

Article

Abietane Diterpenoids from the Bark of *Cryptomeria japonica* and Their Antifungal Activities against Wood Decay Fungi

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Abstract: Phytochemical investigation of the bark of *Cryptomeria japonica* led to the isolation of five new abietane diterpenoids, 5-*epi*-12-hydroxy-6-*nor*-5,6-secoabieta-8,11,13-trien-7,5-olide (1), 12-hydroxy-6 β -methoxy-6,7-secoabieta-8,11,13-trien-7,6-olide (2), 6 β ,12-dihydroxy-7,8-secoabieta-8,11,13-trien-7,8-olide (4), 5,12-dihydroxy-7,8-secoabieta-8,11,13-trien-7,8-olide (5), and 5 α ,8-epoxy-12-hydroxy-7,8-secoabieta-8,11,13-trien-7-al (6), together with one known abietane diterpenoid, obtuanhydride (3). Their structures were elucidated by analysis of spectroscopic data and comparison with the spectral data of known analogs. At the concentration of 100 μ g/mL, compounds 4, 5, and 6 inhibited antifungal activities against wood decay fungi activity by 18.7, 37.2, and 46.7%, respectively.

Keywords: Cupressaceae; *Cryptomeria japonica*; bark; abietane; antifungal



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1. Introduction

Cryptomeria japonica D. Don is a massive evergreen coniferous tree belonging to the monospecific genus *Cryptomeria* in the cypress family Cupressaceae. It is endemic to Japan, known as Japanese cedar or sugi in Japanese [1], and is the main forestry tree species in Japan. This conifer is widely distributed in warm and cool temperate climates. The wood of *C. japonica* is used as a raw material to produce building materials and wood products. Due to its excellent properties, such as aromatic, reddish-pink in color, soft, lightweight yet sturdy, waterproof, and resistant to natural decay, it has become one of the most commercially important plantation forest tree species in several Asian countries, including Japan, Taiwan, Korea, China, India, and Nepal. Researchers have identified a variety of terpenoids, including monoterpenoids, sesquiterpenoids, and diterpenoids from the leaves, heartwood, and barks of this plant [2–24]. Additionally, the crude extracts and secondary metabolites of this plant have been proven to possess a wide range of bioactivities, including cytotoxic [23], antifungal [24], antibacterial [23], antioxidant [25], anti-inflammatory [26], and insect antifeedant [27] and repellent [18] properties. During our ongoing search for new and bioactive metabolites from the bark of *C. japonica*, three sesquiterpenoids [28,29] and ten abietane-type diterpenoids had already been reported by us [29].

Phellinus noxius is an aggressive and destructive pathogen. Trees infected by *P. noxius* could develop brown root disease. The mycelium of *P. noxius* mainly grows in tree roots

an anisotropic effect from the phenyl group. Additionally, the HMBC correlations between H-15/C-12 and C-13; Me-16/C-13; Me-18/C-3; Me-19/C-5; and Me-20/C-1, C-5, and C-9 helped to construct the planar structure of **1**. The relative configurations of stereogenic C-atoms in the tricyclic rings were determined by significant NOE correlations between H_α-1 (δ_{H} 2.36)/H-11, H-5/Me-19, and H-5/Me-20 in the NOESY spectrum (Figure 2). Thus, the structure of **1** was determined as 5-*epi*-12-hydroxy-6-*nor*-5,6-secoabieta-8,11,13-trien-7,5-olide. Complete ¹H and ¹³C NMR chemical shifts were established by ¹H-¹H COSY, HMQC, HMBC, and NOESY spectra.

Table 1. ¹H NMR spectral data of compounds **1**, **2**, and **4–6** (400 MHz in CDCl₃).

