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New Lignans from the Heartwood of *Chamaecyparis obtusa* var. *formosana*

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Four new lignans, 3′,4′-*O,O*-demethylenehinokinin (1), chamalignolide (2), 8′β-hydroxyhinokinin (3) and 7β,8β-epoxyzuonin A (4), as well as (−)-hinokinin (5), and (−)-zuonin A (6), were isolated from the heartwood of *Chamaecyparis obtusa* var. *formosana*. The structures of these lignans were unambiguously determined by spectroscopic methods. And the absolute configuration of 1 was elucidated with a circular dichroism (CD) spectrum.

Key words *Chamaecyparis obtusa* var. *formosana*; Cupressaceae; 3′,4′-*O,O*-demethylenehinokinin; chamalignolide; 8′β-hydroxyhinokinin; 7β,8β-epoxyzuonin A

Chamaecyparis obtusa var. *formosana* is an endemic conifer commonly called "Taiwan Hinoki." It grows at elevations from 1800 to 2600 m in Taiwan's central mountains. Due to its strong resistance to wood-decaying fungi, it is an important building material in Formosa. We have reported on the diterpenes and lignans of its bark.[1)] Phytochemical studies of the wood appear in earlier work,[2—5)] no more than twenty components were observed. The strong anti-fungi characteristic caused us to study the active principles, and we found novel diterpenes, lignans and anthrones.[6—9)] In a continuation of this work, we have now isolated four new lignans, 3′,4′-*O,O*-demethylenehinokinin (**1**), chamalignolide (**2**), 8′β-hydroxyhinokinin (**3**) and 7β,8β-epoxyzuonin A (**4**), together with (−)-hinokinin (**5**)[10)] and (−)-zuonin A (**6**).[11)] In this paper, we describe the structural elucidation of these new compounds; meanwhile, the absolute configuration of **1** was determined using a circular dichroism (CD) spectrum.

The butyrolide, 3′,4′-*O,O*-demethylenehinokinin (**1**), $[M^+]$ *m/z* 342.1100, molecular formula $C_{19}H_{18}O_6$, showed negative optical rotation and IR absorption spectrum with bands for a hydroxyl group (3394 cm^{-1}), γ-butyrolide group (1757 cm^{-1}), and phenyl group (1607, 1517, 1492 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) revealed a methylenedioxy group (δ 5.91, s) attached to a phenyl group, and a 2H at δ 3.83 (1H, dd, J=9.3, 7.0 Hz) and δ 4.06 (1H, dd, J=9.3, 6.7 Hz) characteristic of the methylene group in butyrolide type lactone, as well as two sets of ABX systems (6H) of the phenyl protons presented at δ 6.46—6.79, which could be clearly analyzed. Two benzyl protons were displayed at δ 2.85 (d, J=5.7 Hz), and the other two benzyl protons at δ 2.46 and 2.58 (H-7′) overlapped with signals of H-8 (δ 2.52) and H-8′ (δ 2.45). The MS spectrum of **1** displayed a base peak at *m/z* 135 ($C_8H_7O_2$) and a peak at *m/z* 218 (**7**)[10)]; those are in accordance with a 3,4-methylenedioxybenzyl group on the lactone carbon C-8. Another peak at *m/z* 123 corresponded to a 3,4-dihydroxybenzyl group. The signal at δ 2.85 was assigned as H-7 due to lower field location than H-7′, and to its having a heteronuclear multiple-bond (HMBC) correlation to lactone carbonyl carbon (δ 179.4, Table 1), C-1, and C-2. H-7′ also has an HMBC correlation to C-9′, C-1′, and C-2′. The HMBC technique, as well as Overhauser enhancement and exchange spectroscopy (NOESY) correlation [H-7/δ 6.66 (H-2); H-7′/δ 6.46 (H-2′)], confirmed the structure of **1** as shown. Comparison of ^{1}H- and ^{13}C-NMR data of **1** and hinokinin (**5**) also verified the structure. Lopes[10)] had reported that H_2-9′ were nonequivalent, as two benzyl groups were in *trans*-configuration. Therefore, **1** was in *trans*-configuration. The specific rotation of **1** is −21.8 near (−)-hinokinin (**5**) {$[\alpha]_D$ −26.3°} that confirmed the 8*R*,8*R*′ configuration.[10)] The CD spectra of **1** {$[\Delta\varepsilon]_{262}$ −2.59, $[\Delta\varepsilon]_{293}$ +6.24, $[\Delta\varepsilon]_{318}$ −3.05]} coincided with the ORD data [$[\phi]_{250}$ −10900, $[\phi]_{285}$ +400, $[\phi]_{300}$ −4850] of (8*R*,8′*R*)-(−)-hinokinin,[10)] leading to the conclusion that **1** was (8*R*,8′*R*)-3′,4′-*O,O*-demethylenehinokinin.

