

## Quinones from *Chirita eburnea*

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Five new quinone derivatives, (*R*)-7-hydroxy- $\alpha$ -dunnione (**1**), (*R*)-8-hydroxy- $\alpha$ -dunnione (**2**), (*R*)-7,8-dihydroxy- $\alpha$ -dunnione (**3**), (*R*)-7-methoxy-6,8-dihydroxy- $\alpha$ -dunnione (**4**), and 1,7-dihydroxy-2-hydroxymethylanthraquinone (**5**), along with seven known compounds, were isolated from *Chirita eburnea*. All structures were elucidated by spectroscopic techniques (NMR, MS, UV, and IR). The EtOAc fraction of the EtOH extract and compounds **3** and **4** showed free radical (DPPH) scavenging activity, with IC<sub>50</sub> values of 101.7  $\pm$  5.2  $\mu$ g/mL, 124.82  $\pm$  8.4  $\mu$ M, and 45.72  $\pm$  3.6  $\mu$ M, respectively, compared with 86.91  $\pm$  6.8  $\mu$ M for ascorbate.

*Chirita eburnea* Hance, a species of the family Gesneriaceae, is distributed in Yunnan, Guangxi, Guangdong, and Sichuan Provinces of China.<sup>1</sup> It has been used as folk medicine to treat cough with bleeding and other immunodeficiency diseases.<sup>2</sup> In previous papers the naphthoquinones,  $\alpha$ , $\beta$ -dunnione and  $\beta$ -dunnione derivative were described from *Streptocarpus dunnii* and anthraquinones were obtained from other genera of the family Gesneriaceae.<sup>3–5</sup> We investigated *C. eburnea* because there were no chemical constituents published for this plant and no  $\alpha$ -dunnione derivatives had been found. Four new  $\alpha$ -dunnione derivatives (**1–4**), an anthraquinone, 1,7-dihydroxy-2-hydroxymethylanthraquinone (**5**), and seven known compounds, (*R*)- $\alpha$ -dunnione (**6**),<sup>3</sup> 1-hydroxy-2-hydroxymethylanthraquinone (**7**),<sup>6</sup> 1,4-dihydroxy-2-hydroxymethylanthraquinone (**8**),<sup>7</sup> 1,7-dihydroxy-2-methylanthraquinone (**9**),<sup>8</sup> 1,7-dihydroxy-6-methoxy-2-methylanthraquinone (**10**),<sup>9</sup> 1-hydroxy-2-methoxy-7-methylanthraquinone (**11**),<sup>8</sup> and 1,2-dihydroxy-6-methylanthraquinone (**12**),<sup>10</sup> were identified. The structures of the known compounds were identified by comparison of their MS and <sup>1</sup>H and <sup>13</sup>C NMR data with those reported, and new compounds were determined on the basis of spectroscopic evidence, especially HMBC spectra. The occurrence of  $\alpha$ -dunnione in both this plant and *Streptocarpus dunnii* supports their close taxonomic relationship. Each compound except **6** was evaluated for its antioxidant properties using DPPH assay. This paper describes the isolation, identification, and antioxidant effects of these compounds.

Compound **1** possessed the molecular formula C<sub>15</sub>H<sub>14</sub>O<sub>4</sub> as inferred from EIMS, <sup>1</sup>H and <sup>13</sup>C NMR, and DEPT data. Its UV spectrum exhibited characteristic absorption peaks of the conjugated carbonyl moiety at 266, 307, 360, and 384 nm. The IR spectrum revealed absorption bands for hydroxyl (3350 cm<sup>-1</sup>) and carbonyl groups (1680 cm<sup>-1</sup>). The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **1** showed signals of two conjugated carbonyl carbons [ $\delta$ <sub>C</sub> 182.2, 178.9], eight olefinic carbons, a quaternary carbon ( $\delta$ <sub>C</sub> 45.8), an oxygenated tertiary carbon ( $\delta$ <sub>C</sub> 91.6), and three methyls ( $\delta$ <sub>C</sub> 26.1, 20.7, 14.4). These data suggested that **1** was an  $\alpha$ -dunnione derivative.<sup>3</sup> The <sup>1</sup>H NMR spectrum of **1** showed three protons arising from an aromatic moiety at  $\delta$ <sub>H</sub> 7.86 (1H, dd, *J* = 8.2, 0.25 Hz), 7.37 (1H, dd, *J* = 2.5, 0.25 Hz), and

