Article

Secondary Metabolites from the Roots of *Beilschmiedia tsangii* and Their Anti-Inflammatory Activities

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Abstract: Four new endiandric acid analogues, tsangibeilin C (**1**), tsangibeilin D (**2**), tricyclotsangibeilin (**3**) and endiandric acid M (**4**), one new lignan, beilschminol B (**5**) and two new sesquiterpenes, (+)-5-hydroxybarbatenal (**6**) and (4*R*,5*R*)-4,5-dihydroxycaryophyll-8(13)-ene (**7**), together with four known compounds (**8–11**), were isolated from the roots of *Beilschmiedia tsangii* (Lauraceae). The structures of **1–7** were determined by spectroscopic techniques. Among the isolates, endiandric acid M (**4**) exhibited moderate iNOS inhibitory activity, with an IC_{50} value of 31.70 µM.

Keywords: *Beilschmiedia tsangii*; Lauraceae; root; endiandric acid; anti-inflammatory activity

1. Introduction

Over 40 species of Formosan lauraceous plants have been screened for anti-inflammatory activity using an inducible nitric oxide synthase (iNOS) assay. A methanolic extract of the roots of *B. tsangii* Merr. (Lauraceae) has shown potent inhibition of NO production, with no cytotoxicity against RAW 264.7 cells. *B. tsangii* is a medium-sized evergreen tree, distributed throughout Tonkin, Vietnam, southern and western China and southern Taiwan. About 200 Beilschmiedia species are found in these tropical regions, including two species in Taiwan [1]. Our previous study reported two new tetrahydrofuran-type lignans, beilschmin A and beilschmin B, two new 1-phenylbutyl benzoates, tsangin A and tsangin B, together with thirteen known compounds isolated from the stem of this plant [2]. One year later, three new epoxyfuranoid lignans, 4α,5α-epoxybeilschmin A, 4α,5α-epoxybeilschmin B and beilschmin D, together with nine known compounds, were obtained from the leaves [3]. More recently, six new endiandric acid analogues, tsangibeilin A, tsangibeilin B, endiandramide A, endiandric acid K, endiandric acid L and endiandramide B, two new lignans, beilschminol A and tsangin C, and six known compounds have been obtained from the roots of this species [4]. In this continuation of our research, four new endiandric acid analogues, tsangibeilin C (**1**), tsangibeilin D (**2**), tricyclotsangibeilin (**3**) and endiandric acid M (**4**), one new lignan, beilschminol B (**5**) and two new sesquiterpenes: (+)-5-hydroxybarbatenal (**6**) and (4*R*,5*R*)-4,5-dihydroxycaryophyll-8(13)-ene (**7**), together with four known compounds (**8–11**), have been isolated from the roots of this plant. The structures of **1–7** were determined by spectroscopic techniques. This paper elucidates the structures of **1–7** (Figure 1). Endiandric acid M (**4**) exhibited moderate iNOS inhibitory activity, with an IC_{50} value of 31.70 μM.

Figure 1. Structures of compounds **1–11**.

Figure 1. *Cont.*

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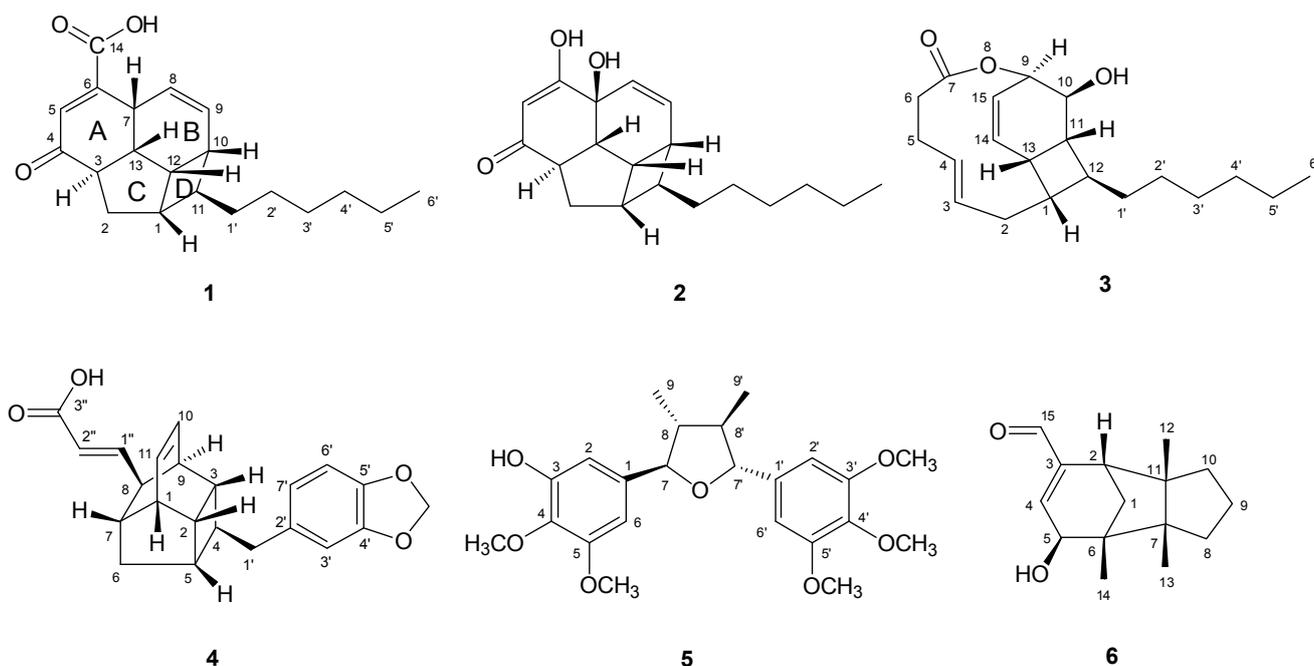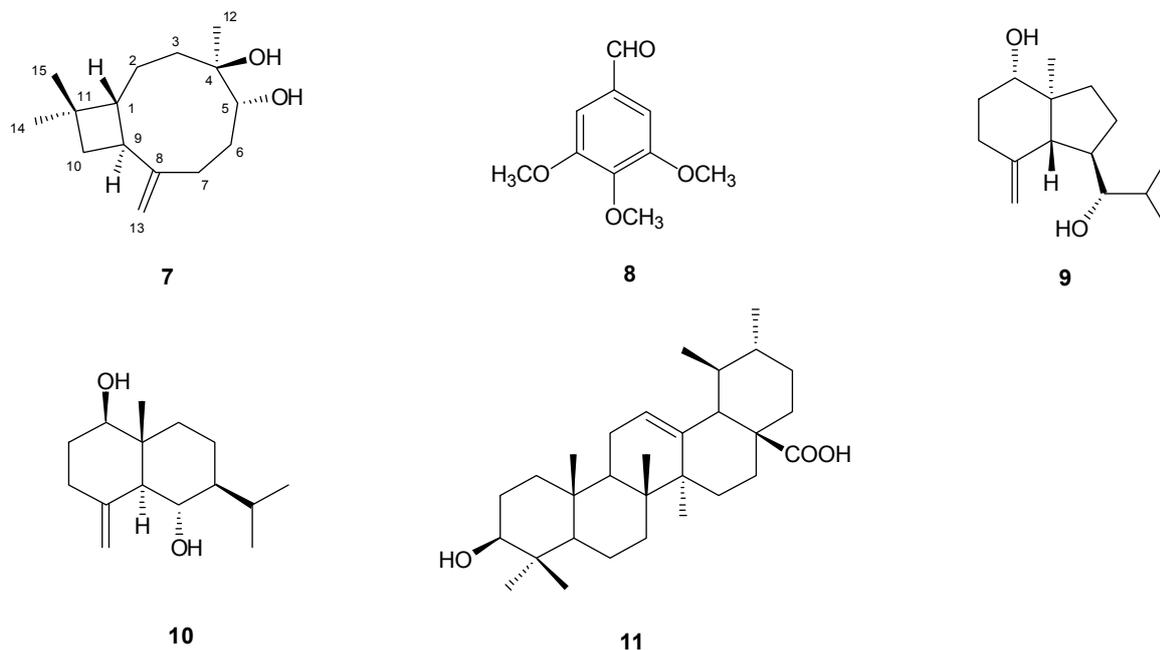
2. Results and Discussion