No.	1	2	4	5	6
1	1.42 m, 2.36 m	1.82 m, 1.78 m	1.74 m, 1.77 m	1.39 m, 1.62 br d (13.2)	1.32 m, 1.72 td (12.8, 3.2)
2	1.40 m, 1.41 m	1.67 m, 1.80 m	1.62 m, 1.66 m	1.32 m, 1.40 m	1.16 m, 1.38 m
3	1.27 m, 1.43 m	1.23 m, 1.54 br d (12.8)	1.25 m, 1.53 br d (14.0)	1.34 m, 1.71 td (12.4, 3.6),	1.49 m, 1.57 m
5	4.05 s	1.69 d (1.6)	1.62 s		
6		5.11 d (1.6),	4.53 s	2.57 d (16.8), 2.61 d (16.8)	2.61 d (3.6)
7					9.38 t (3.6)
11	6.65 s	6.69 s	6.71 s	6.46 s	6.38 s
14	7.91 s	7.65 s	6.97 s	6.72 s	6.65 s
15	3.14 sept (7.2)	3.11 sept (6.8)	3.11 sept (6.8)	3.13 sept (6.8)	3.13 sept (6.8)
16	1.25 d (7.2)	1.25 d (6.8)	1.23 d (6.8)	1.21 d (6.8)	1.18 d (6.8)
17	1.26 d (7.2)	1.25 d (6.8)	1.22 d (6.8)	1.20 d (6.8)	1.17 d (6.8)
18	0.35 s	0.91 s	1.00 s	1.20 s	1.17 s
19	1.08 s	1.12 s	1.06 s	1.16 s	1.05 s
20	1.28 s	1.44 s	1.45 s	1.37 s	1.38 s
11-OH	5.71 s	5.24 s	4.94 br s		5.11 s
6-OCH ₃		3.34 s			

Table 2. ¹³C NMR spectral data of compounds **1**, **2**, and **4–6** (100 MHz in CDCl₃).

No.	1	2	4	5	6
1	35.2	40.0	39.6	40.8	38.1
2	18.7	19.0	18.9	17.9	18.1
3	40.5	41.5	41.6	38.4	41.0
4	37.2	35.0	35.7	37.4	37.0
5	91.6	61.4	67.6	96.0	96.3,
6		105.1	73.4	36.6	47.0
7	164.6	170.1	171.1	172.4	200.8
8	118.4	124.2	143.7	147.3	149.7
9	144.3	148.9	137.3	136.8	136.4
10	37.1	39.8	38.4	48.6	47.9
11	109.0	110.4	111.4	109.2	108.6
12	157.4	155.7	149.4	147.7	147.0
13	132.7	131.6	133.9	133.7	134.0
14	129.0	130.0	117.9	107.9	107.3
15	27.2	27.1	27.2	27.6	27.4
16	22.7	22.8	22.5	22.9	23.1
17	22.7	22.7	22.7	23.0	23.0
18	22.0	32.8	32.6	28.5	28.1
19	30.9	22.6	22.6	26.0	25.6
20	34.0	22.8	20.3	19.9	19.5
6-OCH ₃		56.5			