The MS spectrum of chamalignolide (**2**) gave an exact mass at *m/z* 326.1158, indicating the molecular formula $C_{19}H_{18}O_5$. It also exhibited negative optical rotation, while the IR spectrum of **2** exhibited bands attributable to hydroxyl, aromatic and γ-lactone groups. The ^{1}H-NMR (Table 1) spectrum was consistent with a dibenzyl butyrolide lignan for this compound. The base peak of the MS spectrum at *m/z* 135 ($C_8H_7O_2$), a peak at *m/z* 192 (**8**) and ^{1}H-NMR [δ 2.83 (1H, dd, J=14.0, 6.7 Hz, H_a-7), 2.94 (1H, dd, J=14.0, 4.8 Hz, H_b-7), 6.59 (1H, s, H-2), 6.57, 6.70 (1H each, d, J=7.8 Hz, H-6, -5), 5.91 (2H, s, $-OCH_2O-$)] revealed the presence of a 3,4-methylenedioxybenzyl moiety on the lactone carbon C-8.[10)] The existence of 4-hydroxybenzyl functionality was proved from the MS fragment peak at *m/z* 107 (33%, C_7H_7O) and spectral data [3395 cm^{-1}; δ 6.86, 6.72 (2H each, d, J=8.4 Hz, H-2′, -6′; H-3′, -5′), 2.46, 2.59 (1H each, H_2-7′, overlapping with H-8 and -8′), and an exchangeable proton (δ 5.50)]. H_2-9′ were nonequivalent [δ 3.83 (1H, dd, J=9.3, 6.7 Hz), 4.09 (1H, dd, J=9.3, 6.6 Hz)], indicating two benzyl groups being in *trans*-relation. HMBC correlation [H-7/C-9; H-7′/C-9′] and NOESY correlation [H-7/H-2; H-7′/H-2′] determined the correct situation of two benzyl groups. The specific rotation of **2** was −23.2°, and the data was near to (8*R*,8′*R*)-(−)-hinokinin; therefore, compound **2** was the same 8*R*,8′*R*-configuration as compound **1**. Thus, chamalignolide was (8*R*,8′*R*)-2β-(3′,4′-methylenedioxybenzyl)-3α-(4″-hydroxybenzyl)-γ-butyrolactone.

Compound **3** had the absorption spectral data of a γ-butyrolactone (1770 cm^{-1}, lactone C=O) with a hydroxyl group (3465 cm^{-1}) and $[M]^+$ *m/z* 370.1048 molecular formula $C_{20}H_{18}O_7$. Its ^{1}H-NMR spectrum (Table 1) showed signals for two sets of ABX systems of phenylprotons and one singlet (4H) due to two $-OCH_2O-$ groups, corresponding to two piperonyl groups. The base peak of MS *m/z* was at 135 ($C_8H_7O_2$) for a piperonyl group on C-8 and -8′ of γ-lactone moiety. Comparing ^{1}H- and ^{13}C-NMR (Table 1) data of **3** with those of (−)-hinokinin, the only difference was one additional hydroxyl group in **3**. H-9′ presented an AX system

