Podocarpane-Type Trinorditerpenes from the Bark of *Taiwania cryptomerioides*

Yueh-Hsiung Kuo* and Chi-I Chang

Department of Chemistry, National Taiwan University, Taipei, Taiwan, Republic of China

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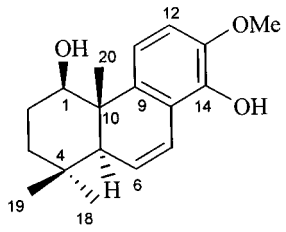
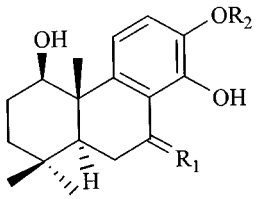
Five new podocarpane-type trinorditerpenes were isolated from the bark of *Taiwania cryptomerioides*. Structures of 1β,14-dihydroxy-13-methoxy-6,8,11,13-podocarpatetraene (**1**), 1β,14-dihydroxy-13-methoxy-8,11,13-podocarpatriene (**2**), 13-hydroxy-12-methoxy-8,11,13-podocarpatrien-3-one (**3**), 14-hydroxy-13-methoxy-1,8,11,13-podocarpatetraen-3-one (**4**), and 13-methoxy-8,12-podocarpatriene-11,14-dione (**5**) were determined by NMR, MS, and other spectral and chemical evidence.

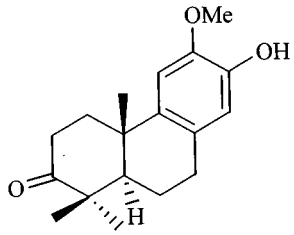
Taiwania cryptomerioides Hayata (Taxodiaceae) is a decay-resistant, economically important tree species indigenous to Taiwan. We previously investigated its heartwood[1−4] and bark,[5−7] and found various sesquiterpenes, lignans, and abietane-type diterpenes. Kamil[8] has reported on bisflavones found in its leaves. Many novel compounds obtained from this plant were described by Lin,[9−12] including 1β,13,14-trihydroxy-8,11,13-podocarpatrien-7-one.[12] We now report five new podocarpane-type trinorditerpenes (**1**−**5**) from the bark of *T. cryptomerioides*.



1



2 $R_1 = H_2$, R_2 = Me
6 R_1 = O, R_2 = H



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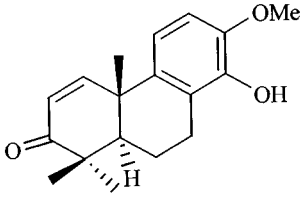

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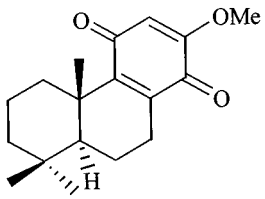