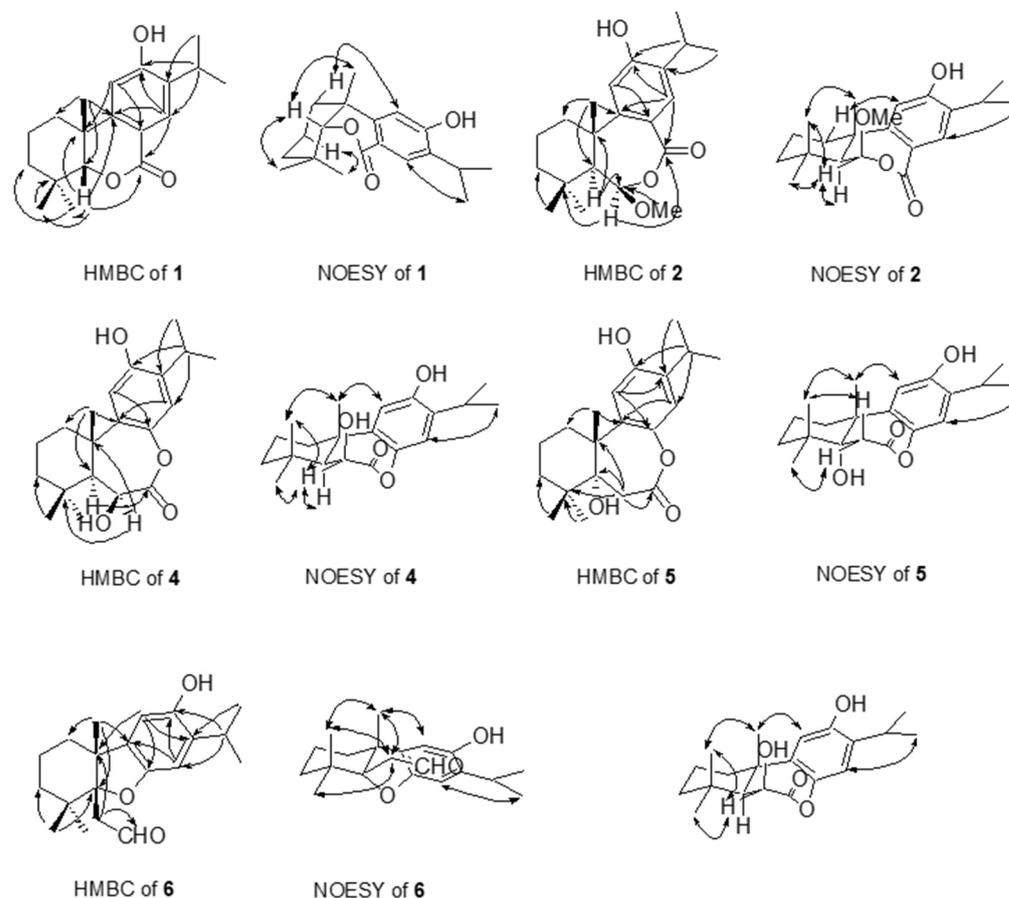


Figure 2. Selected HMBC and NOE correlations of **1**, **2**, and **4–6**.

The UV maximum (271 nm) and IR absorptions (1679, 1606, and 1460 cm^{-1}) of **2** indicated the presence of the benzoyl moiety [32]. An IR absorption at 3337 cm^{-1} for the hydroxyl group was also observed. The molecular formula was established to be $\text{C}_{21}\text{H}_{30}\text{O}_4$ from its HR-EI-MS molecular ion at m/z 346.2150 and its ^{13}C NMR data, indicating seven degrees of unsaturation. The ^1H NMR spectrum of **2** (Table 1) showed resonances for three tertiary-linked methyls [δ_{H} 0.91, 1.12, and 1.44 (each 3H, s, Me-18, Me-19, and Me-20)], two *para*-oriented aromatic protons [δ_{H} 6.69 (1H, s) and 7.65 (1H, s)], an isopropyl group [δ_{H} 1.25 (3H \times 2, d, J = 6.8 Hz) and 3.11 (1H, sept, J = 6.8 Hz)], an oxymethine [δ_{H} 5.11 (1H, d, 1.6 Hz)], a methoxy [δ_{H} 3.34 (3H, s)], and a phenolic proton [δ_{H} 5.24 (1H, s, D_2O exchange)]. A total of 21 carbon signals were observed in the ^{13}C NMR spectrum of **2** and were differentiated by DEPT experiments as five aliphatic methyl, three aliphatic methylene, two aliphatic methine, two aliphatic quaternary, one oxygenated methine, two olefinic methine, four quaternary olefinic, one methoxy, and one lactone carbonyl carbons. From the above evidence, compound **2** was suggested as a dehydroabietane diterpene [32]. After subtracting the 6 degrees of unsaturation derived from the rings A and C and the carbonyl group, the remaining one degree of unsaturation, together with the downshifted H-14 [δ_{H} 7.65 (1H, s)], suggested that the conjugated lactone carbonyl (δ_{C} 170.1) was located at C-7 and linked via an oxygen atom to the hemiacetal carbon, C-6 (δ_{C} 105.1). The hemiacetal proton, H-6 [δ_{H} 5.11 (1H, d, J = 1.6 Hz, H-6)], showed both ^1H - ^1H COSY correlation with H-5 with a small coupling constant, 1.6 Hz, and NOESY correlations with Me-18 and Me-19 confirmed the methoxyl group attached to C-6 in β orientation (Figure 2). In addition, H-5 showed a NOESY correlation with Me-18 (δ_{H} 0.91), while no NOESY correlation with Me-19 implied the *trans*-ring junction between rings A and B. From the above evidence, compound **2** was thus formulated as 12-hydroxy-6 β -methoxy-6,7-secoabietane-8,11,13-trien-7,6-olide.