2.1. Structure Elucidation

Compound **1** was isolated as a light yellowish oil. Its molecular formula was established as $C_{20}H_{26}O_3$ by ESIMS and HRESIMS, with eight unsaturated degrees. The IR spectrum showed absorptions at 1696 cm^{-1} for carbonyl groups (δ 201.6, C-4 and δ 168.3, C-14) and at 3432 cm^{-1} for a hydroxy group of carboxylic acid. These findings were supported by ^{13}C NMR spectrum. The ^{1}H, ^{13}C NMR (Table 1), COSY (Figure 2), HSQC and HMBC (Figure 2) spectra of **1** were similar to those of beilschmiedic acid D [5] and also contained 13 skeletal signals of an endiandric acid moiety. The characteristic two *cis* olefinic protons at δ 5.56 (ddd, *J* = 10.2, 3.0, 1.8 Hz, H-8) and 5.85 (ddd, *J* = 10.2, 4.2, 3.0 Hz, H-9) in **1** were similar to those of beilschmiedic acid D, but the signal for another olefinic proton in **1** was shifted upfield to δ 6.70 (d, *J* = 1.2 Hz, H-5), because a carbonyl group (δ_C 201.6, C-4) in **1** replaced a methylene group [δ 2.06 (m, H_a-4) and 2.54 (dt, *J* = 8.8, 3.1 Hz, H_b-4)] in beilschmiedic acid D. The length of the alkyl side chain at C-11 of **1** was two methylenes less than beilschmiedic acid D, as supported by the molecular formula of **1** ($C_{20}H_{26}O_3$). The rigid tetracyclic skeleton was indicated by HMBC correlations, including: H-5 to C-3, C-6, C-7 and C-14, H-3 to C-4, and C-7, H-13 to C-8 and C-10, H-8 to C-6 and C-10, H-9 to C-7, H-2 to C-3, C-4, C-11 and C-13, H-1 to C-3 and C-13, H-12 to C-3, C-9 and C-11 and H-11 to C-9. The relative configuration of **1**, *rel*-(1*S*,3*R*,7*R*,10*R*,11*S*,12*S*,13*S*), was supported on the basis of NOESY (Figure 3) and structural similarity with beilschmiedic acid D [5]. There are seven chiral centers in **1**. However, in view of the observed optical rotation ($[\alpha]_D^{23} \pm 0$), **1** should be racemic, as are many endiandric acid analogues [6–9]. According to the above data, the structure of **1** was elucidated and named tsangibeilin C.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **1** (acetone-d_6) and **2** (CD$_3$OD).

position	1		2	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	41.6 (CH)	2.36, m	42.0 (CH)	2.41, br dd (12.0, 6.0)
2	31.5 (CH$_2$)	1.56, td (12.0, 6.0) 1.65, br dd (12.0, 5.4)	31.0 (CH$_2$)	1.57, td (12.0, 6.0) 1.62, br dd (12.0, 5.4)
3	50.5 (CH)	3.05, ddd (13.5, 12.0, 5.4)	54.3 (CH)	2.94, ddd (13.2, 12.0, 5.2)
4	201.6 (C)	-	203.7 (C)	-
5	135.8 (CH)	6.70, d (1.2)	132.3 (CH)	6.54, s
6	151.5 (C)	-	158.5 (C)	-
7	36.2 (CH)	3.51, br s	71.2 (C)	-
8	123.5 (CH)	5.56, ddd (10.2, 3.0, 1.8)	125.9 (CH)	6.01, br d (10.2)
9	131.5 (CH)	5.85, ddd (10.2, 4.2, 3.0)	132.6 (CH)	5.87, dd (10.2, 4.2)
10	35.8 (CH)	2.40, m	35.9 (CH)	2.47, m
11	48.0 (CH)	1.47, m	47.9 (CH)	1.45, m
12	35.2 (CH)	2.87, m	32.9 (CH)	2.97, br dd (8.4, 6.0)
13	44.8 (CH)	2.35, m	51.9 (CH)	2.24, ddd (13.2, 8.4, 1.2)
14	168.3 (C)	-	-	-
1'	38.4 (CH$_2$)	1.54, m	38.2 (CH$_2$)	1.54, m
2'	28.3 (CH$_2$)	1.28, m	28.0 (CH$_2$)	1.25, m
3'	31.0 (CH$_2$)	1.28, m	30.4 (CH$_2$)	1.28, m
4'	33.2 (CH$_2$)	1.28, m	33.0 (CH$_2$)	1.28, m
5'	23.9 (CH$_2$)	1.28, m	23.7 (CH$_2$)	1.30, m
6'	15.0 (CH$_3$)	0.88, t (7.2)	14.4 (CH$_3$)	0.90, t (6.9)

