Cytotoxic Lignan Esters from *Cinnamomum osmophloeum*

Authors Tai-Hung Chen[1], Yu-Hao Huang[1], Jhih-Jhang Lin[1], Bing-Chung Liau[1], Sheng-Yang Wang[2], Yang-Chang Wu[3], Ting-Ting Jong[1]*

Affiliations
[1] Department of Chemistry, National Chung-Hsing University, Taichung, Taiwan
[2] Department of Forestry, National Chung-Hsing University, Taichung, Taiwan
[3] Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan

Key words
- *Cinnamomum osmophloeum*
- Lauraceae
- 9,9′-di-*O*-feruloyl-(+)-5,5′-dimethoxysecoisolariciresinol
- (7′*S*,8′*R*,8*R*)-lyoniresinol-9-*O*-(*E*)-feruloyl ester
- (7′*S*,8′*R*,8*R*)-lyoniresinol-9,9′-di-*O*-(*E*)-feruloyl ester

Abstract

The bark and roots of *Cinnamomum osmophloeum* are widely used in Taiwan as spice substitutes for *C. cassia*. We have isolated three novel lignan esters, one dibenzylbutane-type ligan ester [9,9′-di-*O*-feruloyl-(+)-5,5′-dimethoxy secoisolariciresinol (**3**)] and two cyclolignan esters [(7′*S*,8′*R*,8*R*)-lyoniresinol-9-*O*-(*E*)-feruloyl ester (**5**) and (7′*S*,8′*R*,8*R*)-lyoniresinol-9,9′-di-*O*-(*E*)-feruloyl ester (**6**)], and several known lignans from the heartwood and roots of *C. osmophloeum*. We identified these compounds using 1D and 2D NMR spectroscopy and mass spectrometry. Cytotoxicity assays of these novel lignan esters revealed that compound **6** has strong activities against human liver cancer (HepG2 and Hep3B) and oral cancer (Ca9-22) cells, with IC_{50} values of 7.87, 4.31, and 2.51 µg/mL, respectively.

Abbreviations

Hepa-G2:	human hepatoma cell line
Hep3B:	human hepatocellular carcinoma (human hepatoma cell line)
Ca9-22:	human oral cancer cell line (gingival cancer)
SAR:	structure-activity relationship
IC_{50}:	half-maximal inhibitory concentration

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

Cinnamomum osmophloeum Kanehhira (Lauraceae) is an indigenous shrub that grows at altitudes of 400–1500 m in the mountains of Taiwan. Because of its strong fragrance, the bark and roots of *C. osmophloeum* are used in Taiwan as spice substitutes for *C. cassia* [1]. The chemical constituents in leaf essential oils from *C. osmophloeum* are similar to those in bark oils from *C. cassia*; they possess considerable antibacterial [2,3], antitermitic [4], antifungal [2], antimite [2,5], and mosquito larvicidal [2] bioactivities. HPLC analysis performed in our lab has revealed that the major components in *C. osmophloeum* leaf oils are cinnamaldehyde and its derivatives, including cinnamyl alcohol and acetate. Four kaempferol glycosides isolated from the methanol extract of the leaves of *C. osmophloeum* exhibit potent anti-inflammatory activities [6].

Because the bioactivities of the extracts from the leaves of *C. osmophloeum* have been investigated quite thoroughly, in this study we focused on other parts of this plant, the heartwood and roots, for which no chemical or biological studies have previously been reported. To extend the chemical and biological analysis of the bioactive components of Taiwan's indigenous cinnamon tree, we extracted meshed chunks from both the heartwood and roots of *C. osmophloeum*.

This paper describes the isolation and characterization of three novel structurally related lignan esters, one secolignan ester (**3**) and two cyclolignan (or aryltetralin lignan) esters (**5** and **6**), isolated from the ethanol extracts of the heartwood and roots. Their structures were elucidated based on 1D and 2D NMR spectra, and comparisons with literature data. The cytotoxicities of these three novel lignan esters were evaluated.

received February 11, 2009
revised October 7, 2009
accepted October 24, 2009

Bibliography
DOI http://dx.doi.org/10.1055/s-0029-1240634
Published online
Planta Med © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

Correspondence
Prof. Ting-Ting Jong
Department of Chemistry
National Chung Hsing University
250 Kuo-Kuang Road
402 Taichung
Taiwan, R.O.C.
Phone: + 88 64 22 84 04 11 ext. 701
ttjong@mail.nchu.edu.tw

