Further studies on the bark of *Taiwania cryptomerioides* found four new podocarpane derivatives, 1β,13-dihydroxy-8,11,13-podocarpane (1), 14,18-dihydroxy-13-methoxy-8,11,13-podocarpane (2), 1β,14-dihydroxy-13-methoxy-8,11,13-podocarpane-2,7-dione (3), and 3β,14-dihydroxy-13-methoxy-8,11,13-podocarpan-7-one (4), together with a known 1β,13,14-trihydroxy-8,11,13-podocarpatrien-7-one (5). Those structures were elucidated principally from spectral evidence.

**Key words** *Taiwania cryptomerioides*; Taxodiaceae; bark; trinorditerpene; 1-hydroxy-2-oxodehydropodocarpane

*Taiwania cryptomerioides* Hayata (Taxodiaceae) is an endemic plant and also an important building material. It grows at elevations from 1800 to 2600 m in the central mountains in Taiwan. The heartwood of *T. cryptomerioides* is yellowish-red with distinct purplish-pink streaks. In the previous investigation, various sesquiterpenes, lignans, and abietane-type diterpenes were analyzed from the heartwood [1–3] and bark [4,5] of this plant.

Lignans are distributed widely in the plant kingdom. Much interest has been focused on their effectiveness as antineoplastic agents. Taiwanin E methyl ester isolated from *Justicia ciliata* exhibited significant in vitro cytotoxicity against human cervical carcinoma. [6] α-Cardinol isolated from *T. cryptomerioides* showed selectivity for human colon tumor cell lines. [7] The lignans and sesquiterpenes from *T. cryptomerioides* heartwood isolated by us were investigated for cytotoxic activity. [9] Because it is a decay-resistant tree, the anti-fungal characterics were also investigated. α-Cardinol has the highest activity against *Coriolus versicolor* (brown-rot fungi) and *Laetiporus sulphureus* (brown-rot fungi) with 100% antifungal indices at 100 ppm. [9]

Podocarpane diterpenes do not occur extensively in nature but are present in several genera, such as *Azadirachta* [10–14] *Humirianther* [15], *Micrandropsis* [16], and *Podocarpus* [17]. Lin et al. [18] first isolated the podocarpane derivative 1β,13,14-trihydroxy-8,11,13-podocarpan-7-one from the leaves of this plant. Many novel skeleton compounds were also found in Lin et al.’s research [18–21] and we were encouraged to investigate its bark. Fourteen new podocarpane derivatives [22–24] were discovered. In the present study of the more polar fraction, four new compounds, 1β,13-dihydroxy-8,11,13-podocarpan-7-one (1), 14,18-dihydroxy-13-methoxy-8,11,13-podocarpan-7-one (2), 1β,14-dihydroxy-13-methoxy-8,11,13-podocarpan-2,7-dione (3), and 3β,14-dihydroxy-13-methoxy-8,11,13-podocarpan-7-one (4), together with the known 1β,13,14-trihydroxy-8,11,13-podocarpan-7-one (5) [18] were observed. In this paper we deal with their structural elucidation.

Compound 1 has the molecular formula C17H24O2 (based on peak matching of the molecular ion and 13C-NMR spectroscopy). The IR spectrum of 1 displayed peaks for hydroxyl (3421 cm⁻¹) and aromatic (1615, 1498 cm⁻¹) groups. The 1H-NMR (Table 1) spectrum showed three singlet methyl groups. One exchangeable phenolic proton, and three ABX system phenyl protons at δ 8.04 (1H, d, J=8.7 Hz, H-11), 6.57 (1H, dd, J=8.7, 2.7 Hz, H-12), and 6.48 (1H, d, J=2.7 Hz, H-14) indicated the presence of a 1,3,4-trisubstituted phenyl group carrying one hydroxyl group. No isopropyl group and no typical H₆F₁ signal (δ 2.00–2.40) of dehydroabietane and dehydropodocarpane-type derivatives [18,22] were observed. Among 17 13C-NMR signals (Table 1), six belong to the phenyl carbons. Three (singlet) of six phenyl signals appear at lower fields, δ 137.5, 142.1, and 153.2, and those signals were assigned to C-8, C-9, and C-13, respectively. A carbinyl methine signal at δ 3.82 (dd, J=9.6, 6.2 Hz) was assigned to H₆₁ (axial), which had nuclear Overhauser enhancement and exchange spectroscopy (NOESY) correlation with H-S (δ 1.24) and H-11. H-11 in 1 appeared at a relatively lower field, as 5[18,23] and 1β,14-dihydroxy-13-methoxy-8,11,13-podocarpane (6) [23] due to the deshielding by the C-1 equatorial hydroxyl group. The only difference is an additional hydroxyl group in compound 1. The signal at δ 8.04 exhibiting NOESY correlation with H-20 (δ 1.18) was unambiguously consistent with H-11. Therefore compound 1 is 1β,13-dihydroxy-8,11,13-podocarpan-7-one.