Figure 2. COSY (——) and HMBC (⌒) connectivities for compounds **1**–**7**.
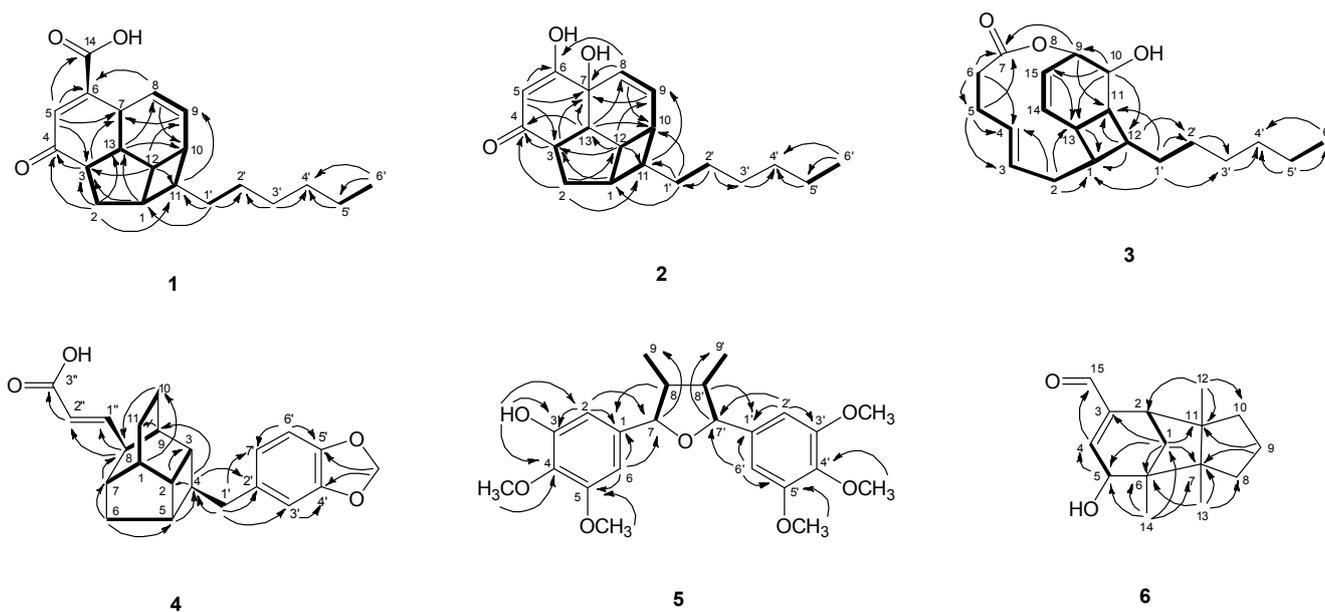
Figure 2. *Cont.*
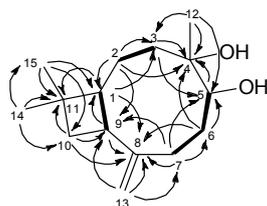
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Analysis of the ESIMS and HREIMS of **2** revealed a molecular formula of $C_{19}H_{26}O_3$, representing seven degrees of unsaturation, one carbon and one degree of unsaturation less than compound **1** ($C_{20}H_{26}O_3$). The IR spectrum indicated the presence of a hydroxy group (3397 cm^{-1}) and a carbonyl group (1692 cm^{-1}). The rigid tetracyclic skeleton of **2** was the same as that of **1**, according to its ^{1}H and ^{13}C NMR spectra (Table 1), inclusive of COSY (Figure 2), NOESY (Figure 3), HSQC and HMBC (Figure 2) experiments. The major differences between **1** and **2** were two hydroxy groups at C-6 and C-7 in **2** were substituted for the carboxyl group (δ 168.3, C-14) and H-7 [δ_H 3.51 (1H, br s)] at C-6 and C-7 in **1**, as supported by HRESIMS, IR and DEPT spectra. The NOESY spectrum (Figure 3) showed correlations between H_a-2, H-3 and H-11, but these three protons showed no correlations with H-1, H_b-2, H-10, H-12 and H-13. This suggested that H_a-2, H-3 and H-11 are on the same side of the molecule, and that H-1, H_b-2, H-10, H-12 and H-13 are on the opposite side of the molecule. The β-orientation of the hydroxy group at C-7 was attributed according to the structural similarity with endiandric acid analogues and biogenetic consideration, where the rings A/B, B/C, C/D and B/D were *cis*-fusion and ring A/C was *trans*-fusion [4,8,9]. The relative configuration of **2**, *rel*-(1*S*,3*R*,7*R*,10*R*,11*S*,12*S*,13*S*) was supported on the basis of NOESY and structural similarity with beilschmiedic acid D [5]. On the basis of the observations above, compound **2** was elucidated and named tsangibeilin D.

Figure 3. NOESY (⌒) connectivities for compounds **1**–**7**.