The molecular formula of **4** was assigned as $C_{20}H_{28}O_4$ by HR-EI-MS at m/z 332.1978, representing seven degrees of unsaturation. The IR absorptions indicated the presence of a hydroxyl (3429 cm^{-1}) group and a lactone carbonyl group (1725 cm^{-1}). The ^1H NMR spectrum of **4** (Table 1) displayed the signals for three tertiary-linked methyls [δ_{H} 1.00, 1.06, and 1.45 (each 3H, s, Me-18, Me-19, and Me-20)], one oxymethine [δ_{H} 4.53 (1H, s, H-6)], two *para*-oriented aromatic protons [δ_{H} 6.71 (1H, s) and 6.97 (1H, s)], an isopropyl group on the benzene ring [δ_{H} 1.22 (3H, d, $J = 6.8$ Hz), 1.23 (3H, d, $J = 6.8$ Hz), and 3.11 (1H, sept, $J = 6.8$ Hz)], and a phenolic proton [δ_{H} 4.94 (1H, s, D_2O exchange)]. A total of 20 carbon signals were found in the ^{13}C NMR spectrum of **4** and were assigned by a DEPT experiment as five aliphatic methyl, three aliphatic methylene, two aliphatic methine, two aliphatic quaternary, one oxygenated methine, two olefinic methine, four quaternary olefinic, and one lactone carbonyl carbons. From the above evidence, compound **4** was proposed to be a dehydroabietane diterpene [32]. A downshifted oxymethine [δ_{H} 4.53 (1H, s, H-6)] neighboring to the carbonyl group (δ_{C} 171.1) and an upshifted phenyl proton H-14 [δ_{H} 6.97 (1H, s)] were observed, which suggested that the carbonyl group was situated at C-7, linking to C-8 via an oxygen atom. The *trans*-ring junction between rings A and B was confirmed by the NOESY correlation between H-5/H-6, H-6/Me-18, H-6/Me-19, Me-19/Me-20, and H-11/Me-20 (Figure 2). Furthermore, the HMBC correlations between H-6/C-4 and C-10 and the NOESY correlations between H-6/Me-18 and Me-19 hinted at the hydroxyl group attached to C-6 in β -axial orientation. Thus, compound **4** was identified as 6 β ,12-dihydroxy-7,8-secoabieta-8,11,13-trien-7,8-olide.