7.17 (1H, dd, *J* = 8.2, 2.5 Hz). This <sup>1</sup>H NMR pattern indicated the location of the hydroxyl group at C-7 or C-6. Signals of  $\delta$ <sub>C</sub> 182.2 and 178.9 in the <sup>13</sup>C NMR spectrum of **1** could be assigned to C-4 and C-9, respectively, by comparison of the chemical shifts for C-4 ( $\delta$ <sub>C</sub> 182.2) and C-9 ( $\delta$ <sub>C</sub> 178.9) with those of  $\alpha$ -dunnione,<sup>3</sup> which was also supported by correlation between  $\delta$ <sub>H</sub> 1.44 (CH<sub>3</sub>-3 $\alpha$ ) and  $\delta$ <sub>C</sub> 182.2 in the HMBC spectrum. In the HMBC spectrum of **1** the cross-peaks [ $\delta$ <sub>H</sub> 7.86 with  $\delta$ <sub>C</sub> 182.2 (C-4), 162.6 and 134.6;  $\delta$ <sub>H</sub> 7.37 with  $\delta$ <sub>C</sub> 121.0, 126.7, and 178.9 (C-9)] suggested that the hydroxyl group was not located at C-6. The correlation patterns [ $\delta$ <sub>H</sub> 1.44 and 1.26 (3-CH<sub>3</sub>) with  $\delta$ <sub>C</sub> 130.3 (C-3a), 91.6 (C-2), and 45.8 (C-3), respectively;  $\delta$ <sub>H</sub> 1.38 (2-CH<sub>3</sub>) with  $\delta$ <sub>C</sub> 91.6 and 45.8;  $\delta$ <sub>H</sub> 1.26 with  $\delta$ <sub>C</sub> 26.1 (3-CH<sub>3</sub>);  $\delta$ <sub>H</sub> 1.44 with  $\delta$ <sub>C</sub> 20.7 (3-CH<sub>3</sub>)] in the HMBC spectrum also confirmed the structure of the 2,3,3-trimethylnaphtho[2,3-*b*]furan moiety. Compound **1** is thus 7-hydroxy- $\alpha$ -dunnione.

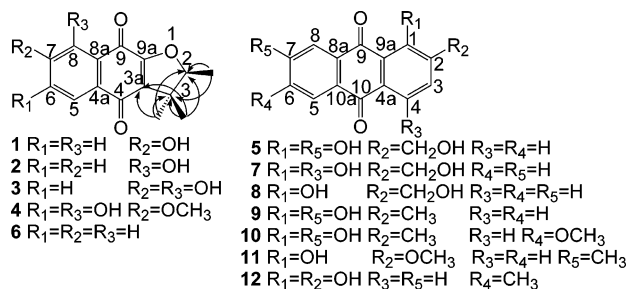
Compound **2** had the molecular formula C<sub>15</sub>H<sub>14</sub>O<sub>4</sub> according to its EIMS, <sup>1</sup>H and <sup>13</sup>C NMR, and DEPT data. The UV spectrum of **2** exhibited absorption peaks of a conjugated carbonyl moiety at 271, 314, 375, and 402 nm. Its IR spectrum indicated the presence of hydroxyl groups (3424 cm<sup>-1</sup>) and carbonyl groups (1675, 1641 cm<sup>-1</sup>). The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **2** showed similarities to those of **1** with the exception of the location of the hydroxyl group. In the <sup>1</sup>H NMR spectrum, three doublets of doublets [ $\delta$ <sub>H</sub> 8.03 (1H, dd, *J* = 7.5, 2.5 Hz), 7.65 (1H, dd, *J* = 8.2, 7.5 Hz), 7.16 (1H, dd, *J* = 8.2, 2.5 Hz)] suggested that the three hydrogens were adjacent and that the hydroxyl group was at either C-5 or 8. The C-9 resonance shifted downfield from  $\delta$ <sub>C</sub> 178.1 to 183.3 in the <sup>13</sup>C NMR spectrum of **2** by comparison of the chemical shifts of  $\alpha$ -dunnione,<sup>3</sup> which placed the hydroxyl at C-8 rather than C-5. Hence compound **2** is 8-hydroxy- $\alpha$ -dunnione.

