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## Three New Sesquiterpenes from *Croton arboreous*

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Received November 6, 2003

Three new sesquiterpenes, 5 $\alpha$ ,7 $\alpha$ ,10 $\beta$ -H-3-patchoulen-2-one (**1**), 5 $\alpha$ ,7 $\alpha$ ,10 $\beta$ -H-4(14)-patchoulen-2 $\alpha$ -ol (**2**), and 9 $\alpha$ ,10 $\beta$ -dihydroxy-2 $\beta$ ,4 $\beta$ -peroxy-1 $\alpha$ ,5 $\beta$ ,7 $\alpha$ -H-guaiane (**3**), were isolated from the aerial parts of *Croton arboreous* along with 14 known compounds. The structures of these compounds were determined on the bases of their spectroscopic data (IR, UV, OR, 1D and 2D NMR, and MS). The anti-inflammatory activity against ear edema in mice produced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was evaluated for all the pure compounds and showed that compounds **4–7** are active.

The aerial parts of *Croton arboreous* (Euphorbiaceae), common name “cascarillo”, are the source of a popular beverage used in Tabasco and Chiapas, Mexico, as an auxiliary anti-inflammatory agent in the treatment of respiratory ailments. The *n*-hexane, acetone, and methanol extracts of this plant were analyzed in the anti-inflammatory assay against ear edema in mice produced by TPA (0.5 mg/ear),<sup>1,2</sup> and the *n*-hexane and acetone extracts were active. The *n*-hexane and acetone extracts were analyzed on TLC, and on the basis of their similar chemical composition and anti-inflammatory activity these extracts were combined. Purification of the nonactive fractions yielded 10 known natural products and three new sesquiterpenes, 5 $\alpha$ ,7 $\alpha$ ,10 $\beta$ -H-3-patchoulen-2-one (**1**), 5 $\alpha$ ,7 $\alpha$ ,10 $\beta$ -H-4(14)-patchoulen-2 $\alpha$ -ol (**2**), and 9 $\alpha$ ,10 $\beta$ -dihydroxy-2 $\beta$ ,4 $\beta$ -peroxy-1 $\alpha$ ,5 $\beta$ ,7 $\alpha$ -H-guaiane (**3**), whose structure elucidation is described herein. Fractionation of the active residue on CC afforded three active groups of fractions, whose further purification yielded four anti-inflammatory compounds, 5 $\alpha$ ,10 $\beta$ -4(15)-eudesmen-1 $\beta$ ,6 $\beta$ -diol (**4**),<sup>3</sup> spathulenol (**5**),<sup>4</sup> 5 $\alpha$ ,10 $\beta$ -3-eudesmen-1 $\beta$ ,6 $\alpha$ -diol (**6**),<sup>5</sup> and junceic acid (**7**).<sup>6</sup>