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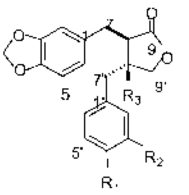
Table 1. ^1H- and ^{13}C-NMR Spectral Data of **1**—**3**, **4** and **6** (300 and 75 MHz in $CDCl_3$)

No.	**1**		**2**		**3**		**4**		**6**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		131.6		131.3		132.7		133.1		134.6
2	6.66 d (1.5)	108.9	6.59 s	109.5	6.81 s	109.6	7.14 d (1.7)	108.4	6.99 d (1.3)	108.1
3		147.8		147.8		147.9		148.2		147.8
4		146.3		146.4		146.3		147.6		147.1
5	6.68 d (8.0)	108.4	6.70 d (7.8)	108.3	6.77 d (7.9)	108.3	6.80 d (7.8)	107.9	6.79 d (8.0)	107.8
6	6.53 dd (8.0, 1.5)	122.8	6.57 d (7.8)	122.3	6.75 d (7.9)	122.2	7.15 dd (7.8, 1.7)	121.2	6.90 dd (8.0, 1.3)	120.2
7	2.85 br d (5.7)	34.1	2.83 dd (14.0, 6.7) 2.94 dd (14.0, 4.8)	34.3	2.92 dd (14.3, 8.9) 3.13 dd (14.3, 4.9)	30.0		108.0	4.30 d (9.2)	82.9
8	2.52[a)]	46.5	2.52[b)]	46.5	2.65 dd (8.9, 4.9)	50.3		81.9	1.68 m	45.9
9		179.4		178.7		177.1	1.28 s	19.9	0.64 d (7.1)	15.2
1′		130.1		130.0		128.2		135.4		135.0
2′	6.46 s	116.1	6.86 d (8.4)	129.8	6.47 d (1.4)	109.9	7.02 d (1.5)	107.4	6.84 br s	107.5
3′		143.8	6.72 d (8.4)	115.5		148.1		147.9		147.4
4′		142.8		154.4		147.1		147.2		146.5
5′	6.76 d (8.0)	115.3	6.72 d (8.4)	115.5	6.72 d (7.9)	108.6	6.76 d (7.9)	107.8	6.77 br s	106.9
6′	6.45 d (8.0)	121.6	6.86 d (8.4)	129.8	6.44 dd (7.9, 1.4)	122.8	6.87 dd (7.9, 1.5)	120.9	6.77 br s	120.1
7′	2.46[a)] 2.58[a)]	38.2	2.46[b)] 2.59[b)]	37.7	2.56 br s	43.1	4.82 d (10.1)	87.4	5.05 d (8.7)	87.4
8′	2.45[a)]	41.0	2.45[b)]	41.2		78.2	2.40 dq (10.1, 6.7)	49.3	2.16 m	48.6
9′	3.83 dd (9.3, 7.0) 4.06 dd (9.3, 6.7)	71.4	3.83 dd (9.3, 6.7) 4.09 dd (9.3, 6.6)	71.2	3.86 d (9.9) 4.15 d (9.9)	76.7	0.97 d (6.7)	8.3	1.01 d (6.7)	14.9
OCH_2O	5.91 s	101.0	5.91 s	108.9	5.93 s	101.0 101.2	5.93 s 5.96 s	101.0 101.3	5.93 s 5.95 s	100.9 101.0

a, b) Overlapping each other.

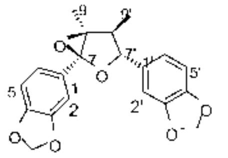


1 $R_1=R_2=OH$, $R_3=H$

2 $R_1=OH$, $R_2=R_3=H$

3 $R_1+R_2=-OCH_2O-$, $R_3=OH$

5 $R_1+R_2=-OCH_2O-$, $R_3=H$



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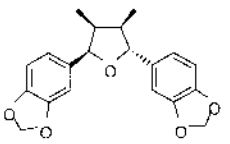

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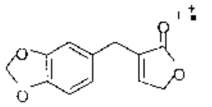
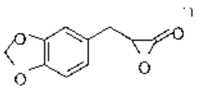