Compound **3** possessed the molecular formula C<sub>15</sub>H<sub>14</sub>O<sub>5</sub> as inferred from EIMS, <sup>1</sup>H and <sup>13</sup>C NMR, and DEPT data. The UV spectrum of **3** exhibited absorption peaks of the conjugated carbonyl moiety at 268, 328, 380, and 421 nm. Its IR spectrum indicated the presence of hydroxyl (3235 cm<sup>-1</sup>) and carbonyl groups (1660, 1634 cm<sup>-1</sup>). <sup>1</sup>H and <sup>13</sup>C NMR spectra of **3** were very similar to those of **2** except for one more hydroxyl group. In the <sup>1</sup>H NMR spectrum of **3**, coupling constants of two doublets [ $\delta$ <sub>H</sub> 7.55 (1H, d, *J* = 8 Hz),  $\delta$ <sub>H</sub> 7.14 (1H, d, *J* = 8 Hz)] showed that two hydrogens were adjacent. The extra hydroxyl group was tentatively positioned at C-7. In the HMBC spectrum of **3**, correlations [ $\delta$ <sub>H</sub> 7.55 (H-5) with  $\delta$ <sub>C</sub> 180.9 (C-4), 150.1 (C-7), and 114.8

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**Figure 1.** Structures of compounds 1–12.**Table 1.** Free Radical Scavenging Activity of EtOAc-Soluble Fraction, **3**, **4**, and Ascorbate in the DPPH Assay<sup>a</sup>

sample	IC <sub>50</sub> (μM)
EtOAc-soluble fraction	101.7 ± 5.2 μg/mL
<b>3</b>	124.82 ± 8.4
<b>4</b>	45.72 ± 3.6
ascorbate	86.91 ± 6.8

<sup>a</sup> Results are given as the mean ± SD (*n* = 4).

(C-8a), δ<sub>H</sub> 7.14 (H-6) with δ<sub>C</sub> 125.3 (C-4a) and 148.8 (C-8)] support the above conclusions. Compound **3** was thus established as 7,8-dihydroxy-α-dunnione.

Compound **4** had the molecular formula C<sub>16</sub>H<sub>16</sub>O<sub>6</sub>, according to EIMS, <sup>1</sup>H and <sup>13</sup>C NMR, and DEPT data. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **4** were similar to those of **3** with the exception for one additional O-methyl group. In the HMBC spectra, the correlation of δ<sub>H</sub> 7.03 with δ<sub>C</sub> 181.2 (C-4), 157.4 (C-6), 139.1 (C-7), and 130.8 (C-4a) assigned δ<sub>H</sub> 7.03 as H-5, and the correlation between δ<sub>H</sub> 3.88 and δ<sub>C</sub> 139.1 (C-7) placed the O-methyl group at C-7. The structure of **4** was thus established as 7-methoxy-6,8-dihydroxy-α-dunnione.

The CD spectrum of **4** showed a negative Cotton effect at 302 nm (*θ* = 4.2 × 10<sup>5</sup>) contributed by a C-4 carbonyl group, with a positive Cotton effect at 380 nm (*θ* = 2.8 × 10<sup>5</sup>) caused by a C-9 carbonyl group.<sup>11–13</sup> The absolute configuration of C-2 of **4** was determined as *R* from a positive Cotton effect of C-9 by using octant rules for ketone, which was supported by those of C-4. The optical rotation values of compounds **1–4** and **6** were –133°, –33.3°, –125°, –120°, and –90°, respectively, indicating that they had the same configuration. Thus compounds **1–4** and **6** were defined as (*R*)-7-hydroxy-α-dunnione (**1**), (*R*)-8-hydroxy-α-dunnione (**2**), (*R*)-7,8-dihydroxy-α-dunnione (**3**), (*R*)-7-methoxy-6,8-dihydroxy-α-dunnione (**4**), and (*R*)-α-dunnione (**6**). Full assignments of <sup>1</sup>H and <sup>13</sup>C NMR of **1–4** are determined by 2D NMR spectra (Table 2).