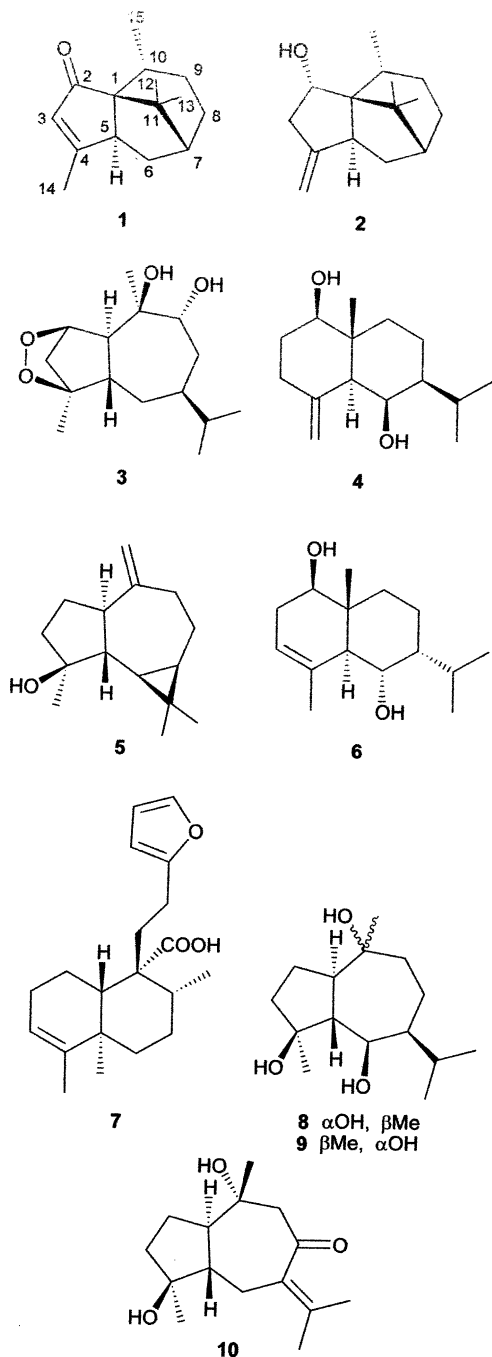
Compound **1** was isolated as a yellowish oil. The HREIMS data indicated a molecular formula C<sub>15</sub>H<sub>22</sub>O on the basis of the *m/z* 218.3383 and five unsaturation degrees, two of them due to the presence of a  $\alpha,\beta$ -unsaturated ketone in accord with the absorptions at 1669 and 1457 cm<sup>-1</sup> in the IR spectrum. The <sup>13</sup>C NMR and DEPT spectra of **1** showed 15 carbon resonances, corresponding to four CH<sub>3</sub>, three CH<sub>2</sub>, four CH, and four C, establishing a patchoulene skeleton for this natural product. This deduction was supported by the <sup>1</sup>H NMR and HMQC spectra, where **1** showed signals of four methyl groups, two singlets at  $\delta$  1.14 ( $\delta$ <sub>C</sub> 27.1, C-13) and 1.01 ( $\delta$ <sub>C</sub> 28.0, C-12) and two doublets at  $\delta$  1.29 (d, *J* = 7.1 Hz,  $\delta$ <sub>C</sub> 15.3, C-15) and  $\delta$  1.98 (d, *J* = 1.2 Hz,  $\delta$ <sub>C</sub> 25.5, C-14), one vinyl hydrogen at  $\delta$  5.65 (q, *J* = 1.2 Hz,  $\delta$ <sub>C</sub> 125.6, C-3), and the bridged hydrogen H-7 at  $\delta$  1.99–1.91 (m,  $\delta$ <sub>C</sub> 58.3). Long-range <sup>1</sup>H–<sup>13</sup>C couplings observed in the HMBC spectrum supported the structure shown for **1**: the coupling between  $\delta$  2.33 (H-5) and the signals at  $\delta$ <sub>C</sub> 65.6 (C-1), 165.3 (C-4), 125.6 (C-3), 25.5 (C-14), and 58.3 (C-7) revealed that the  $\alpha,\beta$ -unsaturated ketone is located in the A ring; that between  $\delta$  1.14 (H-13) and 1.01 (H-12) and the signals at  $\delta$ <sub>C</sub> 42.5 (C-11) and 58.3 (C-7) confirmed the connectivity of the five-membered B ring, and that between  $\delta$  1.29 (H-15) and the signals at  $\delta$ <sub>C</sub>

65.6 (C-1), 44.0 (C-10), and 39.7 (C-9) revealed the connectivity of the six-membered C ring. The configuration of **1** was assigned by the observation of three important correlations in the NOESY spectrum (Figure 1). The signal at  $\delta$  2.33 (H-5) showed correlation to H-6a ( $\delta$ 1.75), while the signal at  $\delta$  1.94 (H-6b) showed correlation to H-12 ( $\delta$ 1.01). Additionally the methyl group C-13 showed strong correlations to protons H-10 ( $\delta$ 1.69) and H-8b ( $\delta$ 1.49). Thus, the structure of **1** was established as 5 $\alpha$ ,7 $\alpha$ ,10 $\beta$ -H-3-patchoulen-2-one.

