New Abietane and Seco-abietane Diterpenes from the Roots of *Taiwania cryptomerioides*

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Four new diterpenes, 3-oxosaprorthoquinone (**1**), 3-oxomicrostegiol (**2**), 3-oxoisotaxodione (**3**), and taiwaninal (**4**), together with two known compounds, 3-oxosapriparaquinone (**5**) and 6-dehydrohinokiol (**6**), were isolated from the roots of *Taiwania cryptomerioides*. The structures of **1**–**4** were principle elucidated based on spectral evidence.

Key words *Taiwania cryptomerioides*; Taxodiaceae; 3-oxosaprorthoquinone; 3-oxomicrostegiol; 3-oxoisotaxodione; taiwaninal

Taiwania cryptomerioides (Taxodiaceae) is one genus and one species of endemic plants in Taiwan. It contains essential oil (more than 6%)¹⁾ in its heartwood. Because of its antifungal and decay-resistant characteristics as well as beautiful yellowish-red color with distinct purplish-pink streaks, it is an important building material with high value. Previously, we investigated the chemical components of the heartwood^{2–4)} and bark^{5–9)} of this plant. α -Cadinol, a major component in the heartwood, shows selectively for human colon tumor cell lines.¹⁰⁾ It also has potent activity against wood-decay fungi.¹¹⁾ Because of interesting structures in addition to those conferring biological activities, we were encouraged to study the diterpene constituents of its roots. We report here four new diterpenes, 3-oxosaprorthoquinone (**1**), 3-oxomicrostegiol (**2**), 3-oxoisotaxodione (**3**), and taiwaninal (**4**), together with the two known compounds 3-oxosaprorthoquinone (**5**)¹²⁾ and 6-dehydrohinokiol (**6**).¹³⁾

3-Oxosaprorthoquinone (**1**) was isolated as red crystal needles; its molecular formula of $C_{20}H_{24}O_3$ was established through ^{13}C -NMR and high-resolution impact mass spectral (HR-EI-MS) data. The index of hydrogen deficiency (IHD) of **1** is 9. The IR and UV spectra of **1** confirmed to the presence of an orthonaphthoquinone group ($\nu_{\max}$ 1664, 1637, 1571 cm^{-1} , $\lambda_{\max}$ 260, 353, 432 nm)¹⁴⁾ and an isolated ketone (1712 cm^{-1}). The 1H -NMR spectrum (Table 1) exhibited signals for two isopropyl groups [δ 1.11 (6H, d, $J=7.2$ Hz, H-18, -19), 2.69 (1H, sep, $J=7.2$ Hz, H-4), 1.14 (6H, d, $J=6.8$ Hz, H-16, -17), 2.99 (1H, sep, $J=6.8$ Hz, H-15)], one aromatic methyl group (δ 2.34, s, H-20), and two methylene groups [δ 3.21 (2H, dd, $J=9.2, 6.8$ Hz, H-1) and 2.63–2.72 (2H, m, H-2, overlapping with H-4)] linked between carbonyl and aromatic groups. In addition, there were signals for three aromatic protons at δ 7.06 (1H, s, H-14), 7.04, and 7.35 (each 1H, d, $J=7.6$ Hz, H-7, -6). Twenty ^{13}C -NMR signals (Table 2) included three carbonyl signals at δ 213.9 (C-3), 182.3 (C-11), and 181.2 (C-12). The former is an isolated ketone, and latter two are orthonaphthoquinone carbonyls. The red color and UV absorption together with eight aromatic signals indicate that **1** is an orthonaphthoquinone derivative. Three methyl signals at δ 18.3 ($2 \times CH_3$), 21.4 ($2 \times CH_3$), and 19.8 (C-20) were assigned as two isopropyl and one aromatic methyl groups. Comparison of the all physical data with those of aethiopinone (**7**)¹⁴⁾ showed that the difference is a side chain of orthonaphthoquinone. The het-

eronuclear multiple-bond correlation spectroscopy (HMBC) (see structure **8**) spectrum confirmed the assigned structure, and the nuclear Overhauser enhancement exchange spectroscopy (NOESY) spectrum (see structure **9**) clarified the

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Table 1. ^1H -NMR Spectral Data of **1**–**4** (400 MHz in CDCl_3)