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Results and Discussion

The molecular formula of compound **1** was established as $C_{18}H_{24}O_3$ by HREIMS. The IR spectrum of **1** showed bands attributable to a hydroxyl group (3416 cm^{-1}), aromatic groups (1626, 1500 cm^{-1}), and an olefinic group (1651 cm^{-1}). UV absorptions at λ_{max} 225, 270, and 308 (sh) nm (log ϵ 4.10, 3.30, and 3.10, respectively) suggested that **1** had a double bond conjugated with an aromatic group. The ^{1}H NMR spectrum showed three methyl singlets at δ 0.92, 1.07, and 1.10 (H-18, H-20, and H-19). Two ortho aromatic protons resonated at δ 6.66 (d, J = 8.5 Hz, H-12) and 7.48 (d, J = 8.5 Hz, H-11). No isopropyl group was observed in its ^{1}H NMR spectrum. Comparison of ^{13}C NMR data with those of the known 1β,13,14-trihydroxy-8,11,13-podocarpatrien-7-one (**6**)[12] suggested that **1** possesses the same carbon skeleton. Eight low-field signals between δ 100 and 145 indicated that **1** contained aromatic and olefinic functionalities. Three downfield ^{13}C NMR signals at δ 140.8, 144.6, and 140.8 were assigned as C-9, C-13, and C-14, respectively. C-13 and C-14 are vicinal oxygenated phenyl carbons bonded to a hydroxyl (δ 5.64, exchangeable with D_2O) and a methoxyl group (δ_H 3.84 and δ_C 56.0). Two olefinic carbon signals appeared at δ 128.7 (C-6) and 121.7 (C-7), and the corresponding proton signal appeared at δ 5.90 (dd, J = 9.7, 2.8 Hz, H-6) and 6.91 (dd, J = 9.7, 2.8 Hz, H-7). Comparison of coupling patterns of H-6 and H-7 in **1** with those of the known Δ^6-deoxocryptojaponol[13] suggested that the H-5 (δ 2.06, t, J = 2.8 Hz) is axial, as allylic coupling with H-7 was observed. H-7 (δ 6.91, dd, J = 9.7, 2.8 Hz) in **1** appeared at lower field than the corresponding proton in Δ^6-deoxocryptojaponol (δ 6.30) due to deshielding from the C-14 hydroxyl group. Because the methoxyl group had a NOE correlation with the phenyl proton, this placed the methoxyl at C-13. A typical H_β-1 signal (δ 2.00−2.40) for dehydroabietane- and dehydropodocarpane-type derivatives[12,14−16] was not observed. A carbinol signal at δ 4.08 (dd, J = 12.9, 4.0 Hz) was assigned as H_α-1 (axial), which had NOE correlation with H-5 (δ 2.06). H-11 (δ 7.48) in **1** also appeared at relatively lower field, as in 1β,13,14-trihydroxy-8,11,13-podocarpatriene, due to the deshielding by the C-1 equatorial hydroxyl group. The above evidence established the structure of **1** as 1β,14-dihydrohydroxy-13-methoxy-6,8,11,13-podocarpatetraene.

Compound **2** ($C_{18}H_{26}O_3$, HREIMS) had a tricyclic diterpenoid skeleton similar to that of **1**. Three methyl singlets, one methoxyl singlet, and two ortho phenyl proton signals, as in **1**, were observed in its ^{1}H NMR spectrum. Compound **2** exhibited six aromatic ^{13}C NMR signals, of which three downfield signals (δ 142.2, 143.7, and 143.4) were assigned to C-9 and the two ortho oxygenated carbons (attached to

* To whom correspondence should be addressed. Fax: 866-2-23636359. E-mail:yhkuo@ccms.ntu.edu.tw.

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a hydroxy group and a methoxyl group). The phenyl proton signal at δ 7.72 (as in **1**) was assigned to H-11 due to deshielding by the C_β-1 hydroxyl group. The methoxyl group was placed at C-13 due to the NOE correlation between methoxyl group and phenyl proton. Comparison of ^{1}H and ^{13}C NMR data of **2** with those of **1** indicated that **2** is 1β,14-dihydroxy-13-methoxy-8,11,13-podocarpatriene. Hydrogenation of **1** with Pd/C in MeOH gave a product that was also identified as **2**.

Compound **3** had the molecular formula $C_{18}H_{24}O_3$ (HREIMS). Hydroxyl, isolated ketone, and aromatic absorptions were present in its IR spectrum. Four singlet methyl groups resonating at δ 1.10, 1.14, 1.26, and 3.83, and two singlet phenyl protons resonating at δ 6.68 (H-11) and 6.58 (H-14) in its ^{1}H NMR spectrum indicated that **3** was also a dehydropodocarpane with substitutions at both C-12 and C-13. A characteristic H_β-1 signal, indicating a dehydropodocarpane molecule, was observed at δ 2.40.[14,15] Two downfield ^{13}C NMR signals (δ 145.0 and 143.6) were assigned as C-12 and C-13, attached to a methoxyl and a hydroxyl group, respectively. The phenyl proton at δ 6.68 exhibited an NOE correlation with H_β-1 and a methoxyl group, confirming that the methoxyl group is at C-12. H-18 (δ 1.14) and H-19 (δ 1.10) exhibited HMBC correlation with the carbonyl signal (δ 217.7), and showed the carbonyl group positions at C-3. Thus, the structure of **3** was elucidated as indicated.

