JOURNAL OF NATURAL PRODUCTS

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Anti-inflammatory Endiandric Acid Analogues from the Roots of *Beilschmiedia tsangii*

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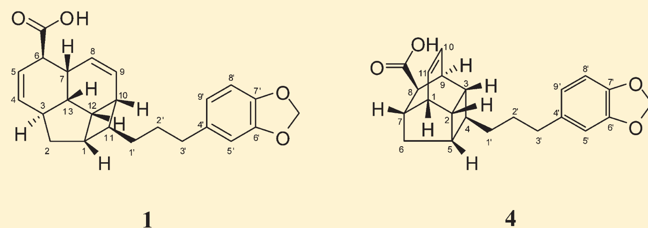
S *Supporting Information*

ABSTRACT: Bioassay-guided fractionation of roots of *Beilschmiedia tsangii* led to the isolation of six new endiandric acid analogues: tsangibeilin A (**1**), tsangibeilin B (**2**), endiandramide A (**3**), endiandric acid K (**4**), endiandric acid L (**5**), and endiandramide B (**6**). Also isolated were two new lignans, beilschminol A (**7**) and tsangin C (**8**), and six known compounds. The structures of **1**−**8** were determined by spectroscopic techniques. Compounds **3** and **6** exhibited potent iNOS inhibitory activity, with IC_{50} values of 9.59 and 16.40 μM, respectively.



*B*eilschmiedia tsangii Merr. (Lauraceae) is a medium-sized evergreen tree, distributed throughout Tonkin, Vietnam, southern and western China, and southern Taiwan. There are about 200 *Beilschmiedia* species found in these tropical regions, including two species in Taiwan.[1] Previous studies on 11 *Beilschmiedia* species revealed endiandric acids,[2−6] 7,7′-epoxylignans,[7,8] 7′,8′-seco-7,7′-epoxylignans,[7] flavonoids,[9,10] alkaloids,[11−13] benzopyrans,[2,3] arylpropanoids,[3,14] amides,[4] sesquiterpenoids,[2−4] and steroids.[3,4] Several isolates showed antibacterial,[5,10] antimalarial,[11] antitubercular,[4,8] or cytotoxic activity.[7] Over 40 species of Formosan lauraceous plants have been screened for anti-inflammatory activity using an inducible nitric oxide synthase (iNOS) assay, and a methanolic extract of roots of *B. tsangii* showed potent inhibition of NO production, with no cytotoxicity against RAW 264.7 cells. Bioassay-guided fractionation of the extract led to the isolation of eight new compounds (**1**−**8**) and six known compounds. The structures of compounds **1**−**8** were established by spectroscopic analyses, and assessment of their anti-inflammatory activity is described herein.

RESULTS AND DISCUSSION

Compounds **1**−**6** were isolated as optically inactive yellowish oils, considered as racemic mixtures, and all structures were determined by ^{13}C NMR, NOESY, COSY, HMQC, and HMBC experiments (see Figures 1 and 2, Supporting Information). Compound **1**, $C_{24}H_{26}O_4$ by ESIMS and HRESIMS analysis, had UV bands at 232 sh and 288 nm and IR absorptions at 3432 (OH), 1696 (carbonyl), and 1040, 934 cm^{-1} (methylenedioxy), similar to those of erythrophloin F.[4] The ^{1}H NMR and ^{13}C NMR spectra of **1** (Table 1) were also similar to those of erythrophloin F,[4] except for a trimethylene group [δ 1.51 (4H, m, H-1′, H-2′), 2.52 (2H, t, J = 5.6 Hz, H-3′)] in **1** instead of a pentamethylene group in erythrophloin F[4] between C-11 of the tetracyclic acid moiety and C-4′ of the benzenoid moiety. Two fewer methylenes in the alkyl side chain in **1** was supported by the molecular formula of **1** ($C_{24}H_{26}O_4$) compared to that of erythrophloin F[4] ($C_{26}H_{30}O_4$). The rigid tetracyclic skeleton was indicated by HMBC correlations, including H-5 to C-6 and C-3, H-4 to C-6, H-13 to C-3, C-4, and C-8, H-8 to C-6, H-9 to C-8, H-12 to C-3, H-2 to C-3 and C-4, and H-10 and H-1 to C-11. The relative configuration of **1**, *rel*-(1*S*,3*S*,6*R*,7*R*,10*R*,11*S*,12*S*,13*S*), the same as that of erythrophloin F,[4] was supported by the NOESY spectrum (Figure 1, Supporting Information). There are eight chiral centers in **1**. However, in view of the observed optical rotation ($[\alpha]^{25}_D$ ±0), **1** was identical to that of many endiandric acid analogues.[3,4,15,16] Thus, **1** should be racemic. On the basis of the above data, compound **1** was named tsangibellin A.

