

## Quinone-type Podocarpanes from the Bark of *Taiwania cryptomerioides*

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Received February 23, 2001; accepted May 8, 2001

**Three quinone-type podocarpanes, 3 $\beta$ -hydroxy-13-methoxy-8,12-podocarpadiene-11,14-dione (1), 18-hydroxy-13-methoxy-8,12-podocarpadiene-11,14-dione (2), and 13-methoxy-8,12-podocarpadiene-2,11,14-trione (3) were isolated from the bark of *Taiwania cryptomerioides*. Their structures were elucidated using spectral methods.**

**Key words** *Taiwania cryptomerioides*; Taxodiaceae; trinorditerpene; podocarpane

In a previous investigation, we analyzed various sesquiterpenes, lignans, and abietane-type diterpenes from the heartwood<sup>1–3)</sup> and bark<sup>4,5)</sup> of *Taiwania cryptomerioides*. Because it is a decay-resistant tree, the antifungus characteristics were also investigated.  $\alpha$ -Cardinol has the best activity against *Coriolus versicolor* (white-rot fungi) and *Laetiporus sulphurens* (brown-rot fungi) with 100% antifungal indices at 100 ppm.<sup>6)</sup> Podocarpane-type diterpenes do not occur extensively in nature. They are presented in the genus of *Azadirachta*,<sup>7–11)</sup> *Humirianther*,<sup>12)</sup> *Micrandropsis*,<sup>13)</sup> and *Podocarpus*.<sup>14)</sup> The podocarpane derivative 1 $\beta$ ,13,14-trihydroxy-8,11,13-podocarpatrien-7-one was first discovered from leaves of *T. cryptomerioides* in 1998.<sup>15)</sup> Because many interesting novel skeletal components have been isolated from its leaves,<sup>15–18)</sup> we were encouraged to study the chemical constituents of its bark again. Recently, we have reported 11 new podocarpane derivatives from the bark of this plant<sup>19,20)</sup> from which only one quinone-type podocarpane derivative, 13-methoxy-8,12-podocarpadiene-11,14-dione,<sup>20)</sup> has been isolated and elucidated. Now we continue to investigate the more polar fraction of same extract and have isolated three new quinone-type podocarpane derivatives. In this paper we deal with the structure elucidation of 3 $\beta$ -hydroxy-13-methoxy-8,12-podocarpadiene-11,14-dione (**1**), 18-hydroxy-13-methoxy-8,12-podocarpadiene-11,14-dione (**2**), and 13-methoxy-8,12-podocarpadiene-2,11,14-trione (**3**).

Compound **1** is a yellow powder with the molecular formula C<sub>18</sub>H<sub>24</sub>O<sub>4</sub> based on high-resolution mass spectroscopy (HR-EI-MS). Analysis of its IR spectrum suggested that **1** contains a quinone group (1671, 1642, 1596 cm<sup>-1</sup>). The UV spectrum ( $\lambda_{\max}$  273.5 nm) and <sup>13</sup>C-NMR (Table 1) data were also consistent with a quinone moiety. In addition to one methoxyl signal ( $\delta_{\text{C}}$  56.0), **1** has seventeen <sup>13</sup>C-NMR signals including three CH<sub>3</sub> signals at  $\delta_{\text{C}}$  15.7, 20.3, and 28.3. This evidence suggests that **1** is a podocarpane derivative. Three singlet methyl groups (Table 1;  $\delta$  0.85, 1.03, and 1.27) and a methoxyl signal ( $\delta$  3.74) were observed, with the latter placed in the quinone moiety. A typical H $\beta$ -1 signal at  $\delta$  2.80 (dt,  $J=13.5$ , 3.5 Hz) was similar to that observed in royleanone<sup>21)</sup> and compound **4**,<sup>20)</sup> and the latter was also isolated from the same source. Comparison of the <sup>1</sup>H- and <sup>13</sup>C-NMR (Table 1) data of **1** with that of compound **4**<sup>20)</sup> suggests that **1** has the same skeletal structure. In proton-detected heteronuclear multiple-bond correlation (HMBC) experiments, H-7 and H-20 signals confirmed the C-8 ( $\delta_{\text{C}}$  140.6), C-9 ( $\delta_{\text{C}}$  151.1), and C-14 ( $\delta_{\text{C}}$  183.1). The olefinic proton had nuclear

