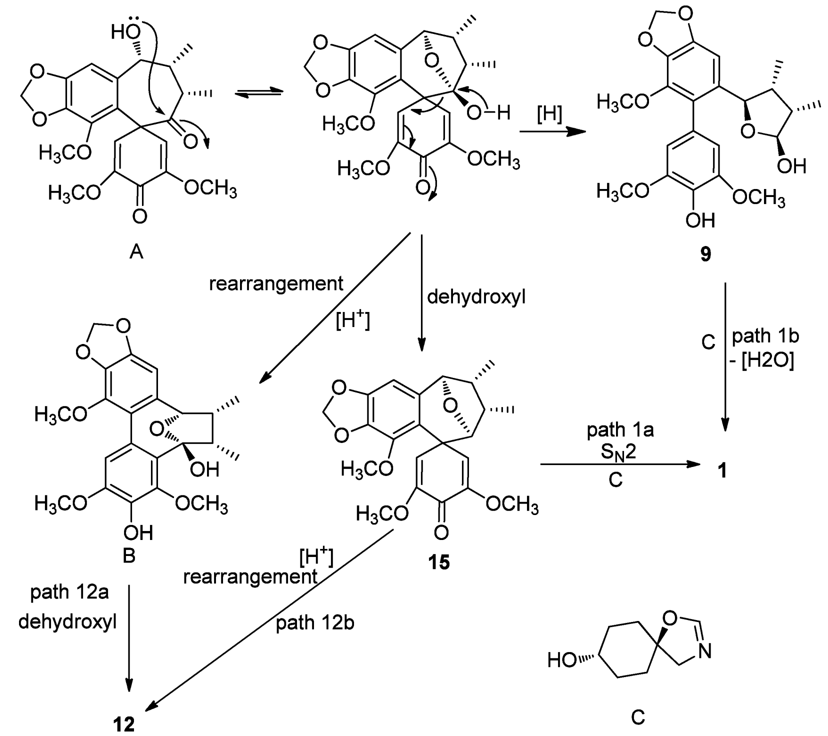


The chemical transformation of **14** to yield **8** allowed us to establish the absolute configuration of the eupomatilone family,[32] which was consistent with that of eupomatilone-6 assigned previously.[12,16] It was reported that eupomatilone was derived from eupodienone (Scheme 3).[6] Chemical transformation in vitro buttressed the plausible biosynthetic pathways of eupodienone to eupomatilone mentioned above. The absolute configuration of eupomatilone-3 was therefore proposed as 3*S*,4*S*,5*R*. Considering that compounds **1**−**11**

Scheme 3. Plausible Biosynthetic Pathways of Eupomatilone-3[6]



Scheme 4. Plausible Biosynthetic Pathways of 1, 9, 12, and 15



uniformly demonstrated *R*-configuration at C-5 regardless of whether its ^{13}C NMR chemical shift was near δ 79 ppm, the revised structure of synthesized 5-*epi*-eupomatilone-6[19,20] to 3,5-bis-*epi*-eupomatilone-6[18] should again be 4-*epi*-eupomatilone-6[15] by our establishment of the absolute configuration.

The discovery of the three arrays of new lignans is an example of chemical diversity, extending the lignan family by derivatives formed by ring cleavage, oxidation, and esterification. Compounds **1**, **9**, **12**, and **15** were considered to be derived from the same parent compound (Scheme 4). Compound **1** may biogenetically be derived from precursor A via intermediates **9** and **15** (two pathways, paths 1a and 1b).[8,33] Compound **12** was probably derived from precursor A via intermediates B or **15** (paths 12a and 12b) by dehydoxyl reaction and rearrangement.[8] These compounds were tested for cytotoxic activity on HepG2 and Bcl7404 cell lines using MTT[34] methods, while **3** exhibited moderate cytotoxicity against HepG2 and Bcl7404 cells, with IC_{50} values of 15 and 17.5 μg/mL, respectively (Table S1, Supporting Information). The seco-lignans **1**−**11** are rare natural products. Compounds **1**−**11**, except **3** and **8**, demonstrate *trans*-H-4−H-5, which were isolated as natural products for the first time and were mistaken as enantiomers of eupomatilones according to previous studies.[19,20] From now on, it is feasible to determine the absolute configuration for C-5 of the eupomatilone family with *trans*-H-4−H-5. In summary, the three skeletons of lignans are proposed to be derived from the same parent compound.