Received: April 1, 2011
Published: August 16, 2011

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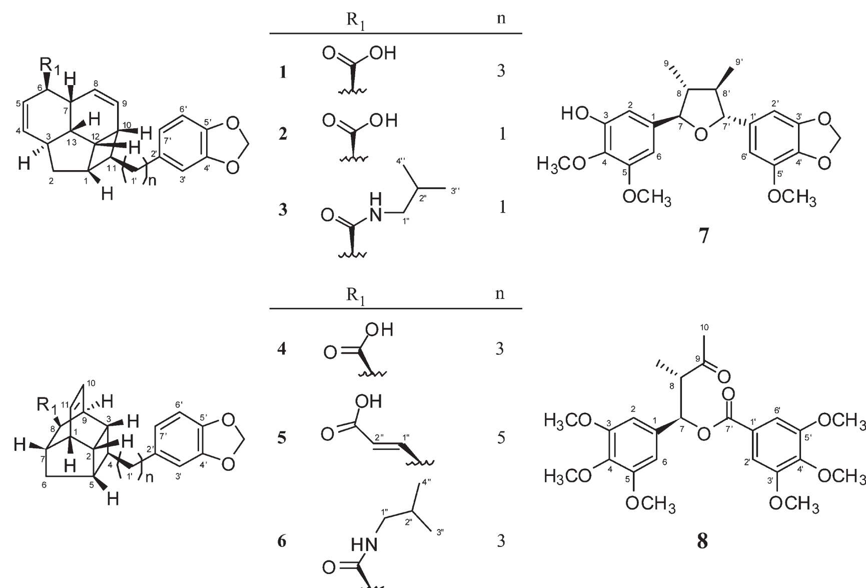
Chart 1



ESIMS and HRESIMS data of compound **2** indicated the molecular formula $C_{22}H_{22}O_4$, C_2H_4 less than that of **1** with 12 degrees of unsaturation. The UV, IR, ^{1}H NMR (Table 1), and ^{13}C NMR (Table 1) spectra were similar to those of **1**, except that a methylene group [δ 2.72 (1H, br d, J = 8.2 Hz, H-1′b), 2.73 (1H, dd, J = 17.0, 8.2 Hz, H-1′a)] in **2** replaced the trimethylene group present in **1**. The relative configuration of **2** was determined to be the same as that of **1** from the NOESY spectrum. Thus, compound **2** was named tsangibellin B.

The molecular formula of compound **4** was determined to be $C_{22}H_{24}O_4$ by ESIMS and HRESIMS, with 11 degrees of unsaturation and two fewer methylenes than endiandric acid I[3] ($C_{24}H_{28}O_4$). UV absorptions showed the presence of a benzenoid moiety at 233 and 286 nm, and the IR spectrum indicated the presence of OH (3431 cm^{-1}), carbonyl (1701 cm^{-1}), and methylenedioxy (1040 and 936 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra of **4** (Table 2) were similar to those of endiandric acid I,[3] except for three methylene groups [δ 1.53 (2H, m, H-1′), 1.54 (2H, m, H-2′), 2.53 (2H, t, J = 7.0 Hz, H-3′)] in **4** instead of a pentamethylene group in endiandric acid I,[3] between a methylenedioxyphenyl group and the main endiandric skeleton. HMBC correlations, H-7 to C-1, C-2, C-5, and C-8, H-9 to C-8, H-3 to C-2, H-8 to C-10, H-6 to C-8, H-5 to C-4, and H-2 to C-4, revealed the endiandric skeleton. The relative configuration of **4** was assigned as *rel*-(1*R*,2*R*,3*R*,4*S*,5*S*,7*S*,8*R*,9*S*) on the basis of a NOESY spectrum (Figure 1, Supporting Information), the same as in endiandric acid I[3] and beilcyclone A.[4] Compound **4** was named endiandric acid K.

Compound **5**, with the molecular formula $C_{26}H_{30}O_4$ with 12 unsaturations deduced from its ESIMS and HRESIMS, showed two methines more than endiandric acid I[3] ($C_{24}H_{28}O_4$). The UV, IR, ^{1}H NMR, and ^{13}C NMR spectra were similar to those of endiandric acid I,[3] except that an α,β-unsaturated carboxylic acid group [δ_H 6.87 (1H, dd, J = 15.8, 8.0 Hz, H-1″), 5.69 (1H, d, J = 15.8 Hz, H-2″), δ_C 171.7 (C=O)] at C-8 in **5** took the place of a carboxylic acid group at C-8 in endiandric acid I.[3] The carboxylic acid group connected to C-8 was supported by the COSY spectrum (Figure 2, Supporting Information) correlations between H-1″/H-2″, H-8 (δ 2.78) and H-8/H-9 (δ 2.55), H-7 (δ 1.90). The HMBC spectrum (Figure 2, Supporting Information) showed correlations between H-1″ (δ 6.87)/C-2″ (δ 118.8), C=O (δ 171.7), C-8 (δ 47.3), and C-7 (δ 41.7) and further proved the location of the α,β-unsaturated carboxylic acid group. The NOESY spectrum of **5** supported the configuration *rel*-(1*R*,2*R*,3*R*,4*S*,5*S*,7*S*,8*R*,9*S*) as in endiandric acid I[3] and beilcyclone A.[4] Compound **5** was named endiandric acid L.