The HR-EI-MS of **5** gave a molecular ion at m/z 332.1977, consistent with the molecular formula of $C_{20}H_{28}O_4$, implying seven degrees of unsaturation. The IR absorptions indicated the presence of a hydroxyl (3416 cm^{-1}) group and a lactone carbonyl group (1699 cm^{-1}). The ^1H NMR spectrum of **5** (Table 1) displayed the signals for three tertiary-linked methyls [δ_{H} 1.16, 1.20, and 1.37 (each 3H, s, Me-19, Me-18, and Me-20)], two *para*-oriented aromatic protons [δ_{H} 6.46 (1H, s) and 6.72 (1H, s)], an isopropyl group on the benzene ring [δ_{H} 1.20 (3H, d, $J = 6.8$ Hz), 1.21 (3H, d, $J = 6.8$ Hz), and 3.13 (1H, sept, $J = 6.8$ Hz)], and a typical AB-type methylene neighboring to a carbonyl group [δ_{H} 2.57 (1H, d, $J = 16.8$ Hz) and 2.61 (1H, d, $J = 16.8$ Hz)]. A total of 20 carbon signals were found in the ^{13}C NMR spectrum of **5** and were assigned by a DEPT experiment as five aliphatic methyl, four aliphatic methylene, one aliphatic methine, two aliphatic quaternary, one oxygenated quaternary, two olefinic methine, four quaternary olefinic, and one lactone carbonyl carbons. Compound **5** showed identical NMR characteristics to that of **4**, and the only difference was in the ring B part. The hydroxyl group was attached to C-5 in **5** instead of C-6 in **4**, which was assured by the HMBC correlations between H-6 with C-4, C-5, C-7, and C-10. The NOESY correlation between H_{α} -6 (δ_{H} 2.57)/Me-18, H_{β} -6 (δ_{H} 2.61)/Me-19, Me-20/Me-19, and Me-20/H-11 assured the *trans*-ring junction between rings A and B (Figure 2). Thus, the structure of **5** was determined as 5,12-dihydroxy-7,8-secoabieta-8,11,13-trien-7,8-olide.

The HR-EI-MS of compound **6** showed an $[\text{M}]^+$ ion at m/z 316.2033, which was consistent with the molecular formula $C_{20}H_{28}O_3$, indicating seven degrees of unsaturation. The IR spectrum indicated the presence of a hydroxyl (3423 cm^{-1}) group and an aldehyde carbonyl group (1706 cm^{-1}). In the ^1H NMR spectra of **6** (Table 1), the signals for three tertiary-linked methyls [δ_{H} 1.05, 1.17, and 1.38 (each 3H, s, Me-19, Me-18, and Me-20)], two *para*-oriented aromatic protons [δ_{H} 6.38 (1H, s) and 6.65 (1H, s)], an isopropyl group on the benzene ring [δ_{H} 1.17 (3H, d, $J = 6.8$ Hz), 1.18 (3H, d, $J = 6.8$ Hz), and 3.13 (1H, sept, $J = 6.8$ Hz)], a phenolic proton [δ_{H} 5.11 (1H, s, D_2O exchange)], and an A_2X coupling system of a methylene neighboring to an aldehyde group [δ_{H} 2.61 (2H, d, $J = 3.6$ Hz) and 9.38 (1H, t, $J = 3.6$ Hz)]. The ^{13}C -NMR spectrum of **6** revealed twenty skeletal carbon resonances, including five aliphatic methyl, four aliphatic methylene, one aliphatic methine, three aliphatic quaternary, two olefinic methine, four quaternary olefinic, and one aldehyde carbonyl carbons. From the above evidence, compound **6** was also proposed to be a dehydroabietane diterpene. An unusual oxygenated quaternary carbon signal (δ_{C} 96.3), together with an upshifted H-14 (δ_{H} 6.65), suggested that C-5 linked to C-8 via an oxygen

atom. The HMBC correlations between H-6 (δ_{H} 2.61)/C-7 and C-10 and the NOESY correlations between H-6/Me-18, H-6/Me-19, H-6/Me-20, Me-20/Me-19, and Me-20/H-11 indicated that the 2-oxoethyl moiety was attached to C-5 in β orientation (Figure 2). Therefore, compound **6** was characterized as 5 α ,8-epoxy-12-hydroxy-7,8-secoabieta-8,11,13-trien-7-al.

Compound **3** was identified as a known abietane diterpenoid, obtuanhydride (**3**), by comparing their spectral data of NMR and mass with those described in the literature [31].

The brown root rot fungus *P. noxius* was used to evaluate the antifungal activity of *C. japonica*'s compounds. The antifungal indices of the diterpenoids at the dosage of 100 $\mu\text{g}/\text{mL}$ are listed in Table 3. Among these compounds, 5,12-dihydroxy-7,8-secoabieta-8,11,13-trien-7,8-olide (**5**) and 5 α ,8-epoxy-12-hydroxy-7,8-secoabieta-8,11,13-trien-7-al (**6**) exhibited the stronger antifungal activity against *P. noxius* with antifungal indices of 37.2 and 46.7%, respectively, compared to compounds **1–4**. The commercial fungicide, didecylmethylammonium chloride (DDAC), was used as a positive control at a concentration of 10 $\mu\text{g}/\text{mL}$ with an antifungal index of 51.1%.

