

Note

New Lignans from the Heartwood of *Cunninghamia lanceolata*

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Two new lignans, including the aryltetralin-type lignan, lanceoline (1), and the diaryl butyrolactone-type lignan, 5-methoxytrachelogenin (2), together with 5-methoxywikstromol (3), were isolated from the low-polar layer of a heartwood extract of *Cunninghamia lanceolata* as their acetylated derivatives 1a, 2a and 3a, respectively, and were identified by spectroscopic analyses. The ¹³C-NMR data for 3a are reported for the first time in this paper.

Key words: *Cunninghamia lanceolata*; Taxodiaceae; heartwood; lanceoline; 5-methoxytrachelogenin

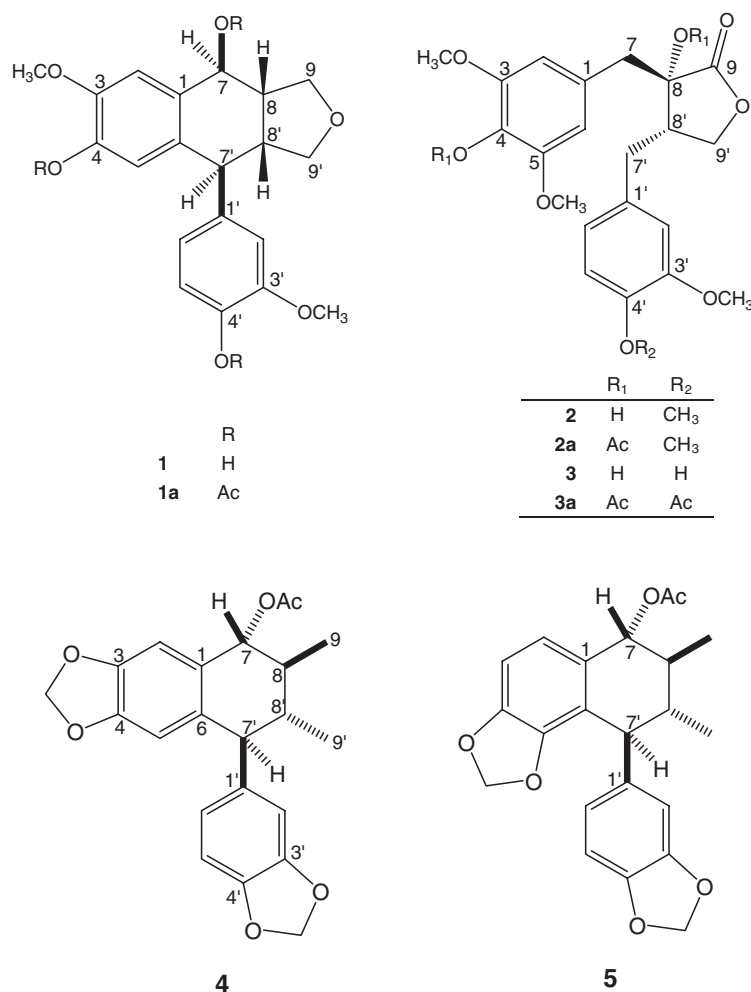
Of the genus *Cunninghamia* (Taxodiaceae), one transplantation (*C. konishii*) and one aboriginal species (*C. lanceolata*) are widely grown in Taiwan.¹⁾ *C. lanceolata*, a large evergreen tree, is cultivated at low altitudes in the north and central Taiwan, and has long been used as a folk medicine for the treatment of asthma, as an analgesic, and of arthritis.²⁾ Previous phytochemical examinations on this plant have revealed a collection of monoterpene essential oils and a series of bis-flavonoids from its wood²⁾ and leaves,³⁾ respectively. However, there are still many other constituents worth investigating to discover and develop potential therapeutic agents. Further studies on the medium-polar layer of a methanolic extract of its heartwood yielded two new lignans accompanied by a known one. This paper deals with the isolation and structural elucidation of these compounds.

