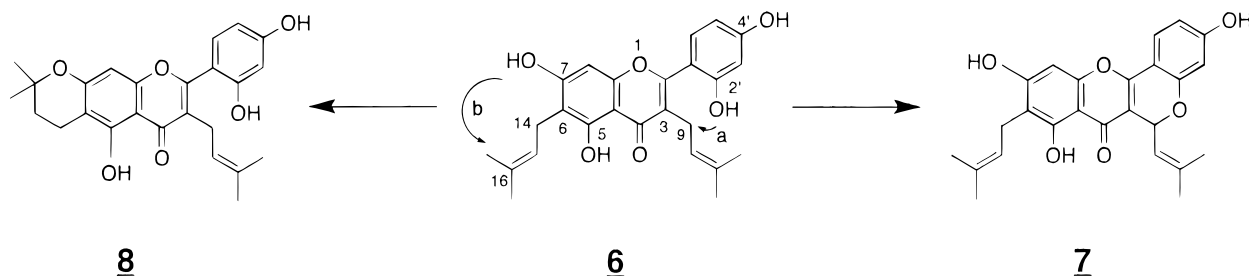




Scheme 2

**Table 1.** Anti-HIV Testing Results of Compounds **1**, **2**, and **4–8**

compound	HIV-inhibitory activity EC <sub>50</sub> (μg/mL)	toxicity IC <sub>50</sub> (μg/mL)
<b>1</b>	1–2	2.2–3.3
<b>2</b>	1.3–2.2	3.7
<b>4</b>	I <sup>a</sup>	5.3
<b>5</b>	I <sup>a</sup>	4.9
<b>6</b>	I <sup>a</sup>	5
<b>7</b>	7.5	17
<b>8</b>	I <sup>a</sup>	12

<sup>a</sup> Inactive.

An ortho coupling constant was measured between two protons at  $\delta_H$  6.92 and 7.69, and the positioning of those aromatic protons at H-7 and H-8, respectively, was supported by an HMBC correlation between the signal at  $\delta_H$  7.69 (H-8) and the carbonyl signal at  $\delta_C$  180.9 (C-9). The spectral assignments of **2** were based on comparison with the reported NMR data of gerontoxanthone I.<sup>10</sup> We also isolated two other known bis-prenylated xanthones, gartanin (**4**) and 8-desoxygartanin (**5**).<sup>11</sup>

Isoprenylated flavones are also very common in the family Moraceae.<sup>7,12</sup> In our investigation we have isolated three such compounds (Scheme 2). NMR and spectral comparisons showed that two of those were reported earlier; cudraflavone C (**6**) was a constituent of *C. tricuspidata*,<sup>13</sup> while isocyclomulberrin (**7**) was isolated from *Artocarpus altilis*.<sup>14</sup> The third compound (**8**), C<sub>25</sub>H<sub>26</sub>O<sub>6</sub>, was found to be a new natural product. 2D NMR experiments showed similarities to **6** in many aspects; however, instead of the two C<sub>5</sub> units, **8** contained only one such unit. The other C<sub>5</sub> unit comprised a 2,2'-dimethylchromane ring system with the 7-OH, as indicated by the chemical shifts of the geminal methyls ( $\delta_H$  1.31) and the oxygen-bearing carbon C-16 ( $\delta_C$  76.4). A closely related compound, cudraflavone B,<sup>15,16</sup> was found to differ from **8** by a  $\Delta^{14}$  double bond, while compound **8** had two methylenes at C-14 and C-15 (triplets at  $\delta_H$  2.62 and 1.81,  $\delta_C$  16.1 and 31.3, respectively). It is interesting to note that **6** could be the precursor of both **7** and **8**; a ring closure between 2'-OH and the allylic C-9 forms compound **7** (Scheme 2, pathway a), while pathway b illustrates the formation of compound **8**.

