5-Deoxyflavones with Cytotoxic Activity from *Mimosa diplotricha*

Lie-Chwen Lin,*†‡ Chun-Tang Chiou,* and Jing-Jy Cheng†

†National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China
‡Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan, Republic of China

**ABSTRACT:** Bioassay-guided isolation of *Mimosa diplotricha* led to the isolation of four new 5-deoxyflavones, diplotrins A–C (1–3) and diplotasin (4), together with 12 known flavonoids, flavonolignans, and triterpenoids. On the basis of spectroscopic evidence, compounds 1–4 were characterized as 2′,S′-dihydroxy-3,7,8,4′-tetramethoxyflavone (1), 3′-hydroxy-3,7,8,4′-tetramethoxyflavone (2), 2′-hydroxy-7,4′S′-trimethoxyflavone (3), and 4-hydroxy-3,10,11-trimethoxyisochromeno-[4,3-b]-chromen-7(5H)-one (4). The cytotoxic effects of these isolated compounds were evaluated against the A549, AGS, HT-29, and PC3 human cancer cell lines. Compounds 2 and S′-methoxyhydrcarpin-D (5) showed the most potent antiproliferative activity.

*Mimosa diplotricha* C. Wright [syn.: *Mimosa invisa* Mart. ex Colla (Leguminosae)], a subshrub with multibranched vines, is native to tropical America. This species has been introduced as a fertilizing plant by agricultural authorities in Taiwan and is naturalized in uncultivated land in central and southern regions. The roots or the whole plant of *M. diplotricha* have been used as an analgesic, antiscorbutic, antitumoral, antiviral, antihelminthic, and tranquillizer in Formosan folk medicine. A variety of chemical components, including alkaloids, flavonoids, 2-phenoxycoumarins, diterpenoids, and triterpenoids, have been identified in *Mimosa* species, but these have received little biological study previously. The chemical constituents of *M. diplotricha* and their biological activities have not been reported. A preliminary biological screen revealed that an EtOH extract of *M. diplotricha* exhibited cytotoxic activity against various human tumor cell lines, prompting a more detailed investigation. Described herein are the isolation of four new 5-deoxyflavones (1–4) from *M. diplotricha* and their evaluation against four human cancer cell lines.

The CHCl₃ and n-BuOH extracts of *M. diplotricha* were subjected individually to a combination of silica gel, Sephadex LH-20, and C₁₈ chromatography, yielding 16 compounds. Of these compounds, 12 were known previously and were identified from their ¹H and ¹³C NMR and MS data as 5′-methoxyhydrcarpin-D (5), 7,3′,4′-trihydroxy-3,8-dimethoxyflavone, 10 2′-hydroxy-3,7,8,4′-pentamethoxyflavone, 11 hernandrost-3(20)-en-7β,11α-diol, 12 5,3′-di-O-methyl-β-caryophyllene, 13 betulinic acid, luteolin, quercetin, quercetin-3-O-sulfopyranoside, 14 quercetin-3-O-arabinofuranoside, 15 myricetin-3-O-sulfopyranoside, 14 and myricetin-3-O-arabinofuranoside. 14

