

Two New Lignans from the Wood of *Cunninghamia konishii*Chi-I Chang^a, Yen-Cheng Li^b, Ching-Chuan Kuo^c, Che-Yi Chao^d, Hsun-Shuo Chang^e, Jyh-Horng Wu^f, Sheng-Yang Wang^{g,**} and Yueh-Hsiung Kuo^{g,h,*}^aDepartment of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung 912, Taiwan^bDepartment of Chemistry, National Taiwan University, Taipei 106, Taiwan^cNational Institute of Cancer Research, National Health Research Institutes, Tainan 707, Taiwan^dDepartment of Health and Nutrition Biotechnology, Asia University, Taichung 413, Taiwan^eGraduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan^fDepartment of Forestry, National Chung Hsing University, Taichung 402, Taiwan^gDepartment of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University, Taichung 404, Taiwan^hTsuzuki Institute for Traditional Medicine, College of Pharmacy, China Medical University, Taichung 404, Taiwan

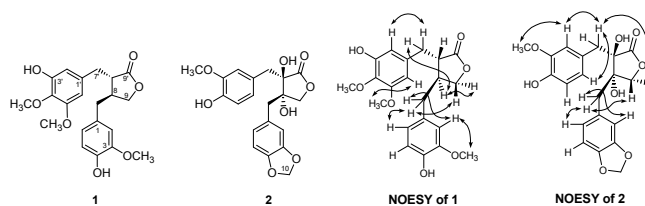
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Received: February 6th, 2013; Accepted: April 4th, 2013Two new lignans, (8*S*,8'*S*)-4,3'-dihydroxy-3,4',5'-trimethoxylignan-9',9-olide (**1**) and *trans*-4',8,8'-trihydroxy-3'-methoxy-3,4-methylenedioxy lignan-9',9-olide (**2**), were isolated from the wood of *Cunninghamia konishii*. Their structures were determined by analysis of spectroscopic data.**Keywords:** *Cunninghamia konishii*, Taxodiaceae, Lignan, (8*S*,8'*S*)-4,3'-Dihydroxy-3,4',5'-trimethoxylignan-9',9-olide, *trans*-4',8,8'-Trihydroxy-3'-methoxy-3,4-methylenedioxy lignan-9',9-olide.

The *Cunninghamia* genus (Taxodiaceae) has two species growing in eastern Asia, one of which is *Cunninghamia konishii* Hayata, an endemic coniferous tree distributed in the northern and central part of Taiwan at altitudes of 1,300–2,700 m [1]. Previous investigations on *C. konishii* have shown that it contains monoterpenes, sesquiterpenes, and diterpenes [2a-k]. Some isolates of this plant exhibit antifungal activity [2g-i] and cytotoxicity [2k]. In the continuing phytochemical investigation [2e,f,i], we further identified two new lignans (**1** and **2**) from the wood of *C. konishii*.

The molecular formula of compound **1**, C₂₁H₂₄O₇, was determined by HR-ESI-MS [M]⁺, *m/z* 388.1519]. The ¹H and ¹³C NMR spectra of **1** revealed a typical 2,3-dibenzyl- γ -butyrolactone skeleton, characterized by the signals at δ_C 178.6 (C-9'), 41.3 (C-8'); H-8, δ_H 2.47, 46.3 (C-8; H-8, δ_H 2.51), and 71.3 (C-9; H₂-9, δ_H 3.88 and 4.13) [3a]. One of the benzyl moiety was assigned as 4-hydroxy-3-methoxybenzyl by the NMR signals including a set of ABX coupling aromatic protons [δ_H 6.77 (1H, d, *J* = 8.0 Hz), 6.49 (1H, dd, *J* = 8.0, 2.0 Hz), and 6.46 (1H, d, *J* = 2.0 Hz)], one methoxy [δ_H 3.82 (3H, s)], one hydroxy [δ_H 5.52 (1H, brs)] and one benzylic methylene [δ_H 2.46 m, 2.59 m]. The other one was identified as 3-hydroxy-4,5-dimethoxybenzyl by a set of *meta* coupling aromatic protons [δ_H 6.26 (1H, d, *J* = 2.0 Hz) and 6.36 (1H, d, *J* = 2.0 Hz)], two methoxy [δ_H 3.78 (3H, s); 3.84 (3H, s)], one hydroxy [δ_H 5.79 (1H, brs)], and one methylene [δ_H 2.81 (1H, dd, *J* = 13.6, 6.8 Hz), 2.90 (1H, dd, *J* = 13.6, 4.8 Hz)]. Two benzyl moieties were attached on C-8 and C-8', respectively, confirmed by the HMBC correlations between H-7 (δ_H 2.46, 2.59) and C-1 (δ_C 129.8), C-2 (δ_C 111.0), C-6 (δ_C 121.2), C-8 (δ_C 46.3), C-9 (δ_C 71.3) and C-8' (δ_C 41.3); H-7' (δ_H 2.81, 2.90) and C-1' (δ_C 133.9), C-2' (δ_C 108.9), C-6' (δ_C 104.9), C-8' (δ_C 41.3), C-9' (δ_C 178.6) and C-8. Relative configuration of

Figure 1: Structures of compounds **1** and **2** and selected NOE correlations.Table 1: ¹H and ¹³C NMR data for **1** and **2** (400 and 100 MHz in CDCl₃).