7 *m/z* 218 (5%)



8 *m/z* 192 (16%)

pattern [δ 3.86, 4.15 (1H each, d, J=9.9 Hz)], suggesting a hydroxyl group situated at C-8′. ABX system signals at δ 2.92 (1H, dd, J=14.3, 8.9 Hz), 3.13 (1H, dd, J=14.3, 4.9 Hz), and 2.65 (1H, dd, J=8.9, 4.9 Hz) were assigned as H_2-7 and H-8, respectively. These protons had an HMBC correlation with C-9, which was further confirmed by the location of hydroxyl a group at C-8′. H-7′ (δ 2.56, 2H, br s) exhibited an HMBC correlation with C-8′ and -9′, and NOESY correlation with H-2′ and -6′. Finally, two benzyl groups were verified as *trans*-configuration by NOESY correlation between H-8 and H_2-7′. Therefore, the structure of **3** was confirmed as 8′β-hydroxyhinokinin.

7β,8β-Epoxyzuonin A (**4**) had high resolution mass spectroscopy and ^{13}C-NMR data consistent with the molecular formula $C_{20}H_{18}O_6$, indicating a twelve index of hydrogen deficiency (IHD). Analysis of the IR spectrum of **4** suggested that it contained an aromatic ring attached with a methylenedioxyl group (3045, 1601, 1508, 1251, 1040, 935 cm^{-1}). The ^1H-NMR spectrum revealed two ABX system aromatic protons [δ 6.80 (d, J=7.8 Hz, H-5)] 7.14 (d, J=1.7 Hz, H-2), and 7.15 (dd, J=7.8, 1.7 Hz, H-6); 6.76 (d, J=7.9 Hz, H-5′), 6.87 (dd, J=7.9, 1.5 Hz, H-6′), and 7.02 (d, J=1.5 Hz, H-2′)], two methylenedioxyl groups attached to different aromatic groups [δ_H 5.93 (2H, br s, δ_C 101.0; δ_H 5.96 (2H, s), δ_C 101.3)], a secondary methyl group [δ 0.97 (d, J=6.7 Hz, H_3-9′)], a tertiary methyl group attached to a carbon carrying oxygen atom (δ 1.28, s, H_3-9), and two consecutive methine protons [δ 4.82 (d, J=10.1 Hz, H-7′) and 2.40 (dq, J=10.1, 6.7 Hz, H-8′)]. Twelve low field signals between 107.4 and 148.2 (Table 1) indicated that **4** contained two aromatic rings carried with a methylenedioxyl group. Benzylic methine proton H-7′, revealed from HMBC correlation with C-1′, -2′ and 8′, resonated at δ_C 87.4. The consecutive H-7′, H-8′ and H_3-9′ was proven by COSY correlation. Comparison of the

^{13}C-NMR data (Table 1) of **4** with those of zuonin A (**6**) (isolated from the same source) had the identical skeleton as **6**; the only difference was an additional epoxide on **4**. The oxide located between C-7 and C-8 was discerned from two quaternary carbon signals at δ_C 108.0 and 81.9, respectively. H$_3$-9 exhibited an HMBC correlation with C-8′, C-7 and C-8. The following NOESY correlations, H$_3$-9/H-6, -8′, H$_3$-9′/H-7′, and H-7′/H-2′, -6′, confirmed the relative configuration of **4**. Therefore, compound **4** was assigned as 7β,8β-epoxyzuonin A.

Experimental

General Experimental Procedures Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 983G spectrophotometer. ^{1}H and ^{13}C spectra were run on a Bruker AM-300 spectrometer with tetramethylsilane as an internal standard. Electron impact-mass spectra (EI-MS), specific rotations and UV were taken on a JEOL JMS-HX 300 mass spectrometer, a JASCO DIP-1000 digital polarimeter, and Hitachi S-3200, respectively. Extracts were chromatographed on silica gel (Merck 70—230 mesh, 230—400 mesh, ASTM).