The methanolic extract of the heartwood of *C. lan-*

ceolata was concentrated to give a residue which was partitioned between EtOAc and water. The combined EtOAc-soluble layer was then sequentially separated by Si-gravity column chromatography to give a fraction containing 1–3, then peracetylated due until no acetyl group could be observed in their ¹H-NMR spectra, and finally chromatographed by HPLC to yield three pure acetylated lignan derivatives 1a–3a.

Compound 1a was obtained as a colorless oil, whose molecular formula was confirmed to be C₂₆H₂₈O₉ as deduced from HR-EI-MS and ¹³C-NMR spectral data. Its IR spectrum exhibited the characteristic absorption of carbonyl groups at 1765 and 1745 cm^{−1} and an aromatic nucleus at 1600 and 1507 cm^{−1}. The ¹H- and ¹³C-NMR spectra of 1a exhibited the general features of an aryltetralin-type lignan,⁴⁾ with two methoxy groups and two acetoxy functionalities on aromatic rings. In the ¹H-NMR spectrum of 1a (Table 1), a multiplet at δ_H 2.51, a singlet at δ_H 4.74, a singlet at δ_H 4.18, and a multiplet at δ_H 2.55 were attributed respectively to H-8, H-7, H-7', and H-8', while four double doublets at δ_H 4.11, 3.77, 4.02, and 3.87 were respectively assigned to be H-9β, H-9α, H-9'β, and H-9'α. The signals for five singlets at δ_H 1.98, 2.23, 2.28, 3.70, and 3.84 corresponded to three acetoxy (one alkyl acetoxy and two aryl acetoxy) and two methoxy groups. ABX-type resonances at δ_H 6.56 (1H, s), 6.54 (1H, d, *J* = 8.4 Hz), and 6.92 (1H, d, *J* = 8.4 Hz) were attributed to a 1,3,4-trisubstituted aryl group, and two singlets at δ_H 6.71 and 6.83 were characterized as being the other 1,2,4,5-tetrasubstituted aromatic ring. The ¹³C-NMR spectrum revealed 26 carbon resonances, which were further assigned by the

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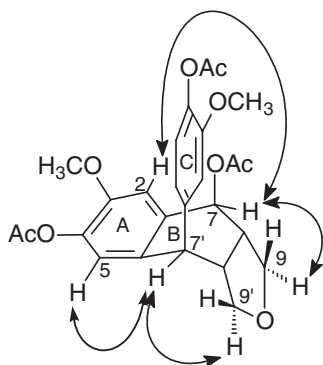
DEPT spectrum as two methoxy carbons, three acetoxy methyl carbons, three acetoxy quaternary carbons, four methine carbons, two alcoholic methylene carbons, five phenyl methine carbons, and seven phenyl quaternary carbons. In the HMBC spectrum of **1a**, the long-range ^{13}C - ^1H correlations, including C-3 (δ_{C} 150.0) with one methoxy (δ_{H} 3.84), C-3' (δ_{C} 150.9) with the other methoxy (δ_{H} 3.70), one acetoxy-bearing carbon signal C-7 (δ_{C} 77.9) with H-2 [δ_{H} 6.83 (1H, s)] and H-9 α [δ_{H} 3.77 (1H, dd, J = 9.2, 5.2 Hz)], another acetoxy-bearing carbon C-4 (δ_{C} 139.7) with H-2 [δ_{H} 6.83 (1H, s)] and H-5 [δ_{H} 6.71 (1H, s)], and the other acetoxy-bearing carbon C-4' (δ_{C} 138.2) with H-2' [δ_{H} 6.56 (1H, s)] and H-5' [δ_{H} 6.92 (1H, d, J = 8.4 Hz)] established the locations of two methoxy and three acetoxy groups. H-7' (δ_{H} 4.18) of **1a** exhibited a lower field shift than that of **4** ($\delta_{\text{H-7'}}$ 3.44) and **5** ($\delta_{\text{H-7'}}$ 3.51).⁵⁾ Meanwhile, H-5 (δ_{H} 6.71) of **1a** exhibited a NOESY correlation with H-7', and was also shifted to a lower field than its corresponding proton in **4** ($\delta_{\text{H-5}}$ 6.12). These data suggested 7'-aryl of **1a** to be in β -pseudo-axial orientation due to escaping the peri-steric hindrance observed from molecular modeling. H-7 (δ_{H} 4.74) also exhibited a NOESY correlation with H-2 (δ_{H} 6.83), suggesting that it was in pseudo-equatorial orientation, as shown in Fig. 1. The acetoxy group