No.	1		2	
	δ_C	δ_H	δ_C	δ_H
1	129.8		128.0	
2	111.0	6.46 d (2.0)	110.4	6.67 d (1.6)
3	146.6		148.1	
4	144.4		147.1	
5	114.4	6.77 d (8.0)	108.7	6.77 d (8.0)
6	121.2	6.49 dd (8.0, 2.0)	123.2	6.61 dd (8.0, 1.6)
7	38.2	2.46 m 2.59 m	37.7	2.81 d (14.1) 2.77 d (14.1)
8	46.3	2.51 m	79.0	
9	71.3	3.88 dd (9.2, 6.0) 4.13 dd (9.2, 7.2)	74.2	4.32 d (9.6) 3.76 d (9.6)
10			101.2	5.94 s
1'	133.9		125.1	
2'	108.9	6.36 d (2.0)	113.8	6.90 d (1.6)
3'	149.1		146.5	
4'	133.9		145.1	
5'	152.5		114.3	6.85 d (8.0)
6'	104.9	6.26 d (2.0)	124.0	6.78 dd (8.0, 1.6)
7'	35.0	2.81 dd (13.6, 6.8) 2.90 dd (13.6, 4.8)	37.1	3.07 d (14.2) 3.03 d (14.2)
8'	41.3	2.47 m	76.9	
9'	178.6		177.0	
3-OMe	55.8	3.82 s		
3'-OMe			56.0	3.87 s
4'-OMe	61.0	3.84 s		
5'-OMe	55.8	3.78 s		
4-OH		5.52 brs		
4'-OH		5.79 brs		5.62 brs

^a) Coupling constants are presented in Hz.

butyrolactone ring was determined by significant NOE correlations between H-8/H_a-9 (δ_{H} 3.88) and H-8/H₂-7' in the NOESY spectrum (Figure 1). Compound **1** showed a positive specific rotation ($[\alpha]_{\text{D}}^{26} = +33.5$), which is opposite to that of 2*R*,3*R*-dibenzylbutyrolactone [3b], indicating a (8*S*,8'*S*) configuration. Accordingly, compound **1** was determined to be (8*S*,8'*S*)-4,3'-dihydroxy-3,4',5'-trimethoxyignan-9',9-olide (**1**).

Compound **2**, C₂₀H₂₀O₈ ($[\text{M}]^+$ at m/z 388.1155), displayed IR absorption bands corresponding to hydroxyl (3436 cm⁻¹), γ -lactone (1766 cm⁻¹), and aromatic (1613 and 1520 cm⁻¹), and methylenedioxy (1043 and 941 cm⁻¹) groups. The ¹H NMR revealed an unusual 2,3-dihydroxy-2,3-dibenzyl- γ -butyrolactone skeleton, characterized by the signals at δ_{C} 74.2 (C-9; H₂-9, δ_{H} 3.76 and 4.32), 76.9 (C-8'), 79.0 (C-8), and 177.0 (C-9') [4]. The ¹H and ¹³C NMR data revealed that one of benzyl moiety was 3,4-methylenedioxybenzyl constructed by the NMR signals of a set of ABX coupling aromatic protons [δ_{H} 6.77 (1H, d, $J = 8.0$ Hz), 6.61 (1H, dd, $J = 8.0, 1.6$ Hz), and 6.67 (1H, d, $J = 1.6$ Hz)], one methylenedioxy [δ_{H} 5.94 (2H, s)], and one benzylic methylene [δ_{H} 2.77 (1H, d, $J = 14.1$ Hz), 2.81 (1H, d, $J = 14.1$ Hz)]. The other benzyl moiety as 4-hydroxy-3-methoxybenzyl was confirmed by a set of ABX pattern aromatic protons [δ_{H} 6.85 (1H, d, $J = 8.0$ Hz), 6.78 (1H, dd, $J = 8.0, 1.6$ Hz), and 6.90 (1H, d, $J = 1.6$ Hz)], one methoxy [δ_{H} 3.87 (3H, s)], one hydroxy [δ_{H} 5.62 (1H, brs)], and a benzylic methylene [δ_{H} 3.03 (1H, d, $J = 14.0$ Hz), 3.07 (1H, d, $J = 14.0$ Hz)]. 3,4-Methylenedioxybenzyl and 4-hydroxy-3-methoxybenzyl moieties were attached on C-8 and C-8', respectively, assured by the HMBC correlations H-7 (δ_{H} 2.77, 2.81) and C-1 (δ_{C} 128.0), C-2 (δ_{C} 110.4), C-6 (δ_{C} 123.2), C-8 (δ_{C} 79.0), C-9 (δ_{C} 74.2) and C-8' (δ_{C} 76.9); between H-7' (δ_{H} 3.03, 3.07) and C-1' (δ_{C} 125.1), C-2' (δ_{C} 113.8), C-6' (δ_{C} 124.0), C-8', and C-9' (δ_{C} 177.0). The *trans*-configuration at C-8 and C-8' was judged from two reasons: one is that significant NOE correlations between H₂-7/H_a-9 (δ_{H} 4.32) and H₂-7'/H_a-9 (δ_{H} 3.76) were observed in the NOESY spectrum, and the other is that no NOE correlation was found between H₂-7 and H₂-7' in the NOESY spectrum (Figure 1). Accordingly, compound **2** was determined to be *trans*-4',8,8'-trihydroxy-3'-methoxy-3,4-methylenedioxyignan-9',9-olide.