Proton	1	2	3	4
1 α	3.21 (dd, 9.2, 6.8)	3.68 (ddd, 14.7, 12.0, 3.6)	1.73 (ddd, 14.4, 12.0, 3.6)	1.49 (ddd, 13.4, 12.8, 3.2)
1 β		3.00 (m)	3.29 (dt, 14.4, 4.4)	2.39 (ddd, 12.8, 3.6, 3.6)
2 α	2.66 (m)	3.00 (m)	2.72 (td, 14.4, 4.4)	1.99 (m)
2 β		2.48 (ddd, 13.5, 12.0, 4.4)	2.37 (ddd, 14.4, 4.4, 3.6)	1.72 (tdd, 13.4, 8.0, 3.6)
3				3.25 ^{a)}
4	2.69 (sep, 7.2)			
5			2.43 (s)	1.57 (br s)
6	7.35 (d, 7.6)	7.16 (d, 7.6)		5.98 (br s)
7	7.04 (d, 7.6)	7.00 (d, 7.6)	6.32 (s)	10.41 (s)
14	7.06 (s)	7.04 (d, 1.2)	6.98 (s)	7.40 (s)
15	2.99 (sep, d, 6.8)	2.90 (sep d, 7.2, 1.2)	3.08 (sep, d, 6.8)	3.25 ^{a)}
16	1.14 (d, 6.8)	1.17 (d, 7.2)	1.19 (d, 6.8)	1.22 (d, 6.8)
17	1.14 (d, 6.8)	1.20 (d, 7.2)	1.19 (d, 6.8)	1.23 (d, 6.8)
18	1.11 (d, 7.2)	0.83 (s)	0.88 (s)	1.18 (s)
19	1.11 (d, 7.2)	1.00 (s)	1.10 (s)	1.24 (s)
20	2.34 (s)	2.37 (s)	1.25 (s)	1.92 (s)
OH			7.61 (s)	2.90 (br s), 6.27 (br s), 7.94 (s)

a) Overlap each.

relative location. Zhang *et al.*¹⁵⁾ oxidized compound **7** with *m*-chloroperbenzoic acid to yield the corresponding epoxide and then treated it with 5% perchloric acid. Four products were isolated, and compound **1** was one of their products, but no physical data were observed. Cryptotriene (**1**) has a 4,5-seco-20(10 $\rightarrow$ 5)-abeoabietane skeleton, the first time such a compound was isolated in this genus.

Based on the HR-EI-MS and ^{13}C -NMR data (Table 2), compound **2** has the molecular formula $\text{C}_{20}\text{H}_{24}\text{O}_3$ with an IHD of 9. The ^1H -NMR spectrum (Table 1) indicated the presence of an isopropyl group [δ 1.17, 1.20 (3H each, d, $J=7.2$ Hz, H-16, H-17), 2.90 (1H, sep d, $J=7.2$, 1.2 Hz)], an aromatic methyl (δ 2.37, s), a gem-dimethyl [δ 0.83, 1.00 (3H each, s, H-18, -19)], an exchangeable hydroxy group (δ 4.86, s), and three aromatic protons [δ 7.00, 7.16 (1H each, d, $J=7.6$ Hz, H-7, H-6), 7.04 (H, d, $J=1.2$ Hz, H-14)], suggesting the existence of an abietane-type diterpene skeleton. H₃-20 (aromatic methyl) showed NOSEY (see structure **10**) correlation with H-6, and H-14 showed correlation with H-16 (and H-17). Based on the above evidence, compound **2** was considered to have a 4,5-seco-20(10 $\rightarrow$ 5)abeoabietane skeleton like compound **1**. Two carbonyl absorption bands (1705, 1660 cm^{-1}) in its IR spectrum indicated that one is cycloheptanone and the second is a conjugated ketone. The UV absorption bands at λ_{max} 245, 250, and 336 nm suggested the presence of a conjugated ketone. It also contained two methylene groups located between the ketone and aromatic groups as revealed by the signals at δ 3.68 (1H, ddd, $J=14.7$, 12.0, 3.6 Hz, H $_{\alpha}$ -1), 3.00 (1H, m, H $_{\beta}$ -1), 2.48 (1H, ddd, $J=13.5$, 12.0, 4.4 Hz, H $_{\beta}$ -2), and 3.00 (1H, m, H $_{\alpha}$ -2). Twenty ^{13}C -NMR signals including two carbonyl signals at δ_{C} 203.5 (C-12) and 210.5 (C-3), five methyl signals at δ_{C} 21.5, 22.0 (isopropyl moiety), 21.2, 21.4 (geminal dimethyl), and 21.23 (aromatic methyl) as well as eight aromatic signals and one quaternary carbon carrying a hydroxy group (δ_{C} 81.9, C-11). The difference between compounds **2** and **1** are a geminal dimethyl and tertiary alcohol in **2** instead of an isopropyl and a ketone of orthonaphthoquinone in **1**. The HMBC correlations of, C-3/H-1, H-2, H-18, H-19; C-11/H-18, H-19, OH; and C-12/OH, H-14 clarified the location of consecutive of C-1, -2, -3, -4, -11, and -12. NOESY (see structure **10**) confirmed