1　　　　　　　　　　　**2**　　　　　　　　　　　**3**
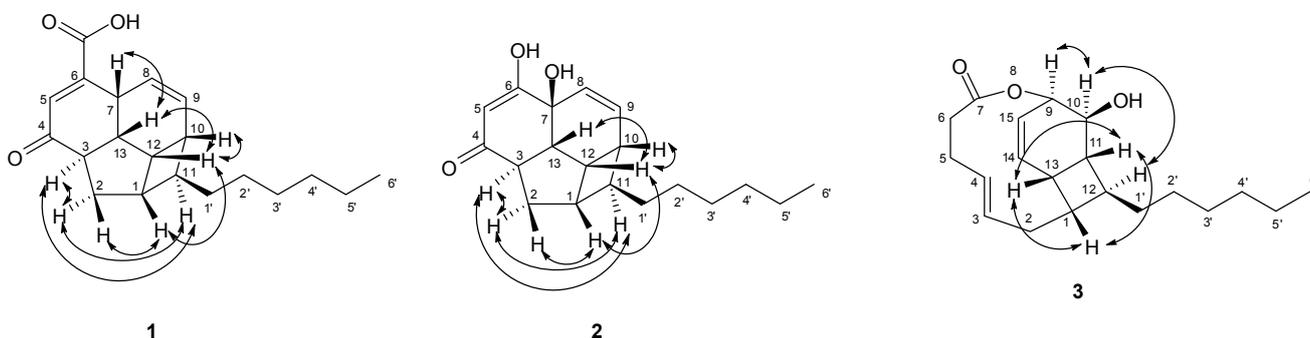
Figure 3. *Cont.*

Compound **3** was obtained as an optically active light yellowish oil with $[\alpha]_D^{24}$ + 36.3 (*c* 0.024, CHCl$_3$). IR absorption bands at 3422 cm^{-1} (OH) and 1729 cm^{-1} (ester carbonyl) were observed. The ESIMS analysis of **3** showed the [M+Na]$^+$ ion at *m/z* 341, in agreement with the molecular formula of C$_{20}$H$_{30}$O$_3$, with six degrees of unsaturation as confirmed by HRESIMS. The ^{13}C NMR (Table 2) and DEPT spectra indicated that **3** contains one methyl, eight methylenes, ten methines and one quaternary carbon. The HSQC and COSY (Figure 2) spectra revealed three fragments, C1-C2-C3-C4-C5-C6, C1-C13-C11-C12-C1 and C-13-C14-C15-C9-C10, and the HMBC (Figure 2) correlations, H-10 to C-9, C-12, C-13 and C-15 and H-9 to C-11, connected the fragments C1-C13-C11-C12-C1 and C-13-C14-C15-C9-C10 to form a cyclohexane ring fused with a cyclobutane ring. The carboxyl group (*δ* 173.5, C-7) connected the fragment C1-C2-C3-C4-C5-C6 with the cyclohexane ring, as supported by HMBC correlations, H-6 to C-4, C-5 and C-7, H-5 to C-3 and C-7 and H-9 to C-7. According to the above evidence, the carboxyl group, the fragment C1-C2-C3-C4-C5-C6, the cyclohexane ring and the cyclobutane ring become a cyclododecane ring, which is also supported by six degrees of unsaturation. The HMBC spectrum showed correlations between H-1' and C-1, C-11, C-2' and C-3', thus establishing the presence of an alkyl chain (C-1'~C-6') to be connected at C-12. Finally, the HRESIMS, IR, and DEPT spectra indicated the presence of a hydroxy group located at C-10. The NOESY spectrum (Figure 3) showed correlations between H-9, H-10 and H-12, suggesting these three protons were on the same side of the molecule, but H-1, H-11 and H-13 showed no correlations with H-9, H-10 and H-12, which should therefore be on the opposite side of the molecule. The relative configuration of **3**, *rel*-(1*S*,9*R*,10*S*,11*R*,12*S*,13*S*), was supported on the basis of NOESY and its structural similarity with know endiandric acid analogues. Based on further spectrographic evidence, the structure of **3** was elucidated and designated as tricyclotsangibeilin.

Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **3** (CDCl$_3$) and **4** (acetone-d_6).

position	3		4	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	41.9 (CH)	2.37, m	43.7 (CH)	2.72, br dd (12.0, 5.4)
2	31.8 (CH$_2$)	1.28, m	41.3 (CH)	2.45, dt (9.0, 5.4)
		1.99, ddd (14.4, 9.6, 4.8)		
3	132.3 (CH)	5.50, ddd (15.6, 9.6, 6.0)	41.1 (CH)	1.72, m
4	130.1 (CH)	5.24, ddd (15.6, 9.6, 5.4)	41.2 (CH)	2.25, br t (7.8)
5	31.2 (CH$_2$)	2.38, m	41.0 (CH)	2.36, m
6	34.3 (CH$_2$)	2.29, ddd (11.4, 5.4, 2.4)	39.9 (CH$_2$)	1.63, br d (12.3)
		2.22, m		1.87, br dd (12.3, 5.4)
7	173.5 (C)	-	43.4 (CH)	1.89, br dd (9.0, 5.4)
8	-	-	48.2 (CH)	2.91, br dd (8.2, 3.6)
9	70.1 (CH)	4.87, dt (5.6, 2.4)	38.8 (CH)	2.53, m
10	67.2 (CH)	3.96, br d (7.2)	134.2 (CH)	6.19, br t (7.2)
11	40.8 (CH)	2.21, m	131.9 (CH)	6.25, br t (7.2)
12	33.3 (CH)	3.18, br dt (15.6, 7.5)	-	-
13	30.9 (CH)	3.02, m	-	-
14	136.0 (CH)	6.16, dd (10.4, 6.0)	-	-
15	123.9 (CH)	6.25, dd (10.4, 5.6)	-	-
1'	36.3 (CH$_2$)	1.47, q (7.5)	43.1 (CH$_2$)	2.78, dd (15.6, 7.8)
				2.81, br d (7.8)
2'	27.7 (CH$_2$)	1.27-1.33, m	136.5 (C)	-
3'	29.5 (CH$_2$)	1.27-1.33, m	110.5 (CH)	6.75, d (1.8)
4'	31.9 (CH$_2$)	2.24, m	149.2 (C)	-
5'	22.7 (CH$_2$)	1.27-1.33, m	147.3 (C)	-
6'	14.1 (CH$_3$)	0.90, t (6.9)	109.3 (CH)	6.74, d (7.8)
7'	-	-	123.1 (CH)	6.68, dd (7.8, 1.8)
1"	-	-	155.3 (CH)	6.72, dd (15.6, 8.2)
2"	-	-	121.3 (CH)	5.71, dd (15.6, 0.9)
3"	-	-	168.4 (C)	-
OCH$_2$O	-	-	102.3 (CH$_2$)	5.94, s