The seven compounds were tested in the primary anti-HIV screen and three compounds exhibited consistent activity (see Table 1). Macluraxanthones B (**1**) and C (**2**) showed the best potential, with EC<sub>50</sub> levels of 1–2.2 μg/mL; isocyclomulberrin (**7**) had an EC<sub>50</sub> level of 7.5 μg/mL, although dihydrocudraflavone B (**8**) showed only marginal activity. The catechol functionality of **1** and **2** would appear to proffer enhanced HIV-inhibitory activity. However, much like many other prenylated phenolic compounds discovered in our laboratory, such as the guttiferones<sup>17–19</sup> or vismiphenone D,<sup>20</sup> all seven compounds from *M. tinctoria*

exhibited very high toxicity toward the CEM-SS host cells, with IC<sub>50</sub> levels of 2.2–17 μg/mL.

## Experimental Section

**General Experimental Procedures.** NMR spectra were recorded on a Varian VXR-500 spectrometer using CDCl<sub>3</sub>, DMSO-d<sub>6</sub>, and CD<sub>3</sub>OD as solvents and internal standards. Infrared spectra were measured on a Perkin-Elmer 267 spectrometer, and ultraviolet spectra were obtained with a Beckman 34 spectrophotometer. Mass spectra were recorded on VG Micromass ZAB 2F and Finnigan Mat 95 mass spectrometers.

**Plant Material.** The bark of *M. tinctoria* (L.) Steud was collected by D. C. Daly, under contract to the National Cancer Institute, near Loreto, Prov. Maynas (longitude 73° 45.0' W, latitude 4° 55.02' S), Peru, in February 1988. A voucher specimen (Q65-T405) is maintained at the New York Botanical Garden. Extraction of 179 g dried, ground plant material with CH<sub>2</sub>Cl<sub>2</sub>–MeOH (1:1) and MeOH yielded 11.12 g crude extract.

**Fractionation of the Crude Extract.** The crude organic extract (8.46 g) was partitioned according to the following protocol: partition between 90% aqueous MeOH and hexane (affording 2.97 g), 80% aqueous MeOH and CCl<sub>4</sub> (0.75 g), and 70% MeOH/H<sub>2</sub>O and CHCl<sub>3</sub> (0.91 g). Then the MeOH was removed in vacuo, and the aqueous residue was extracted with EtOAc to give 0.78 g of organic soluble material. Finally, the H<sub>2</sub>O phase was freeze-dried to give a residue of 3.01 g. The antiviral activity was tracked to the CCl<sub>4</sub> (EC<sub>50</sub> 10 μg/mL) and the CHCl<sub>3</sub> (EC<sub>50</sub> 7 μg/mL) fractions.

**Separation of the CCl<sub>4</sub> Fraction and Isolation of Compounds **1**, **2**, **4**, **5**, and **8**.** The CCl<sub>4</sub> fraction (748 mg) was first subjected to gel permeation through a Sephadex LH-20 column (elution with CH<sub>2</sub>Cl<sub>2</sub>–MeOH–hexane, 5:1:2); 30 fractions were collected. The two most active ones (55 mg, EC<sub>50</sub> 2.5 μg/mL) were combined and further separated by flash chromatography over a short column containing bulk cyano-bonded adsorbent. Elution with mixtures of hexane–iPrOH led to the isolation of two active xanthones, **1** (7 mg) in pure form, and **2** (20 mg), as a mixture with **1**, respectively. A cytotoxic fraction from the Sephadex LH-20 separation (92 mg, IC<sub>50</sub> 12 μg/mL) was separated by two subsequent chromatographies over Si gel and bulk C<sub>18</sub>-bonded phase and HPLC (Rainin Dynamax-NH<sub>2</sub> column, elution with CHCl<sub>3</sub>–MeOH, 17:3) to give two other prenylated xanthones, gartanin (**4**, 8.4 mg) and desoxygartanin (**5**, 5.5 mg). Finally, another anti-HIV active fraction from the initial Sephadex LH-20 column (16 mg, EC<sub>50</sub> 12 μg/mL) was separated by flash cyano-bonded-phase liquid chromatography, followed by HPLC separation (Rainin Dynamax-CN column, elution with hexane–iPrOH, 3:1). Pure dihydrocudraflavone B (**8**, 7 mg) was obtained.