The molecular formula of **5** was established by <sup>1</sup>H NMR, <sup>13</sup>C NMR, DEPT, and EIMS spectra as C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>. Its UV spectrum exhibited characteristic absorption peaks of anthraquinone at 228 (4.05), 264 (4.15), 356 (3.60), and 425 (3.50) nm. The IR spectrum suggested the presence of hydroxyl (3400 cm<sup>-1</sup>) and carbonyl groups (1685, 1670 cm<sup>-1</sup>). The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **5** showed the presence of two conjugated carbonyl carbons (δ<sub>C</sub> 189.7, 181.1), six double bonds, a hydroxymethyl (δ<sub>C</sub> 67.1), and two hydroxyl groups (δ<sub>H</sub> 12.81, 5.45). These signals suggested that **5** was an anthraquinone, 1,7(or 6)-dihydroxy-2-hydroxymethylanthraquinone, by comparison with the <sup>1</sup>H NMR spectra of **7** and **8**.<sup>6,7</sup> The signals at δ<sub>C</sub> 189.73 and 181.14 in **5** were assigned to C-9 and C-10, respectively, also by comparison with known compounds.<sup>4,5</sup> In the HMBC spectrum of **5**, correlations [δ<sub>H</sub> 7.16 (H-3) with δ<sub>C</sub> 135.7 (C-4a), 159.4 (C-1), δ<sub>H</sub> 8.06 (H-4) with δ<sub>C</sub> 138.4 (C-2), 181.1 (C-10), and 115.6 (C-9a), δ<sub>H</sub> 8.38 (H-5) with δ<sub>C</sub>

**Table 2.** <sup>13</sup>C NMR Data for Compounds 1–5<sup>a</sup>

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
<b>1</b>					159.4 s
<b>2</b>	91.6 d	91.9 d	91.8 d	92.1 d	138.4 s
<b>3</b>	45.8 s	45.2 s	45.4 s	45.7 s	134.2 d
<b>3a</b>	130.3 s	131.8 s	132.8 s	131.4 s	
<b>4</b>	182.2 s	181.5 s	180.9 s	181.2 s	119.2 d
<b>4a</b>	126.7 s	133.6 s	125.3 s	130.8 s	135.7 s
<b>5</b>	128.9 d	118.9 d	119.4 d	109.4 d	130.2 d
<b>6</b>	121.0 d	136.9 d	120.5 d	157.4 s	122.6 d
<b>7</b>	162.6 s	123.8 d	150.1 s	139.1 s	164.1 s
<b>8</b>	112.7 d	161.8 s	148.8 s	157.5 s	112.7 d
<b>8a</b>	134.6 s	114.6 s	114.8 s	109.4 s	132.2 s
<b>9</b>	178.9 s	183.3 s	183.7 s	182.7 s	189.7 s
<b>9a</b>	159.1 s	158.4 s	157.8 s	159.8 s	115.6 s
<b>10</b>					181.1 s
<b>10a</b>					126.1 s
–CH <sub>2</sub> OH					67.1 t
–OCH <sub>3</sub>				60.8 q	
2β-CH <sub>3</sub>	14.4 q	14.2 q	14.2 q	14.4 q	
3α-CH <sub>3</sub>	26.1 q	25.7 q	25.7 q	25.9 q	
3β-CH <sub>3</sub>	20.7 q	20.5 q	20.5 q	20.6 q	

<sup>a</sup> Compounds **1** and **2** were measured in acetone-*d*<sub>6</sub>, **3** and **4** in CDCl<sub>3</sub>, and **5** in pyridine-*d*<sub>5</sub>.