ESIMS and HRESIMS data of compound **4** indicated the molecular formula C$_{22}$H$_{22}$O$_4$, four methylenes less than endiandric acid L [4] with 12 degrees of unsaturation, which was the same as endiandric acid L. The UV, IR, ^{1}H NMR (Table 2) and ^{13}C NMR (Table 2) spectra were similar to those of endiandric acid L, except that a methylene group [δ 2.78 (1H, dd, *J* = 15.6, 7.8 Hz, H$_a$-1'), 2.81 (1H, br d, *J* = 7.8 Hz, H$_b$-1')] in **4** replaced the pentamethylene group in endiandric acid L. The relative configuration of **4** was supported by NOESY spectrum and its structural similarity with endiandric acid L [4]. Thus, compound **4** was named endiandric acid M. Compounds **1**, **2** and **4** were racemic, and all structures were determined by ^{13}C NMR, NOESY, COSY, HSQC and HMBC experiments (see Figures 2 and 3).

Compound **5** was obtained as an optically active light yellowish oil, $[\alpha]_D^{25}$ −18.6 (*c* 0.18, MeOH), and its molecular formula was established as C$_{23}$H$_{30}$O$_7$ by ESIMS and HRESIMS. UV absorptions at 209, 240 sh, and 270 nm, in conjunction with a bathochromic shift after the addition of alkali,

indicated a phenolic benzenoid moiety. An IR absorption band at 3415 cm^{-1} (OH) was also observed. The ^{1}H NMR signals for a 7,7'-epoxylignan moiety [δ 1.07 (3H, d, *J* = 6.0 Hz, H-9'), 1.08 (3H, d, *J* = 6.0 Hz, H-9), 1.78 (1H, m, H-8), 1.78 (1H, m, H-8'), 4.61 (1H, d, *J* = 8.8 Hz, H-7) and 4.63 (1H, d, *J* = 9.2 Hz, H-7')] were similar to those of beilschminol A [4], except that OCH$_3$-3' [δ 3.88 (3H, s)] and OCH$_3$-4' [δ 3.83 (3H, s)] in **5** replaced a methylenedioxy group [δ 5.96 (2H, each d, *J* = 1.4 Hz)] in beilschminol A. Thus, the planar structure of **5** was proposed to be 3-hydroxy-4,5,3',4',5'-pentamethoxy-7,7'-epoxylignan. The relative configuration of **5** was based on the NOESY spectrum and its structural similarity with (7*R*,8*R*,7'*R*,8'*R*)-3-hydroxy-3',4'-methylenedioxy-4,5,5'-trimethoxy-7,7'-epoxylign [4]. Thus, compound **5** was elucidated as *rel*-(7*R*,8*R*,7'*R*,8'*R*)-3-hydroxy-4,5,3',4',5'-pentamethoxy-7,7'-epoxylignan and named beilschminol B.

Compound **6** was isolated as an optically active light yellowish oil, $[\alpha]_D^{25}$ + 123.8 (*c* 0.06, CHCl$_3$). The HRESIMS analysis of **6** gave a quasi-molecular ion peak at *m/z* 257.1516 [M+Na]$^+$ (calcd. for C$_{15}$H$_{22}$O$_2$Na, 257.1517), consistent with a molecular formula of C$_{15}$H$_{22}$O$_2$ with five degrees of unsaturation. The IR, ^{1}H, and ^{13}C NMR (Table 3) spectra resembled those of (+)-barbatenal [10], except that a hydroxy group and an oxymethine proton [δ 4.09 (1H, d, *J* = 3.6, H-5)] in C-5 (δ$_c$ 71.2) of **6** replaced a methylene [δ 1.65 (1H, dd, *J* = 21.1, 3.3 Hz, H$_a$-5), 2.04 (1H, dd, *J* = 20.1, 2.8 Hz, H$_b$-5)] in C-5 (δ$_c$ 41.9) of (+)-barbatenal, resulting in the downshifting of the olefinic proton (δ$_H$ 6.60, H-4) in **6** from δ$_H$ 5.87 (H-4) in (+)-barbatenal. The relative configuration of **6** was determined by NOESY spectrum, where H$_a$-1 showed correlations with H-2, H-12, H-13 and H-14, but no correlation with H-5. It has been suggested that H$_a$-1, H-2, H-12, H-13 and H-14 are on the same side of the molecule and that H$_b$-1 and H-5 are on the opposite side of the molecule. Furthermore, H-5 showed a correlation with H-14 due to a spatial distance between these two protons of <4 Å, as supported by simulating the ChemDraw 3D Ultra (version 10.0). Based on the NOESY spectrum, the optical rotation and comparison to values in the literature, the relative configuration of **6** was determined to be *rel*-(2β, 5α, 12β, 13β, 14β). Hence, the structure of **6** was elucidated and designated as (+)-5-hydroxybarbatenal, which was further confirmed by DEPT, HSQC, NOESY (Figure 3) and HMBC (Figure 2) techniques.

