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

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Received November 12, 2001; accepted January 7, 2002

Further studies on the bark of *Taiwania cryptomerioides* found four new podocarpane derivatives, 1β,13-dihydroxy-8,11,13-podocarpatriene (1), 14,18-dihydroxy-13-methoxy-8,11,13-podocarpatriene (2), 1β,14-dihydroxy-13-methoxy-8,11,13-podocarpatriene-2,7-dione (3), and 3β,14-dihydroxy-13-methoxy-8,11,13-podocarpatrien-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

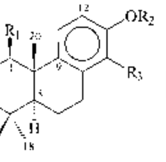
Taiwania cryptomerioides Hayata (Taxodiaceae) is an endemic plant and also an important building material. It grows at elevations from 1800 to 2600 m in the central mountains in Taiwan. The heartwood of *T. cryptomerioides* is yellowish-red with distinct purplish-pink streaks. In the previous investigation, 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.[8] Because it is a decay-resistant tree, the antifungal characterics were also investigated. α-Cardinol has the highest activity against *Coriolus versicolor* (white-rot fungi) and *Laetiporous suphurens* (brown-rot fungi) with 100% antifungal indices at 100 ppm.[9]

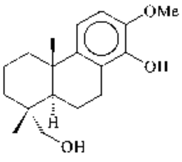
Podocarpane diterpenes do not occur extensively in nature but are present in several genera, such as *Azadirachta*,[10—14] *Humirianther*,[15] *Micrandropsis*,[16] and *Podocarpus*.[17] Lin *et al.*[18] first isolated the podocarpane derivative 1β,13,14-trihydroxy-8,11,13-podocarpatrien-7-one from the 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-podocarpatriene (**1**), 14,18-dihydroxy-13-methoxy-8,11,13-podocarpatriene (**2**), 1β,14-dihydroxy-13-methoxy-8,11,13-podocarpatriene-2,7-dione (**3**), and 3β,14-dihydroxy-13-methoxy-8,11,13-podocarpatrien-7-one (**4**), together with the known, 1β,13,14-trihydroxy-8,11,13-podocarpatrien-7-one (**5**),[18] were observed. In this paper we deal with their structural elucidation.

Compound **1** has the molecular formula $C_{17}H_{24}O_2$ (based on peak matching of the molecular ion and ^{13}C-NMR spectroscopy). The IR spectrum of **1** displayed peaks for hydroxyl (3421 cm^{-1}) and aromatic (1615, 1498 cm^{-1}) groups. The ^{1}H-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 H_β-1 signal (δ 2.00—2.40) of dehydroabietane and dehydropodocarpane-type derivatives[18,22] were observed. Among 17 ^{13}C-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 H_α-1 (axial), which had nuclear Overhauser enhancement and exchange spectroscopy (NOESY) correlation with H-5 (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-podocarpatriene (**6**),[23] due to the deshielding by the C-1 equatorial hydroxyl group. When comparing the ^{1}H- and ^{13}C-NMR data between **1** and **7**,[21] 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-podocarpatriene.

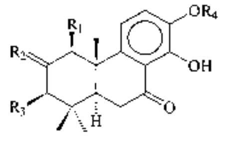
Eighteen ^{13}C-NMR signals and exact mass spectrum data confirmed the molecular formula of **2** to be $C_{18}H_{26}O_3$. Aromatic and hydroxyl absorptions are present in its IR spectrum. Three singlet methyl groups at δ 0.86 (H-19), 1.18 (H-20), and 3.82 (–OCH_3) and two *ortho*-coupling phenyl protons at δ 6.78 and 6.67 (d, J=8.6 Hz, H-11, -12) (Table 1) in its ^{1}H-NMR spectrum were observed. The signals at δ 3.21 and 3.46 (1H each, d, J=10.9 Hz) and δ_C 72.1 (Table 1) indicated a hydroxymethyl group attached to a quarternary 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