■ EXPERIMENTAL SECTION

General Experimental Procedures. NMR spectra were recorded at 300 K (600 MHz for ^1H and 150 MHz for ^{13}C) with the CD_3COCD_3 (δ 2.05/29.8) solvent as internal standard. The 1D and 2D NMR spectra were performed using standard software. The HRESIMS was performed on a Q-TOF mass spectrometer. Preparative HPLC was performed on a liquid chromatograph equipped with an UV detector and semipreparative column (C_{18}, 5 μm, 19 × 250 mm). Column chromatography was carried out on silica gel (160−200 mesh), MCI CHP-20 gel (75−150 μm), ODS (40−63 μm), and Sephadex LH-20. TLC was performed on precoated plates (GF254), and spots were detected on TLC under UV and by heating after spraying with color reagents: vanillic aldehyde (15 g) + ethanol (250 mL) + H_2SO_4 (2.5 mL). X-ray crystallographic analyses were carried out using Cu Kα radiation (λ = 1.54178 Å) at 298 K and Mo Kα radiation (λ = 0.71073 Å) at 153 K. The structures were solved by direct methods using the SHELXS-97 program.

Plant Material. The plant was collected from Jinfoshan in Chongqing City, People's Republic of China, in July 2010 and identified as *G. chinensis* by Prof. S. R. Yi. A voucher specimen (T57) has been deposited at the herbarium of Chengdu Institute of Biology, Chinese Academy of Science.

Extraction and Isolation. Dried and powdered whole plants of *G. chinensis* (1.4 kg) were extracted with ethanol at room temperature (3 × 7 days) to give an extract (146 g), which was suspended in H_2O and extracted with petroleum ether and ethyl acetate (3 × 0.5 L, 3 h each) successively. The ethyl acetate extract (16 g) was separated by column chromatography on MCI CHP-20 (6 × 30 cm) with a gradient system of aqueous methanol (7:3, 1 L, 8:2, 2 L, 9:1, 1 L, and 10:0, 1 L) to yield three fractions (A−C). Fraction B (8 g) was subjected to silica gel chromatography (4 × 60 cm) with chloroform/methanol mixtures of increasing polarity (35:1 to 1:1) to give fractions (BA−BH) and **20**. Fraction BG was separated on a semipreparative column (2−13 min, 40−95%, 13−16 min, 95−100%) to afford **17** (13 mg, t_R 9.4 min) and **16** (18 mg, t_R 11.7 min). Fraction BD was purified on a semipreparative column (2−15 min, 40−95%, 15−18 min, 100%) to afford **18** (21 mg, t_R 9.1 min). Fraction BB (1.2−1.5 L, 3 g) was subjected to repeated silica gel chromatography with petroleum ether/acetone mixtures of increasing polarity to give a fraction, which was separated on a semipreparative column (2−16 min, 60−95% aqueous methanol, flow rate, t_R 14.3 min) to afford **1** (15 mg), as well as a mixture (2−8 min, 70−80%, 8−16 min, 80%, 16−18 min, 95% aqueous methanol) which afforded **2** (6 mg, t_R 13.2 min) and **3** (2 mg, t_R 12.8 min). Thereafter, fraction BBD was separated on a semipreparative column (2−8 min, 20−85%, 8−12 min, 85−95%, 12−18 min, 95% aqueous methanol, flow rate, 16 mL/min) to afford **14** (25 mg, t_R 11.5 min), **15** (30 mg, t_R 11.8 min), and **12** (8 mg, t_R