164.1 (C-7), 132.2 (C-8a), and 181.1, δ<sub>H</sub> 7.47 (H-6) with δ<sub>C</sub> 112.7 (C-8) and 126.1 (C-10a), δ<sub>H</sub> 8.01 (H-8) with δ<sub>C</sub> 122.6 (C-6), 126.1 and 189.7 (C-9)] supported that compound **5** was 1,7-dihydroxy-2-hydroxymethylanthraquinone.

For the screening and evaluation of antioxidant activity of crude extracts and each pure compound except **6**, a DPPH assay was adopted,<sup>14–16</sup> with ascorbate as a positive control. In the DPPH assay, the free radical scavenging activities of tested samples were expressed as IC<sub>50</sub>. The EtOAc fraction and compounds **3** and **4** showed powerful free radical scavenging activity (Table 1) compared to ascorbate. Scavenger activities of other compounds were weak and not strongly linear to their concentrations. Compounds **3** and **4** showed stronger activities from the effects of the carbonyl group and the interactions from the two hydroxyl groups. The activity of **4** was more powerful than that for **3**, possibly because an additional O-methyl group increased the activity of the two hydroxyl groups. Those quinones are especially active compounds and may account for the plant's ability to treat the diseases mentioned above.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. CD spectra were obtained using a JASCO J-810 spectropolarimeter (Japan Spectroscopic, Tokyo, Japan) in CDCl<sub>3</sub> solution. UV spectra were recorded on a Shimadzu double-beam 210A spectrophotometer. IR (KBr) spectra were obtained on Bio-Rac FTS-135 infrared spectrophotometer. <sup>1</sup>H, <sup>13</sup>C, and 2D NMR spectra were recorded on a DRX-500 MHz NMR spectrometer with TMS as internal standard. MS data were obtained on VG Autospec-3000 spectrometer, 70 eV for EI. Silica gel (200–300 mesh) for column chromatography and GF<sub>254</sub> for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. Rp 18 silica gel (40–65 μm) was bought from Merck Co., Germany. 1,1-Diphenylpicrylhydrazyl (DPPH) was purchased from Sigma Co.

**Plant Materials.** The whole plant of *C. eburnea* was collected and identified by Dr. De-Shan Deng in August 2002 in Guangxi Province, People's Republic of China.

**Extraction and Isolation.** Air-dried whole plant (4.0 kg) was crushed and extracted with 90% aqueous EtOH (12 L × 4) at room temperature (48 h × 4) to yield an EtOH extract. After removal of the EtOH under reduced pressure, the viscous

concentration was partitioned with EtOAc (4 × 10 L) to afford an EtOAc extract (80 g). The EtOAc fraction was adsorbed on 120 g of silica gel and chromatographed on a prepacked Si gel (1.0 kg) column, eluting with a mixture of CHCl<sub>3</sub>–Me<sub>2</sub>CO [from CHCl<sub>3</sub> to CHCl<sub>3</sub>–Me<sub>2</sub>CO (1:1)], to give eight fractions (I–VIII) according to differences in composition monitored by TLC (Si gel GF<sub>254</sub>). Fraction III (15.8 g) was further purified by repeated column chromatography over silica gel (280 g) using petroleum ether–EtOAc and petroleum ether–CHCl<sub>3</sub>, which gave three fractions (A–C) and compound **3** (10 mg). Fraction A (650 mg) was subjected to RP<sub>18</sub> gel (80 g) CC, eluting with MeOH–H<sub>2</sub>O (from 8:2 to 9:1) to afford **2** (120 mg) and **6** (6 mg). Repeated column chromatography of fraction B (1.0 g) afforded **1** (15 mg). Fraction IV (25.5 g) was loaded on column chromatography over silica gel (400 g), eluted by petroleum ether–Me<sub>2</sub>CO (from 8:2 to 7:3) to obtain fractions D (95.6 mg) and E (76.5 mg), which were subjected to RP<sub>18</sub> gel (80 g) and eluted with MeOH–H<sub>2</sub>O (from 7:3 to 8:2) to afford **8** (13 mg) and **11** (14 mg), respectively. Fraction V (18.9 g) was rechromatographed on a Si gel (300 g) column and eluted with petroleum ether–Me<sub>2</sub>CO (from 3:1 to 2:1) and CHCl<sub>3</sub>–Me<sub>2</sub>CO (from 5:1 to 4:1) to yield **7** (8 mg), **9** (10 mg), **10** (15 mg), and **12** (21 mg). Fraction VI (6.0 g) was also subjected to column chromatography over silica gel (100 g) using CHCl<sub>3</sub>–Me<sub>2</sub>CO (from 4:1 to 3:1) as eluent to afford fractions F and G, and fractions F (75.2 mg) and G (98.3 mg) were eluted by MeOH–H<sub>2</sub>O (from 7:3 to 8:2) over RP<sub>18</sub> gel (80 g) CC to afford **4** (18 mg) and **5** (21 mg), respectively.