Diplotrin A (1) was obtained as yellow needles from MeOH. The molecular formula of 1 was consistent with C₁₀H₁₄O₃ from the HRESIMS at m/z 375.1066 [M + H]+ and in agreement with the carbon and proton numbers as deduced from the ¹³C and ¹H NMR spectra. The ¹H NMR spectrum of 1 exhibited a pair of aromatic doublets at δ 7.95 and 7.02 (each 1H, d, J = 9.0 Hz), characteristic of H-5 and H-6 in 5-dehydroxyflavones. 11 Two other aromatic proton singlets at δ 6.59 (H-3′) and 7.32 (H-6′) and four aromatic methoxy signals at δ 3.84, 3.91, 3.95, and 3.97 were also observed. The addition of the shift reagents, AlCl₃ and NaOAc, had no effect on the UV spectra, indicating no free hydroxy group at C-3 or C-5, 15 or at C-7 or C-4′, respectively. Examination of the ¹³C NMR spectrum of 1 (Table 1) showed in addition 14 signals for aromatic carbons including eight oxygenated carbons, the presence of four methoxy groups in the range δ 56.0–61.8, and a carbonyl group at δ 173.4. These data suggested that 1 is a flavone with a 3,7,8,2′,4′,5′-hexahydroxy substitution pattern. From HMBC (Figure 1) and NOESY experiments, the locations of the methoxy substitutions were inferred to be at C-3, C-7, C-8, and C-4′. A HMBC experiment showed correlations from –OCH₃ (δ 3.95) and H-6 to C-8, from –OCH₃ (δ 3.97) and H-5 to C-7, and from –OCH₃ (δ 3.91), H-3′, and H-5′ to C-4′, suggesting the placement of methoxy groups at C-7, C-8, and C-4′. The fourth methoxy group was located at C-3 on the basis of the low-field shift of C-3 (δC 138.2) observed and a relevant HMBC correlation (δH 3.91/δC 138.2). The NOESY experiment performed on 1

Received: April 13, 2011
Published: August 29, 2011
showed interactions between the OCH$_3$-7 and H-6 signals and between those of OCH$_3$-4 and H-3', further supporting the above deductions. Therefore, compound 1 (diplotrin A) was deduced as 2',5',6'-dihydroxy-3,7,8,4'-tetramethoxyflavone.

Diplotrin B (2) was obtained as a light yellow, amorphous powder. The $^1$H and $^{13}$C NMR spectra of 2 resembled those of 1 except for the presence of ABX-type aromatic signals [($\delta$ 7.70 (d, $J$ = 1.8 Hz, H-2'), 7.09 (d, $J$ = 8.4 Hz, H-5')] instead of having two singlet aromatic signals on the B-ring. The HRESIMS of 2 revealed a protonated molecular ion at m/z 359.1120 [M + H]$,^+$, consistent with the molecular formula C$_{19}$H$_{18}$O$_7$ and in agreement with the presence of one less hydroxy group in ring B than in compound 1. A NOESY experiment on 2 showed an interaction between the OCH$_3$-4 and H-5' resonances. Accordingly, compound 2 (diplotrin B) was deduced as 3'-hydroxy-3,7,8,4'-tetramethoxyflavone.

Diplotrin C (3) was obtained as a light yellow, amorphous powder. The ESIMS exhibited a [M + H]$^+$ ion at m/z 329 and, together with the $^{13}$C NMR data, suggested a molecular formula of C$_{19}$H$_{16}$O$_6$. The $^{13}$C NMR spectrum of 3 (Table 1) showed 14 signals for the aromatic carbons of a flavone, including six oxygenated carbons, together with resonances for three methoxy groups and a carbonyl group. The $^1$H NMR spectrum exhibited an aromatic ABC-type pattern [($\delta$ 8.01 (d, $J$ = 9.0 Hz, H-5), 7.04 (dd, $J$ = 9.0, 2.4 Hz, H-6), and 7.22 (d, $J$ = 2.4 Hz, H-8)], a characteristic low-field H-5 signal, and a HMBC correlation between H-5 and $\delta$C 180.7, indicating the molecule of 3 to be a 5-dehydroxyflavone substituted at C-7. Two singlets, at $\delta$ 6.58 and $\delta$ 7.52, were assignable to the two para-position aromatic protons, H-3' and H-6', suggesting a 1,2,4,5-substituted B-ring system. The other aromatic singlet at $\delta$ 7.33 was placed at C-3. The positions of three methoxy groups and one hydroxy group were established by HMBC and NOESY experiments. A HMBC experiment showed correlations of $\delta$ 3.96 (OCH$_3$-7), H-5/$\delta$ 166.2 (C-7), $\delta$ 3.87 ($-$OCH$_3$), H-3', H-6'/$\delta$ 154.9, $\delta$ 3.88 ($-$OCH$_3$), H-3', H-6'/$\delta$ 144.1, and H-3', H-6'/$\delta$ 154.6, indicating the placements of a methoxy group at C7 and two methoxy groups and one hydroxy group on the B-ring. Key NOESY correlations of $\delta$ 6.58 (H-3')/\$\delta_7$ 3.87 and $\delta$ 7.52 (H-6')/\$\delta_7$ 3.88 were observed, but not for $\delta$ 7.33 (H-3), implying the presence of OCH$_3$-4', OCH$_3$-5', and OH-2'. On the basis of the above data, compound 3 was deduced as 2'-hydroxy-7,4',5'-trimethoxyflavone.