The UV, IR, ^{1}H NMR, and ^{13}C NMR data of compounds **3** and **6** were similar to those of **2** and **4**, respectively, but they both showed an *N*-isobutylamido moiety [δ_H 0.89 (6H, d, J = 6.8 Hz, CH$_3$-3″,4″), 1.77 (1H, m, H-2″), 3.03 (1H, br dd, J = 13.2, 6.4 Hz, H-1b″), 3.10 (1H, br dd, J = 13.2, 6.0 Hz, H-1a″), 5.73 (1H, br t, J = 6.0 Hz, NH), δ_C 174.7 (C=O)] in **3**; [δ_H 0.89 (6H, d, J = 6.8 Hz, CH$_3$-3″,4″), 1.71 (1H, m, H-2″), 2.99 (1H, br dd, J = 13.3, 6.1 Hz, H-1″b), 3.05 (1H, br dd, J = 13.3, 6.0 Hz, H-1″a), 5.43 (1H, br t, J = 6.0 Hz, NH), δ_C 174.5 (C=O)] in **6**, respectively, which had replaced the carboxylic acid group in **2** and the carboxylic acid group in **4**. The configuration of **3** was determined to be *rel*-(1*S*,3*S*,6*R*,7*R*,10*R*,11*S*,12*S*,13*S*), the same as **2**; and the configuration of **6** was determined to be *rel*-(1*R*,2*R*,3*R*,4*S*,5*S*,7*S*,8*R*,9*S*), the same as **4**. Compounds **3** and **6** were named endiandramide A and endiandramide B, respectively.

Compound **7** was isolated as an optically active yellowish oil, $[\alpha]^{28}_D$ −43.4 (*c* 0.07, MeOH). HRESIMS of **7** gave an [M + Na]$^+$ ion at *m/z* 425.1574, consistent with a molecular formula of $C_{22}H_{26}O_7$. UV absorptions at 210, 243 sh, and 275 nm suggested the presence of a benzenoid moiety. IR absorption bands at 3440 cm^{-1} (OH) and 1034, 934 cm^{-1} (methylenedioxy) were observed. The ^{1}H NMR signals characteristic for a 7,7′-epoxylignan moiety [δ 1.04 (3H, d, J = 6.0 Hz, H-9′), 1.06 (3H, d, J = 6.0 Hz, H-9), 1.72 (1H, m, H-8), 1.79 (1H, m, H-8′), 4.57 (1H, d, J = 9.2 Hz, H-7), 4.58 (1H, d, J = 9.2 Hz, H-7′)] were similar to those of *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan,[17] except that an OH [δ 5.74 (1H, br s, OH-3)] was present in **7** rather than a methoxy group [δ 3.80 (3H, s, OCH$_3$-3)]. Thus, the planar

Table 1. NMR Data (400 MHz, $CDCl_3$) for Compounds 1, 2, and 3

	1		2		3	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	34.8	2.24, m	34.4	2.39, m	34.4	2.36, m
2	34.7	1.32, td (12.3, 6.0)	34.7	1.31, td (12.1, 6.0)	34.6	1.31, td (12.5, 6.0)
		1.57, dd (12.3, 5.4)		1.54, dd (12.1, 5.4)		1.53, dd (12.5, 5.4)
3	36.9	2.56, m	36.8	2.56, m	36.6	2.63, m
4	134.5	6.22, dt (9.8, 2.4)	134.3	6.20, dt (9.6, 2.2)	135.2	6.24, dt (9.8, 2.2)
5	123.9	5.73, dt (9.8, 3.0)	123.9	5.72, dt (9.6, 2.8)	124.9	5.59, dt (9.8, 3.0)
6	49.1	3.01, m	49.2	3.01, m	51.9	2.83, m
7	32.8	2.99, m	32.8	2.99, m	34.7	2.80, m
8	129.7	5.46, br, dd (10.0, 1.2)	129.8	5.42, br, d (10.6)	130.6	5.45, br, dd (10.0, 1.4)
9	129.3	5.64, ddd (10.0, 6.8, 3.6)	129.0	5.46, br, dd (10.6, 2.8)	128.7	5.41, ddd (10.0, 6.4, 3.2)
10	41.1	2.27, m	40.9	2.41, m	40.6	2.38, m
11	45.8	1.47, m	47.1	1.78, m	47.1	1.75, m
12	33.1	2.64, q (7.6)	32.9	2.70, br, d (7.6)	33.0	2.68, br, d (7.2)
13	42.0	1.72, ddd (12.0, 7.6, 5.6)	41.9	1.72, m	41.7	1.73, m
1′	36.6	1.51, m	42.5	2.72, br, d (8.2)	42.5	2.71, br, d (8.0)
				2.73, dd (17.0, 8.2)		2.72, dd (16.0, 8.2)
2′	29.1	1.51, m	134.5		134.5	
3′	35.6	2.52, t (5.6)	108.9	6.65, d (1.6)	108.9	6.64, d (1.6)
4′	136.5		147.4		147.4	
5′	108.8	6.66, d (1.4)	145.6		145.5	
6′	147.5		108.0	6.71, d (8.0)	108.0	6.70, d (8.0)
7′	145.4		121.3	6.61, dd (8.0, 1.6)	121.3	6.60, dd (8.0, 1.6)
8′	108.0	6.72, d (7.8)				
9′	121.0	6.61, dd (7.8, 1.4)				
1″					46.9	3.03, br, dd (13.2, 6.4)
						3.10, br, dd (13.2, 6.0)
2″					28.5	1.77, m
3″					20.03	0.89, d (6.8)
4″					20.01	0.89, d (6.8)
NH						5.73, br, t (6.0)
OCH_2O	100.7	5.92, s	100.7	5.91, s	100.7	5.90, s
C=O	180.0		180.5		174.7	