1 R_1 = OH, R_2 = R_3 = H
6 R_1 = OH, R_2 = Me, R_3 = OH
7 R_1 = R_2 = R_3 = H



2



3 R_1 = OH, R_2 = O, R_3 = H, R_4 = Me
4 R_1 = H, R_2 = H_2, R_3 = OH, R_4 = Me
5 R_1 = OH, R_2 = H_2, R_3 = R_4 = H

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1**—**4** (400 MHz and 100 MHz in $CDCl_3$)

No	**1**		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	77.8	3.82 dd (9.6, 6.2)	38.7	1.36 m (α) 2.24 br d (12.7)	80.8	4.54 s	36.2	1.64 td (12.6, 4.2, α) 2.32 dt (12.6, 3.1, β)
2	30.2	1.73 m	18.6	1.69 m	209.1		27.5	1.80 m
3	39.7	1.35 m	35.0	1.41 m	52.9	2.44[a] 2.51[a]	78.5	3.33 dd (10.7, 4.6)
4	33.3		37.7		49.6		38.8	
5	49.7	1.24[a]	43.4	1.57 d (12.8)	47.6	2.47[a]	48.4	1.80 m
6	18.8	1.84 m	18.0	1.81 m	35.8	2.83 d (8.4)	35.9	2.72 dd (18.3, 6.6, α) 2.75 dd (18.3, 11.0, β)
7	29.7	2.74 ddd (17.1, 8.7, 8.9) 2.85 ddd (17.1, 7.5, 3.4)	23.7	2.66 m (α) 2.89 dd (17.9, 6.6)	204.7		206.2	
8	137.5		122.1		115.7		115.2	
9	142.1		144.0		145.0		147.5	
10	43.3		37.1		38.9		37.4	
11	123.2	8.04 d (8.7)	114.8	6.78 d (8.6)	115.7	7.55 d (8.5)	113.1	6.69 d (8.3)
12	112.7	6.57 dd (8.7, 2.7)	108.1	6.67 d (8.6)	118.0	7.03 d (8.5)	117.9	6.99 d (8.3)
13	153.2		143.3		147.1		146.5	
14	114.7	6.48 d (2.7)	142.4		153.1		153.1	
18	32.7	0.89 s	72.1	3.21 d (10.9) 3.46 d (10.9)	32.0	1.14 s	27.5	1.04 s
19	21.2	0.91 s	17.3	0.86 s	21.9	0.97 s	15.0	0.94 s
20	17.5	1.18 s	25.4	1.18 s	18.0	1.08 s	23.6	1.19 s
$-OCH_3$			55.9	3.82 s	56.2	3.87 s	56.2	3.86 s
Ar-OH		6.47 s		4.50 s		12.89 s		12.96 s

[a] Obscured by another signal.

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_β-1 signal of dehydroabietane appeared at δ 2.24 (1H, br d, 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-11. The hydroxyl group located at C-18 showed no NOESY correlation with H-20. Therefore **2** was identified as 14,18-dihydroxy-13-methoxy-8,11,13-podocarpatriene.

The molecular formula ($C_{18}H_{22}O_5$), and UV (λ_{max} 221.0, 271.5, 364.0 nm) and IR (3449, 3200—2700, 1715, 1646, 1586, 1474 cm^{-1}) spectra of compound **3** suggest 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).[13)] Two equivalent methylene protons (δ 2.83, d, J=8.4 Hz) showing 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 *ortho*-phenyl protons are present at δ 7.03 (d, J=8.5 Hz, H-12) and 7.55 (d, J=8.5 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 attributable to the deshielding of the C-1_β hydroxyl group, as in compounds **1** and **6**.[18,23)] 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_2-3 (δ_C 52.9) and H-5 (δ_C 47.6) were present between δ 2.42 and 2.51. The NOESY correlation (H-1/H_α-3, 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-podocarpatriene-2,7-dione.