The UV spectrum of compound **4** ($C_{18}H_{22}O_3$) showed conjugated ketone absorption bands at λ_{max} 223 and 282 nm. Its IR spectrum exhibited hydroxyl, conjugated carbonyl, and aromatic absorptions. The ^{1}H NMR spectrum contained three singlet methyl signals (δ 1.16, 1.20, 1.36), a phenolic OH (δ 5.71, br s, exchangeable with D_2O), a phenolic methyl (δ 3.86), *ortho*-phenyl protons (δ 6.75 and 6.90), vicinal olefinic protons (δ 5.98 and 7.55), and a $-CHCH_2CH_2-$ moiety. These signals were very similar to those of totarolenone.[17] A downfield signal (δ 7.55, H-1) can be reasonably explained in terms of strong deshielding by the phenyl group. H-1 and H-12 signals had NOE correlations with H-11 and the methoxyl group and placed the methoxyl and hydroxyl groups at C-13 and C-14, respectively. Comparison of ^{1}H and ^{13}C NMR data of **4** with those of **3** indicated that **4** is 14-hydroxy-13-methoxy-1,8,11,13-podocarpatetraen-3-one. This conclusion was fully supported by ^{13}C NMR, HMQC, and HMBC data.

Compound **5** showed a molecular ion in its MS spectrum at *m/z* 288.1735 corresponding to molecular formula $C_{18}H_{24}O_3$. Analysis of its IR spectrum suggested that **5** contained a quinone group (1670, 1610, 1586 cm^{-1}). The UV spectrum and ^{13}C NMR data were also consistent with a quinone. Three singlet methyl signals (δ 0.87, 0.90, and 1.27) and a methoxyl signal (δ 3.73) were observed, with the latter placed on the quinone moiety. A typical H_β-1 signal at δ 2.74 was similar to that observed in royleanone.[18] H-7 (δ 2.28) had HMBC correlations with δ 140.5 (C-8), 151.9 (C-9), and 183.3 (C-14), and H-20 (δ 1.27) had an HMBC correlation with δ 36.6 (C-1), 51.7 (C-5), 151.9 (C-9), and 38.9 (C-10). Thus, signals at δ 140.5, 151.9, and 183.3 were confirmed to be C-8, C-9, and C-14, respectively. The olefinic proton (δ 5.71) had one HMBC correlation with δ 151.9 (C-9) and none with δ 140.5 (C-8). The observation that H_3-18 and H-5; H_3-19 and H_3-20 had NOE correlations indicated that the AB ring is *trans*-fused. Thus, **5** was thus assigned as 13-methoxy-8,12-podocarpadiene-11,14-dione.

Experimental Section

General Experimental Procedures. Melting points were determined with a Yanagimato micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on Bruker AM-300 at 300 and 75 MHz in $CDCl_3$ solution with tetramethysilane (TMS) as an internal standard. EIMS, HREIMS, UV, and specific rotations were taken on a Finnigan TSQ-46C, a JEOL SX-102A, a Hitachi S-3200 spectrometer, and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on Si gel (Merk 3374, 70−230 mesh).

Plant Material. The bark of *T. cryptomerioides* was collected in Tai-Chun, Taiwan, in 1996. The plant 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.

Extraction and Isolation. Air-dried pieces of bark of *T. cryptomerioides* (12 kg) were extracted three times with acetone (60 L) at room temperature (7 days each time). The combined acetone extracts were evaporated in vacuo to leave a black residue, which was suspended in H_2O (8 L) and then partitioned (3×) with 1 L of ethyl acetate. The EtOAc fraction (360 g) was chromatographed on Si gel using *n*-hexane and EtOAc of increasing polarity and further purfied by HPLC (Lichrosorb Si-60, 7 μm, 250 mm × 25 mm), eluting with CH_2Cl_2−EtOAc (50:1). Five components, **1** (6.4 mg), **2** (6.2 mg), **3** (7.1 mg), **4** (8.5 mg), and **5** (3.6 mg), were obtained in pure form.

