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

Chiou-Feng CHYU, Hsiu-Chuan LIN, and Yueh-Hsiung KUO*

Department of Chemistry, National Taiwan University; Taipei, Taiwan 106, Republic of China.
Received April 5, 2004; accepted September 25, 2004

Four new diterpenes, 3-oxosaprhoorthoquinone (1), 3-oxomicrostegiol (2), 3-oxoisotaxodione (3), and tawaiinal (4), together with two known compounds, 3-oxasariparaquinone (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-oxosaprhoorthoquinone; 3-oxomicrostegial; 3-oxoisotaxodione; tawaiinal

*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. \(\alpha\)-Cadino, 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-oxosaprhoorthoquinone (1), 3-oxomicrostegiol (2), 3-oxoisotaxodione (3), and tawaiinal (4), together with the two known compounds 3-oxasaripparaquinone (5)\(^12\) and 6-dehydrohinokiol (6).\(^13\)

3-Oxosaprhoorthoquinone (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-El-MS) data. The index of hydrogen deficiency (IHD) of 1 is 9. The IR and UV spectra of 1 confirmed the presence of an orthonaphthoquinone group (\(\nu_{\text{max}}\) 1664, 1637, 1571 cm\(^{-1}\), \(\lambda_{\text{max}}\) 260, 353, 432 nm)\(^14\) and an isolated ketone (1712 cm\(^{-1}\)). The \(^1\)H-NMR spectrum (Table 1) exhibited signals for two isopropyl groups [\(\delta\) 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 (\(\delta\) 2.34, s, H-20), and two methylene groups [\(\delta\) 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 \(\delta\) 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 \(\delta\) 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 \(\delta\) 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 heteronuclear multiple-bond correlation spectroscopy (HMBC) (see structure 8) spectrum confirmed the assigned structure, and the nuclear Overhause enhancement exchange spectroscopy (NOESY) spectrum (see structure 9) clarified the...
groups as revealed by the signals at methylene groups located between the ketone and aromatic the presence of a conjugated ketone. It also contained two (3H each, s, H-18, -19)], an exchangeable hydroxy group (d
IHD of 9. The 1H-NMR spectrum (Table 1) indicated the H3-20 (aromatic methyl) showed NOSEY (see structure gesting the existence of an abietane-type diterpene skeleton. presence of an isopropyl group [d
13C-NMR signals including two carbonyl signals at (isopropyl moiety), 21.2, 21.4 (geminal dimethyl), and 21.23 relative location. Zhang et al.15 oxidized compound 7 with m-chloroperoxybenzoic acid to yield the corresponding exopoxide and then treated it with 5% perchloric acid. Four products were isolated, and compound I was one of their products, but no physical data were observed. Crytometrione (1) has a 4,5-seco-2(10→5)-abietane skeleton, the first time such a compound was isolated in this genus.

Based on the HR-EI-MS and 13C-NMR data (Table 2), compound 2 has the molecular formula C20H24O4 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. H-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-2(10→5)abietane skeleton like compound I. 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 λmax 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, Hα-1), 3.00 (1H, m, Hβ-1), 2.48 (1H, ddd, J=13.5, 12.0, 4.4 Hz, Hβ-2), and 3.00 (1H, m, Hβ-2). Twenty 13C-NMR signals including two carbonyl signals at δ 203.5 (C-12) and 210.5 (C-3), five methyl signals at δC 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 quartenary carbon carring a hydroxy group (δC 81.9, C-11). The difference between compounds 2 and I are a geminal dimethyl and tertiary alcohol in 2 instead of an isopropyl and a ketone of orthoanilinoquinone in I. 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 consective of C-1, -2, -3, -4, -11, and -12. NOESY (see structure 10) confirmed 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-oxosapapthorquinone (1), and the pathway was sketched as in Chart 1. Compound 2 is an aldol condensation product of I via enol 12.

Compound 3 is also a diterpene based on its molecular formula of C28H34O3, which was deduced from the HR-EI-MS and 13C-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 13C-NMR data (Table 2) and distor- tionless enhancement by polarization transfer (DEPT) spectroscopy analysis showed 20 signals including five CH3 (δC 21.4, 21.4, 23.9, 24.3, 30.7), three carbonyl [δC 181.3 (C-12), 199.6 (C-6), 213.1 (C-3)], six olefinic carbons (2×CH,
Three singlet methyl groups [δ 1.25 (H-20)], an isopropyl group attached on aromatic signals (δ 1.92), and 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. Taiwanese (4) has a 5,6-secoabietane-type skeleton and its biotransformation was proposed from compound 6.10

**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 Si60 (7 μm)] 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 twice 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 11 of ethyl acetate. The EtOAc fraction (360 g) was 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 Si60 (7 μm)] 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 twice 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 11 of ethyl acetate. The EtOAc fraction (360 g) was 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 Si60 (7 μm)] 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 twice 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 11 of ethyl acetate. The EtOAc fraction (360 g) was 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 Si60 (7 μm)] 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 twice 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 11 of ethyl acetate. The EtOAc fraction (360 g) was 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 Si60 (7 μm)] 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 twice 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 11 of ethyl acetate. The EtOAc fraction (360 g) was 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 Si60 (7 μm)] 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 twice 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 11 of ethyl acetate. The EtOAc fraction (360 g) was 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 Si60 (7 μm)] 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 twice 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 11 of ethyl acetate. The EtOAc fraction (360 g) was 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 Si60 (7 μm)] 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 twice 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 11 of ethyl acetate. The EtOAc fraction (360 g) was 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 Si60 (7 μm)] 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 twice 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 11 of ethyl acetate. The EtOAc fraction (360 g) was 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 Si60 (7 μm)] 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.
(Calcd for C_{20}H_{24}O_{4}, 328.1675).

Tawaninal (4): Light yellow solid, mp 160—162 °C; [α]_{D}^{25} = −23.5° (c=0.19, CHCl_{3}). UV \lambda_{\text{max}}(\log e) 235 (410), 295 (3.99) nm. IR (KBr) \nu_{\text{max}} 3408, 2876, 1668, 1603, 1567, 1374, 1293, 1245 cm^{-1}. 1H- and 13C-

NMR (CD_{3}COCD_{3}, 400, 100 MHz ) data see: Tables 1 and 2. EI-MS (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).

Acknowledgment This research was supported by the National Science Council of the Republic of China.

References and Notes