The HRESIMS of 4 showed a pseudomolecular ion at m/z 357.0956 [M + H]$^+$, in agreement with a molecular formula of C$_{19}$H$_{16}$O$_7$, and indicating 12 degrees of unsaturation. The $^1$H NMR spectrum exhibited two pairs of aromatic doublets at $\delta$ 8.00/7.00 (J = 9.0 Hz, H-8/9) and $\delta$ 7.42/6.90 (J = 8.4 Hz, H-5/6) and three aromatic methoxy groups at $\delta$ 3.95, 3.98, and 4.03. In addition, the appearance of a downfield methylene proton as a singlet at $\delta$ 5.34, consistent with an ether oxygen, and an aromatic moiety placed vicinal to the methylene carbon were characteristic of a peltogynoid derivative. The $^{13}$C NMR spectrum of 4 (see Experimental Section) showed 14 signals for aromatic carbons, of which seven are oxygenated, three methoxy groups in the range $\delta$ 62.8–61.6, and a carbonyl group at $\delta$ 171.4. Another distinctive feature was the observation of an oxygenated methylene carbon at $\delta$ 63.2. Diagnostic HMBC correlations were

### Table 1. $^1$H and $^{13}$C NMR Data of Compounds 1–3

<table>
<thead>
<tr>
<th>position</th>
<th>$^1$H</th>
<th>$^{13}$C</th>
<th>$^2$H</th>
<th>$^{13}$C</th>
<th>$^3$H</th>
<th>$^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta_C$</td>
<td>$\delta_H$</td>
<td>$\delta_C$</td>
<td>$\delta_H$</td>
<td>$\delta_C$</td>
<td>$\delta_H$</td>
</tr>
<tr>
<td>2</td>
<td>2'</td>
<td>3'</td>
<td>4'</td>
<td>5'</td>
<td>6'</td>
<td>OCH$_3$-3</td>
</tr>
<tr>
<td>3</td>
<td>150.2</td>
<td>157.6</td>
<td>163.6</td>
<td>171.4</td>
<td>180.7</td>
<td>61.8</td>
</tr>
<tr>
<td>4</td>
<td>138.2</td>
<td>141.4</td>
<td>110.6</td>
<td>119.6</td>
<td>110.0</td>
<td>60.4</td>
</tr>
<tr>
<td>5</td>
<td>173.4</td>
<td>176.7</td>
<td>180.7</td>
<td>115.9</td>
<td>115.9</td>
<td>63.2</td>
</tr>
<tr>
<td>6</td>
<td>121.0</td>
<td>7.95 (d, 9.0)</td>
<td>117.1</td>
<td>7.52 (d, 9.0)</td>
<td>124.6</td>
<td>113.0</td>
</tr>
<tr>
<td>7</td>
<td>156.6</td>
<td>158.3</td>
<td>166.2</td>
<td>101.5</td>
<td>154.6</td>
<td>64.0</td>
</tr>
<tr>
<td>8</td>
<td>136.8</td>
<td>138.1</td>
<td>107.2</td>
<td>144.1</td>
<td>154.9</td>
<td>3.80 s</td>
</tr>
<tr>
<td>9</td>
<td>150.0</td>
<td>150.9</td>
<td>159.7</td>
<td>118.0</td>
<td>114.4</td>
<td>3.88 s</td>
</tr>
<tr>
<td>10</td>
<td>118.6</td>
<td>119.6</td>
<td>110.0</td>
<td>110.0</td>
<td>115.4</td>
<td>3.88 s</td>
</tr>
<tr>
<td>1'</td>
<td>110.2</td>
<td>124.6</td>
<td>110.0</td>
<td>110.0</td>
<td>115.6</td>
<td>3.88 s</td>
</tr>
<tr>
<td>2'</td>
<td>155.4</td>
<td>116.2</td>
<td>7.70 (d, 1.8)</td>
<td>114.1</td>
<td>115.9</td>
<td>64.0</td>
</tr>
<tr>
<td>3'</td>
<td>102.2</td>
<td>6.59 s</td>
<td>147.7</td>
<td>150.9</td>
<td>154.9</td>
<td>3.88 s</td>
</tr>
<tr>
<td>4'</td>
<td>150.9</td>
<td>151.8</td>
<td>154.9</td>
<td>113.0</td>
<td>7.52 s</td>
<td>3.88 s</td>
</tr>
<tr>
<td>5'</td>
<td>139.8</td>
<td>112.4</td>
<td>7.09 (d, 8.4)</td>
<td>112.3</td>
<td>7.75</td>
<td>64.0</td>
</tr>
<tr>
<td>6'</td>
<td>113.5</td>
<td>7.32 s</td>
<td>122.3</td>
<td>113.0</td>
<td>7.52 s</td>
<td>3.88 s</td>
</tr>
</tbody>
</table>