Overhauser enhancement and exchange spectroscopy (NOESY) correlation to the methoxyl group ( $\delta$  3.74) and HMBC correlation with C-9, not with C-8. This evidence identified the olefinic proton at C-12. A carbonyl proton at  $\delta$  3.24 (dd,  $J=8.2$ , 7.9 Hz) was assigned to be the C-3  $\alpha$ -axial based on its coupling pattern, NOESY correlation to H-5 ( $\delta$  1.04, d,  $J=13.0$  Hz), and HMBC correlation to C-1, C-4, C-18, and C-19. Therefore compound **1** is 3 $\beta$ -hydroxy-13-methoxy-8,12-podocarpadiene-11,14-dione.

Compound **2** has the same molecular formula as **1**, C<sub>18</sub>H<sub>24</sub>O<sub>4</sub>, as established by HR-EI-MS. It is a yellow amorphous solid containing a quinone group, and its functionality was determined from UV absorption at  $\lambda_{\max}$  274.0 nm and IR absorption bands at 1670, 1646, and 1596 cm<sup>-1</sup>. Eighteen <sup>13</sup>C-NMR signals contained four olefinic carbons ( $\delta_{\text{C}}$  140.5, 151.8, 108.8, and 157.2), two quinone carbonyl carbons ( $\delta_{\text{C}}$  187.4 and 183.2), and a methoxyl carbon ( $\delta_{\text{C}}$  55.9). This evidence suggests that **2** is a podocarpane derivative. However, two singlet methyl groups ( $\delta_{\text{H}}$  0.82 [ $\delta_{\text{C}}$  17.7] and 1.31 [ $\delta_{\text{C}}$  20.8]) and one hydroxymethyl group ( $\delta_{\text{H}}$  3.14 and 3.45 [1H each,  $J=10.8$  Hz;  $\delta_{\text{C}}$  71.8]) were also observed. The chemical shift of the methyl group at  $\delta$  1.31 similar to the corresponding proton in **1** and **4** led us to assume that it was H-20. The results of NOE correlation between  $\delta$  0.82 and 1.31 led to the conclusion that the carbonyl proton is H-18, which has NOESY correlation to H-5 ( $\delta$  1.44). H-5 exhibited no NOE correlation with H-20 and H-19, indicating that the ring is *trans*-fused. Comparison of <sup>1</sup>H- and <sup>13</sup>C-NMR data of **1**, **2**, and **4** as well as HMBC and NOESY methods confirmed the structure of **2** to be 18-hydroxy-13-methoxy-8,12-podocarpadiene-11,14-dione.

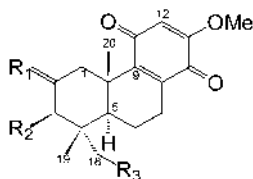
Compound **3**, a yellow amorphous solid has 18 <sup>13</sup>C-NMR signals and the molecular formula C<sub>18</sub>H<sub>22</sub>O<sub>4</sub> based on its exact mass spectrum. A *p*-benzoquinone moiety attached to a methoxyl group ( $\nu_{\max}$  1675, 1644, 1601 cm<sup>-1</sup>;  $\delta_{\text{H}}$  5.76 [1H, s], 3.75 [3H, s];  $\delta_{\text{C}}$  140.7, 148.8, 186.7, 108.8, 157.4, 182.8, 56.1) and a cyclohexanone ( $\nu_{\max}$  1707 cm<sup>-1</sup>;  $\delta_{\text{C}}$  210.9) moiety were revealed from its spectrum. Comparison of <sup>1</sup>H- and <sup>13</sup>C-NMR data of **3** and **4** suggested that compound **3** has an additional ketone that **4** does not. The chemical shift of H-18 and H-19 at  $\delta$  1.07 and 0.99, respectively, excluded the oxo function at C-3. The typical H $\beta$ -1 signal in quinone-type podocarpane derivatives is near  $\delta$  2.8 ( $\delta$  2.80 in **1**, 2.74 in **2**, 2.74 in **4**<sup>20)</sup>). However, the H $\beta$ -1 signal of **3** at low-field  $\delta$  3.48 (d,  $J=14.8$  Hz) indicated that the oxo function is situated at C-2. AB system signals displayed at  $\delta$  2.27 and 2.39