12.6 min), as well as a mixture, which was further purified on a semipreparative column (2−15 min, 85−95%) to afford **19** (15 mg, t_R 9.7 min). Fraction BBE was then subjected to silica gel chromatography (3 × 60 cm) eluted with gradient polarity of petroleum ester/acetone (8:1 to 1:1) to give fraction BBEC and was further separated on a semipreparative column (2−15 min, 63−82%, 15−19 min, 82%, 19−23 min, 82−90%) to afford **9** (5 mg, t_R 6.4 min), **10** (3 mg, t_R 10.0 min), **14** (13 mg, t_R 11.3 min), **15** (9 mg, t_R 12.5 min), **6** (8 mg, t_R 13.4 min), **8** (8 mg, t_R 15.2 min), **7** (8 mg, t_R 15.5 min), and **12** (2 mg, t_R 16.1 min). Fraction BBF was subjected to silica gel chromatography (3 × 60 cm) eluted with 80/20 petroleum ether/ethyl acetate (5% *i*-PrOH) to give **8** and a mixture, which was further separated on a semipreparative column (2−15 min, 60−95%, 15−18 min, 95−100%) to afford **4** (8 mg, t_R 11.4 min) and **5** (7 mg, t_R 12.1 min). Fraction BBG was separated by silica gel chromatography with petroleum ether/ethyl acetate mixtures of increasing polarity to give three fractions (BBGA−BBGC). Compound **13** (6 mg, t_R 13.6 min) was obtained from fraction BBGB (2−15 min, 60−95%, 15−18 min, 95%), whereas **11** (4 mg, t_R 15.6 min) was obtained from fraction BBGA (2−6 min, 30−60%, 6−6.2 min, 60−80%, 6.2−18 min, 80−95%). Semipreparative column chromatography solvent, aqueous MeOH; flow rate, 16 mL/min.

Crystal Structure Determination of 1. Diffraction data (φ and ω scans) were collected using Cu Kα radiation (λ = 1.54178 Å). $C_{30}H_{37}NO_9$, MW = 555.61, orthorhombic, a = 10.3937(13) Å, b = 10.5073(13) Å, c = 25.914(3) Å, $\alpha = \beta = \gamma$ = 90.00°, V = 2830.0(6) Å^3, T = 298(2) K, space group $P2_12_12_1$, Z = 4, d = 1.304 g/cm^3, $F(000)$ = 1184, 6996 reflections measured, 4123 independent reflections (R_{int} = 0.1332). The final R_1 values were 0.0603 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1795 ($I > 2\sigma(I)$). The final R_1 values were 0.1954 (all data). The final $wR(F^2)$ values were 0.2478 (all data). The goodness of fit on F^2 was 1.175. Flack parameter = 0.2(3). Crystallographic data for gymnothelignan A (**1**) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 857186). These data can be obtained free of charge from the Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.

Crystal Structure Determination of 4. Diffraction data (φ and ω scans) were collected using Cu Kα radiation (λ = 1.54178 Å). Compound **4**, $C_{24}H_{32}O_8$, MW = 448.50, was monoclinic and space group $P2_1$ with a = 10.8975(4) Å, b = 18.7065(8) Å, c = 11.7408(5) Å, $\alpha = \gamma$ = 90°, β = 92.968(3)°, V = 2390.20(17) Å^3, Z = 4, d = 1.246 g/cm^3, $F(000)$ = 960; crystal size 0.22 × 0.15 × 0.13 mm; 14 004 reflections measured, 6996 reflections unique, θ_{max} = 67.49°. The final R_1 values were 0.0482 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1253 ($I > 2\sigma(I)$). The final R_1 values were 0.0960 (all data). The final $wR(F^2)$ values were 0.1870 (all data). The goodness of fit on F^2 was 0.990. Flack parameter = 0.1(3). The structure was solved by direct methods (SHELXS-97) and expanded using SHELXL-97. Crystallographic data for gymnothelignan D (**4**) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 857185). These data can be obtained free of charge from the Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.

Crystal Structure Determination of 8. Diffraction data (φ and ω scans) were collected using Mo Kα radiation (λ = 0.71073 Å). Compound **8**, $C_{23}H_{28}O_8$, MW = 432.45, was orthorhombic and space group $P2_12_12_1$ with a = 6.9969(17) Å, b = 11.484(3) Å, c = 27.382(7) Å, $\alpha = \beta = \gamma$ = 90°, V = 2200.1(9) Å^3, Z = 4, d = 1.306 g/cm^3, $F(000)$ = 920; crystal size 0.48 × 0.35 × 0.08 mm; 19 484 reflections measured, 3370 reflections unique (R_{int} = 0.0477). The final R_1 values were 0.0438 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0906 ($I > 2\sigma(I)$). The final R_1 values were 0.0511 (all data). The final $wR(F^2)$ values were 0.0944 (all data), θ_{max} = 29.12°, goodness of fit was 0.999. The structure was solved by direct methods (SHELXS-97) and expanded using SHELXL-97. Crystallographic data for gymnothelignan H (**8**) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 870519). These data can be obtained free of charge from the Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.