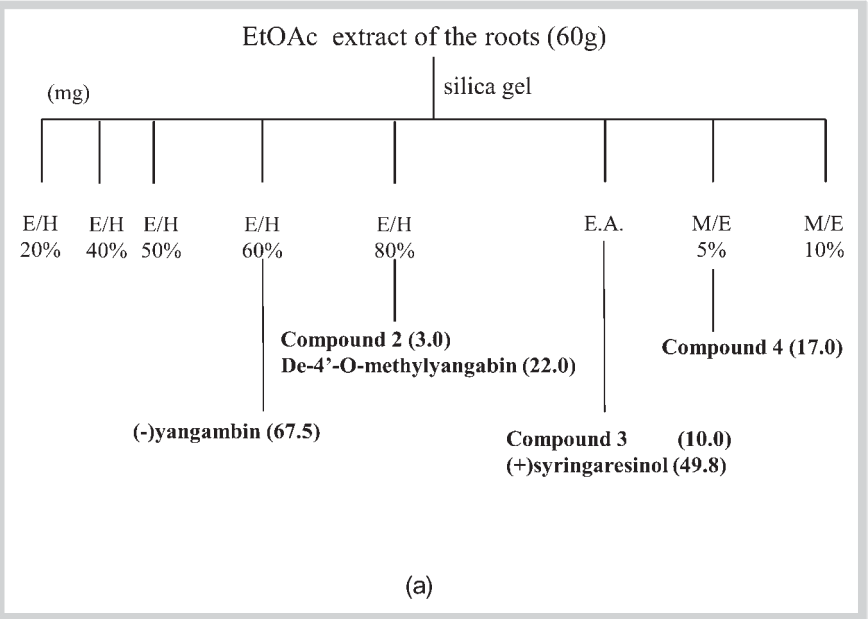

Fig. 1 Isolation of the (**a**) EtOAc and (**b**) *n*-BuOH extracts from the roots of *C. osmophloeum*.

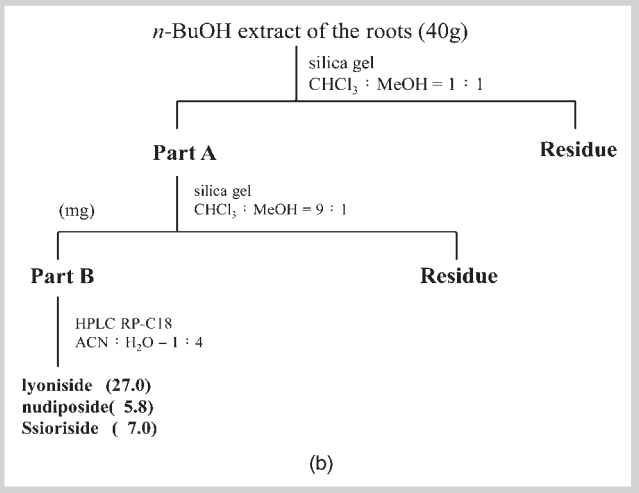

Fig. 1 b *n*-BuOH extracts.

Materials and Methods

General experimental procedures

Optical rotations were measured using a Perkin-Elmer 241 polarimeter. Fourier transform infrared (FTIR) spectra were recorded using a Perkin-Elmer Spectrum 100 FT-IR spectrometer. UV spectra were recorded using a Cintra UV 101 spectrometer. ^{1}H NMR spectra were recorded using Varian Unity Inova 400 and 600 MHz FT-NMR spectrometers. The chemical shifts (δ) are expressed in ppm; coupling constants (J), in Hz. High-resolution fast atom bombardment mass spectrometry (HR-FAB/MS) was performed using a Finnigan Thermo Quest MAT 95XL MS spectrometer. Silica gel 60 F_{254} (70–230 and 230–400 mesh; Merck) was used as the stationary phase for column chromatography (CC). Thin layer chromatography (TLC) and preparative TLC were performed using plates precoated with silica gel; the developed plates were viewed under UV light at 254 and 365 nm. Preparative high-performance liquid chromatography (HPLC) was performed using an Agilent 1100 series system equipped with a UV detector.

Plant material

A whole plant of *C. osmophloeum* was collected from a farm in Nanto County in August 2006; it was identified by Dr. Sen-Song Zheng of the Experimental Forest of the National Taiwan University, Taipei, Taiwan. A voucher specimen (BP8) has been deposited in the herbarium of the Forest Department of the National Chung Hsing University, Taichung, Taiwan.

Extraction and isolation

Air-dried heartwood chips (10.0 kg) were exhaustively extracted with 95% ethanol (30 L) at ambient temperature. The extracts were concentrated under vacuum to yield the ethanol extract (390 g), which was partitioned between EtOAc and H_2O (1 : 1) to separate the EtOAc- and H_2O-soluble fractions. The EtOAc phase was concentrated (90 g), coated with silica gel (90 g), and subjected to CC (SiO_2, 70–230 mesh, 4.5 × 46 cm), eluting with a gradient of hexane, EtOAc, and MeOH (hexane/EtOAc, from 1 : 0, 9 : 1, 4 : 6, to 0 : 1; then EtOAc/MeOH, from 7 : 3 to 0 : 1; each 2 L) to yield six fractions. Fraction 3 was subjected to CC (SiO_2; $CHCl_3$) to afford a crude portion, which was then purified through recrystallization to obtain compound **3** (80 mg). Fraction 4 was further subjected to CC [(i) SiO_2; $CHCl_3$/MeOH, 98 : 2; (ii) Sephadex LH-20 (100 g); 80% MeOH] to obtain compounds **1** (2 mg), **5** (13 mg), and **6** (28 mg).

Air-dried roots (10.0 kg) were exhaustively extracted with 95% ethanol (30 L) at ambient temperature. The extracts were concentrated under vacuum to yield the ethanol extract (540 g), which was partitioned between EtOAc and H_2O (1 : 1) to separate the EtOAc- and H_2O-soluble fractions; the latter was further partitioned with *n*-BuOH to yield the *n*-BuOH extract (40 g). The EtOAc phase was concentrated (60 g), coated with silica gel, and subjected to CC [SiO_2 (60 g); gradient eluting with hexane, EtOAc, and MeOH] to yield several fractions. The isolation processes for the EtOAc and *n*-BuOH extracts are depicted schematically in **Fig. 1 a** and **b**.