structure of **7** was proposed to be 3-hydroxy-3′,4′-methylenedioxy-4,5,5′-trimethoxy-7,7′-epoxylignan. The relative configuration of **7** was identical to that of *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan[17,18] on the basis of the optical rotation, values of coupling constants, and the chemical shift of protons 7, 7′, 8, 8′, 9, and 9′ observed in the ^{1}H NMR spectrum of **7**. Thus, compound **7**, named beilschminol A, was *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3-hydroxy-3′,4′-methylenedioxy-4,5,5′-trimethoxy-7,7′-epoxylignan.

Compound **8**, $[\alpha]^{26}_D$ −10.2 (*c* 0.07, $CHCl_3$), had the molecular formula $C_{24}H_{30}O_9$. UV absorptions suggested the presence of a benzenoid moiety. The IR spectrum showed absorption indicating an ester carbonyl group. The ^{1}H NMR spectrum of **8** was similar to that of tsangin A,[7] except for two methoxy groups [δ 3.88 (3H, s, OCH_3-5′), 3.89 (3H, s, OCH_3-4′)] rather than a methylenedioxy group at C-4′ and C-5′ of tsangin A. Thus, the planar structure of **8** was proposed to be 8-methyl-9-oxo-7-(3,4,5-trimethoxyphenyl)butyl-3′,4′,5′-trimethoxybenzoate. The NOESY spectrum showed marked correlation between H-7 (δ 5.90) and CH_3-8 (δ 1.00) and minor correlation between H-7 (δ 5.90) and H-8 (δ 3.21) in **8**, suggesting that H-7 and H-8 had different orientations. This phenomenon was the same as that of tsangin A, but opposite of that of tsangin B. However, the optical rotation of **8** was opposite of that of tsangin A (7*S*,8*R*), indicating that the configuration of **8** should be (7*R*,8*S*). Therefore, the structure of **8**, named tsangin C, was elucidated as (7*R*,8*S*)-8-methyl-9-oxo-7-(3,4,5-trimethoxyphenyl)butyl-3′,4′,5′-trimethoxybenzoate.

The known compounds β-sitosterol,[7] 6β-hydroxystigmast-4-en-3-one,[4] *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan,[17] *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan,[17] *rel*-(7*S*,8*S*,7′*R*,8′*R*)-3,3′,4,4′,5,5′-hexamethoxylignan,[19,20] and vanillin[4] were identified by comparison of their physical and spectroscopic data with values reported in the literature.

The genus *Beilschmiedia* has only two species in Taiwan, *B. tsangii* and *B. erythrophloia*. Phytochemical studies of the roots of *Beilschmiedia* species have been restricted so far to our research on the roots of *B. erythrophloia*[3,4] and *B. tsangii*.[7,8] These two species both possess endiandric acid derivatives.