Plant Material The heartwood of *C. obtusa* var. *formosana* was collected from Taichung, Taiwan, in 1996. The plant was identified by Mr. Muh-Tsuen Gun, formerly a technician of the Department of Botany, National Taiwan University. A voucher specimen has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation The dried heartwood of *C. obtusa* var. *formosana* (11 kg) was extracted with Me$_2$CO (120 l) at room temperature (7 d×2). To the evaporated Me$_2$CO extract was added H$_2$O to 1 l, and then this phase was partitioned with ethyl acetate (1 l×3). The combined ethyl acetate layer afforded a black syrup (680 g) that was subsequently chromatographed over Si gel with a hexane–EtOAc gradient solvent system. Compounds **4** (8.4 mg), **6** (16.5 mg) (25% EtOAc in hexane), **3** (7.8 mg), **5** (602 mg) (30% EtOAc in hexane), **1** (9.5 mg), and **2** (9.7 mg) (50% EtOAc in hexane) were eluted with continuous orders.

3′,4′-*O*,*O*-Demethylenehinokinin (**1**): Pale yellow, amorphous. $[\alpha]_D^{26}$ $-21.8°$ (c=0.61, CHCl$_3$). UV λ_{max} (MeOH) nm (log ε): 233 (3.75), 286 (3.66). IR ν_{max} (KBr) cm^{-1}: 3394, 1757, 1607, 1517, 1492, 1260, 1038. ^{1}H- and ^{13}C-NMR: Table 1. CD (c=5.0×10^{-6}, MeOH) $\Delta\varepsilon$=-2.59 (262 nm), $\Delta\varepsilon$=+6.24 (293 nm), $\Delta\varepsilon$=-3.05 (318 nm). EI-MS (70 eV) *m/z* (rel. int. %): 342 (M$^+$, 46), 280 (14), 218 (5), 162 (14), 135 (100), 123 (48), 77 (22). High resolution (HR)-EI-MS *m/z* 342.1100 (Calcd for $C_{19}H_{18}O_6$: 342.1103).

Chamalignolide (**2**): Pale yellow liquid. $[\alpha]_D^{22}$ $-23.2°$ (c=0.41, CHCl$_3$). UV λ_{max} (MeOH) nm (log ε): 225 (3.91), 285 (3.58). IR ν_{max} (KBr) cm^{-1}: 3395, 1763, 1616, 1507, 1490, 1249, 1039, 932. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS (70 eV) *m/z* (rel. int. %): 326 (M$^+$, 15), 192 (16), 135 (100), 107 (33), 77 (32). HR-EI-MS *m/z* 326.1158 (Calcd for $C_{19}H_{18}O_5$: 326.1154).

8′-Hydroxyhinokinin (**3**): Pale yellow liquid. $[\alpha]_D^{19}$ $-89.3°$ (c=0.85, CHCl$_3$). UV λ_{max} (MeOH) nm (log ε): 234 (3.70), 286 (3.58). IR ν_{max} (KBr) cm^{-1}: 3465, 3070, 1770, 1611, 1507, 1491, 1249, 1039, 928. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS (70 eV) *m/z* (rel. int.): 370 (10) [M]$^+$, 135 (100), 77 (32), 51 (16). HR-EI-MS *m/z* 370.1048 (Calcd for $C_{20}H_{18}O_7$: 370.1053).

7β,8β-Epoxyzuonin A (**4**): Liquid. $[\alpha]_D^{25}$ $-14.4°$ (c=0.31, CHCl$_3$). UV λ_{max} (MeOH) nm (log ε): 237 (3.52), 287 (3.43). IR ν_{max} (film) cm^{-1}: 3045, 1601, 1508, 1490, 1251, 1040, 935, 810. ^{1}H- and ^{13}C-NMR: Table 1. EI-MS (70 eV) *m/z* (rel. int. %): 354 (M$^+$, 5), 162 (25), 149 (100), 135 (20). HR-EI-MS *m/z* 354.1102 (Calcd for $C_{20}H_{18}O_6$: 354.1103).

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