Table 1 ^{1}H and ^{13}C NMR spectral data for compounds **3, 5**, and **6** (600 MHz)[a].

Position	Compound 3 (in DMSO-d_6)		Compound 5 (in $CDCl_3$)		Compound 6 (in $CDCl_3$)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		130.0		128.4		128.1
2	6.37 (1H, s)	106.1	6.50 (1H, s)	106.1	6.51 (1H, s)	105.9
3		147.8		146.2		146.3
4		133.6		137.0		137.1
5		147.8		145.5		145.4
6	6.37 (1H, s)	106.1		124.8		124.7
7	2.58 (1H, dd, 7.8, 13.8)	34.4	2.67 (1H, dd, 11.4,15) H_{ax}	32.9	2.76 (2H, m)	33.1
	2.74 (1H, dd, 6, 13.8)		2.73 (1H, dd, 4.8,15) H_{eq}			
8	2.20 (1H, m)	40.0	1.98 (1H, m)	36.2	2.16 (1H, m)	36.3
9	4.09 (1H, dd, 5.4, 11.4 Hz)	63.8	4.16 (1H, dd, 6.6, 11.0) Ha	67.4	4.21–4.34 (2H, m)	66.9
	4.32 (1H, dd, 6.6, 11.4 Hz)		4.31 (1H, dd, 4.8, 11.0) Hb			
1′		130.0		137.6		137.6
2′	6.37 (1H, s)	106.1	6.35 (1H, s)	105.0	6.36 (1H, s)	105.0
3′		147.8		146.8		146.8
4′		133.6		132.7		132.9
5′		147.8		146.8		146.8
6′	6.37 (1H, s)	106.1	6.35 (1H, s)	105.0	6.36 (1H, s)	105.0
7′	2.58 (1H, dd, 7.8, 13.8)	34.4	4.38 (1H, d, 5.4 Hz)	41.3	4.21–4.34 (1H, m)	42.5
	2.74 (1H, dd, 6.0, 13.8)					
8′	2.20 (1H, m)	40.0	2.06 (1H, m)	47.6	2.24 (1H, m)	45.4
9′	4.32 (1H, dd, 6.6, 11.4)	63.8	3.63 (2H, m)	63.4	4.21–4.34 (2H, m)	64.3
	4.09 (1H, dd, 5.4, 11.4)					
1′′		125.5		126.7		126.8
2′′	7.29 (1H, d, 1.2)	111.1	7.02 (1H, d, 1.8)	109.3	6.96 (1H, d, 1.8)	109.2
3′′		147.9		146.8		146.7
4′′		149.4		148.1		148.0
5′′	6.78 (1H, d, 7.8)	115.5	6.91 (1H, d, 8.4)	114.7	6.88 (1H, d, 8.4)	114.6
6′′	7.09 (1H, dd, 1.2, 7.8)	123.2	7.06 (1H, dd, 1.8, 8.4)	123.2	7.01 (1H, dd, 1.8, 8.4)	123.1
7′′	7.53 (1H, d, 15.6)	145.1	7.58 (1H, d, 16.0)	145.3	7.55 (1H, d, 15.6)	145.1
8′′	6.48 (1H, d, 15.6)	114.4	6.23 (1H, d, 16.0)	114.9	6.27 (1H, d, 15.6)	115.1
9′′		166.7		167.4		167.2
1′′′		125.5				126.8
2′′′	7.29 (1H, d, 1.2)	111.1			6.96 (1H, d, 1.8)	109.2
3′′′		147.9				146.7
4′′′		149.4				148.0
5′′′	6.78 (1H, d, 7.8)	115.5			6.88 (1H, d, 8.4)	114.6
6′′′	7.09 (1H, dd, 1.2, 7.8)	123.2			7.01 (1H, dd, 1.8, 8.4)	123.1
7′′′	7.53 (1H, d, 15.6)	145.1			7.55 (1H, d, 15.6)	145.1
8′′′	6.48 (1H, d, 15.6)	114.4			6.22 (1H, d, 15.6)	115.1
9′′′		166.7				167.2
3-OMe	3.66 (3H, s)	55.7	3.89 (3H, s)	56.1	3.89 (3H, s)	56.1
5-OMe	3.66 (3H, s)	55.7	3.41 (3H, s)	59.9	3.30 (3H, s)	59.6
3′-OMe	3.80 (3H, s)	55.7	3.78 (3H, s)	56.3	3.80 (3H, s)	55.9
5′-OMe	3.66 (3H, s)	55.7	3.78 (3H, s)	56.3	3.80 (3H, s)	55.9
3′′-OMe	3.66 (3H, s)	55.7	3.93 (3H, s)	55.9	3.90 (3H, s)	56.4
3′′′-OMe	3.80 (3H, s)	55.7			3.90 (3H, s)	56.4

[a] Chemical shifts are represented in ppm; number of hydrogen atoms, splitting pattern, and coupling constants (Hz) are given in parentheses; assignments were confirmed through HSQC, HMBC, 1D-NOE, and ^{1}H-^{1}H COSY experiments

Experimental data for the novel lignan esters

*9,9′-Di-O-feruloyl-5,5′-dimethoxysecoisolariciresinol (**3**)*: White powder; m.p.: 214–216 °C; $[\alpha]_D^{24}$ = +56.8 (*c* 0.5, DMSO); UV (MeOH) λ_{max} (log ε) 326 (5.03), 297 (4.93), 234 (5.00), 208 (5.47), 192 (5.25) nm; IR (KBr) ν_{max} (cm^{-1}): 3420, 1693, 1604, 1511; FAB/MS (rel. int., %) *m*/*z* 775 $[M + H]^+$ (3.8); HR-FAB/MS $[M + H]^+$ *m*/*z*, 775.2975 (calcd. for $C_{42}H_{47}O_{14}$, 775.2966). ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz) spectra of **3** were depicted in ◉ **Table 1**.