Crystal Structure Determination of 14. Diffraction data (φ and ω scans) were collected using Cu Kα radiation (λ = 1.54178 Å). Compound **14**, $C_{24}H_{24}O_7$, MW = 400.41, was monoclinic and space group $P2_1$ with a = 10.068(3) Å, b = 9.308(2) Å, c = 11.584(3) Å, $\alpha = \gamma$ = 90°, β = 108.829(10)°, V = 1027.5(5) Å^3, Z = 2, d = 1.294 g/cm^3, $F(000)$ = 424; crystal size 0.28 × 0.22 × 0.19 mm; 4103 reflections measured, 3642 independent reflections (R_{int} = 0.0266). The final R_1 values were 0.0714 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.2309 ($I > 2\sigma(I)$). The final R_1 values were 0.1246 (all data). The final $wR(F^2)$ values were 0.2943 (all data). The goodness of fit on F^2 was 1.156. Flack parameter = −0.3(6). The structure was solved by direct methods (SHELXS-97) and expanded using SHELXL-97. Crystallographic data for gymnothelignan N (**14**) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 870520). These data can be obtained free of charge from the Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.

Gymnothelignan A (1): colorless crystals; $[\alpha]_D^{20}$ −9 (c 0.09, CH_3OH); mp 171−172 °C; UV λ_{max} (CH_3OH) 284 nm; IR (KBr) ν_{max} 3437, 1619 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); HRESIMS m/z 578.2368 [M + Na]$^+$ (calcd for $C_{30}H_{37}NO_9Na$ 578.2361).

Gymnothelignan B (2): off-white powder; $[\alpha]_D^{20}$ −65 (c 0.04, CH_3OH); UV λ_{max} (CH_3OH) 281 nm; IR (KBr) ν_{max} 3436, 1615 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); HRESIMS m/z 578.2382 [M + Na]$^+$ (calcd for $C_{30}H_{37}NO_9Na$ 578.2361).

Gymnothelignan C (3): off-white powder; $[\alpha]_D^{20}$ −30 (c 0.03, CH_3OH); UV λ_{max} (CH_3OH) 280 nm; IR (KBr) ν_{max} 3401, 1635 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); HRESIMS m/z 578.2385 [M + Na]$^+$ (calcd for $C_{30}H_{37}NO_9Na$ 578.2361).

Gymnothelignan D (4): colorless crystals; $[\alpha]_D^{20}$ −12 (c 0.03, CH_3OH); mp 133−134 °C; UV λ_{max} (CH_3OH) 276 nm; IR (KBr) ν_{max} 3392, 2960, 2933, 1607, 1490, 1461, 1097 cm^{-1}; ^1H and ^{13}C NMR data (Table 4); HRESIMS m/z 471.1978 [M + Na]$^+$ (calcd for $C_{24}H_{32}O_8Na$ 471.1989).

Gymnothelignan E (5): white powder; $[\alpha]_D^{20}$ −7 (c 0.35, Me_2CO); UV λ_{max} (CH_3OH) 277 nm; IR (KBr) ν_{max} 3429, 2935, 1608, 1488, 1463, 1102 cm^{-1}; ^1H and ^{13}C NMR data (Table 4); HRESIMS m/z 471.1976 [M + Na]$^+$ (calcd for $C_{24}H_{32}O_8Na$ 471.1989).

Gymnothelignan F (6): yellowish powder; $[\alpha]_D^{20}$ −2 (c 0.20, Me_2CO); UV λ_{max} (CH_3OH) 287 nm; IR (KBr) ν_{max} 3430, 2962, 2936, 1614, 1519, 1476, 1112 cm^{-1}; ^1H and ^{13}C NMR data (Tables 2 and 3); HRESIMS m/z 455.1690 [M + Na]$^+$ (calcd for $C_{23}H_{28}O_8Na$ 455.1676).