Table 2. ^{13}C -NMR Spectral Data of **1**–**4** (100 MHz in CDCl_3)

No.	1	2	3	4 ^{a)}
1	24.9	24.7	35.9	41.8
2	38.5	40.3	36.0	29.3
3	213.9	210.5	213.1	80.0
4	40.8	55.7	47.2	39.7
5	140.2	137.9	66.2	53.6
6	136.9	130.8	199.6	93.2
7	128.4	127.5	132.6	191.8
8	134.9	129.1	143.2	128.1
9	128.5	138.3	118.8	134.1
10	147.2	140.5	40.4	37.1
11	182.3	81.9	145.8	139.1
12	181.2	203.5	181.3	149.2
13	144.8	141.2	146.4	132.3
14	140.1	141.1	136.2	124.4
15	26.8	27.3	27.4	28.0
16	21.4	21.5	21.4	22.5
17	21.4	22.0	21.4	22.6
18	18.3	21.2	24.3	28.5
19	18.3	21.4	23.9	17.5
20	19.8	21.2	30.7	23.2

a) In CD_3COCD_3 .

the relative configuration. Comparison of the physical data between **2** and microstegiol (**11**)¹⁶⁾ allowed the structure of **2** to be assigned as 3-oxomicrostegiol. The biotransformation of **2** was proposed from 3-oxosaporthoquinone (**1**), and the pathway was sketched as in Chart 1. Compound **2** is an aldol condensation product of **1** via enol **12**.

Compound **3** is also a diterpene based on its molecular formula of $\text{C}_{20}\text{H}_{24}\text{O}_4$, which was deduced from the HR-EI-MS and ^{13}C -NMR data. It has an IHD of 9 due to its molecular formula. The IR spectrum shows absorption bands at 3329, 1714, 1668, 1634, and 1620 cm^{-1} , referring to hydroxyl, cyclohexanone, conjugated cyclohexanone, cyclohexanone with a hydrogen bond, and conjugated double bond, respectively. The ^{13}C -NMR data (Table 2) and distortionless enhancement by polarization transfer (DEPT) spectroscopy analysis showed 20 signals including five CH_3 (δ_{C} 21.4, 21.4, 23.9, 24.3, 30.7), three carbonyl [δ_{C} 181.3 (C-12), 199.6 (C-6), 213.1 (C-3)], six olefinic carbons (2 $\times\text{CH}$,

Chart 1. Proposed Biogenic Pathway of 2

Chart 2. Proposed Biogenic Pathway of 4

4×C), two CH₂, two CH, and two C. The UV spectrum indicated conjugated carbonyl absorption ($\lambda_{\max}$ 323, 336 nm). Three singlet methyl groups [δ 0.88 (H-18), 1.10 (H-19), 1.25 (H-20)] in addition to an isopropyl attached on *sp*² carbon [δ 1.19 (6H, d, J =6.8 Hz, H-16, H-17), 3.08 (1H, sep, J =6.8 Hz, H-15)] suggested that **3** is an abietane-type diterpene. An exchangeable enol proton at δ 7.61 indicated chelation with carbonyl. H-15 has an HMBC correlation with δ_C 181.3 and 136.2 [resonating with δ 6.98 (s)], and therefore they were assigned to be C-12 and C-14, respectively. The signal at δ 2.42 was assigned to be H-5, which exhibited HMBC correlations with C-18 (δ_C 24.3), C-19 (δ_C 23.9), C-6 (δ_C 199.6), C-4 (δ_C 47.2), and C-9 (δ_C 118.8). The HMBC correlations of HO/C-12, -11, -9 and H-20/C-10, -9, -5, -1 confirmed the hydroxyl group placed at C-11. Two singlet olefinic protons (δ 6.98, 6.32) exhibited the NOESY correlations (see structure **13**), and thus δ 6.32 is at C-7. The third carbonyl was located at C-3 due to HMBC correlations with H-18 and H-19. Comparison of the spectral data of the B, C ring of taxodione¹⁷ and the B, C ring of compound **3** showed that all are similar. The difference is an additional ketone on C-3 in compound **3**, but compound **3** is not 3-oxotaxidione. It was assigned to be 3-oxoisotaxidione due to the *cis*-fused A, B ring revealed by the NOESY correlation (see structure **13**). Because H-20 (δ 1.25) has NOESY correlations with H-5 (δ 2.42), H _{α} -1 [3.29 (1H, dt, J =14.4, 4.4 Hz)] and H _{β} -1 [1.73 (1H, td, J =14.4, 3.6 Hz)], H _{α} -1 was observed at a lower field than is generally case due to the deshielding effect from C-11 OH. The *cis*-fused A, B ring is unique among abietane-type diterpenes.