Compound **2** was an alcohol in accord with its absorption at 3408 cm<sup>-1</sup> in the IR spectrum and the signal at  $\delta$  3.84 in <sup>1</sup>H NMR. The spectroscopic data in the <sup>1</sup>H, <sup>13</sup>C NMR and <sup>13</sup>C DEPT spectra were very similar with those of compound **1**. However, **2** showed only three signals for methyl groups [singlets at  $\delta$  1.01 (H-13) and 0.96 (H-12) and a doublet at  $\delta$  1.14 (H-15)] and one AB system at  $\delta$  4.59 and 4.55, which was assigned to an exocyclic methylene (H-14). In the COSY spectrum, both signals for H-14 showed correlation with the signal at  $\delta$  2.14–2.05 (m, H-3a) and also with the signal for H-3b at  $\delta$  2.69 and between the latter two with the signal at  $\delta$  3.84, establishing that the alcohol was located at C-2. The set of correlations shown in the NOESY spectrum for **1** were also observed for **2**, in addition to the correlation between H-10 ( $\delta$ 1.61) and H-2 ( $\delta$  3.84), establishing an  $\alpha$  orientation of the hydroxyl group on C-2 and the structure 5 $\alpha$ ,7 $\alpha$ ,10 $\beta$ -H-4(14)-patchoulen-2 $\alpha$ -ol for compound **2**.

Compound **3** was a yellowish oil with molecular formula C<sub>15</sub>H<sub>26</sub>O<sub>4</sub> (*M*<sup>+</sup> – O<sub>2</sub>, *m/z* 238.1933), indicating three unsaturation degrees. The <sup>13</sup>C NMR spectrum of **3** showed 15 carbon resonances, corresponding to four CH<sub>3</sub>, three CH<sub>2</sub>, six CH, and two C in accord with the DEPT spectrum. The two quaternary carbons of **3** showed binding to oxygen, in accord with their chemical shifts at  $\delta$ <sub>C</sub> 87.4 (s) and 77.4 (s). This characteristic and the multiplicity of the 13 other carbons established a totally saturated guaiane skeleton for this natural product, which justified two unsaturation degrees. The two quaternary carbons corresponded to C-4 and C-10 in accord with the chemical shift for the methyl groups C-14 and C-15 at  $\delta$  1.49 (s,  $\delta$ <sub>C</sub> 21.5) and 1.17 (s,  $\delta$ <sub>C</sub> 32.1), respectively, in the <sup>1</sup>H, <sup>13</sup>C NMR and HMQC spectra. The presence of two additional tertiary carbons joined to oxygen was established by the signals at  $\delta$  3.91 and 3.80. The first one corresponds to H-9 in accord with a correlation of this signal with the signal at  $\delta$  1.68 (H-8a) in the COSY spectrum and with the long-range <sup>1</sup>H–<sup>13</sup>C coupling of the

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signal at  $\delta$  1.17 (H-15) with the signal at  $\delta$  86.8 (C-9) in the HMBC spectrum. The second one corresponded to H-2 in accord with the correlation of the signal with the signals at  $\delta$  0.98 (H-3a) and 1.41 (H-3b) in the COSY spectrum. The downfield shift observed in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra for H-14 ( $\Delta\delta$  1.49–1.20 = 0.29) and C-4 ( $\Delta\delta$  87.4–81.2 = 7.2) with respect to teuclatriol (**8**)<sup>7</sup> and  $\Delta\delta$  1.49–1.26 = 0.23 and  $\Delta\delta$  87.4–81.1 = 7.3 with respect to 10-epiteuclatriol (**9**)<sup>7</sup> established a peroxide function on C<sub>4</sub>–C<sub>2</sub>, justifying the third unsaturation in this natural product. The other two oxygenated functions correspond to hydroxyl groups, in accord with the absorption at 3398  $\text{cm}^{-1}$  in the IR spectrum. The junction of the guaiane rings was *trans*, in accord with a  $J_{\text{H1-H5}}$  = 12.8 Hz.<sup>7,8</sup> The relative configuration at C-10 was established by comparison of the chemical shift due to C-15 in **3** with those of zedoarondiol (**10**,  $\delta_{\text{C15}}$  20.6), a guaiane whose 10 $\alpha$ -hydroxy configuration has been determined by X-ray analysis,<sup>9</sup> and the epimeric compounds at C-10 teuclatriol (**8**, 10 $\alpha$ -hydroxy,  $\delta_{\text{C15}}$  22.2)

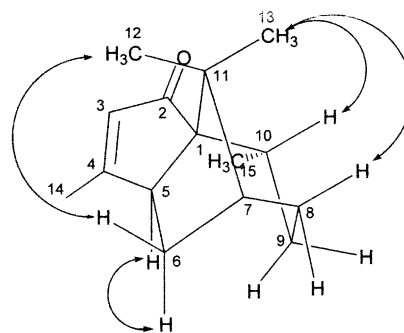


Figure 1. Spatial correlations observed from NOESY spectra of **1** and **2**.