Compound **7** was isolated as an optically active light yellowish oil with $[\alpha]_D^{25}$ −23.9 (*c* 0.032, CHCl$_3$). IR absorption bands at 3396 cm^{-1} (OH) and 1637 cm^{-1} (C=C) were observed. From the HRESIMS data, the molecular formula was determined to be C$_{15}$H$_{26}$O$_2$ (*m/z* 261.1828 [M+Na]$^+$; calcd. 261.1830). The ^{13}C NMR (Table 3) and DEPT spectra indicated that **7** contains three methyls, six methylenes, three methines and three quaternary carbons, suggesting a sesquiterpene skeleton. The HSQC and COSY (Figure 2) spectra revealed the correlations from C1-C9, C2-C3 and C-5-C6-C7 and the HMBC (Figure 2) correlations, H-1 to C-3, C-8 and C-11, H-10 to C-8, C-9 and C-11, H-9 to C-1 and C-8, H-7 to C-5, C-6, C-8 and C-9, H-6 to C-4, C-5 and C-8, H-3 to C-4 and C-5 and H-2 to C-1, C-3, C-4 and C-9, connected the fragments C1-C9, C2-C3 and C-5-C6-C7 to form a cyclononane ring and a cyclobutane ring. HMBC correlations, H-15 to C-1, C-10 and C-11, H-14 to C-10, C-11 and C-15, H-12 to C-3, C-4 and C-5, H-13 to C-7, C-8 and C-9, confirmed that three methyl groups were connected at C-4 and C-11 and that a terminal double bond was present at C-8. Finally, the HRESIMS, IR, DEPT spectra indicated two hydroxy groups located at C-4 and C-5, respectively. The NOESY spectrum (Figure 3) showed correlations between H$_b$-7, H-9, H-12 and H-14, suggesting these three protons were on the same side of the molecule, but H-1, H-5, H$_a$-7 and H-15 showed no correlations with H$_b$-7, H-9, H-12 and H-14 and, therefore, should be on the opposite side of the molecule. The

structure of **7** was elucidated as (4*R*, 5*R*)-4,5-dihydroxycaryophyll-8(13)-ene and identified with its synthetic compound [11]. Though compound **7** has previously been reported as a reduction intermediate [11], our study is the first time to have isolated this compound from a natural source.

Table 3. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **6** (CDCl$_3$) and **7** (CDCl$_3$).

position	6		7	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	36.9 (CH$_2$)	1.37, br d (12.0)	56.9 (CH)	1.65, ddd (10.2, 3.6, 1.8)
		1.69, dd (12.0, 4.2)		
2	42.8 (CH)	2.64, d (4.2)	23.2 (CH$_2$)	1.33, m
				1.66, m
3	147.7 (C)	-	40.8 (CH$_2$)	1.56, ddd (15.0, 8.4, 1.8)
				1.93, ddd (15.0, 11.1, 2.1)
4	147.5 (CH)	6.60, dd (3.6, 0.6)	75.1 (C)	-
5	71.2 (CH)	4.09, d (3.6)	73.3 (CH)	3.60, br dd (6.0, 2.4)
6	48.7 (C)	-	32.6 (CH$_2$)	1.57, m
				1.75, m
7	56.9 (C)	-	34.7 (CH$_2$)	2.05, m
				2.43, ddd (13.2, 9.0, 4.2)
8	36.2 (CH$_2$)	1.10, m	151.8 (C)	-
		1.58, m		
9	38.6 (CH$_2$)	1.25, m	42.3 (CH)	2.37, q (10.2)
10	27.1 (CH$_2$)	1.32, br dd (6.9, 0.9)	36.0 (CH$_2$)	1.58, m
		1.54, m		1.74, t (10.2)
11	54.9 (C)	-	34.1 (C)	-
12	26.9 (CH$_3$)	1.089, s	21.5 (CH$_3$)	1.14, s
13	24.2 (CH$_3$)	0.96, s	110.5 (CH$_2$)	4.92, d (1.2)
				4.94, d (1.2)
14	18.7 (CH$_3$)	1.087, s	22.1 (CH$_3$)	0.98, s
15	193.3 (CH)	9.54, s	30.1 (CH$_3$)	1.00, s
OH	-	-	-	2.07, br s
OH	-	-	-	2.29, br d (3.0)

The known compounds, 3,4,5-trimethoxybenzaldehyde (**8**) [12], octahydro-4-hydroxy-3α-methyl-7-methylene-α-(1-methylethyl)-1*H*-indene-1-methanol (**9**) [13,14], eudesm-4(15)-ene-1β,6α-diol (**10**) [15,16] and ursolic acid (**11**) [17] were identified by comparison of their physical and spectroscopic data with values reported in the literature.

2.2. Anti-inflammatory Activities

E$_{max}$ (%) and IC$_{50}$ (μM) values of iNOS inhibitory activity of five compounds, including compounds **4**, **5** and **11**, and the two compounds, 6β-hydroxystigmast-4-en-3-one and *rel*-(7*S*,8*S*,7'*R*,8'*R*)-3,3',4,4',5,5'-hexmethoxylignan, reported by Huang *et al.*, 2011 [4], were obtained at the concentration range of 0.1 to 100 μM. Results are shown in Table 4. The technique for the

anti-iNOS activity assay was the same as in our previous study [4]. Among the isolates, endiandric acid M (**4**) exhibited moderate iNOS inhibitory activity, with IC_{50} value of 31.70 μM. Endiandramide B [4], endiandric acids K [4], L [4] and M, with the same skeleton but with different substituents at C-8, exhibited ascending degrees of iNOS inhibitory activity in this order: endiandramide B (16.40 μM) > endiandric acid M (**4**) (31.70 μM) > endiandric acid L (39.56 μM) > endiandric acid K (58.21 μM). This suggests that the potency of the substituent at C-8 was an *N*-isobutylamido group > an α,β-unsaturated carboxylic acid group > a carboxylic acid group. Endiandric acid M (**4**), with four fewer methylenes than endiandric acid L, showed stronger potency than endiandric acid L. This suggests that fewer methylenes in the alkyl side of endiandric acid analogues result in greater potency of iNOS inhibitory activity.

Table 4. Mean E_{max} and IC_{50} of isolates from roots of *B. tsangii* on nitrite production induced by LPS in RAW 264.7 cells.