Eighteen 13C-NMR signals and exact mass spectrum data confirmed the molecular formula of 2 to be C₁₇H₂₃O₃. Aromatic and hydroxyl absorptions are present in its IR spectrum. Three singlet methyl groups at δ 0.86 (H-19), 1.18 (H-20), and 3.82 (–OCH₃) and two ortho-coupling phenyl protons at δ 6.78 and 6.67 (d, J=8.6 Hz, H-11, -12) (Table 1) in its 1H-NMR spectrum were observed. The signals at δ 3.21 and 3.46 (1H each, d, J=10.9 Hz) and δc 72.1 (Table 1) indicated a hydroxymethyl group attached to a quaternary carbon. Three (singlet) of six phenyl signals appearing at δ 144.0, 143.3, and 142.4 were assigned to C-9, C-13, and C-14, respectively. An exchangeable phenolic proton present at...
δ 4.50 (1H, br s), and the methoxy group showing a NOESY correlation with H-12 suggested methoxy and hydroxyl groups located at C-13 and -14, respectively. A typical H-1 signal of dehydroabietaene appeared at δ 2.24 (1H, brd, J = 12.7 Hz), which exhibited NOESY correlation with H-11 (δ 6.78). The signal at δ 1.18 was assigned to H-20 due to its NOESY correlation with H-20 and H-11 (δ 6.69). Therefore, 2 was identified as 14,18-dihydroxy-13-methoxy-8,11,13-podocarpantriene.

The molecular formula (C_{18}H_{22}O_{5}), and UV (λ_{max} 221.0, 3035, 1580, 1479 cm⁻¹) and IR (3449, 3200—2700, 1715, 1646, 1571, 364.0 nm) spectra of compound 3 suggested that it contains conjugated and isolated carbonyl, aromatic, and hydroxyl groups. Two carbonyl groups (δ_{C} 209.1, 204.7) were observed, and one is located at C-7 (δ 204.7) (Table 1) causing a strong hydrogen bond with the C-14 hydroxyl group (δ 12.89). Three singlet methyl groups present at δ 1.14 (H-18). 0.97 (H-19) and 1.08 (H-20) are in a higher field than those in nimosone (12-hydroxy-8,11,13-abietatriene-3,7-dione). Two equivalent methylene protons (δ 2.83, δ 5.45 s) showed correlated spectroscopy (COSY) correlation with H-5, NOESY correlation with H-5, H-19, and H-20, and heteronuclear multiple bond connectivity (HMBC) correlation with C-4, C-5, and C-7 were assigned to H-6. Two orthophenyl protons are present at δ 7.03 (d, J = 8.3 Hz, H-12) and 7.55 (d, J = 8.3 Hz, H-11). The former phenyl proton had NOESY correlation with the phenolic methyl (δ 3.87) group, and the latter phenyl proton occurring in the low field was attributed to the deshielding of the C-1,2,2’ hydroxyl group, as in compounds 1 and 6. The H-1 (δ 4.54, s) occurred at a lower field than that in 1 (δ 3.82) and 6 (δ 3.98) due to the neighboring C-2 (δ 209.1) carbonyl group. The signals of H-3 (δ 3.19) and H-5 (δ 47.6) were present between δ 2.22 and 2.51. The NOESY correlation (H-1/H, δ 3.12, H-5) and HMBC correlation (H-1/C-2, C-9, C-20; H-18, H-19/C-3, C-5) confirmed the structure of 3 to be 1β,14-dihydroxy-13-methoxy-8,11,13-podocarpantriene-2,7-dione.