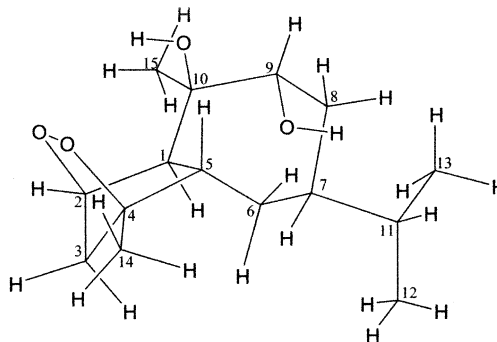


Figure 2. Energy-minimized molecular model of **3**.

and epiteuclatriol (**9**, 10 $\beta$ -hydroxy,  $\delta_{\text{C15}}$  29.9).<sup>7</sup> Compound **3** showed a chemical shift for C-15 similar to **9** at  $\delta$  32.1, establishing a  $\beta$  orientation for the hydroxyl group and an  $\alpha$  orientation for C-15. A *trans* and *syn* relationship between H-1 and H-2 should have a  $J_{\text{H1}\alpha\text{-H2}\beta}$  = 9.0 Hz and  $J_{\text{H1}\alpha\text{-H2}\alpha}$  = 5.0 Hz, respectively.<sup>8</sup> Compound **3** showed a  $J_{\text{H1}\alpha\text{-H2}\alpha}$  = 4.8 Hz, corresponding to a *syn* relationship, establishing that the C<sub>4</sub>–C<sub>2</sub> peroxide was  $\beta$ . Additionally observed coupling constants were  $J_{\text{H5}\beta\text{-H6}\alpha}$  = 9.2 Hz and  $J_{\text{H5}\beta\text{-H6}\beta}$  = 4.0 Hz,  $J_{\text{H6}\alpha\text{-H7}\alpha}$  = 5.2 Hz, and  $J_{\text{H8}\alpha\text{-H9}\beta}$  = 5.2 Hz, which could be explained in accord with the H–H dihedral angles of 147°, 27°, 47°, and 48°, respectively, obtained from the energy-minimized molecular model calculation (Figure 2). Thus, the structure of **3** was established as 9 $\alpha$ ,10 $\beta$ -dihydroxy-2 $\beta$ ,4 $\beta$ -peroxy-1 $\alpha$ ,5 $\beta$ ,7 $\alpha$ -guaiane and was confirmed by the HMBC spectrum, where **3** showed long-range  $^1\text{H}$ – $^{13}\text{C}$  couplings between  $\delta$  0.99 (H-13) and 0.98 (H-12) and the signals at  $\delta$  47.5 (C-7) and 28.5 (C-11); between  $\delta$  1.49 (H-14) and the signals at  $\delta$  87.4 (C-4), 30.2 (C-3), and 61.8 (C-5); between  $\delta$  1.17 (H-15) and the signals at  $\delta$  41.6 (C-1) and 86.8 (C-9); between  $\delta$  1.30 (H-6) and 61.8 (C-5); and between  $\delta$  1.24 (H-8) and 86.8 (C-9).