*a* Measured in CDCl$_3$, *b* Measured in MeOH-d$_4$.  

![Figure 1. Key HMBC corrections of compound 1.](image-url)
observed between H-8 (δ 8.00) and C-7 (δ 171.4), C-10 (δ 156.1), and C-11a (δ 149.1), between H-9 (δ 7.00) and C-11 (δ 136.6) and C-7a (δ 119.2), between OCH3-11 (δ 4.03) and C-11, and between OCH3-10 (δ 3.98) and C-10 of the aromatic ring A. Other HMBC correlations were observed for H-5 (δ 5.34) with C-6a (δ 135.8, C-4 (δ 140.7), and C-12b (δ 118.5); H-1 (δ 7.42) with C-3 (δ 149.2), C-4a (δ 118.1), and C-12a (δ 147.7); H2 (δ 6.90) with C-4 (δ 140.7) and C-12b (δ 118.5); and OCH3-3 (δ 3.95) with C-3. On the basis of the above evidence, compound 4 (diplotisin) was deduced as 4-hydroxy-3,10,11-trimethoxyisochromen-4,3-b-chromen-7(SH)-one.

Thirteen of the compounds isolated were evaluated for their antiproliferative activity against the A549, AGS, HT-29, and PC3 human tumor cell lines. Compounds 2 and 5 exhibited antiproliferative activity against one or more tumor cell lines. Compounds 2 and 6 were the most effective, with GI50 values of 2.7, 1.7, 7.5, and 20.8 μM, respectively. The GI50 data for 5 were, in turn, 20.3, 24.8, 4.1, and 2.3 μM. The GI50 values against the four human cancer cell lines of the remaining compounds were all >10 μM.