**(R)-7-Hydroxy- $\alpha$ -dunnione (1):** red amorphous powder;  $[\alpha]_D^{25} -133^\circ$  (c 0.075, CH<sub>3</sub>OH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 266 (4.25), 307 (4.02), 360 (3.55), 384 (2.64) nm; IR (KBr)  $\nu_{\max}$  3350, 2950, 1680, 1600, 1550 cm<sup>-1</sup>; <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>, 500 MHz)  $\delta$  7.86 (1H, dd, *J* = 8.2, 0.25 Hz, H-5), 7.37 (1H, dd, *J* = 2.5, 0.25 Hz, H-8), 7.17 (1H, dd, *J* = 8.2, 2.5 Hz, H-6), 4.57 (1H, q, *J* = 6.4 Hz, H-2), 1.44 (3H, s, 3 $\alpha$ -CH<sub>3</sub>), 1.26 (3H, s, 3 $\beta$ -CH<sub>3</sub>), 1.38 (3H, d, *J* = 6.4 Hz, 2 $\beta$ -CH<sub>3</sub>); EIMS (70 eV) *m/z* 258 [M]<sup>+</sup> (60), 243 (100), 215 (10); <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>, 125 MHz), see Table 2.

**(R)-8-Hydroxy- $\alpha$ -dunnione (2):** red amorphous powder;  $[\alpha]_D^{25} -33.3^\circ$  (c 0.09, CH<sub>3</sub>OH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 232 (4.13), 271 (4.20), 314 (3.90), 375 (3.52), 402 (2.98) nm; IR (KBr)  $\nu_{\max}$  3424, 2968, 1675, 1641, 1609, 1451 cm<sup>-1</sup>; <sup>1</sup>H NMR (Me<sub>2</sub>CO, 500 MHz)  $\delta$  11.63 (1H, s, -OH), 8.03 (1H, dd, *J* = 7.5, 2.5 Hz, H-5), 7.65 (1H, dd, *J* = 8.2, 7.5 Hz, H-6), 7.16 (1H, dd, *J* = 8.2, 2.5 Hz, H-7), 4.57 (1H, q, *J* = 6.4 Hz, H-2), 1.46 (3H, s, 3 $\alpha$ -CH<sub>3</sub>), 1.26 (3H, s, 3 $\beta$ -CH<sub>3</sub>), 1.42 (3H, d, *J* = 6.4 Hz, 2-CH<sub>3</sub>); EIMS (70 eV) *m/z* 258 [M]<sup>+</sup> (21), 243 (100), 227 (50); <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>, 125 MHz), see Table 2.