The high-resolution mass spectrum and 13C-NMR data (Table 1) of compound 4 showed that it has the formula C_{18}H_{23}O_{4}. Its UV spectrum suggested that 4 had a phenone moiety. The IR absorptions were attributable to a hydroxyl group (3450 cm⁻¹), a strong hydrogen-bonding hydroxyl group (3300—2700 cm⁻¹), an aromatic group (3035, 1580, 1479 cm⁻¹), and a conjugated carbonyl (1635 cm⁻¹). The 1H-NMR spectrum showed three singlet methyl and one phenolic methyl groups. Two ortho aromatic protons resonated at δ 6.69, 6.99 (1H each, d, J = 8.3 Hz), and no isopropyl group was observed in its 1H-NMR spectrum. Three (singlet each at δ_{C} 147.5, 146.5, 153.1) of six aromatic carbon signals were assigned to C-9, -13, and -14, respectively. The above evidence confirmed that the structure of 4 is an 8,11,13-podocarpantriene with one hydroxyl and one methoxy group attached to the aromatic ring. A low-field singlet at δ_{H} 12.96 indicated a hydrogen bond signal between the C-14 OH and C-7 carbonyl. A carbonyl methine proton at δ 3.33 (dd, J = 10.7, 4.6 Hz) was assigned to have an H-3 α-axial orientation due to its coupling constant and NOESY correlation with H-18. H_{4}-1 was present at δ 2.32 (dt, J = 12.6, 3.1 Hz) showing NOESY correlation with H-20 and H-11 (δ 6.69). Finally, MeO-13 and H-12 (δ 6.99) showed correlations in their NOESY spectra, establishing the structure of 4 to be 3β,14-dihydroxy-13-methoxy-8,11,13-podocarpantriene-7-one. Podocarpane-type diterpenes do not occur extensively in nature. It is interesting that many podocarpane derivatives are found in the bark and leaves, but not in the heartwood of T.
cryptomerioides. The oxidation at C-3 in tricyclic diterpenoids is very general, but that at C-1 or C-2 is rare. The simultaneous oxidation at C-1 and C-2 as acyloan functionality (as in 3) is very unique.

The structure determination and data assignment of the above four new podocarpane derivatives were aided by proton-detected heteronuclear multiple-quantum coherence (HMQC) and proton-detected HMBC experiments.

Experimental

General Experimental Procedures Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. UV spectra were recorded on a Perkin-Elmer 781 spectrophotometer. 1H- and 13C-NMR spectra were recorded on a Bruker DMX-400 at 400 and 100 MHz (HMQC) and proton-detected HMBC experiments. Structures were characterized on silica gel (Merck 70—230 mesh, 230—400 mesh, ASTM).

Extraction and Isolation Air-dried pieces of the bark of T. cryptomerioides (12 kg) were extracted three times with acetone (60 l) at room temperature (7 d each time). The acetone extract was evaporated in vacuo to leave a black residue, which was suspended in H2O (81), and then partitioned (3 × 11) with 11 of ethyl acetate. The EtOAc fraction (360 g) was chromatographed on silica gel (Merck 70—230 mesh, 230—400 mesh, ASTM). Extracts were chromatographed on silica gel (Merck 70—230 mesh, 230—400 mesh, ASTM).

T. cryptomerioides was collected in Tai-Chin, Taiwan, in 1996. The plant material was identified by Mr. Muh-Tsuen Gun, formerly a technician of the Department of Botany, National Taiwan University. A voucher specimen (no. 013542) has been deposited at the Herbarium of the Department of Botany of the National Taiwan University, Taipei, Taiwan.

Table 1; EI-MS (70 eV) (rel. int. %)

\[
\begin{array}{ccc}
\text{Compound} & \text{EI-MS (70 eV) (rel. int. %)} \\
\hline
1 & \text{M}^+ (100), 271 (36), 203 (60); HR-EI-MS m/z 304.1667 (M+ Caled for C14H18O7, 318.1461). \\
2 & 3\beta,14-Dihydroxy-13-methoxy-8,11,13-podocarpatrien-7-one (4): Yellow crystal; mp 87—89°C; [\alpha]_D^29 +9.4° (c=0.35, CHCl3); UV \lambda_{	ext{abs}} \text{nm (log } \varepsilon) 224.5 (4.00), 269.5 (3.80), 356.0 (3.36); IR (film) \nu_{\text{max}} 3300—2700, 3035, 1580, 1479 cm\(^{-1}\); 1H- and 13C-NMR, see Table 1; EI-MS (70 eV) (rel. int. %) m/z 304 [M+] (100), 271 (36), 203 (60); HR-EI-MS m/z 304.1668 (M+ Caled for C14H18O7, 318.1461). \\
\hline
\end{array}
\]

Acknowledgments

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

References