**Separation of the CHCl<sub>3</sub> Fraction and Isolation of Compounds **6–8**.** Gel permeation chromatography of the CHCl<sub>3</sub> fraction (913 mg) through a Sephadex LH-20 column (elution with MeOH) gave two fractions with significant anti-HIV activity. The first fraction (168 mg, EC<sub>50</sub> 4–5 μg/mL) was subjected to a separation scheme that included another Sephadex LH-20 chromatography (elution with CHCl<sub>3</sub>/MeOH, 1:1), followed by flash chromatography over a cyano-bonded phase column (elution with mixtures of hexane–iPrOH) and HPLC purification (on a 1 × 25 cm Rainin Dynamax-CN

column; elution with hexane–iPrOH, 3:1). This led to the isolation of two known compounds, cudraflavone C (**6**, 15 mg) and isocyclomulberrin (**7**, 9 mg) in pure form. The second active fraction from the initial Sephadex LH-20 chromatography of the CHCl<sub>3</sub>-soluble portion (257 mg, EC<sub>50</sub> 3 µg/mL), was subjected to the same separation methodology, which led to the isolation of more dihydrocudraflavone B (**8**, 10 mg).

**Macluraxanthone B (1):** yellow amorphous solid, UV (MeOH),  $\lambda_{\max}$  nm (log  $\epsilon$ ) 376 (3.99), 320 (4.16), 259 (4.38), 236 (4.36), 206 (4.44); IR (CH<sub>2</sub>Cl<sub>2</sub>)  $\nu_{\max}$  3390, 2929, 1634, 1587, 1480, 1294, 1200, 1168, 1074, 935, 847 cm<sup>-1</sup>; HREIMS  $m/z$  396.1525 (M<sup>+</sup>, calcd for C<sub>23</sub>H<sub>24</sub>O<sub>6</sub>: 396.1572); EIMS  $m/z$  (relative abundance) 396 (M<sup>+</sup>, 100), 381 (89), 353 (15), 341 (39), 328 (32), 313 (42), 285 (24), 273 (30); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  180.0 (C-9), 160.8 (C-3), 159.8 (C-1), 154.4 (C-7), 152.9 (C-4a), 150.9 (C-4b), 150.7 (C-14), 144.0 (C-6), 131.0 (C-18), 122.6 (C-17), 115.3 (C-2), 111.8 (C-8a), 108.7 (C-15), 108.4 (C-8), 106.6 (C-4), 102.7 (C-5), 102.3 (C-9a), 41.1 (C-11), 29.0 (C-12 & C-13), 25.8 (C-20), 21.8 (C-16), 18.1 (C-19); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  14.2 s, 10.70 br s, 9.70 br s, 9.10 s (4 D<sub>2</sub>O exchangeable protons, 1-OH, 6-OH, 7-OH & 3-OH, respectively), 7.37 s (H-8), 6.86 s (H-5), 6.35 (dd,  $J$  = 17.9, 10.7 Hz, H-14), 5.10 (br t, 7.1, 1.4, H-17), 4.95 (dd, 17.9, <1, H-15), 4.90 (dd, 10.5, <1, H-15'), 3.45 (d, 7.1, 2H, H-16, 16'), 1.84 (br s, 3H, Me-19), 1.63 (br s, 3H, Me-20) and 1.57 (br s, 6H, Me-12, -13).