Gymnothelignan G (7): off-white powder; $[\alpha]_D^{20}$ −10 (c 0.25, Me_2CO); UV λ_{max} (CH_3OH) 296 nm; IR (KBr) ν_{max} 3442, 2928, 1606, 1469, 1115 cm^{-1}; ^1H and ^{13}C NMR data (Tables 2 and 3); HRESIMS m/z 455.1673 [M + Na]$^+$ (calcd for $C_{23}H_{28}O_8Na$ 455.1676).

Gymnothelignan H (8): colorless crystals; $[\alpha]_D^{20}$ −80 (c 0.03, Me_2CO); mp 184−185 °C; UV λ_{max} (CH_3OH) 301 nm; IR (KBr) ν_{max} 3393, 2958, 2934, 1607, 1475, 1465, 1115, 1026 cm^{-1}; ^1H and ^{13}C NMR data (Tables 2 and 3); HRESIMS m/z 455.1675 [M + Na]$^+$ (calcd for $C_{23}H_{28}O_8Na$ 455.1676).

Gymnothelignan I (9): yellowish powder; $[\alpha]_D^{20}$ −8 (c 0.10, Me_2CO); UV λ_{max} (CH_3OH) 302 nm; IR (KBr) ν_{max} 3436, 2964, 2937, 1614, 1476, 1113 cm^{-1}; ^1H and ^{13}C NMR data (Tables 2 and 3); HRESIMS m/z 441.1522 [M + Na]$^+$ (calcd for $C_{22}H_{26}O_8Na$ 441.1520).

Gymnothelignan J (10): yellowish powder; $[\alpha]_D^{20}$ −4 (c 0.10, Me_2CO); UV λ_{max} (CH_3OH) 298 nm; IR (KBr) ν_{max} 3426, 2962, 2936, 1608, 1460, 1100 cm^{-1}; ^1H and ^{13}C NMR data (Table 4); HRESIMS m/z 457.1852 [M + Na]$^+$ (calcd for $C_{23}H_{30}O_8Na$ 457.1833).

Gymnothelignan K (11): white powder; $[\alpha]_D^{20}$ −10 (c 0.09, CH_3OH); UV λ_{max} (CH_3OH) 280 nm; IR (KBr) ν_{max} 3435, 2935, 1615, 1476, 1114 cm^{-1}; ^1H and ^{13}C NMR data (Tables 2 and 3); HRESIMS m/z 841.3069 [M + Na]$^+$ (calcd for $C_{44}H_{50}O_{15}Na$ 841.3043).

Gymnothelignan L (12): yellow powder; $[\alpha]_D^{20}$ −2 (c 0.20, Me_2CO); UV λ_{max} (CH_3OH) 307 nm; IR (KBr) ν_{max} 3429, 2961, 2934, 1616, 1479, 1101 cm^{-1}; ^{1}H and ^{13}C NMR data (Tables 5 and 6); HRESIMS m/z 401.1595 $[M + H]^+$ (calcd for $C_{22}H_{25}O_7$ 401.1594).

Gymnothelignan M (13): yellow powder; $[\alpha]_D^{20}$ +8 (c 0.03, CH_3OH); UV λ_{max} (CH_3OH) 300 nm; IR (KBr) ν_{max} 3428, 2957, 2928, 1617, 1451, 1079 cm^{-1}; ^{1}H and ^{13}C NMR data (Tables 5 and 6); HRESIMS m/z 401.1597 $[M + H]^+$ (calcd for $C_{22}H_{25}O_7$ 401.1594).

Gymnothelignan N (14): colorless crystals; $[\alpha]_D^{20}$ +13 (c 0.04, CH_3OH); mp 201−202 °C; UV λ_{max} (CH_3OH) 290 nm; IR (KBr) ν_{max} 2958, 2936, 1668, 1614, 1475, 1112 cm^{-1}; ^{1}H and ^{13}C NMR data (Tables 5 and 6); HRESIMS m/z 423.1423 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_7Na$ 423.1414).