Compounds	E_{max} (%) [a]	IC_{50} (μM) [b]
endiandric acid M (**4**)	97.03 ± 1.30	31.70 ± 0.25
beilschminol B (**5**)	55.62 ± 1.96	95.37 ± 0.52
ursolic acid (**11**)	36.55 ± 4.66	>100
6β-hydroxystigmast-4-en-3-one [c]	11.69 ± 2.91	>100
rel-(7*S*,8*S*,7'*R*,8'*R*)-3,4,5,3',4',5'-hexmethoxylignan[c]	52.67 ± 5.05	98.26 ± 0.13
aminoguanidine [d] (a selective iNOS inhibitor)	80.35 ± 0.26	26.55 ± 0.48
N^{ω}-nitro-L-arginine [d] (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] This compound was reported in Huang *et al.*, 2011 [4]; [d] positive control.

3. Experimental Section

3.1. General Experimental Procedures

All melting points were measured on a Yanaco micro-melting apparatus and were uncorrected. Optical rotations were measured on a Jasco P-1020 digital polarimeter. The UV spectra were obtained with a Jasco V-530 UV/VIS spectrophotometer, and 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$_3$ or acetone-d_6 or CD$_3$OD 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, 150 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) or acetone-d_6 (^{1}H, δ 2.05; ^{13}C, δ 205.1) or CD$_3$OD (^{1}H, δ 3.31; ^{13}C, δ 49.0), with TMS as the internal standard. ESIMS were obtained on an API 3000 mass spectrometer (Applied Biosystems) and HRESIMS 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.

3.2. 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.

3.3. Extraction and Isolation

The dried roots (7.9 kg) of *B. tsangii* were sliced and extracted three times with cold MeOH (10 L) at room temperature. The concentrated MeOH extract (280 g) inhibited nitrite production below 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 CC and eluted with *n*-hexane-EtOAc gradient solvent system (15:1 to 100% EtOAc) to obtain 13 fractions (A-1–A-13). Fractions A-4–A-13 showed inhibition of nitrite production using the anti-iNOS assay, and the H_2O-soluble fraction showed no inhibitory activity. CC of fraction A-8 (9.6 g) on silica gel, eluting with a gradient of CH_2Cl_2–EtOAc, yielded fractions A-8-1 to A-8-10. Fraction A-8-5 (416 mg) was subjected to MPLC (RP-18), eluting with acetone:H_2O (3:2), to obtain fractions A-8-5-1 to A-8-5-14. Fraction 8-5-6 (11.7 mg) was applied to preparative TLC (*n*-hexane:acetone, 3:1) to obtain **6** (2.8 mg). Fraction A-8-5-14 (210 mg) was applied to MPLC (RP-18) eluting with acetone:H_2O (2:1) to give 20 fractions (A-8-5-14-1–A-8-5-14-20). Fraction A-8-5-14-10 (20.8 mg) was separated over MPLC eluting with *n*-hexane:acetone (8:1) to afford **3** (1.2 mg). Fraction A-8-7 (1.97 g) was submitted to MPLC (RP-18), eluting with MeOH:H_2O (10:1), to obtain fractions A-8-7-1 to A-8-7-14. Fraction A-8-7-6 (223 mg) was applied to MPLC and eluted with *n*-hexane: acetone (4:1) to obtain nine fractions (A-8-7-6-1–A-8-7-6-9). Fraction A-8-7-6-7 (5.5 mg) was purified by preparative TLC (RP-18) developed with MeOH:H_2O (15:1) to give **4** (4.4 mg). Fraction A-8-7-11 was subjected to MPLC and eluted with *n*-hexane: acetone (5:1) to yield **11** (5.7 mg). Fraction A-9 (17.8 g), on silica gel CC, eluting with a gradient of *n*-hexane–acetone, provided fractions A-9-1 to A-9-16. Fraction A-9-4 (104 mg) was applied to MPLC (RP-18) and eluted with acetone:H_2O (1:1) to provide **7** (1.6 mg) and 15 fractions (A-9-4-1–A-9-4-15). Fraction A-9-4-12 (3.6 mg) was purified by preparative TLC (RP-18) and developed with *n*-hexane:EtOAc (2:1) to give **10** (3.2 mg). Fraction A-9-4-4 was treated with the same step as fraction A-9-4-12 to yield **9** (1.1 mg). Fraction A-9-9 (587 mg) was separated over MPLC (RP-18), eluting with a system of acetone:H_2O (1:1), to afford fractions A-9-9-1 to A-9-9-21. Fraction A-9-9-2 (5 mg) and fraction A-9-9-10 (13.3 mg) were applied by preparative TLC developed with CH_2Cl_2:MeOH (70:1) and CH_2Cl_2:EtOAc (8:1) to give **8** (2.5 mg) and **5** (8.9 mg), respectively. Fraction A-9-9-20 (462 mg) was submitted to MPLC (RP-18) and eluted with acetone:H_2O (3:1) to afford fractions A-9-9-20-1 to A-9-9-20-15. Fraction A-9-9-20-6 (63.8 mg) was subjected to MPLC (RP-18) and eluted with acetone:H_2O (2:1) to afford fractions A-9-9-20-6-1 to A-9-9-20-6-12. Fraction A-9-9-20-6-3 (3.8 mg) and fraction A-9-9-20-6-7 (13.3 mg) were purified by preparative TLC (RP-18), developed with MeOH:H_2O (5:1) and acetone: H_2O (2:1), to give **1** (3.7 mg) and **2** (3.5 mg), respectively.

Tsangibeilin C (**1**): Light yellowish oil; $[\alpha]_D^{23} \pm 0$ (*c* 0.07, CHCl$_3$); IR (neat) ν_{max} cm^{-1}: 3432 (hydroxy group of carboxylic acid), 1696 (C=O); for ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; ESIMS: *m/z* 337 [M + Na]$^+$; HRESIMS: *m/z* 337.1782 [M + Na]$^+$ (calcd. for C$_{20}$H$_{26}$O$_3$Na, 337.1780).