**Experimental Section**

**General Experimental Procedures.** Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. UV spectra were measured on a Hitachi U-3200 spectrophotometer in MeOH. IR spectra were obtained on a Nicolet Avatar 320 IR spectrometer. 1H, 13C, and 2D NMR spectra were recorded on a Varian INOVA-500 spectrometer with deuterated solvents used as internal standards. ESIMS and HRESIMS were measured with a Finnigan LCQ and Shimadzu LCMS-IT-TOF spectrometers, respectively. Column chromatography was performed on Sephadex LH-20 (4.8 × 100 cm), with step gradient mixtures of EtOAc and CHCl3/MeOH, flow rate: 15 mL/min) to yield 1 (11.7 mg) and 2 (63.5 mg). A solid precipitate that separated from Fr.C8-4 was recrystallized with MeOH to give hernicancarin (6.4 mg). The filtrate of Fr.C8-4 (250 mg) was chromatographed over Sephadex LH-20 (MeOH) to give 1 (46.9 mg) and 4 (1.7 mg). Fr.C9 (3.23 g) was chromatographed over Sephadex LH-20 (4.8 × 80 cm), using EtOAc elution, to yield four fractions (Fr.C9-1–C9-4). Two solid precipitates separated individually from Fr.C9-2 and Fr.C9-4 and were recrystallized with MeOH to give 3 (2.1 mg) and 5 (3.2 mg). The filtrate of Fr.C9-4 (250 mg) was chromatographed over a Sephadex LH-20 column (MeOH) to give luteolin (34.6 mg). Further purification of Fr.C9-3 (78.7 mg) by semi-preparative HPLC (solvent: 60% MeOH/H2O, flow rate: 15 mL/min) gave 7,3',4'-trihydroxy-3,8-dimethoxyflavone (14.0 mg). Fr.C10 (1.10 g) was chromatographed over Sephadex LH-20, by elution with 5% CHCl3/MeOH, to afford five subfractions, and 5,3-di-O-methyluteolin (2.9 mg) was obtained from the fifth subfraction. (c) Workup of the n-BuOH extract: The n-BuOH extract (73 g) was subjected to column chromatography over Sephadex LH-20 (8 × 100 cm) eluted with MeOH and yielded eight fractions (Fr.B1–B8). Purification of Fr.B6 (5.58 g) over a Sephadex LH-20 column (MeOH) gave quercetin-3-O-xylopyranoside (34.0 mg), quercetin-3-O-arabino-furanoside (49.6 mg), myricetin-3-O-xylopyranoside (16.8 mg), and myricetin-3-O-arabinofuranoside (12.4 mg). In the same way, luteolin (46.4 mg) and quercetin (69.1 mg) were obtained from Fr.B7 (790.5 mg) and Fr.B8 (595.4 mg), respectively.

**Diplotisin A (1):** Yellow crystals from MeOH, mp 113 °C; UV (MeOH) λmax (log ε) 226 (4.30), 242 (4.33), 298 (4.08), 338 (3.99) nm; +NaOMe 236 (4.47), 293 (4.19) nm; IR νmax (KBr) 3298, 1638, 1601, 1515, 1438, 1357, 1121, 1087 cm⁻¹; ¹H NMR and ¹³C NMR, see Table 1; ESIMS m/z 375 [M + H]+; HRESIMS m/z 375.1066 [M + H]+ (calcld for C19H17O7, 375.1071).

**Diplotisin B (2):** Yellow crystals from MeOH, mp 159 °C; UV (MeOH) λmax (log ε) 250 (4.35), 263 (4.17), 308 (4.11), 347 (4.30) nm; +NaOMe 256 (4.35), 310 (4.10), 385 (4.02) nm; IR (KBr) νmax 3430, 1638, 1601, 1515, 1438, 1357, 1121, 1087 cm⁻¹; ¹H NMR and ¹³C NMR data, see Table 1; ESIMS m/z 359 [M + H]+; HRESIMS m/z 359.1120 [M + H]+ (calcld for C18H16O7, 359.1131).

**Diplotisin C (3):** Yellow powder from MeOH, mp 170 °C; UV (MeOH) λmax (log ε) 234 (4.19), 249 (4.22), 302 (4.12), 361 (4.24) nm; +NaOMe 232 (4.58), 258 (4.28), 305 (4.28), 422 (4.29) nm; IR (KBr) νmax 3380, 1625, 1561, 1405, 1218, 1168, 1093 cm⁻¹; ¹H NMR and ¹³C NMR, see Table 1; ESIMS m/z 329 [M + H]+; HRESIMS m/z 329.1003 [M + H]+ (calcld for C19H15O7, 329.1025).