Experimental

Plant material: The wood of *C. konishii* was collected at Luantashan, Nantau County, Taiwan, in December 1996. The plant material was identified by Prof. Shao-Shun Ying, Department of

Forestry, NTU. A voucher specimen (013492) has been deposited at the Herbarium of National Taiwan University, Taipei, Taiwan.

Extraction and isolation: Dried wood (6.5 kg) of *C. konishii* was crushed into pieces and extracted with MeOH (60 L) three times (7 days each time) at room temperature. After removal of the solvent under vacuum, the extract (60.2 g) was suspended in water (500 mL), and then partitioned sequentially using *n*-hexane (500 mL \times 3), EtOAc (500 mL \times 4), and *n*-BuOH (500 mL \times 3). The EtOAc fraction (15.6 g) was chromatographed on silica gel (450 g) using *n*-hexane–EtOAc and EtOAc–MeOH mixtures as solvent systems to obtain 11 fractions. HPLC of fr. 5 from *n*-hexane/EtOAc (2/3) elution on a Merck LiChrosorb Si 60 column with *n*-hexane–CH₂Cl₂–EtOAc–*i*-PrOH (5:5:1:0.2) as eluent yielded **1** (3.7 mg). Fr. 9 from *n*-hexane–EtOAc (1:4) elution was further purified by HPLC to give **2** (3.1 mg) using CH₂Cl₂–acetone–*i*-PrOH (3:1:0.2).

(8*S*,8'*S*)-4,3'-Dihydroxy-3,4',5'-trimethoxyignan-9',9-olide (**1**)

Yellowish oil.

$[\alpha]_{\text{D}}^{26}$: +33.5 (c 0.31, CHCl₃).

IR: 3397, 1766, 1573, 1484, 1381, 1162, 844, 758 cm⁻¹.

UV λ_{max} (MeOH) nm (log ϵ): 207 (4.42), 238 (sh, 3.94), 280 (3.39).

¹H and ¹³C NMR: Table 1.

EI-MS m/z (rel. int.): 388 [$\text{M}]^+$ (83), 378 (4), 368 (6), 354 (3), 344 (2), 332 (3), 328 (6), 316 (7).

HR- EI-MS: m/z 388.1519 (calcd for C₂₁H₂₄O₇ 388.1522, [$\text{M}]^+$).

trans-4',8,8'-Trihydroxy-3'-methoxy-3,4-methylenedioxyignan-9',9-olide (**2**)

Yellowish oil.

$[\alpha]_{\text{D}}^{26}$: -124.7 (c 0.41, CHCl₃).

IR: 3436, 1766, 1613, 1520, 1434, 1043, 941, 917 cm⁻¹.

UV λ_{max} (MeOH) nm (log ϵ): 207 (4.40), 230 (4.05), 283 (3.81).

¹H and ¹³C NMR: Table 1.

EI-MS m/z (rel. int.): 388 [$\text{M}]^+$ (100), 379 (4), 369 (6), 354 (5), 343 (3), 332 (3), 329 (7), 317 (8).

HR- EI-MS: m/z 388.1155 (calcd for C₂₀H₂₀O₈ 388.1158, [$\text{M}]^+$).

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