Table 3. Antifungal index of diterpenoids from *C. japonica* bark against *Phellinus noxius*.

No.	1	2	3	4	5	6	DDAC
Antifungal index (%)	4.5	11.3	3.4	18.7	37.2	46.7	51.1

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were recorded on a Jasco-DIP-180 polarimeter (JASCO Co., Tokyo, Japan). UV and IR spectra were recorded on a Shimadzu UV-1601 (Shimadzu, Kyoto, Japan) and a Perkin-Elmer 983 G (PerkinElmer Ltd., Bucks, UK) spectrophotometer, respectively. ^1H and ^{13}C NMR and 2D NMR spectra were obtained on a Varian-Unity-Plus-400 spectrometer (Varian Inc., Palo Alto, CA, USA). Chemical shifts are referenced to residual solvent signals. EI-MS and HR-EI-MS were obtained on a Jeol-JMS-HX300 mass spectrometer (JEOL Ltd., Tokyo, Japan). Column chromatography (CC) was performed by using Merck Silica gel 60 (230–400 mesh) (Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) analyses were carried out on pre-coated silica gel plates (silica gel 60 F₂₅₄) (Merck, Darmstadt, Germany). HPLC was performed by using a normal phase column (Purospher STAR Si, 5 μm , 250 \times 10 mm) (Merck, Darmstadt, Germany) on an LDC Analytical-III system (LDC Analytical, Gelnhausen, Germany).

3.2. Plant Material

The bark of *C. japonica* D. Don was collected in Sitou, Taiwan, in June 2000. A voucher specimen (TCF13443) has been deposited at the Herbarium of the Department of Forestry, NCHU, Taiwan. Species identification was confirmed by Dr. Yen-Hsueh Tseng, Department of Forestry, National Chung-Hsing University (NCHU).

3.3. Extraction, Isolation, and Identification

The air-dried bark of *C. japonica* (16.0 kg) was extracted by soaking in MeOH (100 L \times 3) at room temperature for 7 days each time in a closed container. The extracts were combined and concentrated under reduced pressure at 45 $^{\circ}\text{C}$ to produce 480 g of a brown crude residue, which was suspended in H₂O (1 L) and then partitioned sequentially with EtOAc (1 L) and *n*-BuOH (1 L) to afford EtOAc, *n*-BuOH, and H₂O soluble fractions, respectively. The EtOAc fraction (430 g) was subjected to column chromatography on a silica gel (4.0 kg) column, eluted with a gradient of *n*-hexane–EtOAc, followed by an EtOAc–MeOH gradient of increasing polarity to obtain 11 fractions, fr. 1 (2.6 g), 2 (29.4 g), 3 (47.8 g), 4 (92.4 g), 5 (21.6 g), 6 (18.1 g), 7 (22.5 g), 8 (35.8 g), 9 (19.2 g), 10 (44.2 g), and 11 (72.2 g). Fr. 3 from hexane/AcOEt (9:1) elution (47.8 g) was chromatographed over a silica gel column (7 \times

60 cm) using hexane/CH₂Cl₂ (1:0–0:1) mixtures to afford nine fractions, 3A–3H. Further purification of subfraction 3F by HPLC afforded **5** (1.1 mg) using hexane/AcOEt (9:1). Fr. 4 from *n*-hexane–EtOAc (4:1) elution was rechromatographed over a silica gel column using a gradient mixture of CH₂Cl₂–EtOAc (100:1 to 0:1) to obtain sixteen fractions, 4A–4P. Further purification of subfraction 4I by HPLC afforded **1** (2.8 mg) using *n*-hexane–EtOAc (7:3). Further purification of subfraction 4K by HPLC afforded **6** (3.9 mg) using *n*-hexane–EtOAc (7:3). Further purification of subfraction 4L by HPLC afforded **3** (4.6 mg) using *n*-hexane–EtOAc (7:3). Further purification of subfraction 4M by HPLC afforded **2** (1.4 mg) and **4** (3.1 mg) using *n*-hexane–EtOAc (3:1).