*(7′S,8′R,8R)-Lyoniresinol-9-O-(E)-feruloyl ester (**5**):* Yellow solid; $[\alpha]_D^{22}$ = +10 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 325 (4.36), 285 (4.28), 235(4.55), 206 (4.03), 196 (4.80) nm; IR (KBr) ν_{max} (cm^{-1}): 3420, 1693, 1603, 1512, 1463; EI/MS (rel. int., %) *m*/*z* 596 $[M^+]$ (34), 578 (5.6), 420 (8.5), 402 (84), 371 (61), 344 (21), 217 (54), 194 (65), 177 (100), 167 (75), 145 (47), 117 (26); HR-FAB/MS $[M + H]^+$ *m*/*z* 597.2345 (calcd. for $C_{32}H_{37}O_{11}$, 597.2336). ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz) spectra of **5** were depicted in ◉ **Table 1**.

*(7′S,8′R,8R)-Lyoniresinol-9,9′-di-O-(E)-feruloyl ester (**6**):* Yellow solid; $[\alpha]_D^{22}$ = + 63.3 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 325 (3.75), 285 (4.58), 235 (4.81), 206 (5.23), 194 (4.98) nm; IR (KBr) ν_{max} (cm^{-1}): 3412, 2927, 1693, 1603, 1586, 1512, 1463, 1422; FAB/MS (rel. int., %) *m*/*z* 795 $[M + Na]^+$ (11.5), 773 $[M + H]^+$ (11.6), 772 $[M]^+$ (14.6); HRFAB/MS $[M]^+$ *m*/*z* 772.2721 (calcd. for $C_{42}H_{44}O_{14}$, 772.2731). Mass and HRFAB spectra of **6** were supplied as Supporting Information, see **Fig. 1S** and **2S**. ^{1}H-NMR ($CDCl_3$, 600 MHz)and ^{13}C-NMR ($CDCl_3$, 150 MHz) spectra of **6** were depicted in ◉ **Table 1**.

Tumor cell growth inhibition assay

The assay was performed at the natural Products Research Center and School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, R.O.C. The procedures were as follows: HepG2, Hep3B, and Ca9-22 cells (Food Industry Research and Development Institute) were cultured in minimal essential medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and were maintained at 37 °C under 5% CO_2. The cytotoxicity was examined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [7–9]. Briefly, exponentially growing cells (tumor cells, including HepG2, Hep3B, and Ca9-22) were seeded (1×10^4 cells) into a 96-well plate in triplicate and were then preincubated for 20 h so that they would undergo cell attachment. The medium was then aspirated and fresh medium (100 µL) containing various concentrations (20, 10, 5, 1 µg/mL) of the test compound was added to the cultures. The cells were incubated in the presence of each compound at 37 °C for 72 h under humidified air containing 5% CO_2. Cell survival was evaluated after adding MTT solution (1 mg/mL in PBS; 10 mL). After 4 h of incubation at 37 °C, DMSO (100 µL) was added to dissolve the precipitated MTT. The microplates were shaken for 15 min and then the absorbance was determined at 550 nm using a multi-well scanning spectrophotometer. Because of the limited sample availability and for the preliminary screen, all the samples were tested in duplicate. As an index of cytotoxicity, the value of IC_{50} (the concentration of 50% inhibition) was determined from the dose-response curve to test the drug inhibition of cell growth in each tumor cell line. Doxorubicin (Sigma-Aldrich; purity: > 98%; TLC grade) was used as the positive control.

Supporting information

Original spectra for compound **6** are available as Supporting Information.

Results and Discussion

▼

Three dibenzylbutane-type lignans with the 9(9′) oxygen atom attached, secoisolariciresinol (**1**), isolated as an enantiomeric mixture with its (−) enantiomer in excess (observed [α] − 7.5, *c* 0.1, $CHCl_3$; lit. [α] − 24.6, *c* 0.4, EtOH [8,9]); its diferuloyl esters (**2**), isolated as an enantiomeric mixture with its (−) enantiomer in excess (observed [α] − 12.6, *c* 0.80, $CHCl_3$; lit. [α] − 41.2, *c* 0.80, $CHCl_3$ [10]); and the (+)-secolynoiresinol diferuloyl esters (**3**), and three related cyclolignans, (−)-lyoniresinol (**4**), isolated as an enantiomeric mixture with its (−) enantiomer in excess (observed [α] − 4.0, *c* 1.08, CH_3OH; lit. [α] + 13.3, *c* 0.1, $CHCl_3$ for (+)-lyoniresinol [11,12]), and its mono (**5**) and diferuloyl (**6**) esters, were isolated from the roots and heartwood of Taiwan's indigenous cinnamon tree (*C. osmophloeum*). Among these lignans, (+)-secolyoniresinol diferulate (**3**), (+)-lyoniresinol monoferulate (**5**), and its (+)-diferulate (**6**) are novel lignan esters isolated for the first time. In addition, we also isolated several other known lignans from the extracts of the heartwood and roots of *C. osmophloeum*, including tetrahydrofuran-type lignans {(−)-yangambin [13,14], (+)-syringaresinol [15], and (±)-*de*-4′-*O*-methylyangabin [16]} and three lignan xylosides (lyoniside [17], nudiposide [17], and ssioriside [18]).