Compound **4** has the formula C₂₀H₂₈O₅ based on the HR-EI-MR and ¹³C-NMR data. Lower-field signals at δ 10.41 and IR absorption bands at 2876 and 1668 cm⁻¹ together with UV absorption band $\lambda_{\max}$ of 235.5 and 295.5 nm indicated the presence of a benzaldehyde function in taiwaninal (**4**). Three singlet methyl groups [δ 1.18 (H-18), 1.24 (H-19), 1.92 (H-20)], an isopropyl group attached to aromatic signals [δ 1.22, 1.23 (3H each, d, J =6.8 Hz, H-16, -17), 3.25 (1H, m, H-15)], and six aromatic ¹³C-NMR signals (Table 2) defined **4** as an 8,11,13-dehydroabietane-type diterpene. Three exchangeable hydroxyl protons (in CD₃COCD₃ solvent) at δ 2.90 (br s), 6.27 (br d, J =4.0 Hz), and 7.94 (s) were assigned to be C-3 OH, C-6 OH, and C-12 OH, respectively. A phenyl proton at δ 7.40 (s, H-14) was positioned between aldehyde and isopropyl groups attributable to NOESY correlations

with H-7, H-16, and H-17. The HMBC correlations of H-14/C-7 (δ 191.8) and C-15 (δ 28.0) confirmed the relative position. A lower-field ¹³C-NMR signal at δ_C 93.2 [resonating with δ_H 5.98 (d, J =4.0 Hz)] was assigned to a hemiacetal carbon (C-6). Since H-6 coupled with C-6 OH in its COSY spectrum, this proton also has HMBC correlations with C-11 (δ_C 139.1) and C-10. A signal at δ 1.57 (br s, δ_C 53.6) was assigned as H-5 due to HMBC correlations with C-19, -18, -6, -4, and -3 (δ_C 80.0). A signal at δ 3.25 (1H) showing HMBC correlations with C-19, -18, -5, and -4 was assigned as H-3 based on the above-mentioned evidence. Therefore the structure of **4** was proposed to be 6,7-secoabietane-6,7-dial with C-11 OH, and the C-11 OH reacted with C-5 CHO to form the hemiacetal. The related configuration of **4** was based on the NOESY correlation (see structure **14**). H-5 has NOESY correlation with H-3, H-6, and H₃-18 and no correlation with H-20, and the evidence clarified the *trans*-fused A, B ring. C-6 OH caused the signal of H-20 to shift downfield to δ 1.92 as a result of the 1,3-diaxial relation. The lack of coupling between H-5 and H-6 indicated that H-6 is in α -equatorial orientation. Taiwaninal (**4**) has a 5,6-secoabietane-type skeleton and its biotransformation was proposed from compound **6**.¹⁸⁾

Experimental

General Experimental Procedures Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. Specific rotations were recorded on a JASCO DIP-100 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 983 G spectrometer. ¹H- and ¹³C-NMR spectra were recorded on a Bruker DMX-400 spectrometer. EI-MS were measured with a JEOL JMS-HX 300 mass spectrometer and a JASCO PIP-1000 digital polarimeter. Extracts were chromatographed on silica gel (Merck 70–230 mesh, 230–400 mesh) and purified on a semi-preparative normal-phase HPLC column [250×10 mm, Lichrosorb Si 60 (7 μ m)] carried out with a LDC Refracto Monitor III.