Tsangibeilin D (**2**): Light yellowish oil; $[\alpha]_D^{24} \pm 0$ (*c* 0.07, CHCl$_3$); IR (neat) ν_{max} cm^{-1}: 3397 (OH), 1692 (C=O); for ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; ESIMS: *m/z* 325 [M + Na]$^+$; HREIMS: *m/z* 302.1879 [M]$^+$ (calcd. for C$_{19}$H$_{26}$O$_3$, 302.1882).

Tricyclotsangibeilin (**3**): Light yellowish oil; $[\alpha]_D^{24} + 36.3$ (*c* 0.024, CHCl$_3$); IR (neat) ν_{max} cm^{-1}: 3422 (OH), 1729 (C=O); for ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; ESIMS: *m/z* 341 [M + Na]$^+$; HRESIMS: *m/z* 341.2096 [M + Na]$^+$ (calcd. for C$_{20}$H$_{30}$O$_3$Na, 341.2093).

Endiandric acid M (**4**): Light yellowish oil; $[\alpha]_D^{25} \pm 0$ (*c* 0.09, CHCl$_3$); IR (neat) ν_{max} cm^{-1}: 3461 (hydroxy group of carboxylic acid), 1694 (C=O), 1040, 937 (OCH$_2$O); for ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; ESIMS: *m/z* 373 [M + Na]$^+$; HRESIMS: *m/z* 373.1418 [M + Na]$^+$ (calcd. for C$_{22}$H$_{22}$O$_4$Na, 373.1416).

Beilschminol B (**5**): Light yellowish oil; $[\alpha]_D^{25} -18.6$ (*c* 0.18, MeOH); UV (MeOH) λmax (log ε): 209 (4.63), 240 sh (3.92), 270 (3.12) nm; UV (MeOH+KOH) λmax (log ε): 210 (4.71), 242 sh (3.97), 280 (3.25) nm; IR (neat) ν_{max} cm^{-1}: 3415 (OH), 1593, 1508, 1461 (benzene ring); ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.07 (3H, d, *J* = 6.0 Hz, H-9'), 1.08 (3H, d, *J* = 6.0 Hz, H-9), 1.78 (2H, m, H-8, H-8'), 3.83 (3H, s, OCH$_3$-4'), 3.88 (12H, s, OCH$_3$-3', OCH$_3$-4, OCH$_3$-5, OCH$_3$-5'), 4.61 (1H, d, *J* = 8.8 Hz, H-7), 4.63 (1H, d, *J* = 9.2 Hz, H-7'), 5.78 (1H, br s, D$_2$O exchangeable, OH-3), 6.55 (1H, d, *J* = 1.8 Hz, H-6), 6.61 (2H, s, H-2', H-6'), 6.64 (1H, d, *J* = 1.8 Hz, H-2); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.9 (C-9'), 14.0 (C-9), 50.9 (C-8'), 51.0 (C-8), 55.9 (OCH$_3$-5), 56.1 (OCH$_3$-3', OCH$_3$-5'), 60.8 (OCH$_3$-4'), 60.9 (OCH$_3$-4), 88.2 (C-7), 88.5 (C-7'), 101.6 (C-6), 102.9 (C-2', C-6'), 105.8 (C-2), 134.7 (C-4), 137.2 (C-4'), 138.0 (C-1'), 138.7 (C-1), 149.1 (C-3), 152.4 (C-5), 153.2 (C-3', C-5'); ESIMS: *m/z* 441 [M + Na]$^+$; HRESIMS: *m/z* 441.1887 [M + Na]$^+$ (calcd. for C$_{23}$H$_{30}$O$_7$Na, 441.1889).

(+)-5-Hydroxybarbatenal (**6**): Light yellowish oil; $[\alpha]_D^{25} +123.8$ (c 0.06, CHCl$_3$); IR (neat) ν_{max} cm^{-1}: 3408 (OH), 1681 (C=O); for ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; ESIMS: *m/z* 257 [M + Na]$^+$; HRESIMS: *m/z* 257.1516 [M + Na]$^+$ (calcd. for C$_{15}$H$_{22}$O$_2$Na, 257.1517).

(4*R*,5*R*)-4,5-Dihydroxycaryophyll-8(13)-ene (**7**): Light yellowish oil; $[\alpha]_D^{25} -23.9$ (c 0.032, CHCl$_3$); IR (neat) ν_{max} cm^{-1}: 3396 (OH), 1637 (C=C); for ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; ESIMS: *m/z* 261 [M + Na]$^+$; HRESIMS: *m/z* 261.1828 [M + Na]$^+$ (calcd. for C$_{15}$H$_{26}$O$_2$Na, 261.1830).

4. Conclusions

There are about 200 Beilschmiedia species in the tropical regions mentioned earlier, including two species in Taiwan [1]. Except for the two species in Taiwan, previous studies of nine *Beilschmiedia* species in the world have revealed endiandric acids [5,18,19], flavonoids [20,21], alkaloids [22–24], benzopyrans [18], arylpropanoids [25] and sesquiterpenoids [18]. Several isolates have shown antibacterial [19,21] and antimalarial [22] activities. Phytochemical studies of the two Formosan Beilschmiedia species have been carried out by our group, analyzing the stems [2], the leaves [3] and the roots [4] of *B. tsangii,* together with the roots [8,9] of *B. erythrophloia*. Both species possess endiandric acids, 7,7'-epoxylignans, 7',8'-seco-7,7'-epoxylignans, benzopyrans, arylpropanoids, amides, sesquiterpenoids and steroids. In this study, endiandric acids, 7,7'-epoxylignans, benzenoid,

sesquiterpenoids and triterpenoid were also reported. The constituents of the roots of the two Formosan Beilschmiedia species showed 19 new endiandric acid analogues as key secondary metabolites. The roots of other Beilschmiedia species found in other regions have not yet been studied. However, several isolates from two Formosan Beilschmiedia species have shown cytotoxic [2], anti-tubercular [3,9] or anti-inflammatory activities [4] in previous studies. These three bioactivities have not yet been examined in other members of the Beilschmiedia genus.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

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