Below, we describe the structural elucidation of each of these three novel lignan esters, based on FAB mass spectrometry and 1D and 2D NMR spectroscopy.

We isolated compound **3** as a white powder that was only slightly soluble in $CHCl_3$ and not easily dissolved in other organic solvents. Its molecular formula, $C_{42}H_{46}O_{14}$, determined from its molecular ion (HR-FAB-MS), suggested 20 degrees of unsaturation. The IR spectrum revealed the presence of a hydroxyl group (3420 cm^{-1}), an *α,β*-unsaturated conjugate ester moiety (1693 cm^{-1}), and an aromatic ring (1604, 1511 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **3** revealed only about half of its protons and carbon atoms, suggesting a symmetrical molecule. The major skeleton of **3** exhibits seven signals for carbon atoms in its DEPT ^{13}C NMR spectrum, corresponding to one aromatic ring (four carbon atoms) and three aliphatic carbon atoms, namely one methine (*δ* 40.0), one methylene (*δ* 34.4), and one downfield-shifted methylene (*δ* 63.8), due to the attachment of an oxygen atom.

The ^{1}H NMR spectrum of **3** reveals five protons attached to the aliphatic CH, CH_2, and OCH_2 carbon atoms: at *δ* 2.20 (1H, m); 2.58 (1H, dd, *J* = 7.8, 13.8 Hz), 2.74 (1H, dd, *J* = 6.0, 13.8 Hz), and 4.09 (1H, dd, *J* = 5.4, 11.4 Hz), 4.32 (1H, dd, *J* = 6.6, 11.4 Hz), respectively; we established their inter-couplings through COSY spectroscopy.

Another structural subunit in **3**, the *α,β*-unsaturated conjugate ester, was identified based on the observation of nine additional signals for carbon atoms, including a carbonyl (C=O) carbon at 166.7 ppm, plus signals for trans-olefinic protons at 7.53 (1H, d, *J* = 15.6 Hz) and 6.48 (1H, d, *J* = 15.6 Hz) ppm, and an ABX spin system of an aromatic ring with signals at 7.29 (1H, d, *J* = 1.2 Hz), 7.09 (1H, dd, *J* = 7.8, 1.2 Hz), and 6.78 (1H, d, *J* = 7.8 Hz) ppm in the ^{1}H-NMR spectrum. The HMBC spectrum of **3** connected the above carbon atoms and protons, suggesting that it is a *trans*-feruloyl moiety.

Comparisons of the ^{1}H and ^{13}C NMR data of **3** with those of the known compound **2** [10] indicate that they are both derivatives of **1**, a dibenzylbutane-type lignan, with **3** exhibiting two additional methoxy-substituents at C-5 and -5′. In the HMBC spectrum of **3**, correlations existed between H-9 (*δ* 4.32, 4.09) and the carbonyl C-9″ (*δ* 166.7) and between H-9 (*δ* 4.32, 4.09) and C-7 (*δ* 34.4), establishing the site of esterification at C-9. Supported by HMBC correlations, we assigned the three methoxy carbons at *δ* 3.66 and 3.80 to be located at C-3 (and C-5) and C-3″, respectively.

With regard to its stereochemistry, we eliminated a meso form for **3** because it exhibited a specific rotation (+ 56.8, *c* 0.5, DMSO) opposite to that of the known compound **2** (lit. [α] = − 41.2, *c* 0.80, $CHCl_3$ [10]); therefore, we assigned the configurations at C-8 and C-8′ of **3** as *S* and *S*, respectively. Notably, however, the compound **2** isolated from the same roots in this study was an enantiomeric mixture having its (−) enantiomer in excess. Accordingly, we tentatively establish compound **3** to be (+)-(8*S*,8′*S*)-9,9′-di-*O*-feruloyl-5,5′-dimethoxysecoisolariciresinol.

We isolated compound **5** as a yellow solid. Its molecular formula, $C_{32}H_{36}O_{11}$, determined from its molecular ion (HR-FAB-MS), represents 15 degrees of unsaturation. The IR spectrum revealed the

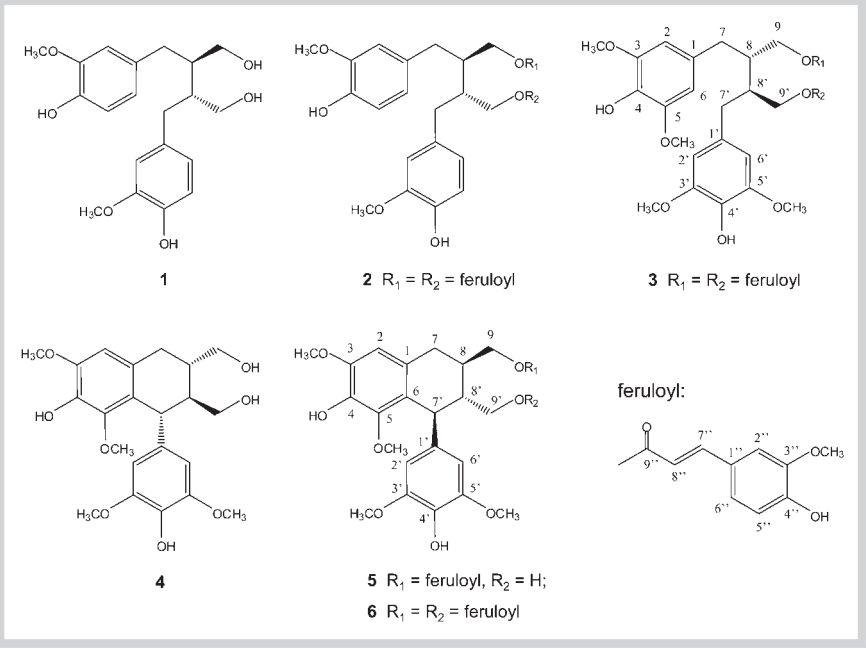

Fig. 2 Structures of compounds **1**–**6**.