The high-resolution mass spectrum and ^{13}C-NMR data (Table 1) of compound **4** showed that it has the formula $C_{18}H_{24}O_4$. 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 ^{1}H-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 ^{1}H-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-podocarpatriene 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 carbinyl methine proton at δ 3.33 (dd, J=10.7, 4.6 Hz) was assigned to have an H-3α-axial orientation due to its coupling constant and NOESY correlation with H-18. H_β-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-podocarpantrien-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 acyloin 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. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DMX-400 at 400 and 100 MHz in $CDCl_3$, with tetramethylsilane as an internal standard. Electron impact (EI)-MS, FAB-MS, UV, and specific rotations were recorded on a JEOL JMS-HX 300, a JOEL JMS-HX 110, a Hitachi S-3200 spectrometer, and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merck 70—230 mesh, 230—400 mesh, ASTM).

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

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 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 as eluent and further purified by HPLC eluted with *n*-hexane : EtOAc (25 : 75). Five components, 1β,13-dihydroxy-8,11,13-podocarpatriene (**1**) (24 mg), 14,18-dihydroxy-13-methoxy-8,11,13-podocarpatriene (**2**) (45 mg), 1β,14-dihydroxy-13-methoxy-8,11,13-podocarpatriene-2,7-dione (**3**) (8 mg), 3β,14-dihydroxy-13-methoxy-8,11,13-podocarpatrien-7-one (**4**) (38 mg), and 1β,13,14-trihydroxy-8,11,13-podocarpatrien-7-one (**5**) (18 mg) were obtained in pure form.

1β,13-Dihydroxy-8,11,13-podocarpatriene (**1**): Yellow amorphous solid; $[\alpha]_D^{23}$ $-3.1°$ (c=0.55, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε) 219.5 (3.84), 281.0 (2.87); IR (film) ν_{max} 3421, 1615, 1498, 1293, 1250, 1016 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table 1; EI-MS (70 eV) (rel. int. %) *m/z* 260 [M$^+$] (60), 201 (100), 160 (29), 145 (24); high resolution (HR)-EI-MS *m/z* 260.1776 (M$^+$ Calcd for $C_{17}H_{24}O_2$, 260.1770).

14,18-Dihydroxy-13-methoxy-8,11,13-podocarpatriene (**2**): Yellow amorphous solid; $[\alpha]_D^{18}$ $+5.3°$ (c=0.42, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε) 223.0 (3.64), 279.0 (3.23); IR (film) ν_{max} 3438, 1587, 1494, 1275, 1050, 756 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table 1; EI-MS (70 eV) (rel. int. %) *m/z* 290 [M$^+$] (45), 275 (43), 257 (62), 177 (25), 163 (33), 84 (100); HR-EI-MS *m/z* 290.1879 (M$^+$ Calcd for $C_{18}H_{26}O_3$, 290.1875).

1β,14-Dihydroxy-13-methoxy-8,11,13-podocarpatriene-2,7-dione (**3**): Yellow amorphous solid; $[\alpha]_D^{24}$ $-35.0°$ (c=0.17, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε) 221.0 (4.04), 271.5 (3.03), 364.0 (3.19); IR (film) ν_{max} 3449, 3200—2700, 1715, 1646, 1586, 1474, 1261, 1049 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table 1; EI-MS (70 eV) (rel. int. %) *m/z* 318 [M$^+$] (100), 302 (47), 245 (80), 205 (49), 149 (30); HR-EI-MS *m/z* 318.1461 (M$^+$ Calcd for $C_{18}H_{22}O_5$, 318.1461).

3β,14-Dihydroxy-13-methoxy-8,11,13-podocarpatrien-7-one (**4**): Yellow crystal; mp 87—89 °C; $[\alpha]_D^{24}$ $-9.4°$ (c=0.35, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε) 224.5 (4.00), 269.5 (3.80), 356.0 (3.36); IR (film) ν_{max} 3450, 3300—2700, 3035, 1580, 1479 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table 1; EI-MS (70 eV) (rel. int. %) *m/z* 304 [M$^+$] (100), 271 (36), 203 (60); HR-EI-MS *m/z* 304.1667 (M$^+$ Calcd for $C_{18}H_{24}O_4$, 304.1668).

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

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