5-Epi-12-hydroxy-6-nor-5,6-secoabieta-8,11,13-trien-7,5-olide (1). Gum; [α]_D²⁵: +20.8 (c 0.5, CHCl₃); IR ν_{\max} : 3297, 1679, 1606, 1513, 1467, 1367, 1261, 1182, 1122, 1056, 678 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ): 224 (4.20), 271 (4.05), 295 (3.72) nm; EI-MS *m/z* (%): 302 (100) [M]⁺, 287 ([M–CH₃]⁺, 29), 271 (25), 271 (39), 220 (41), 203 (76), 247 (20), 55 (21); HR-EI-MS [M]⁺ *m/z* 302.1880 (calcd for C₁₉H₂₆O₃ 302.1883).

12-Hydroxy-6 β -methoxy-6,7-secoabieta-8,11,13-trien-7,6-olide (2). Gum; [α]_D²⁵: +21.8 (c 0.6, CHCl₃); IR ν_{\max} : 3337, 1679, 1606, 1580, 1460, 1407, 1261, 1142, 1062, 976 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ): 221 (4.71), 271 (4.46) nm; EI-MS *m/z* (%): 346 ([M]⁺, 12), 314 ([M–HOCH₃]⁺, 5), 302 (10), 286 (29), 271 (100), 255 (60), 217 (31), 204 (74), 170 (66), 145 (20), 115 (21); HR-EI-MS [M]⁺ *m/z* 346.2150 (calcd for C₂₁H₃₀O₄ 346.2145).

Obtuanhydride (3). Solid; [α]_D²⁵: –17.4 (c 0.18, CHCl₃); IR ν_{\max} : 1788, 1734, 1600, 1511, 1248, 1040 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ): 228 (3.81), 280 (3.52) nm; EI-MS *m/z* (%): 344 ([M]⁺, 65), 329 (22), 301 (78), 285 (32), 243 (18), 218 (100), 69 (32); ¹H-NMR (CDCl₃): δ 6.81 (s, H-11), 7.62 (s, H-14), 5.99 (br s, Ar-OH), 1.98 (br d, 13.0Hz, H-1), 3.13 (sep, 6.8Hz, H-15), 2.72 (s, H-5), 1.50 (s, H-20), 1.21 (d, 6.8, H-17), 1.23 (d, 6.8, H-16), 1.39 (s, H-19), 1.06 (s, H-18); ¹³C-NMR (CDCl₃): 42.1 (C-1), 18.8 (C-2), 40.6 (C-3), 33.4 (C-4), 59.2 (C-5), 166.9 (C-6), 165.8 (C-7), 119.5 (C-8), 151.1 (C-9), 40.8 (C-10), 114.3 (C-11), 157.5 (C-12), 133.7 (C-13), 131.8 (C-14), 26.7 (C-15), 22.0 (C-16), 22.2 (C-17), 32.9 (C-18), 22.3 (C-19), 23.0 (C-20).

6 β ,12-Dihydroxy-7,8-secoabieta-8,11,13-trien-7,8-olide (4). Gum; [α]_D²⁵: +30.5 (c 1.1, CHCl₃); IR ν_{\max} : 3429, 1725, 1507, 1460, 1407, 1241, 1175, 1049, 883, 738 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ): 284 (3.53) nm; EI-MS *m/z* (%): 332 ([M]⁺, 33), 304 ([M–CO]⁺, 100), 289 (10), 273 (17), 271 (20), 299 (17), 203 (13), 192 (8), 179 (17), 152 (11); HR-EI-MS [M]⁺ *m/z* 332.1978 (calcd for C₂₀H₂₈O₄ 332.1988).