Plant Material The roots of *T. cryptomerioides* were collected from Taichung, Taiwan, in August 1996. The plant was identified by Dr. Shang-Tzen Chang, Professor of the Department of Forestry, National Taiwan University. A voucher specimen (no. 013542) has been deposited in the Herbarium of the Department of Botany of National Taiwan University, Taipei, Taiwan.

Extraction and Isolation Air-dried root slices of *T. cryptomerioides* (15 kg) were extracted two times with acetone (125 l) at room temperature (7 d each). The acetone extract was evaporated *in vacuo* to give a black residue, which was suspended in H₂O (7 l), and then partitioned (3 times) with 1 l of ethyl acetate. The EtOAc fraction (365 g) was chromatographed on silica gel using mixtures of hexane and EtOAc of increasing polarity as eluents and further purified with HPLC. Six components, 3-oxoisotaxodione (**3**) (9 mg), 3-oxosaporthothione (**1**) (16 mg), 3-oxosapriparaquinone (**5**) (7.0 mg), 3-oxomicrostegiol (**2**) (8 mg), and 6-dehydrohinokiol (**6**) (9 mg) were eluted with 40% EtOAc in hexane, and taiwaninal (**4**) (10 mg) was eluted with 50% EtOAc in hexane.

3-Oxosaporthothione (1): Red needle, mp 72–73 °C; UV $\lambda_{\max}^{\text{MeOH}}$ (log ϵ) 260 (4.20), 353 (3.38), 432 (3.57) nm. IR (KBr) $\nu_{\max}$ 1712, 1664, 1637, 1571, 1256 cm⁻¹. ¹H- and ¹³C-NMR (CDCl₃, 400, 100 MHz) data: see Tables 1 and 2. EI-MS (70 eV) (rel. int. %) m/z 312 [M⁺] (38), 284 (40), 251 (57), 213 (100), 178 (73), 81 (60); HR-EI-MS m/z 312.1724 (M⁺, Calcd for C₂₀H₂₄O₃, 312.1726).

3-Oxomicrostegiol (2): Yellow solid, mp 77–78 °C; [α]_D²⁵ = +402.2° (c =0.08, CHCl₃). UV $\lambda_{\max}^{\text{MeOH}}$ (log ϵ) 245 (4.12), 250 (4.12), 336 (3.93) nm. IR (KBr) $\nu_{\max}$ 1704, 1660, 1388, 1210, 1170, 1097, 1031, 981 cm⁻¹. ¹H- and ¹³C-NMR (CDCl₃, 400, 100 MHz) data: see Tables 1 and 2. EI-MS (70 eV) (rel. int. %) m/z 312 [M⁺] (76), 228 (100), 213 (28), 259 (100), 149 (41), HR-EI-MS m/z 312.1724 (M⁺, Calcd for C₂₀H₂₄O₄, 312.1726).

3-Oxisotaxodione (3): Yellow gum; [α]_D²⁵ = -108.9° (c =0.44, CHCl₃). UV (MeOH) $\lambda_{\max}$ (log ϵ) 323 (4.26), 336 (4.29) nm. IR (KBr) $\nu_{\max}$ 3329, 1714, 1668, 1634, 1620, 1389, 1236 cm⁻¹. ¹H- and ¹³C-NMR (CDCl₃, 400, 100 MHz) data: see Tables 1 and 2. EI-MS (70 eV) (rel. int. %) m/z 312 [M⁺] (98), 285 (22), 244 (82), 232 (86), 231 (100). HR-EI-MS m/z 328.1667

(Calcd for $C_{20}H_{24}O_4$, 328.1675).

Tawaninal (**4**): Light yellow solid, mp 160—162 °C; $[\alpha]_D^{25} = -23.5^\circ$ ($c=0.19$, $CHCl_3$). UV λ_{max}^{MeOH} (log ϵ) 235 (410), 295 (3.99) nm. IR (KBr) ν_{max} 3408, 2876, 1668, 1603, 1567, 1374, 1293, 1245 cm^{-1} . 1H - and ^{13}C -NMR (CD_3COCD_3 , 400, 100 MHz) data see: Tables 1 and 2. EI-MS (70 eV) (rel. int. %) m/z 348 [M^+] (6), 315 (30), 290 (20), 279 (26), 167 (34), 149 (100). HR-EI-MS m/z 348.1926 (M^+ , Calcd for $C_{20}H_{28}O_5$, 348.1937).

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