(δ_{H} 1.98, s) was shifted to a higher field than the general data, the supposition being that this acetoxy group was located at the same face as ring C due to shielding by ring C. The evidence provides the conclusion that ring B adopted a twisted-boat form of conformation (Fig. 1). Tetrahydrofuran fused at C-8 was not positioned at the β -face, because the 1,3-pseudo-diaxial orientation has more steric hindrance than peri-effect hindrance, which would force ring C into adopting the β -quasi-equatorial orientation as in compounds **4** and **5**. NOESY correlations of H-7/H-9 α (δ_{H} 3.77) and H-7'/H-9' α (δ_{H} 3.87) illustrate the tetrahydrofuran fused in *cis*-form at the α -face (Fig. 1). This structure also coincides with the result of singlet patterns of H-7 and H-7'. The relative configuration of **1a** was therefore unambiguously determined as that shown.

The molecular formula for **2a**, $\text{C}_{26}\text{H}_{30}\text{O}_{10}$, was determined by ^{13}C -NMR and HR-EI-MS data. The IR spectrum of **2** indicated the presence of a γ -lactone and phenyl acetoxy carbonyl (1772 cm^{-1}), alkyl carbonyl (1735 cm^{-1}), and benzene ring (1613 , 1510 cm^{-1}). In the ^1H -NMR spectrum of **2a** (Table 1), ABX-type resonances at δ_{H} 6.38 (1H, d, J = 1.8 Hz), 6.50 (1H, dd, J = 8.0, 1.8 Hz), and 6.73 (1H, d, J = 8.0 Hz) were attributed to a 1,3,4-trisubstituted aryl group, and one

Table 1. ^1H - and ^{13}C -NMR Data for Compounds **1a–3a** (400 and 100 MHz in CDCl_3)

No.	1a		2a		3a	
	δ_{C}	δ_{H} mult. (J/Hz)	δ_{C}	δ_{H} mult. (J/Hz)	δ_{C}	δ_{H} mult. (J/Hz)
1	127.1		132.0		132.0	
2	111.3	6.83 s	107.1	6.43 s	107.1	6.43 s
3	150.0		152.0		152.0	
4	139.7		128.1		128.2	
5	125.4	6.71 s	152.0		152.0	
6	138.2		107.1	6.43 s	107.1	6.43 s
7	77.9	4.74 s	43.5	2.95 d (14.0) 3.15 d (14.0)	43.6	2.93 d (14.0) 3.15 d (14.0)
8	45.0	2.51 m	80.2		80.1	
9	70.9	3.77 dd (9.2, 5.2) 4.11 dd (9.2, 6.0)	174.8		174.7	
1'	143.1		130.2		136.7	
2'	112.7	6.56 s	111.6	6.38 d (1.8)	112.6	6.43 d (1.8)
3'	150.9		147.8		151.1	
4'	138.2		148.9		138.5	
5'	122.6	6.92 d (8.4)	111.2	6.73 d (8.0)	122.9	6.89 d (8.0)
6'	120.8	6.54 d (8.4)	120.3	6.50 dd (8.0, 1.8)	120.3	6.50 dd (8.0, 1.8)
7'	52.1	4.18 s	33.3	2.28 ^a 2.30 ^a	33.7	2.28 ^a 2.30 ^a
8'	40.8	2.55 m	42.4	2.76 m	42.2	2.76 m
9'	63.6	3.87 dd (11.2, 2.8) 4.02 dd (11.2, 9.6)	71.6	4.15 dd (8.2, 8.2) 4.20 dd (8.2, 8.2)	71.7	4.21 d (9.2)
3-OCH ₃	55.9	3.84 s	55.8	3.73 s	55.8	3.73 s
5-OCH ₃			55.8	3.73 s	55.8	3.73 s
3'-OCH ₃	55.9	3.70 s	56.1	3.76 s	56.1	3.76 s
4'-OCH ₃			56.1	3.80 s		
-OCOCH ₃	169.1		168.5		168.5	
-OCOCH ₃	20.6	2.23 s	20.5	2.22 s	20.4	2.22 s
-OCOCH ₃	168.9		170.2		168.9	
-OCOCH ₃	20.6	1.98 s	20.4	2.32 s	20.6	2.26 s
-OCOCH ₃	170.9				170.2	
-OCOCH ₃	20.6	2.28 s			20.5	2.31 s