E_{max} (%) and IC_{50} (μM) values of iNOS inhibitory activity of compounds **1**−**8**, *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-methylenedioxy-3,4,5,

Table 2. NMR Data (400 MHz, $CDCl_3$) for Compounds 4, 5, and 6

	4		5		6	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	41.8	2.68, ddd (7.2, 5.2, 2.0)	42.2	2.66, br, dd (10.6, 5.4)	41.9	2.69, br, ddd (6.4, 5.2, 1.6)
2	40.1	2.34, dt (8.2, 5.6)	40.4	2.36, br, dt (10.8, 5.4)	40.4	2.36, dt (8.8, 5.6)
3	39.5	1.62, m	40.3	1.60, m	39.8	1.62, m
4	39.3	1.63, m	39.0	1.74, br, t (7.6)	39.0	1.73, m
5	40.2	2.22, br, t (6.4)	40.0	2.22, br, t (6.2)	40.2	2.22, t (6.4)
6	38.5	1.51, m	38.9	1.57, m	38.9	1.53, m
		1.89, ddd (12.8, 7.6, 5.6)		1.86, m		1.89, ddd (12.8, 7.6, 5.6)
7	38.2	2.53, t (7.0)	41.7	1.90, m	39.3	2.42, t (4.8)
8	49.0	2.84, d (2.4)	47.3	2.78, dd (8.0, 3.0)	50.2	2.77, d (3.2)
9	34.9	3.00, br, s	37.4	2.55, m	36.1	2.83, dd (9.2, 4.4)
10	132.0	6.21, m	132.5	6.17, br, t (6.8)	132.0	6.20, ddd (8.0, 6.4, 1.2)
11	131.2	6.21, m	130.6	6.23, br, t (6.8)	131.0	6.26, ddd (8.0, 6.4, 1.2)
1′	35.8	1.53, m	36.3	1.48, m	35.8	1.51, m
2′	29.4	1.54, m	27.1	1.29, m	29.4	1.54, m
3′	35.7	2.53, t (7.0)	29.1	1.28, m	35.7	2.53, t (7.0)
4′	136.6		31.7	1.57, m	136.6	
5′	108.8	6.67, d (2.0)	35.6	2.51, t (7.6)	108.8	6.67, d (1.6)
6′	147.4		136.7		147.4	
7′	145.4		108.8	6.67, d (1.6)	145.4	
8′	108.0	6.72, d (8.0)	147.4		108.0	6.72, d (8.0)
9′	121.0	6.62, dd (8.0, 2.0)	145.4		121.0	6.62, dd (8.0, 1.6)
10′			108.0	6.72, d (7.8)		
11′			121.0	6.62, dd (7.8, 1.6)		
1″			157.0	6.87, dd (15.8, 8.0)	46.9	2.99, br, dd (13.3, 6.1)
						3.05, br, dd (13.3, 6.0)
2″			118.8	5.69, d (15.8)	28.5	1.71, m
3″					20.01	0.89, d (6.8)
4″					20.01	0.89, d (6.8)
NH						5.43, br, t (6.0)
OCH_2O	100.7	5.91, s	100.7	5.91, s	100.7	5.91, s
C=O	180.5		171.7		174.5	

5′-tetramethoxy-7,7′-epoxylignan, and *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan were obtained at the concentration range of 0.1 to 100 μM, and the results are shown in Table 3. The anti-inflammatory activities of endiandric acid derivatives (**1**−**3**, **5**, **6**) were greater than those of the lignans. The sensitivity of inhibition on NO production of **6** was higher than that of lignans **7** and **8**. At the lower concentrations (0.1−25 μM), **6** produced a concentration-dependent anti-iNOS effect, but tended to a plateau when concentration was more than 25 μM. On the other hand, the inhibitory patterns of **7** and **8** were similar and only started showing results above 25 μM. The E_{max} value of **6** was 64.21%, and those of **7** and **8** were 15.87% and 18.94%, respectively, at 25 μM. The NO inhibitory activity of **1**, with a different tetracyclic moiety, was stronger than that of **4**. Among the endiandric acid analogues and positive controls, endiandramides A (**3**) and B (**6**), with an *N*-isobutylamido group, displayed the more potent anti-inflammatory effects.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 digital polarimeter. IR spectra (KBr or neat) were taken on a Perkin-Elmer System 2000 FT-IR spectrometer. 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, NOESY, HSQC, HMBC) NMR spectra using $CDCl_3$ as solvent were recorded on Varian Germini 2000-200 (200 MHz for ^{1}H NMR, 50 MHz for ^{13}C NMR), Varian Unity Plus 400 (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR), and Varian VNMRS-600 (600 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) spectrometers. Chemical shifts were internally referenced to the solvent signals in $CDCl_3$ (^{1}H, δ 7.26; ^{13}C, δ 77.0), with TMS as the internal standard. Low-resolution ESIMS were obtained on an API 3000 mass spectrometer (Applied Biosystems) and high-resolution ESIMS on a Bruker Daltonics APEX II 30e mass spectrometer. Silica gels (70-230, 230−400 mesh) (Merck) were used for column chromatography (CC), and silica gel 60 F-254 (Merck) was used for analytical and preparative TLC. A spherical C18 100A column (20−40 μM) (Silicycle) was used for medium-pressure liquid chromatography.