**Diplotisin B (4):** Light yellow, amorphous powder; UV (MeOH) λmax (log ε) 252 (3.77), 311 (3.61), 343 (3.63) nm; IR (KBr) νmax 3389, 1619, 1601, 1438, 1383, 1286, 1097, 1067 cm⁻¹; ¹H NMR (600 MHz, CDCl3) δ 8.00 (1H, d, J = 9.0 Hz, H-8), 7.00 (1H, d, J = 9.0 Hz, H-9), 6.90 (1H, d, J = 8.4 Hz, H-2), 7.42 (1H, d, J = 8.4 Hz, H-1), 5.34 (2H, s, H-3a, H-3b), 3.95 (s, OCH3-3), 3.98 (s, OCH3-10), 4.03 (s, OCH3-11); ¹³C NMR (150 MHz, CDCl3) δ 114.4 (C-1), 109.7 (C-2), 149.2 (C-3), 140.7 (C-4), 118.1 (C-4a), 63.2 (C-5), 153.8 (C-6a), 171.4 (C-7), 119.2 (C-8), 121.4 (C-9), 109.6 (C-10), 165.1 (C-10), 136.6 (C-11), 149.1 (C-11a), 147.7 (C-12a), 118.5 (C-12b), 56.2 (OCH3-3), 56.5 (OCH3-10), 61.6 (OCH3-11); ESIMS m/z 357 [M + H]+; HRESIMS m/z 357.0956 [M + H]+ (calcld for C20H19O7, 357.0974).
Cell Lines and Reagents. Human A549 lung carcinoma cells (BCRC 60074), human AGS gastric adenocarcinoma cells (BCRC 60102), human HT-29 colorectal adenocarcinoma cells (BCRC 67003), and human PC3 prostate carcinoma cells (BCRC 60122) were obtained from the Bioresources Collection and Research Center (BCRC), Hsin-Chu, Taiwan. All cell lines were maintained in RPMI 1640 medium supplied with 10% fetal bovine serum, nonessential amino acid, 100 units/mL penicillin, and 100 units/mL streptomycin. Cell culture reagents were obtained from Invitrogen (Rockville, MD). Sulforhodamine B (SRB), trichloroacetic acid (TCA), and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell Proliferation Assay. The SRB assay was performed with cells seeded in 96-well plates and cultured overnight. Then, six wells of each cell line were fixed in situ with 10% TCA to represent a measurement at the time of drug addition (T₀). In addition, test compounds were added to the cells and incubated for an additional 48 h. The assay was terminated by adding 10% TCA. After rinsing the plates with PBS and air-drying, 0.4% SRB solution (weight per volume in 1% acetic acid) was added to each well, and the plates were incubated for 10 min at room temperature. Unbound dye was removed with 1% acetic acid, and the plates were air-dried. Bound SRB was subsequently solved with 10 mM Tris base. Cell proliferation was determined by measuring the optical density at 515 nm using a M5 microplate reader (Molecular Devices, Sunnyvale, CA). The absorbance measurement of cells at time zero and 0.09 μM against the A549, AGS, HT-29, and PC3 cancer cells, respectively.

ASSOCIATED CONTENT

Supporting Information. 1H NMR and 13C NMR spectra of compounds 1–4 are available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
Tel: +886-2-28201999, ext. 7101. Fax: +886-2-2826 4276. E-mail: lclin@nircm.edu.tw.

ACKNOWLEDGMENT

This work was supported by grants from the National Science Council, Republic of China (NSC 99-2320-B-077-003 and NSC 97-2323-B-077-002).

REFERENCES

(2) Chiu, N. Y.; Change, K. H. The Illustrated Medicinal Plants of Taiwan; Southern Materials Center Inc.: Taipei, 1998; Vol. 5, p 99.