^aSignals without multiplicity were picked up from the COSY or HMQC spectra.**Fig. 1.** Relative Stereochemistry and Key NOESY Data (↔) for Compound **1a**.

singlet at δ_{H} 6.43 (2H, s) was deduced as the other 1,3,4,5-tetrasubstituted aromatic ring with 3,5-symmetry. Its ^{13}C -NMR spectrum (Table 1) also indicated the presence of 12 aromatic carbon resonances, seven of which were substituted, as confirmed by the DEPT experiment. The foregoing data plus the remaining carbons, including two methylene carbons (δ_{C} 33.3, 43.5), one oxymethylene carbon (δ_{C} 71.6), one methine carbon (δ_{C} 42.4), one quaternary oxygenated carbon (δ_{C}

80.2), and one lactone carbonyl carbon (δ_{C} 174.8), suggested a diarylbutyrolactone type of lignan.⁶⁾ Long-range ^{13}C - ^1H correlation (HMBC) data unambiguously established the structure of **2a**. Correlations were observed between H₂-9' (δ_{H} 4.15, dd, J = 8.2, 8.2 Hz) and C-7' (δ_{C} 33.3), the oxygenated quaternary carbon C-8 (δ_{C} 80.2) and the lactone carbonyl carbon C-9 (δ_{C} 174.8) also confirming a diaryl- γ -butyrolactone. Other diagnostic correlations including C-3, -5 (δ_{C} 152.0) with one singlet methoxy [δ_{H} 3.73 (6H)], C-3' (δ_{C} 147.8) with another methoxy (δ_{H} 3.76), C-4' (δ_{C} 148.9) with the other methoxy (δ_{H} 3.80), one acetoxy-bearing carbon C-4 (δ_{C} 128.1) with H-2, -6 [δ_{H} 6.43 (2H, s)], the other acetoxy-bearing carbon C-8 (δ_{C} 80.2) with H-7a [δ_{H} 2.95 (1H, d, J = 14.0 Hz)] and H-7b [δ_{H} 3.15 (1H, d, J = 14.0 Hz)] established the locations of three methoxyl and two acetoxyl groups. In the ^{13}C -NMR spectrum of **2a**, the chemical shifts of C-7 (δ_{C} 43.5), C-8 (δ_{C} 80.2), C-9 (δ_{C} 174.8), C-7' (δ_{C} 33.3), C-8' (δ_{C} 42.4), and C-9' (δ_{C} 71.6), diagnostic for the distinction between 8,8'-*cis*- and 8,8'-*trans*-fused butyrolactone rings, were superimposable on those of a *trans*-fused acetylated butyrolactone such as trachelogenin diacetate.⁷⁾ Thus, compound **2a** was characterized to be 5-methoxytrachelogenin diacetate.

The spectroscopic data for **3a** were interpreted by comparing with those for **2a**. The only structural difference between **3a** and **2a** is a methoxy group located at C-4' of **2a** being substituted by an acetoxy functionality in **3a**, as was evident by the significant down-field shifts of $\delta_{C-3'}$ and $\delta_{C-5'}$, and the up-field shift of $\delta_{C-4'}$. Therefore, **3a** was determined to be a triacetylated derivative of 5-methoxywikstromol, which had previously been isolated from *Thuja occidentalis*⁸⁾ and synthesized *via* Pd-charcoal catalysis of the (–)-*O*-dibenzylhydroxythujaplicatin methyl ester.⁹⁾ However, its ¹³C-NMR data is reported for the first time in this paper.