5,12-Dihydroxy-7,8-secoabieta-8,11,13-trien-7,8-olide (5). Gum; [α]_D²⁵: +11.5 (c 0.3, CHCl₃); IR ν_{\max} : 3416, 1699, 1427, 1381, 1261, 1175, 1043, 870, 744 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ): 232 (3.83), 299 (3.79) nm; EI-MS *m/z* (%): 332 ([M]⁺, 100), 317 ([M–CH₃]⁺, 6), 273 (7), 249 (93), 233 (26), 207 (15), 203 (45), 161 (17); HR-EI-MS [M]⁺ *m/z* 332.1977 (calcd for C₂₀H₂₈O₄ 332.1988).

5 α ,8-Epoxy-12-hydroxy-7,8-secoabieta-8,11,13-trien-7-al (6). Gum; [α]_D²⁵: +22.8 (c 0.9, CHCl₃); IR ν_{\max} : 3423, 1706, 1434, 1255, 1169, 1096, 864, 738 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ): 232 (3.75), 304 (3.79) nm; EI-MS *m/z* (%): 316 ([M]⁺, 100), 301 ([M–CH₃]⁺, 4), 287 (7), 273 (13), 257 (9), 233 (73), 203 (53), 191 (23), 149 (11), 59 (11); HR-EI-MS [M]⁺ *m/z* 316.2033 (calcd for C₂₀H₂₈O₃ 316.2039).

3.4. Antifungal Assay

The antifungal assay was performed in this study to evaluate the antifungal activity of diterpenoids isolated from the bark of *C. japonica* based on the methods used in our previous studies with slight modifications, including the fungus species and positive control [33]. *P. noxius*, an aggressive and destructive pathogen that can cause brown root disease in infected trees, was used in antifungal assays. Antifungal assessment of the isolated compounds was conducted using a mycelial radial growth inhibition technique against *P. noxius*. The tested compounds were added to sterilized potato dextrose agar (PDA) to give 100 ppm concentrations of extractives. The testing plates were incubated at 27 ± 2 °C. When the mycelium of fungi reached the edge of the control plate, the antifungal index was calculated as follows: antifungal index (%) = (1 – D_a/D_b) × 100, where D_a:

diameter of growth zone in the experimental dish (cm), D_b : diameter of growth zone in the control dish (cm). The assays were performed three times, and the data were averaged. The commercial fungicide, didecyldimethylammonium chloride (DDAC), was used as a positive control at the concentration of 10 $\mu\text{g}/\text{mL}$.

4. Conclusions

In this study, five new abietane diterpenoids, 5-*epi*-12-hydroxy-6-*nor*-5,6-secoabieta-8,11,13-trien-7,5-olide (1), 12-hydroxy-6 β -methoxy-6,7-secoabieta-8,11,13-trien-7,6-olide (2), 6 β ,12-dihydroxy-7,8-secoabieta-8,11,13-trien-7,8-olide (4), 5,12-dihydroxy-7,8-secoabieta-8,11,13-trien-7,8-olide (5), and 5 α ,8-epoxy-12-hydroxy-7,8-secoabieta-8,11,13-trien-7-al (6), together with one known abietane diterpenoid, obtuanhydride (3), were isolated and characterized from the bark of *C. japonica*. At a concentration of 100 $\mu\text{g}/\text{mL}$, the antifungal activities of compounds 5 and 6 against *P. noxius* were stronger than those of compounds 1–4, with antifungal indices of 37.2% and 46.7%, respectively. The present findings revealed that compounds 5 and 6 have the potential to be used as natural antifungal agents against *P. noxius*.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/plants13091197/s1>. $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, HMQC, HMBC, $^1\text{H-}^1\text{H}$ COSY, NOESY, EI-MS, infrared, and ultraviolet–visible spectra of compounds 1, 2, and 4–6.

Author Contributions: The study was designed by Y.-H.K. and C.-I.C., C.-C.C., S.-Y.W. and Y.-H.K. performed the experiments, analyzed the data, and wrote the manuscript. All authors contributed to discussions, reviewed the manuscript, and approved the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article and Supplementary Materials.

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