Gymnothelignan O (15): white powder; $[\alpha]_D^{20}$ +15 (c 0.04, CH_3OH); UV λ_{max} (CH_3OH) 283 nm; IR (KBr) ν_{max} 2936, 1668, 1615, 1477, 1113 cm^{-1}; ^{1}H and ^{13}C NMR data (Tables 5 and 6); HRESIMS m/z 423.1435 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_7Na$ 423.1414).

Treatment of 14 and 15 with H_2SO_4. Gymnothelignan N (**14**, 11 mg) was dissolved in methanol (2 mL) and then added 5 mL of 2 M H_2SO_4 and heated at 80 °C under reflux for 1 h. After cooling to room temperature, the solution was neutralized with excess $NaHCO_3$ and filtered, and the filtrate was extracted three times each with 7 mL of ethyl acetate. The combined organic layer was evaporated to dryness and then separated by HPLC with a C_{18} column, using a mixed solvent of methanol/water (0−2 min, 70%, 2−16 min, 70−95%, 16−18 min, 95%) to yield the corresponding **14***a* (1.7 mg, t_R 10.8 min, 15% yield) and **14***b* (0.5 mg, t_R 9.7 min, 5% yield). **14***a*: white powder, $[\alpha]_D^{20}$ −80 (c 0.03, Me_2CO); ^{1}H NMR (600 MHz, acetone-d_6, δ_H 2.05, Figure S66) was in good agreement with that of **8**. **14***b*: yellowish powder, $[\alpha]_D^{20}$ +100 (c 0.02, Me_2CO); ^{1}H NMR (600 MHz, acetone-d_6, δ_H 2.05, Figure S67) δ_H 6.90 (s, H-3′), 6.45, 6.36 (br s, each 1H, H-6″, and 2″), 6.01, 6.00 (br s, each 1H, OCH_2O), 5.11 (d, J = 7.1 Hz, H-5), 4.54 (d, J = 2.6 Hz, H-2), 3.82, 3.80, 3.74, 3.43 (s, each 3H, 3″, 5″, 6′, and 2-OCH_3), 1.69, 1.67 (m, 2H, H-4, and H-3), 0.90 (d, J = 7.1 Hz, H_3-7), 0.71 (d, J = 7.2 Hz, H_3-6). Treatment of **15** (17 mg) as described for **14** afforded corresponding **15***a* (2.8 mg, t_R 16.2 min, 16% yield, solvent, 60−95% aqueous MeOH, flow rate, 16 mL/min). **15***a*: white powder, ^{1}H NMR (600 MHz, acetone-d_6, δ_H 2.05, Figure S68) was in good agreement with that of **6**.

■ ASSOCIATED CONTENT

Supporting Information

IR, 1D, and 2D NMR spectra of compounds **1**−**15**, ^{1}H NMR spectra of **14***a*, **14***b*, and **15***a*, NMR free induction decay (FID) files of compounds **1**, **4**, and **8**, CIFs of compounds **1**, **4**, **8**, and **14**, cytotoxic activity of these compounds on HepG2 and Bcl7404 cell lines. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: xuhongxi88@gmail.com, zhouyan@cib.ac.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by grants from the 2010 technological talents program of the Chinese Academy of Sciences granted to Y. Zhou and the National Natural Sciences Foundation (Nos. 30973634, 30900129 and 81130069) of China.