presence of a hydroxyl group (3420 cm^{-1}), an *α,β*-unsaturated conjugated ester moiety (1693 cm^{-1}), and an aromatic ring (1603, 1512 cm^{-1}). The comparison of the ^{1}H and ^{13}C NMR data of **5** with those of lyoniresinol (**4**) [11, 12] suggested a lignan skeleton (**Fig. 2**). In addition to the 18 carbon signals, including two aromatic rings and six aliphatic carbons belonging to the lignan moiety, the ^{13}C NMR spectrum of **5** revealed 10 other carbon signals, consistent with a *trans*-feruloyl moiety, which we confirmed by the presence of proton signals for the trans-olefinic bond at 7.58 (1H, d, *J* = 16.0 Hz) and 6.23 (1H, d, *J* = 16.0 Hz) ppm, and an ABX spin system for the aromatic ring at 7.06 (1H, dd, *J* = 8.4, 1.8 Hz), 7.02 (1H, d, *J* = 1.8 Hz), and 6.91 (1H, d, *J* = 8.4 Hz) ppm, plus a methoxy proton signal at 3.93 ppm (s, 3H) in its ^{1}H NMR spectrum.

In the DEPT spectrum of **5**, the six aliphatic carbons displayed three methine signals (*δ* 47.6, 41.3, 36.2) and three methylene signals (*δ* 67.4, 63.4, 32.9), two of them bearing oxygen atoms; thus, we assumed the presence of a cyclolignan skeleton. Five signals for methoxy protons appeared at *δ* 3.93 (3H), 3.89 (3H), 3.78 (2 × 3H), 3.41 (3H) in the ^{1}H NMR spectrum of **5**; from HMBC correlations, we established their positions at C-3″, C-3, C-3′ (and C-5′), and C-5, respectively. The proton signal of methoxy at *δ* 3.41 (upfield, at C-5 position) was shielded by the aromatic ring. In the HMBC spectrum of **5**, correlations existed between H-9 (*δ* 4.32, 4.09) and C-9″ (*δ* 166.7), between H-9 (*δ* 4.32, 4.09) and C-7 (*δ* 34.4), and between H-9 (*δ* 4.32, 4.09) and C-8 (*δ* 36.2); these data indicate that the position of esterification is at C-9. **Fig. 3a** presents the major HMBC correlations for compound **5**.

The two vicinal protons H-7′ and H-8′ of **5** had a coupling constant (*J*) of 5.4 Hz; the two vicinal protons H-8′ and H-8 each had values of $W_{1/2}$ greater than 16 Hz (18 Hz for H-8′ 24 Hz for H-8), indicating that H-8′ and H-8 were located in axial positions [19]. Meanwhile, in the NOESY spectrum of **5** (**Fig. 3b**), H-7′ and H-9′ were correlated, as were H-2′ and H-8′, indicating that H-7′, H-8′, and H-8 were all aligned in relative trans configurations. Therefore, we determined compound **5** to be a derivative of lyoniresinol (**4**).

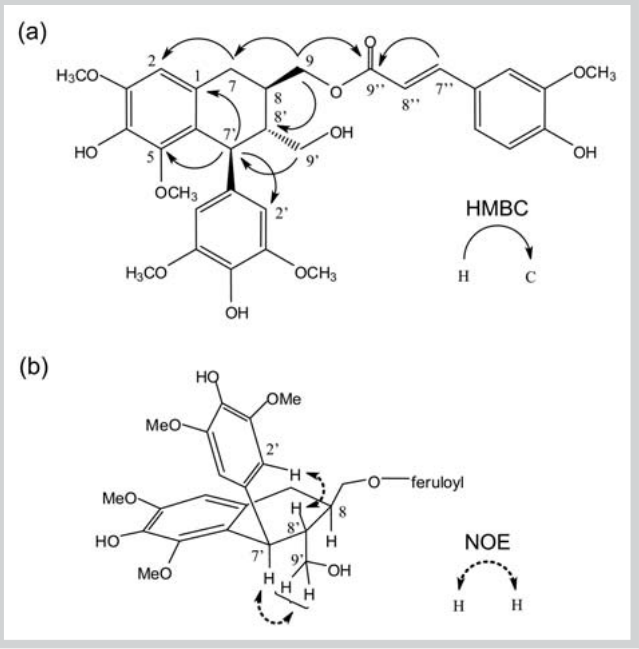

Fig. 3 Major observed (**a**) HMBC and (**b**) ^{1}H-^{1}H NOE correlations for compound **5**.