1β,14-Dihydroxy-13-methoxy-6,8,11,13-podocarpatetraene (1): amorphous solid; $[\alpha]^{22}_D$ −86.6° (*c* 0.70, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 225 (4.10), 270 (3.30), 308 (3.10, sh) nm; IR (dry film) λ_{max} 3416, 3043, 1651, 1626, 1500, 1255, 1096, 910 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.48, 6.66 (each 1H, d, J = 8.5 Hz, H-11, H-12), 6.91, 5.90 (each 1H, dd, J = 9.7, 2.8 Hz, H-7, H-6), 5.64 (1H, br s, −OH, exchangeable with D_2O), 4.08 (1H, d, J = 12.9, 4.0 Hz, H-1), 3.84 (3H, s, $-OCH_3$), 2.06 (1H, t, J = 2.8 Hz, H-5), 1.81 (2H, m, H-2), 1.48, 1.35 (each 1H, m, H-3), 0.92, 1.10, 1.09 (each 3H, s, H-18, -20, -19); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 144.6 (s, C-13), 140.8 (s, C-14), 140.8 (s, C-9), 128.7 (d, C-6), 121.7 (d, C-7), 119.9 (s, C-8), 115.7 (d, C-11), 109.0 (d, C-12), 76.4 (d, C-1), 56.0 (q, $-OCH_3$), 50.4 (d, C-5), 43.6 (s, C-10), 39.0 (t, C-3), 32.5 (s, C-4), 31.8 (q, C-18), 29.9 (t, C-2), 22.0 (q, C-19), 13.5 (q, C-20); EIMS (70 eV) *m/z* 288 $[M]^+$ (100), 270 (96), 255 (76), 223 (65), 180 (100), 157 (43); HREIMS *m/z* 288.1722 (calcd for $C_{18}H_{24}O_3$, 288.1726).

1β,14-Dihydroxy-13-methoxy-8,11,13-podocarpatriene (2): amorphous solid; $[\alpha]^{18}_D$ +17.8° (*c* 0.40, $CHCl_3$); IR (dry film) λ_{max} 3456, 3035, 1633, 1507, 1248, 990, 858, 784 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.72, 6.67 (each 1H, d, J = 8.8 Hz, H-11, -12), 5.50 (1H, br s, −OH, exchangeable with D_2O), 3.83 (3H, s, $-OCH_3$), 3.82 (1H, overlapping with $-OCH_3$, H-1), 2.75 (2H, m, H-7), 1.86, 1.74 (each 1H, m, H-6), 1.74 (2H, m, H-2), 1.42, 1.30 (each 1H, m, H-3), 1.23 (1H, overlapping with other signal, H-5), 1.20, 0.91, 0.91 (each 3H, s, H-11, -18, -19); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.7 (s, C-13), 143.4 (s, C-14), 142.2 (s, C-9), 122.6 (s, C-8), 117.6 (d, C-11), 107.9 (d, C-12), 77.8 (d, C-1), 55.9 (q, OCH_3), 49.2 (d, C-5), 43.4 (s, C-10), 39.6 (t, C-3), 33.2 (s, C-4), 32.7 (q, C-18), 30.3 (t, C-2), 23.2 (t, C-7), 21.2 (q, C-19), 18.2 (t, C-6), 17.7 (q, C-20); EIMS (70 eV) *m/z* 290 $[M]^+$ (56), 272 (38), 257 (62), 231 (100), 192 (19); HREIMS *m/z* 290.1883 (calcd for $C_{18}H_{24}O_3$, 290.1883).

13-Hydroxy-12-methoxy-8,11,13-podocarpatrien-3-one (3): amorphous solid; $[\alpha]^{15}_D$ +25.1° (*c* 0.30, $CHCl_3$); IR (dry film) λ_{max} 3713, 3043, 1706, 1619, 1513, 1281, 1142, 1024, 870 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.68, 6.58 (each 1H, s, H-11, -14), 5.44 (1H, br s, −OH, exchangeable with D_2O), 3.83 (3H, s, $-OCH_3$), 2.78 (2H, m, H-7), 2.60 (2H, m, H-2), 2.40 (1H, br d, J = 13.1 Hz, H-1β), 1.90 (1H, m, H-1α), 1.86 (1H, overlapping with other signal, H-5), 1.74 (2H, m, H-6), 1.26, 1.14, 1.10 (each 3H, s, H-20, -18, -19); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 217.7 (s, C-13), 145.0 (s, C-12), 143.6 (s, C-13), 138.9 (s, C-9), 127.9 (s, C-8), 114.1 (d, C-14), 107.6 (d, C-11), 56.0 (q, $-OCH_3$), 50.6 (d, C-5), 47.3 (s, C-4), 37.8 (t, C-1), 37.1 (s, C-10), 34.6 (t, C-2), 30.3 (t, C-7), 27.0 (q, C-18), 24.7 (q, C-20), 21.0

(q, C-19), 20.3 (t, C-6); EIMS (70 eV) *m/z* 288 $[M]^+$ (90), 273 (100), 257 (38), 231 (77), 206 (37); HREIMS *m/z* 288.1729 (calcd for $C_{18}H_{24}O_3$, 288.1726).