■ REFERENCES

(1) Yang, W.-L.; Tian, J.; Ding, L.-S. *Chin. J. Chin. Mater. Med.* **2001**, *26*, 43.
(2) Alberto Marco, J; Barberá, O.; Sanz, J. F.; Sanchez-Parareda, J. *Phytochemistry* **1985**, *24*, 2471.
(3) Nee'Shukla, R. V.; Misra, K. *Phytochemistry* **1981**, *20*, 339.
(4) González, A. G.; Guillermo, J. A.; Ravelo, A. G.; Jimenez, I. A.; Gupta, M. P. *J. Nat. Prod.* **1994**, *57*, 400.
(5) Bohlmann, F.; Zdero, C.; Schöneweiss, S. *Chem. Ber.* **1976**, *109*, 3366.
(6) Carroll, A.; Taylor, W. *Aust. J. Chem.* **1991**, *44*, 1705.
(7) Carroll, A.; Taylor, W. *Aust. J. Chem.* **1991**, *44*, 1615.
(8) Read, R.; Taylor, W. *Aust. J. Chem.* **1981**, *34*, 1125.
(9) Bowden, B.; Read, R.; Taylor, W. *Aust. J. Chem.* **1981**, *34*, 799.
(10) Hirokawa, Y.; Kitamura, M.; Kato, C.; Kurata, Y.; Maezaki, N. *Tetrahedron Lett.* **2011**, *52*, 581.
(11) Mitra, S.; Gurrala, S. R.; Coleman, R. S. *J. Org. Chem.* **2007**, *72*, 8724.
(12) Johnson, J. B.; Bercot, E. A.; Williams, C. M.; Rovis, T. *Angew. Chem.* **2007**, *119*, 4598.
(13) Rainka, M. P.; Milne, J. E.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2005**, *44*, 6177.
(14) Kabalka, G. W.; Venkataiah, B. *Tetrahedron Lett.* **2005**, *46*, 7325.
(15) Yu, S. H.; Ferguson, M. J.; McDonald, R.; Hall, D. G. *J. Am. Chem. Soc.* **2005**, *127*, 12808.
(16) Gurjar, M. K.; Karumudi, B.; Ramana, C. V. *J. Org. Chem.* **2005**, *70*, 9658.
(17) Gurjar, M. K.; Cherian, J.; Ramana, C. V. *Org. Lett.* **2004**, *6*, 317.
(18) Coleman, R. S.; Gurrala, S. R. *Org. Lett.* **2004**, *6*, 4025.
(19) Hutchison, J. M.; Hong, S.-p.; McIntosh, M. C. *J. Org. Chem.* **2004**, *69*, 4185.
(20) Hong, S.-p.; McIntosh, M. C. *Org. Lett.* **2001**, *4*, 19.
(21) Ward, R. S. *Nat. Prod. Rep.* **1997**, *14*, 43.
(22) Schmidt, B. *J. Org. Chem.* **2004**, *69*, 7672.
(23) Flack, H. D. *Acta Crystallogr., Sect. A* **1983**, *39*, 876.
(24) Hiranrat, A.; Mahabusarakam, W.; Carroll, A. R.; Duffy, S.; Avery, V. M. *J. Org. Chem.* **2011**, *77*, 680.
(25) Crystals were crystallized from the sample that was previously used for NMR spectra.
(26) Hamed, W.; Brajeul, S.; Mahuteau-Betzer, F.; Thoison, O.; Mons, S.; Delpech, B.; Hung, N. V.; Sévenet, T.; Marazano, C. *J. Nat. Prod.* **2006**, *69*, 774.
(27) Spencer, G. F.; Flippen-Anderson, J. L. *Phytochemistry* **1981**, *20*, 2757.
(28) Liu, J.-S.; Li, L. *Phytochemistry* **1995**, *38*, 241.
(29) Smith, D. M.; Tran, M. B.; Woerpel, K. A. *J. Am. Chem. Soc.* **2003**, *125*, 14149.
(30) Larsen, C. H.; Ridgway, B. H.; Shaw, J. T.; Woerpel, K. A. *J. Am. Chem. Soc.* **1999**, *121*, 12208.
(31) The diastereoselectivity of the nucleophilic substitution confirmed the inherent stereoelectronic preference for inside versus outside attack. However, rearrangement products were not isolated.
(32) Compound **14** had the same absolute configuration of eupodienone which was isolated from the genus *Eupomatia*. It was therefore supposed that compound **14** and eupodienone were derived from the same parent compound. See ref 6.
(33) Pelter, A.; Satchwell, P.; Ward, R. S.; Blake, K. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2201.
(34) Wang, X.; Chen, Y.; Han, Q.-b.; Chan, C.-y.; Wang, H.; Liu, Z.; Cheng, C. H.-k.; Yew, D. T.; Lin, M. C. M.; He, M.-l.; Xu, H.-x.; Sung, J. J. Y.; Kung, H.-f. *Proteomics* **2009**, *9*, 242.