Plant Material. Roots of *B. tsangii* were collected at Mudan, Pingtung County, Taiwan, in April 2009, and identified by one of the authors (I.-S.C.). A voucher specimen (Chen 6120) was deposited in the Herbarium of the School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. The dried roots (7.9 kg) of *B. tsangii* were sliced and extracted with cold MeOH (10 L) three times. The concentrated MeOH extract (280 g) inhibited nitrite production below

dx.doi.org/10.1021/np200279r | *J. Nat. Prod.* 2011, 74, 1875–1880

Table 3. Mean E_{max} and IC_{50} of Isolates from Roots of *Beilschmiedia tsangii* on Nitrite Production Induced by LPS in RAW 264.7 Cells

compound	E_{max} (%)[a]	IC_{50} (μM)[b]
tsangibeilin A (**1**)	96.92 ± 0.62	49.59 ± 0.64
tsangibeilin B (**2**)	96.07 ± 0.51	42.30 ± 1.06
endiandric acid K (**4**)	77.94 ± 1.31	58.21 ± 0.19
endiandric acid L (**5**)	91.42 ± 2.03	39.56 ± 2.30
endiandramide A (**3**)	97.32 ± 0.25	9.59 ± 2.28
endiandramide B (**6**)	72.07 ± 1.07	16.40 ± 3.32
beilschminol A (**7**)	86.60 ± 0.29	51.54 ± 0.50
tsangin C (**8**)	85.45 ± 0.08	51.42 ± 1.63
rel-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan	63.41 ± 0.77	48.97 ± 1.68
rel-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-dimethylene-dioxy-5,5′-dimethoxy-7,7′-epoxylignan	65.62 ± 2.48	52.45 ± 3.70
aminoguanidine[c] (a selective iNOS inhibitor)	80.35 ± 0.26	26.55 ± 0.48
N^{ω}-nitro-L-arginine[c] (a nonselective iNOS inhibitor)	43.72 ± 0.76	152.46 ± 10.53

[a] E_{max} indicates mean maximum inhibitory effect, at a concentration of 100 μM, expressed as a percentage inhibition of nitrite production induced by LPS (200 ng/mL) in the presence of vehicle. [b] IC_{50} means the concentration producing 50% E_{max} (n = 4−6 in each group). [c] Positive control.

25%, with no observed cytotoxicity at 100 μg/mL. The MeOH extract was partitioned using EtOAc−H_2O (1:1) to obtain EtOAc-soluble (120 g) and H_2O-soluble (80 g) fractions. The EtOAc solubles (100 g) were applied to a silica gel column (70−230 mesh, 2.2 kg), eluting with a solvent gradient of *n*-hexane−EtOAc to obtain fractions 1−13. Fractions 4−13 showed inhibition of nitrite production using the anti-iNOS assay, and the H_2O-soluble fraction had no inhibitory activity. Fraction 6 (3.0 g) was crystallized from MeOH to obtain β-sitosterol (193 mg). CC of fraction 8 (9.6 g) on silica gel (230−400 mesh, 500 g), eluting with a gradient of CH_2Cl_2−EtOAc yielded fractions 8-1 to 8-10. Fraction 8-2 (31.1 mg) was subjected to a silica gel column (15 g), eluting with *n*-hexane−acetone (10:1), to afford *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan (4.0 mg). Fraction 8-4 (1.03 g) was applied to an RP-C_{18} column (55 g), eluting with MeOH−H_2O (5:1), to provide vanillin (1.5 mg), *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan (800 mg), **3** (15.6 mg), and **6** (13.8 mg). Fraction 8-5 (416 mg) was subjected to RP-C_{18} CC (55 g), eluting with acetone−H_2O (3:2), to obtain fractions 8-5-1 to 8-5-14 and to produce **4** (35.3 mg). Fraction 8-5-6 (11.7 mg) was applied to PTLC (*n*-hexane−acetone, 3:1) to obtain **7** (3.5 mg). Fraction 8-7 (1.97 g) was submitted to RP-C_{18} CC (55 g), eluting with MeOH−H_2O (10:1), to obtain fractions 8-7-1 to 8-7-14. Fraction 8-7-7 (198 mg) was subjected to silica gel CC (30 g), eluting with *n*-hexane−acetone (6:1), to afford **2** (32.9 mg). Fraction 8-7-14 (475 mg) was subjected to the treatment described above to obtain 6β-hydroxystigmast-4-en-3-one (23.5 mg). Fraction 8-7-10 (86.8 mg) was chromatographed on silica gel (15 g), eluting with *n*-hexane−acetone (5:1), to obtain **1** (10.0 mg). Fraction 8-7-11 (89.3 mg) yielded **5** (8.6 mg). Fraction 9 (17.8 g), upon silica gel CC (230−400 mesh, 500 g), eluting with a gradient of *n*-hexane−acetone, provided fractions 9-1 to 9-16. Fraction 9-6 (1.5 g), upon silica gel CC (55 g), eluting with *n*-hexane−EtOAc (5:1), gave fractions 9-6-1 to 9-6-14. Fraction 9-6-8 (24.0 mg), upon PTLC (CH_2Cl_2−MeOH, 40:1), yielded *rel*-(7*S*,8*S*,7′*R*,8′*R*)-3,3′,4,4′,5,5′-hexamethoxylignan (8.8 mg). Fraction 9-9 (587 mg), upon RP-C_{18} CC (55 g), eluting with acetone−H_2O (1:1), yielded fractions 9-9-1 to 9-9-21. Fraction 9-9-8 (6.5 mg) was applied to PTLC (*n*-hexane−EtOAc, 2:1) to obtain **8** (3.4 mg).