Experimental

General. Optical rotation data were measured with Jasco DIP-180 digital polarimeter at room temperature. IR spectra were recorded with a Perkin-Elmer 781 spectrophotometer. ¹H- and ¹³C-NMR spectra were recorded with a Bruker AM-300 instrument, using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ values (ppm), and coupling constants (*J*) are given in hertz (Hz). Electron-impact mass spectra (EI-MS) were obtained by Finnigan TSQ-U6C and Jeol SX-102A mass spectrometers. The extract was chromatographed on silica gel (Merck 3374, 70–230 mesh), and further purified in a semi-preparative normal-phase HPLC column (250 × 10 mm; 7 μ m, Li Chrosorb Si 60).

Plant material. The heartwood of *Cunninghamia lanceolata* (Lamb.) Hook. f. was collected on the Mount of Nantou in Taiwan on 1995 and identified by Prof. Shao-Shun Ying of Department of Forestry at 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 air-dried heartwood of *C. lanceolata* (7.0 kg) was extracted three times with 80 liter of MeOH (7 d each) at room temperature. The combined extract was evaporated under vacuum to give a black residue, which was suspended in 8-liter of water and then partitioned with EtOAc (2-liter × 3). The ethyl acetate layer (120.5 g) was then chromatographed in an Si column, using mixtures of *n*-hexane and EtOAc as the eluent. The relative polar fractions were dissolved in pyridine and Ac₂O, and the mixture left overnight at room temperature. The reaction mixture was then added dropwise to ice-cooled water while stirring, and the resulting suspension was extracted with EtOAc. After the usual work-up, the extract could be readily purified by normal-phase HPLC with 80% EtOAc in *n*-hexane (flow rate of 3 ml/min) to yield pure acetylated derivatives **1a** (6.8 mg), **2a** (8.7 mg) and **3a** (11.5 mg).

Lanceoline triacetate (1a): colorless oil; $[\alpha]_D^{26} +17.2^\circ$ (*c* 0.2, CHCl₃); IR $\nu_{\max}$ (dry film) cm^{−1}: 2952, 1765, 1745, 1600, 1507; ¹H-NMR: see Table 1; ¹³C-

NMR: see Table 1; EI-MS (70 eV, rel. int. %) *m/z*: 484 (M⁺, 10), 443 (15), 442 (45), 400 (100), 309 (20), 149 (18), 57 (55); HR-EI-MS *m/z*: 484.1740 (M⁺, calcd. for C₂₆H₂₈O₉, 484.1733).

5-Methoxytrachelogenin diacetate (2a): colorless oil; $[\alpha]_D^{26} -84.5^\circ$ (*c* 0.3, CHCl₃); IR $\nu_{\max}$ (KBr) cm^{−1}: 2952, 1772, 1735, 1613, 1510, 1188, 1023; ¹H-NMR: see Table 1; ¹³C-NMR: see Table 1; EI-MS (70 eV, rel. int. %) *m/z*: 502 (M⁺, 10), 460 (20), 249 (25), 167 (38), 151 (100), 137 (20), 84 (45), 57 (45); HR-EI-MS *m/z*: 502.1849 (M⁺, calcd. for C₂₆H₃₀O₁₀, 502.1839).

5-Methoxywikstromol triacetate (3a): colorless oil; $[\alpha]_D^{26} -85.2^\circ$ (*c* 0.5, CHCl₃); IR $\nu_{\max}$ (dry film) cm^{−1}: 2945, 1772, 1735, 1606, 1513, 1135, 1023; ¹H-NMR: see Table 1; ¹³C-NMR: see Table 1; EI-MS (70 eV, rel. int. %) *m/z*: 530 (M⁺, 2), 488 (20), 426 (15), 384 (30), 249 (100), 167 (35), 137 (40), 57 (35); HR-EI-MS *m/z*: 530.1777 (M⁺, calcd. for C₂₇H₃₀O₁₁, 530.1788).

Acknowledgments

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