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Table 1. <sup>1</sup>H- and <sup>13</sup>C-NMR Spectral Data of Compounds 1–3 (400 MHz and 100 MHz in CDCl<sub>3</sub>)

No.	1		2		3	
	δ <sub>C</sub>	δ <sub>H</sub>	δ <sub>C</sub>	δ <sub>H</sub>	δ <sub>C</sub>	δ <sub>H</sub>
1	34.6	1.24 m 2.80 dt (13.5, 3.5)	36.1	1.10 td (13.1, 3.6) 2.74 br d (13.1)	52.1	2.17 d (14.8) 3.48 d (14.8)
2	27.8	1.72 m	18.3	1.59 m 1.75 m	210.9	-
3	78.3	3.24 dd (8.2, 7.9)	34.7	1.28 m 1.44 m	54.9	2.27 d (13.9) 2.39 d (13.9)
4	39.1	—	37.8	—	36.6	—
5	51.2	1.04 d (13.0)	45.0	1.44*	50.6	1.54*
6	17.1	1.44 tdd (13.0, 12.3, 5.7)	17.0	1.43 m 1.77 m	17.9	1.55* 1.95 dd (10.6, 6.7)
7	25.8	1.86 dd (13.0, 7.4) 2.30 ddd (20.2, 12.3, 7.4) 2.70 dd (20.2, 5.7)	25.2	2.32 ddd (19.9, 11.3, 7.5) 2.64 dd (19.9, 5.0)	25.2	2.35 m 2.78 dd (19.4, 4.1)
8	140.6	—	140.5	—	140.7	—
9	151.1	—	151.8	—	148.8	—
10	38.6	—	38.8	—	41.7	—
11	187.3	—	187.4	—	186.7	—
12	108.9	5.72 s	108.8	5.71 s	108.8	5.67 s
13	157.2	—	157.2	—	157.4	—
14	183.1	—	183.2	—	182.8	—
18	28.3	1.03 s	71.8	3.14 d (10.8) 3.45 d (10.8)	33.2	1.07 s
19	15.7	0.85 s	17.7	0.82 s	23.9	0.99 s
20	20.3	1.27 s	20.8	1.31 s	21.9	1.36 s
-OCH <sub>3</sub>	56.0	3.74 s	55.9	3.73 s	56.1	3.75 s

\* Overlapped with other signals.



	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
1	H <sub>2</sub>	OH	H
2	H <sub>2</sub>	H	OH
3	O	H	H
4	H <sub>2</sub>	H	H

(1H each, d,  $J=13.9$  Hz, H-3) was assigned as vicinal to the carbonyl group. H<sub>2</sub>-1 ( $\delta$  2.17 and 3.48) and H<sub>2</sub>-3 ( $\delta$  2.27 and 2.39) with HMBC correlation to the carbonyl group ( $\delta_C$  210.9) as well as H<sub>2</sub>-3 with NOESY correlation with H-18 and H-19 are further evidence for the C-2 oxo-function. Based on the above reasons, it was concluded that the structure of **3** is 13-methoxy-8,12-podocarpadiene-2,11,14-trione. The oxidation at C-2 among podocarpane derivatives is unique.