14-Hydroxy-13-methoxy-1,8,11,13-podocarpatetraen-3-one (4): amorphous solid; $[\alpha]^{21}_D$ +16.1° (*c* 0.45, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 223 (4.10), 282 (3.20) nm; IR (dry film) λ_{max} 3462, 3030, 1660, 1617, 1497, 1268, 1241, 1096, 857 cm^{-1}; $_1$H NMR ($CDCl_3$, 300 MHz) δ 7.55, 5.98 (each 1H, d, J = 10.5 Hz, H-1, -2), 6.90, 6.75 (each 1H, d, J = 8.7 Hz, H-11, -12), 5.71 (1H, br s, −OH, exchangeable with D_2O), 3.86 (3H, s, −OCH_3), 2.72 (1H, m, H-7β), 3.01 (1H, dd, J = 16.8, 6.6 Hz, H-7a), 2.10 (1H, dd, J = 12.3, 2.1 Hz), 1.36, 1.20, 1.16 (each 3H, s, H-20, -18, -19), 1.80 (2H, m, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 204.8 (s, C-3), 157.3 (d, C-1), 144.0 (s, C-13), 143.2 (s, C-14), 137.9 (s, C-9), 126.3 (s, C-8), 126.3 (d, C-2), 114.9 (d, C-11), 108.3 (d, C-12), 56.0 (q, −OCH_3), 47.7 (d, C-5), 44.6 (s, C-4), 39.7 (s, C-10), 28.3 (q, C-20), 27.0 (q, C-18), 23.9 (t, C-7), 21.3 (q, C-19), 18.4 (t, C-6); EIMS (70 eV) *m/z* 286 $[M]^+$ (36), 271 (100), 259 (12), 243 (17), 189 (12); HREIMS *m/z* 286.1581 (calcd for $C_{18}H_{22}O_3$, 286.1570).

13-Methoxy-8,12-podocarpadiene-11,14-dione (5): yellow amorphous solid; $[\alpha]^{17}_D$ +10.0° (*c* 0.29, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 274 (4.10) nm; IR (dry film) λ_{max} 3048, 1670, 1610, 1586, 1255, 970, 837 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.71 (1H, s, H-12), 3.73 (3H, s, −OCH_3), 2.74 (1H, br d, J = 12.8 Hz, H-1β), 2.64, 2.28 (each 1H, m, H-7), 1.83, 1.37 (each 1H, m, H-6), 1.69, 1.49 (each 1H, m, H-2), 1.43, 1.16 (each 1H, m, H-3), 1.27, 0.90, 0.87 (each 3H, s, H-20, -18, -19), 1.09 (1H, m, H-1a), 1.03 (1H, overlapping with other signal, H-5); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 187.5 (s, C-11), 183.3 (s, C-14), 157.1 (s, C-13), 151.9 (s, C-9), 140.5 (s, C-8), 108.9 (d, C-12), 55.9 (q, −OCH_3), 51.7 (d, C-5), 41.3 (t, C-3), 38.9 (s, C-10), 36.6 (t, C-1), 33.6 (s, C-4), 33.5 (q, C-18), 25.6 (t, C-7), 21.8 (q, C-19), 20.3 (q, C-20), 18.9 (t, C-2), 17.2 (t, C-6); EIMS (70 eV) *m/z* 288 $[M]^+$ (37), 275 (95), 241 (23), 205 (90), 193 (51), 179 (100); HREIMS *m/z* 288.1735 (calcd for $C_{18}H_{24}O_3$, 288.1726).

Catalytic Hydrogenation of 1 with Pd/C. Compound **1** (4 mg) was hydrogenated in MeOH (2 mL) with 5% Pd/C (5 mg) as a catalyst. After 4 h, the product **2** (3 mg) was obtained, identical in all respects to **2**.

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