*Tsangibeilin A (**1**):* yellowish oil; $[\alpha]^{27}_{D}$ ±0 (c 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 sh (3.59), 288 (3.44) nm; IR (neat) ν_{max} 3432 (OH), 1696 (C═O), 1040, 934 (OCH_2O) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; ESIMS m/z 401 $[M + Na]^+$; HRESIMS m/z 401.1726 $[M + Na]^+$ (calcd for $C_{24}H_{26}O_4Na$, 401.1729).

*Tsangibeilin B (**2**):* yellowish oil; $[\alpha]^{24}_{D}$ ±0 (c 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 235 (3.65), 285 (3.64) nm; IR (neat) ν_{max} 3402 (OH), 1704 (C═O), 1040, 936 (OCH_2O) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; ESIMS m/z 373 $[M + Na]^+$; HRESIMS m/z 373.1418 $[M + Na]^+$ (calcd for $C_{22}H_{22}O_4Na$, 373.1416).

*Endiandramide A (**3**):* yellowish oil; $[\alpha]^{25}_{D}$ ±0 (c 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 233 (3.69), 285 (3.58) nm; IR (neat) ν_{max} 3297 (NH), 1647 (C═O), 1039, 933 (OCH_2O) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; ESIMS m/z 428 $[M + Na]^+$; HRESIMS m/z 428.2200 $[M + Na]^+$ (calcd for $C_{26}H_{31}NO_3Na$, 428.2202).

*Endiandric acid K (**4**):* yellowish oil; $[\alpha]^{25}_{D}$ ±0 (c 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 233 (3.64), 286 (3.57) nm; IR (neat) ν_{max} 3431 (OH), 1701 (C═O), 1040, 936 (OCH_2O) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 2; ESIMS m/z 375 $[M + Na]^+$; HRESIMS m/z 375.1571 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_4Na$, 375.1572).

*Endiandric acid L (**5**):* yellowish oil; $[\alpha]^{26}_{D}$ ±0 (c 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 235 (3.58), 286 (3.31) nm; IR (neat) ν_{max} 3446 (OH), 1693 (C═O), 1040, 936 (OCH_2O) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 2; ESIMS m/z 429 $[M + Na]^+$; HRESIMS m/z 429.2044 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_4Na$, 429.2042).

*Endiandramide B (**6**):* yellowish oil; $[\alpha]^{25}_{D}$ ±0 (c 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 233 (3.58), 286 (3.52) nm; IR (neat) ν_{max} 3314 (NH), 1647 (C═O), 1040, 933 (OCH_2O) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 2; ESIMS m/z 430 $[M + Na]^+$; HRESIMS m/z 430.2356 $[M + Na]^+$ (calcd for $C_{26}H_{33}NO_3Na$, 430.2358).

*Beilschminol A (**7**):* pale yellowish oil; $[\alpha]^{28}_{D}$ −43.4 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.43), 243 sh (3.60), 275 (3.11) nm; IR (neat) ν_{max} 3440 (OH), 1596, 1509, 1455 (aromatic ring), 1034, 932 (OCH_2O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.62 (1H, d, J = 2.0 Hz, H-6), 6.58 (2H, s, H-2′, 6′), 6.54 (1H, d, J = 2.0 Hz, H-2), 5.96 (each 1H, each d, J = 1.4 Hz, OCH_2O), 5.74 (1H, br s, OH, D_2O exchangeable), 4.58 (1H, d, J = 9.2 Hz, H-7′), 4.57 (1H, d, J = 9.2 Hz, H-7), 3.92 (3H, s, OCH_3-5′), 3.88 (3H, s, OCH_3-5), 3.88 (3H, s, OCH_3-4), 1.79 (1H, m, H-8′), 1.72 (1H, m, H-8), 1.06 (3H, d, J = 6.0 Hz, H-9), 1.04 (3H, d, J = 6.0 Hz, H-9′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.4 (C-5), 149.1 (C-1), 148.8 (C-3′), 143.5 (C-5′), 138.6 (C-3), 137.1 (C-1′), 134.8 (C-4), 134.5 (C-4′), 105.8 (C-6), 105.5 (C-2′), 101.8 (C-2), 101.4 (OCH_2O), 100.3 (C-6′), 88.4 (C-7′), 88.2 (C-7), 60.9 (OCH_3-4), 56.6 (OCH_3-5′), 55.9 (OCH_3-5), 51.1 (C-8), 50.8 (C-8′), 14.0 (C-9), 13.8 (C-9′); ESIMS m/z 425 $[M + Na]^+$; HRESIMS m/z 425.1574 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_7Na$, 425.1576).