Compound **5** exhibited positive optical rotation ([*α*] +10, *c* 0.3, MeOH). The circular dichroism (CD) spectrum of **5** displayed positive Cotton effects at 243 and 272 nm and negative Cotton effects at 258 and 289 nm (**Fig. 4**), consistent with the reported CD spectrum of (+)-lynoiresinol (positive Cotton effects at 244 and 274 nm; negative Cotton effect at 285 nm) [11, 12]. Therefore, we identified compound **5** to be (+)-(7′*S*,8′*R*,8*R*)-lyoniresinol-9-*O*-(*E*)-feruloyl ester.

Compound **6** was isolated as a yellow solid. Its molecular formula, $C_{42}H_{44}O_{14}$, obtained from its molecular ion (HR-FAB-MS), represented 21 degrees of unsaturation. The IR spectrum exhibited

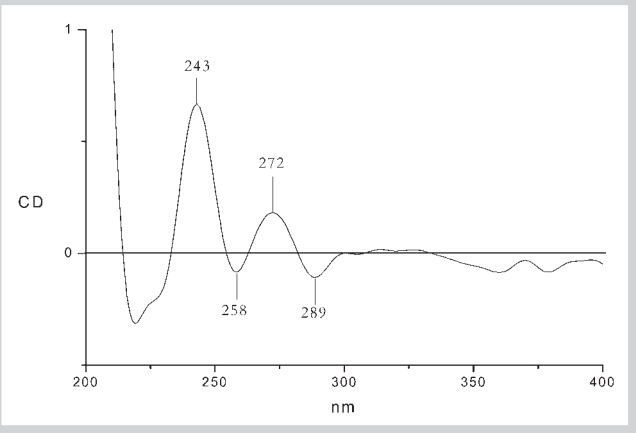

Fig. 4 CD spectrum of compound **5**.

signals for a hydroxyl group (3412 cm^{-1}), an *α,β*-unsaturated conjugated ester moiety (1693 cm^{-1}), and an aromatic ring (1586, 1512 cm^{-1}). A comparison of the ^1H and ^{13}C NMR data (**Table 1**) with those of compounds **5** and **6** indicates that their skeletons are similar (**Fig. 2**). In the ^1H NMR spectrum of **6**, four olefinic protons at 7.55 (2 × 1H, d, *J* = 15.6 Hz), 6.27 (1H, d, *J* = 15.6 Hz), and 6.22 (1H, d, *J* = 15.6 Hz) ppm indicate the existence of two trans-double-bonds. Two ABX spin systems of the aromatic ring appear at 7.02 (1H, dd, *J* = 8.4, 1.8 Hz), 7.01 (1H, dd, *J* = 8.4, 1.8 Hz), 6.96 (2H, d, *J* = 1.8 Hz), 6.89 (1H, d, *J* = 8.4 Hz), and 6.88 (1H, d, *J* = 8.4 Hz) ppm. In the ^{13}C NMR spectrum, the corresponding 10 signals indicated the existence of two *trans*-feruloyl moieties. Six signals for methoxy protons appear at *δ* 3.90 (2 × 3H), 3.89 (3H), 3.80 (2 × 3H), and 3.30 (3H) in the ^1H NMR spectrum; the DEPT spectrum of **6**, together with HMBC correlations, established that these groups were attached at C-3′(and C-5′), C-3, C-3″ (and C-3‴), and C-5, respectively.

In the NOESY experiment of **6**, the relative configuration of protons were correlated between H-7′ and H-9′, and between H-2′ and H-8′, indicating that the three protons (H-7′, H-8′, and H-8) were *trans* from each other. To acquire their coupling constants, a homo-decoupling experiment was performed: when we irradiated at *δ* 4.21–4.34, where the signals for five protons (corresponding to the H-7′, two H-9, and two H-9′ protons) appear, we observed the H-8′ signal to simplify to a doublet with large coupling constant ($J_{8,8'}$ = 9.2 Hz). Thus, we could assign a relative trans configuration to the protons H-8′ and H-8 [11, 12]. In addition, in a 1-D NOE experiment, when we irradiated the aromatic proton H-2′ (H-6′), we obtained an enhanced signal for the proton H-7′, acknowledged as a doublet having a coupling constant ($J_{7',8'}$) of 7.2 Hz. Hence, we determined compound **6** to be a derivative of lyoniresinol, containing two *trans*-feruloyl moieties. In the HMBC spectrum of **6**, the correlations indicated that the positions of esterification were at C-9 and C-9′.

Further, the absolute configuration of **6** was determined by its CD spectrum (observed as positive Cotton effects at 245 and 279 nm, and negative Cotton effect at 291 nm), which was consistent with that of (+)-lyoniresinol (positive Cotton effects at 244 and 274 nm; negative Cotton effect at 285 nm) [11, 12]; consequently, compound **6** was identified as (+)-(7′*S*,8′*R*,8*R*)-lyoniresinol-9,9′-di-*O*-(*E*)-feruloyl ester.

Next, we tested the three novel lignan esters (**3, 5, 6**) isolated from the heartwood and roots extract of *C. osmophloeum* for their cytotoxicities against HepG2, Hep3B, and Ca9-22 cancer cells. **Table 2** presents the structure-activity relationships (SARs) for these lignans. The cyclolignans (i.e., compounds **5** and **6**) were more potent than the dibenzylbutane-type lignan (compound **3**) toward these three cancer cell lines. Additionally, a feruloyl effect was evident: the lignan possessing two feruloyl groups (compound **6**) was more potent than that featuring only one feruloyl group (compound **5**). Thus, feruloyl substituents at both the C9 and C9′ positions enhanced the cytotoxicity of the cyclolignan. The values of IC_{50} of compound **6** against the HepG2, Hep3B, and Ca9-22 cell lines – 7.87, 4.31, and 2.51 µg/mL, respectively – suggest its feasibility for drug development.