## Experimental Section

**General Experimental Procedures.** The *n*-hexane–acetone extract from *C. arboreous* was fractionated using open CC (Merck Kiesel-gel 60 and Supelclean SPE LC-SI 6 mL tubes) and TLC (ALUGRAM SIL G/UV<sub>254</sub> silica gel plates), using mixtures of *n*-hexane–acetone as eluent. In the TLC analysis, the compounds were visualized by UV light and spraying with a 1% solution of  $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4$  in 2 N  $\text{H}_2\text{SO}_4$ ; UV spectra were recorded on a Hewlett-Packard 8453 spectrophotometer in  $\text{CHCl}_3$ . IR spectra were recorded with Bruker Vector 22 IR instrument in  $\text{CHCl}_3$  solution;  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra as well as 2D NMR experiments were recorded in  $\text{CDCl}_3$  on Varian Unity 400 and Varian-Gemini 300 spectrometers, and the chemical shifts were expressed in parts per

million ( $\delta$ ) relative to TMS as internal standard. Mass spectra were measured on a JEOL JMS-AX 505 HA mass spectrometer. Electron impact mass spectra were obtained at 70 eV ionization energy.

**Plant Material.** The aerial parts of *C. arboreous* (6 m tree with red resin) were collected and identified by Biol. Esteban Manuel Martínez on March 13, 2002, at 4 km SE of La Nueva Vida, Camino a Xpujil (18°46'30 N, 89°22'23 W) at 270 m above sea level in Calakmul, Campeche, México. A voucher specimen was deposited at the National Herbarium (MEXU, voucher 27171) at the Instituto de Biología, UNAM, México.

**Extraction and Isolation.** The air-dried parts of *C. arboreous* (3.48 kg) were powdered and exhaustively extracted with *n*-hexane, acetone, and MeOH (12 L  $\times$  3 each) to yield 45.8, 38.6, and 148.1 g of residue, respectively. These extracts were evaluated in the anti-inflammatory assay,<sup>1,2</sup> the *n*-hexane and acetone extracts being active. On the basis of their similar anti-inflammatory activity and chemical composition on TLC, the *n*-hexane and acetone extracts were joined and absorbed on silica gel (85 g) and chromatographed on CC over silica gel 60 (850 g), using a gradient of *n*-hexane–acetone as eluent. The composition of the obtained fractions (500 mL each) was monitored by TLC, and the chromatographically identical fractions were combined, yielding eight groups, G-1 [3.9 g, *n*-hexane 100%], G-2 [9.8 g, *n*-hexane–acetone, 95:5], G-3 [8.3 g, *n*-hexane–acetone, 95:5], G-4 [7.3 g, *n*-hexane–acetone, 9:1], G-5 [2.3 g, *n*-hexane–acetone, 85:15], G-6 [1.7 g, *n*-hexane–acetone, 8:2], G-7 [2.6 g, *n*-hexane–acetone, 7:3], and G-8 [3.1 g, *n*-hexane–acetone, 6:4], which were evaluated in the anti-inflammatory assay.<sup>1,2</sup> G-2, G-3, and G-5 displayed anti-inflammatory activity. Each group was further separated using CC over silica gel 60, with a gradient of *n*-hexane–acetone as eluent. Fraction G-2 yielded junceic acid<sup>6</sup> (7, 236 mg, 0.52%) and  $\beta$ -sitosterol. Fraction G-3 yielded 1 $\beta$ ,10 $\alpha$ -4 $\beta$ ,5 $\alpha$ -diepoxy-7 $\alpha$ H-germacran-6 $\beta$ -ol<sup>10</sup> ( $[\alpha]_D^{25}$  –28.6° (*c* 0.2, CHCl<sub>3</sub>), 96 mg, 0.21%), 1 $\alpha$ ,10 $\beta$ -4 $\beta$ ,5 $\alpha$ -diepoxy-7 $\alpha$ H-germacran-6 $\beta$ -ol<sup>11</sup> ( $[\alpha]_D^{25}$  +152.2° (*c* 0.5, CHCl<sub>3</sub>), 52 mg, 0.11%), 5 $\alpha$ ,10 $\beta$ -4(15)-eudesmen-1 $\beta$ ,6 $\alpha$ -diol<sup>3</sup> ( $[\alpha]_D^{25}$  –51.6° (*c* 0.1, CHCl<sub>3</sub>), 193 mg, 0.42%), and 5 $\alpha$ ,10 $\beta$ -4(15)-eudesmen-1 $\beta$ ,6 $\beta$ -diol<sup>3</sup> ( $[\alpha]_D^{25}$  +15.7° (*c* 0.1, CHCl<sub>3</sub>), 4, 177 mg, 0.39%). Fraction G-5 yielded spathulenol<sup>4</sup> ( $[\alpha]_D^{25}$  +3.7° (*c* 0.01, CHCl<sub>3</sub>), 5, 259 mg, 0.57%), 5 $\alpha$ ,10 $\beta$ -3-eudesmen-1 $\beta$ ,6 $\alpha$ -diol<sup>5</sup> ( $[\alpha]_D^{25}$  +44.0° (*c* 0.52, CHCl<sub>3</sub>), 6, 48 mg, 0.11%), and 9 $\alpha$ ,10 $\beta$ -dihydroxy-2 $\beta$ ,4 $\beta$ -peroxy-1 $\alpha$ ,5 $\beta$ ,7 $\alpha$ H-guaiane ( $[\alpha]_D^{25}$  +8.3° (*c* 0.05, CHCl<sub>3</sub>), 3, 39 mg, 0.08%). Additional constituents of the inactive fractions were 5 $\alpha$ ,7 $\alpha$ ,10 $\beta$ H-3-patchoulene-2-one (1, 47 mg, 0.10%), 5 $\alpha$ ,7 $\alpha$ ,10 $\beta$ H-4(14)-patchoulene-2 $\alpha$ -ol (2, 56 mg, 0.12%), lupenone<sup>12</sup> (286 mg, 0.62%), 4 $\beta$ ,6 $\beta$ -dihydroxy-1 $\alpha$ H,5 $\beta$ H-9-guaiane<sup>13</sup> ( $[\alpha]_D^{25}$  +10.5° (*c* 0.60, CHCl<sub>3</sub>), 88 mg, 0.19%), teucladiol<sup>7</sup> ( $[\alpha]_D^{25}$  +2.1° (*c* 0.43, CHCl<sub>3</sub>), 79 mg, 0.17%), quercetin-3,4'-dimethyl ether<sup>14</sup> (266 mg, 0.58%), 2'-*O*- $\beta$ -D-glucopyranosyl-5,7,5'-trihydroxy-3,4'-dimethoxyflavone<sup>15</sup> (141 mg, 0.31%), and  $\beta$ -sitosterol- $\beta$ -D-glucopyranoside.<sup>16</sup>