**Macluraxanthone C (2):** yellow amorphous solid; HREIMS  $m/z$  396.1589 (M<sup>+</sup>, calcd for C<sub>23</sub>H<sub>24</sub>O<sub>6</sub>: 396.1572); EIMS  $m/z$  (relative abundance) 396 (M<sup>+</sup>, 71), 381 (100), 341 (31), 325 (35), 313 (30), 285 (20); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  180.9 (C-9), 164.0 (C-3), 160.6 (C-1), 154.6 (C-4a), 149.7 (C-14), 149.2 (C-6), 145.0 (C-4b), 131.8 (C-18), 130.3 (C-5), 122.9 (C-17), 118.0 (C-8), 114.1 (C-8a), 113.6 (C-15), 112.4 (C-7), 106.7 (C-2), 103.7 (C-9a), 102.1 (C-4), 43.8 (C-11), 27.0 (C-12 & C-13), 25.6 (C-20), 22.4 (C-16), 17.8 (C-19); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  13.0 s, 7.65 s (D<sub>2</sub>O exchangeable protons, 1-OH and 3-OH, respectively), 7.69 (d,  $J$  = 8.6 Hz, H-8), 6.92 (d, 8.6, H-7), 6.48 (dd, 17.7, 10.5, H-14), 5.49 (d, 17.7, H-15), 5.38 (dd, 10.5, <1, H-15'), 5.27 (br t, 7, H-17), 3.42 (d, 6.8, 2H, H-16, 16'), 1.81 (br s, 3H, Me-19), 1.70 (br s, 3H, Me-20) and 1.63 (br s, 6H, Me-12, -13).

**Dihydrocudraflavone B (8):** viscous yellow solid; UV (MeOH),  $\lambda_{\max}$  nm (log  $\epsilon$ ) 306 (3.78), 262 (4.41), 233 (4.03), 206 (4.36); IR (CH<sub>2</sub>Cl<sub>2</sub>)  $\nu_{\max}$  3567, 1651, 1618, 1457, 1375, 1217, 1158, 1094 cm<sup>-1</sup>; HREIMS  $m/z$  422.1733 (M<sup>+</sup>, calcd for C<sub>25</sub>H<sub>26</sub>O<sub>6</sub>: 422.1729); EIMS  $m/z$  (relative abundance) 422 (M<sup>+</sup>, 30), 405 (18), 379 (70), 323 (20), 293 (21), 167 (16), 149 (100), 71 (38), 69 (37), 57 (44), 55 (38); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  182.0 (C-4), 162.2 (C-2), 160.7 (C-2'), 160.0 (C-7), 158.6 (C-5), 156.7 (C-4'), 155.6 (C-8a), 131.4 (C-11 & C-6'), 121.9 (C-10), 119.9 (C-3), 111.4 (C-1'), 107.0 (C-5'), 104.5 (C-6), 103.5 (C-4a), 102.9 (C-3'), 94.4 (C-8), 76.4 (C-16), 31.3 (C-15), 26.6 (C-17 & C-18), 25.7 (C-13), 23.9 (C-9), 17.6 (C-12), 16.1 (C-14); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  13.42 s, 9.86 s, 9.77 s (3 D<sub>2</sub>O exchangeable protons, H-5, H-2', and H-4', respectively), 7.06 (d,  $J$  = 8.3 Hz, H-6'), 6.42 (d, 2.2, H-3'), 6.33 (dd, 8.3, 2.2, H-5'), 6.29 s (H-8), 5.01 (dt, 7.0, 1.3, H-10), 2.98 (d, 7, 2H, H-9, -9'), 2.62 (t, 6.8,

2H, H-14, -14'), 1.81 (t, 6.8, 2H, H-15, -15'), 1.54 (br s, 3H, Me-13), 1.36 (br s, 3H, Me-12), 1.31 (s, 6H, Me-17, -18).

**Acknowledgment.** We thank the New York Botanical Garden and G. M. Cragg for the plant collection, T. McCloud for extraction, J. B. McMahon and R. J. Gulakowski for the anti-HIV assays, and Glen Gray for the mass spectrometry data.

**Supporting Information Available:** Full spectroanalytical details, including NMR data (COSY, HMQC, and HMBC correlations) and full NMR assignments of signals of compounds **1–2** and **4–8** (Tables 2–8). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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NP000175M