Acknowledgments

We thank Dr. Sheng-Yang Wang for helping with the plant treatment, the Forest Bureau for financial support, and Mr. Peter Glink for editorial assistance.

References

1 *Lin ZP.* The specific topic publication of *Cinnamomum osmophloeum*. Taipei: Taiwan Forestry Research Institute; 1992
2 *Liu JY.* Antibacterial, antifungal, mosquito larvicidal and antimite activities of leaf essential oils from six chemotypes of *Cinnamomum osmophloeum* [dissertation]. Taipei: National Taiwan University; 2005
3 *Chan ST, Chen PF, Chang SC.* Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. J. Ethnopharmacol 2001; 77: 123–127
4 *Chang ST, Cheng SS.* Antitermitic activity of leaf essential oils and components from *Cinnamomum osmophloeum*. J Agric Food Chem 2002; 50: 1389–1392
5 *Chen PF, Chan ST, Wu HH.* Application of leaf essential oils and their constituents from *Cinnamomum osmophloeum* on the manufacture of antimite paper. Q J Chin Forest 2002; 35: 397–403
6 *Fang SH, Rao YK, Tzeng YM.* Inhibitory effects of flavonol glycosides from *Cinnamomum osmophloeum* on inflammatory mediators in LPS/IFN-γ-activated murine macrophages. Bioorg Med Chem 2005; 13: 2381–2388

Table 2 Cytotoxicities of the lignans **3, 5**, and **6** isolated from *Cinnamomum osmophloeum* against three human cancer lines (HepG2, Hep3B, and Ca9-22)[a].

Compound	IC_{50} (µg/mL)[b]		
	HepG2	Hep3B	Ca9-22
9,9′-di-*O*-feruloyl-(+)-5,5′-dimethoxy secoisolariciresinol (**3**)	> 20	> 20	> 20
(7′*S*,8′*R*,8*R*)-lyoniresinol-9-*O*-(*E*)-feruloyl ester (**5**)	16.64 ± 0.20	14.49 ± 0.03	8.51 ± 0.00
(7′*S*,8′*R*,8*R*)-lyoniresinol-9,9′-di-*O*-(*E*)-feruloyl ester (**6**)	7.87 ± 0.04	4.31 ± 0.04	2.51 ± 0.08
doxorubicin[c]	0.05 ± 0.00	0.14 ± 0.00	0.04 ± 0.00

[a] HepG2 and Hep3B are cell lines of human liver carcinomas; Ca9-22 is the cell line of a human oral carcinoma; [b] IC_{50} is the half-maximal inhibitory concentration; [c] Used as positive control

7 *Alley MC, Scudiero DA, Monks A, Hursey ML, Ciezerwinski MJ, Fine DL.* Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 1988; 48: 589–601

8 *Achenbach H, Waibel R, Aeldae-Mensah I.* Lignans and other constituents from *Carissa edulis.* Phytochemistry 1983; 22: 749–753

9 *Inoshiri S, Sasaki M, Kohda H, Ohtsuka H, Yamasaki K.* Aromatic glycosides from *Berchemia racemosa.* Phytochemistry 1987; 26: 2811–2814

10 *Fuchino H, Satoh T, Tanaka N.* Chemical evaluation of *Betula* species in Japan. I. Constituents of *Betula ermanii*. Chem Pharm Bull 1995; 43: 1937–1942

11 *Hanawa F, Shiro M, Hayashi Y.* Heartwood constituents of *Betula maximowicziana*. Phytochemistry 1997; 45: 589–595

12 *Miyamura M, Nohara T, Tomimatsu T, Nishioka I.* Seven aromatic compounds from bark of *Cinnamomum cassia*. Phytochemistry 1983; 22: 215–218

13 *Ahmed AA, Mahmoud AA, Ali ET, Tzakou O, Couladis M, Mabry TJ, Gati T, Toth G.* Two highly oxygenated eudesmanes and 10 lignans from *Achillea holosericea.* Phytochemistry 2002; 59: 851–856

14 *Lee CK, Chang MH.* The chemical constituents from the heartwood of *Eucalyptus citriodora*. J Chin Chem Soc 2000; 47: 555–560

15 *Lo WL, Wu YC, Heieh TJ, Kuo SH, Lin HC, Chen CY.* Chemical constituents from the stems of *Michelia compressa*. Chin Pharm J 2004; 56: 69–75

16 *Miyazawa M, Kasahara H, Kameoka H.* Biotransformation of (+)-magnolin and (+)-yangabin in rat. Phytochemistry 1993; 32: 1421–1424

17 *Smite E, Pan H, Lundgren LN.* Lignan glycosides from inner bark of *Betula pendula*. Phytochemistry 1995; 40: 341–343

18 *Yoshinari K, Sashida Y, Shimomura H.* Two new lignan xylosides from the barks of *Prunus ssiori* and *Prunus padus.* Chem Pharm Bull 1989; 37: 3301–3303

19 *Ouyang MA, Wein YS, Su RK, Kuo YH.* Rhusemialins A–C, new cyclolignan ester from the roots of *Rhus javanica* var. *roxburghiana*. Chem Pharm Bull 2007; 55: 804–807