**5 $\alpha$ ,7 $\alpha$ ,10 $\beta$ H-3-Patchoulene-2-one (1):** yellowish oil;  $[\alpha]_D^{25}$  –53.9° (*c* 3.6, CHCl<sub>3</sub>); UV (CHCl<sub>3</sub>)  $\lambda_{max}$  (log  $\epsilon$ ) 239 (3.68), 205 (3.40) nm; IR (CHCl<sub>3</sub>)  $\nu_{max}$  2946, 2870, 1669 (CO), 1457 (C=C), 1375 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  5.65 (1H, q, *J* = 1.2 Hz, H-3), 2.33 (1H, d, *J* = 3.6 Hz, H-5), 1.99–1.91 (1H, m, H-7), 1.98 (3H, d, *J* = 1.2 Hz, H-14), 1.94 (1H, d, *J* = 11.2 Hz, H-6b), 1.94 (1H, m, H-9a), 1.75 (1H, ddd, *J* = 11.2, 3.6, 3.6 Hz, H-6a), 1.70 (1H, dd, *J* = 11.2, 5.2 Hz, H-8a), 1.69 (1H, ddd, *J* = 8.4, 7.1, 4.8 Hz, H-10), 1.62 (1H, dd, *J* = 10.4, 5.2 Hz, H-9b), 1.49 (1H, dd, *J* = 11.2, 4.4 Hz, H-8b), 1.29 (3H, d, *J* = 7.1 Hz, H-15), 1.14 (3H, s, H-13), 1.01 (3H, s, H-12); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz)  $\delta$  203.8 (C, C-2), 165.3 (C, C-4), 125.6 (CH, C-3), 65.6 (C, C-1), 59.1 (CH, C-5), 58.3 (CH, C-7), 44.8 (CH<sub>2</sub>, C-6), 44.0 (CH, C-10), 42.5 (C, C-11), 39.7 (CH<sub>2</sub>, C-9), 28.0 (CH<sub>3</sub>, C-12), 27.1 (CH<sub>3</sub>, C-13), 26.7 (CH<sub>2</sub>, C-8), 25.5 (CH<sub>3</sub>, C-14), 15.3 (CH<sub>3</sub>, C-15); EIMS *m/z* 218 [M<sup>+</sup>, C<sub>15</sub>H<sub>22</sub>O] (100), 203 [M<sup>+</sup> – CH<sub>3</sub>] (27), 176 [M<sup>+</sup> – C<sub>3</sub>H<sub>6</sub>] (43), 175 [M<sup>+</sup> – C<sub>3</sub>H<sub>6</sub> – H] (42), 161 [M<sup>+</sup> – C<sub>3</sub>H<sub>6</sub> – CH<sub>3</sub>] (51), 147 [M<sup>+</sup> – C<sub>3</sub>H<sub>6</sub> – CH<sub>3</sub> – CH<sub>2</sub>] (20), 136 (29), 135 (28), 121 (28), 95 (15), 91 (13), 81 (9), 55 (9), 41 (12); HREIMS *m/z* 218.3383 (calcd for C<sub>15</sub>H<sub>22</sub>O, 218.3390).