#### 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. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were determined using a Bruker DMX-400 at 400 and 100 MHz in CDCl<sub>3</sub> solution with tetramethylsilane (TMS) as an internal standard. EI-MS, FAB-MS, UV, and specific rotations were determined using a JEOL JMS-HX 300, JOEL JMS-HX 110, Hitachi S-3200 spectrometer, and 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 has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

**Extraction and Isolation** Air-dried pieces of *T. cryptomerioides* bark (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<sub>2</sub>O (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 high-performance liquid chromatography eluting with *n*-hexane:EtOAc (25:75). Three components, 3 $\beta$ -hydroxy-13-methoxy-8,12-podocarpadiene-11,14-dione (**1**) (4.9 mg), 18-hydroxy-13-methoxy-8,12-podocarpadiene-11,14-dione (**2**) (4.4 mg), and 13-methoxy-8,12-podocarpadiene-2,11,14-trione (**3**) (4.1 mg), were obtained in pure form.

3 $\beta$ -Hydroxy-13-methoxy-8,12-podocarpadiene-11,14-dione (**1**): Yellow powder; mp 184–186 °C;  $[\alpha]_D^{24} +11.1^\circ$  ( $c=0.45$ , CHCl<sub>3</sub>); UV  $\lambda_{max}$  nm (log  $\epsilon$ ): 273.5 (4.01); IR (film)  $\nu_{max}$  3436, 1671, 1642, 1596, 1221, 1029 cm<sup>-1</sup>; <sup>1</sup>H- and <sup>13</sup>C-NMR, see Table 1; EI-MS (70 eV)  $m/z$  (rel. int. %) 304 (M<sup>+</sup>, 1), 271 (8), 243 (27), 174 (47), 161 (49), 160 (100), 148 (33); HR-EI-MS  $m/z$  304.1678 (M<sup>+</sup> Calcd for C<sub>18</sub>H<sub>24</sub>O<sub>4</sub>, 304.1668).

18-Hydroxy-13-methoxy-8,12-podocarpadiene-11,14-dione (**2**): Yellow amorphous solid;  $[\alpha]_D^{24} +11.5^\circ$  ( $c=0.40$ , CHCl<sub>3</sub>); UV  $\lambda_{max}$  nm (log  $\epsilon$ ): 274.0 (3.95); IR (film)  $\nu_{max}$  3443, 1670, 1646, 1596, 1220, 1054, 984, 857 cm<sup>-1</sup>; <sup>1</sup>H- and <sup>13</sup>C-NMR, see Table 1; EI-MS (70 eV)  $m/z$  (rel. int. %) 304 (M<sup>+</sup>, 16), 273 (100), 205 (14), 179 (34); HR-EI-MS  $m/z$  304.1681 (M<sup>+</sup> Calcd for C<sub>18</sub>H<sub>24</sub>O<sub>4</sub>, 304.1668).

13-Methoxy-8,12-podocarpadiene-2,11,14-trione (**3**): Yellow amorphous solid;  $[\alpha]_D^{24} +104.0^\circ$  ( $c=0.37$ , CHCl<sub>3</sub>); UV  $\lambda_{max}$  nm (log  $\epsilon$ ): 272.0 (3.96); IR (film)  $\nu_{max}$  1707, 1675, 1644, 1601, 1218, 1076, 987, 864 cm<sup>-1</sup>; <sup>1</sup>H- and <sup>13</sup>C-NMR, see Table 1; EI-MS (70 eV)  $m/z$  (rel. int. %) 302 (M<sup>+</sup>, 11), 289 (100), 287 (76), 205 (52), 149 (25); HR-EI-MS  $m/z$  302.1522 (M<sup>+</sup> Calcd for C<sub>18</sub>H<sub>22</sub>O<sub>4</sub>, 302.1512).

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

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