**(R)- $\alpha$ -7,8-Dihydroxy- $\alpha$ -dunnione (3):** red amorphous powder;  $[\alpha]_D^{25} -125^\circ$  (c 0.10, CH<sub>3</sub>OH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 222 (4.12), 268 (4.01), 328 (3.74), 380 (3.36), 421 (3.05) nm; IR (KBr)  $\nu_{\max}$  3235, 2807, 1660, 1634, 1600, 1548 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  11.84 (1H, br s, OH), 7.55 (1H, d, *J* = 8 Hz, H-5), 7.14 (1H, d, *J* = 8 Hz, H-6), 6.15 (1H, br s, -OH), 4.58 (1H, q, *J* = 6.5 Hz, H-2), 1.46 (3H, s, 3 $\alpha$ -CH<sub>3</sub>), 1.26 (3H, s, 3 $\beta$ -CH<sub>3</sub>), 1.44 (3H, d, *J* = 6.5 Hz, 2-CH<sub>3</sub>); EIMS (70 eV) *m/z* 274 [M]<sup>+</sup> (70), 259 (100), 245 (20); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz), see Table 2.

**(R)-7-Methoxy-6,8-dihydroxy- $\alpha$ -dunnione (4):** red amorphous powder, mp 136–138 °C;  $[\alpha]_D^{25} -120^\circ$  (c 0.10, CH<sub>3</sub>OH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 220 (4.13), 270 (3.95), 293 (3.50), 332 (3.77), 426 (3.12) nm; IR (KBr)  $\nu_{\max}$  3315, 2805, 1670, 1635, 1600, 1550 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  12.30 (1H, br s, 8-OH), 7.03 (1H, q, *J* = 2.0 Hz, H-5), 4.58 (1H, q, 6.5 Hz, H-2), 3.88 (3H, s, -OCH<sub>3</sub>), 1.41 (3H, s, 3 $\alpha$ -CH<sub>3</sub>), 1.22 (3H, s, 3 $\beta$ -CH<sub>3</sub>), 1.38 (3H, d, *J* = 6.5 Hz, 2-CH<sub>3</sub>); EIMS (70 eV) *m/z* 304 [M]<sup>+</sup> (75), 289 (100), 271 (20), 261 (15); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz), see Table 2.

**1,7-Dihydroxy-2-hydroxymethylanthraquinone (5):** yellow amorphous powder; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 228 (4.05), 264 (4.15), 356 (3.60), 425 (3.50) nm; IR (KBr)  $\nu_{\max}$  3400, 3235, 1685, 1670, 1602, 1550 cm<sup>-1</sup>; <sup>1</sup>H NMR (C<sub>5</sub>D<sub>5</sub>N, 500 MHz)  $\delta$  12.81 (1H, br s, 1-OH), 8.38 (1H, dd, *J* = 8.5, 0.25 Hz, H-5), 8.06 (1H, d, *J* = 8.2 Hz, H-4), 7.16 (1H, d, *J* = 8.2 Hz, H-3), 8.01 (1H, dd, *J* = 2.5, 0.25 Hz, H-8), 7.47 (1H, dd, *J* = 8.5, 2.5 Hz, H-6), 5.45 (1H, br s, 7-OH), 4.70 (2H, s, -CH<sub>2</sub>-OH); EIMS (70 eV) *m/z* 270 [M]<sup>+</sup> (62), 241 (100), 224 (30), 196 (25), 168 (30); <sup>13</sup>C NMR (C<sub>5</sub>D<sub>5</sub>N, 125 MHz), see Table 2.

**Evaluation of DPPH Radical Scavenging Activity.**<sup>14–16</sup> Antioxidant activities of the crude extract and each pure compound except **6** were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. A 1.0 mL portion of a 200  $\mu$ M DPPH MeOH solution was added to 1.0 mL of sample solutions of four different concentrations and allowed to react at room temperature. After 30 min the absorbance values were measured at 517 nm using a spectrophotometer and converted into the percentage antioxidant activity (AA) using the following formula:

$$(\text{AA } \%) = 100 - \{[(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100] / \text{Abs}_{\text{control}}\}$$

MeOH (1.0 mL) plus sample solution (1.0 mL) was used as a blank. DPPH solution plus MeOH was used as a negative control, and ascorbate as a positive control. The IC<sub>50</sub> value is the concentration of test sample required to scavenge 50% DPPH free radical.

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