*Tsangin C (**8**):* yellowish oil; $[\alpha]^{26}_{D}$ −10.2 (c 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (4.51), 269 (3.85) nm; IR (neat) ν_{max} 1716 (C═O), 1591, 1505, 1462 (aromatic ring) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.24 (2H, s, H-2′, H-6′), 6.63 (2H, s, H-2, H-6), 5.90 (1H, d,

J = 10.2 Hz, H-7), 3.89 (3H, s, OCH_3-4′), 3.88 (6H, s, OCH_3-3′, OCH_3-5′), 3.87 (6H, s, OCH_3-3, OCH_3-5), 3.82 (3H, s, OCH_3-4), 3.21 (1H, dd, J = 10.2, 7.2 Hz, H-8), 2.25 (3H, s, H-10), 1.00 (3H, d, J = 7.2 Hz, CH_3-8); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 209.6 (C-9), 165.0 (C-7′), 153.4 (C-3, C-5), 153.0 (C-3′, C-5′), 142.5 (C-4′), 138.1 (C-4), 133.6 (C-1), 124.8 (C-1′), 106.9 (C-2′, C-6′), 104.3 (C-2, C- 6), 78.7 (C-7), 60.9 (OCH_3-4′), 60.8 (OCH_3-4), 56.3 (OCH_3-3′, OCH_3-5′), 56.2 (OCH_3-3, OCH_3-5), 52.5 (C-8), 28.5 (C-10), 13.7 (CH_3-8); ESIMS m/z 485 $[M + Na]^+$; HRESIMS m/z 485.1790 $[M + Na]^+$ (calcd for $C_{24}H_{30}O_9Na$, 485.1787).

Anti-iNOS Activity Assay. RAW 264.7 cells, a transformed murine macrophage cell line, obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan), were maintained by once-weekly passage in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum and penicillin− streptomycin.

Nitrite measurement was based on our published technique.[21] Cell aliquots (5×10^5 cells/mL) were grown to confluence on 24-well plates for 24 h. The medium was changed to serum-free DMEM for another 4 h to render the attached cells quiescent. To assess the effects on LPS-induced NO production, compounds and the two positive controls N^{ω}-nitro-L-arginine (L-NNA, a nonselective NOS inhibitor; 100 μM) and aminoguanidine (a selective iNOS inhibitor; 100 μM) or vehicle (DMSO; 0.1%) were added in the absence or presence of LPS (200 ng/mL) to the cells for another 24 h. The culture supernatant was subsequently collected for nitrite assay as a reflection of NO production.[22] An aliquot of supernatant was mixed with an equal volume of Griess reagent (prepared by adding 1 part 0.1% napthylethylenediamine dihydrochloride to 1 part 1% sulfanilamide in 5% phosphoric acid) and incubated at room temperature for 10 min. The absorbance at 550 nm was measured using a microplate spectrophotometer (Bio-Tek Instrument, Inc., Winooski, VT, USA). Fresh medium was used as the blank. The nitrite concentration was determined by reference to a standard curve, using sodium nitrite diluted in the stock culture medium. Results are expressed as percentage of inhibition calculated versus vehicle plus LPS-treated cells.

Cell Viability Assay. A redox indicator, Alamar Blue, was used to measure cytotoxicity as described previously.[23] After culture supernatant was removed for NO measurement as described above, a solution of 10% Alamar Blue in DMEM was added to each well containing RAW 264.7 cells. The plates were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 3 h. Following incubation, the absorbance of the Alamar Blue was read spectrophotometrically at dual wavelengths, 570 and 600 nm, against the blank prepared from cell-free wells. The absorbance in cultures treated with LPS plus vehicle was regarded as indicating 100% cell viability.

Statistical Analysis. For each experimental series, data are given as mean ± SE, with n representing the number of independently performed experiments. All data were analyzed using an IBM-compatible statistical software package (SPSS for Windows, Ver. 10.0). Significance of the concentrations and sample treatments was determined by two-way analysis of variance (ANOVA) with repeated measurements. When significant interactions did take place, the simple main effect of each factor was assessed using Kruskal−Wallis nonparametric ANOVA. Post hoc comparisons were carried out between means, according to the suitability. A p value of less than 0.05 was considered to indicate a statistically significant difference.

■ ASSOCIATED CONTENT

S Supporting Information. 1D and 2D NMR, including Figures 1 and 2, for compounds **1**−**8**. This information is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work was kindly supported by the National Science Council of the Republic of China (NSC 99-2320-B-037-009).

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