**5 $\alpha$ ,7 $\alpha$ ,10 $\beta$ H-4(14)-Patchoulene-2 $\alpha$ -ol (2):** colorless oil;  $[\alpha]_D^{25}$  –16.4° (*c* 1.2, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>)  $\nu_{max}$  3408 (OH), 2927, 2862, 1463 (C=C), 1387 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  4.59 (1H, td, *J* = 3.0, 0.9 Hz, H-14a), 4.55 (1H, dd, *J* = 3.0, 3.0 Hz, H-14b), 3.84 (1H, dd, *J* = 9.3, 6.9 Hz, H-2), 2.69 (1H, dd, *J* = 15.3, 6.9 Hz, H-3b), 2.18 (1H, d, *J* = 4.2 Hz, H-5), 2.14–2.05 (1H, m, H-3a), 1.90 (1H, m, H-9a), 1.72 (1H, dd, *J* = 12.3, 3.2 Hz, H-6b), 1.61 (2H, m, H-7, H-10), 1.59 (1H, m, H-8a), 1.58 (1H, m, H-6a), 1.42 (1H, m, H-9b), 1.40 (1H, m, H-8b), 1.14 (3H, d, *J* = 7.5 Hz, H-15), 1.01 (3H, s, H-13), 0.96 (3H, s, H-12); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz)  $\delta$  148.8 (C, C-4), 108.2 (CH<sub>2</sub>, C-14), 73.4 (CH, C-2), 60.6 (CH, C-7), 58.3 (C, C-1), 52.5 (CH, C-5), 45.3 (CH, C-10), 42.8 (CH<sub>2</sub>, C-6), 41.5 (C, C-11), 40.3 (CH<sub>2</sub>, C-9), 40.2 (CH<sub>2</sub>, C-3), 27.0 (CH<sub>3</sub>, C-13), 26.6 (CH<sub>2</sub>, C-8), 25.6 (CH<sub>3</sub>, C-12), 14.7 (CH<sub>3</sub>, C-15); EIMS *m/z* 220 [M<sup>+</sup>, C<sub>15</sub>H<sub>24</sub>O] (26), 202 [M<sup>+</sup> – H<sub>2</sub>O] (100), 160 [M<sup>+</sup> – H<sub>2</sub>O – C<sub>3</sub>H<sub>6</sub>] (41), 159 [M<sup>+</sup> – H<sub>2</sub>O – C<sub>3</sub>H<sub>6</sub> – H] (29), 137 (14), 97 (8); HREIMS *m/z* 220.3553 (calcd for C<sub>15</sub>H<sub>24</sub>O, 220.3549).

**9 $\alpha$ ,10 $\beta$ -Dihydroxy-2 $\beta$ ,4 $\beta$ -peroxy-1 $\alpha$ ,5 $\beta$ ,7 $\alpha$ H-guaiane (3):** yellowish oil;  $[\alpha]_D^{25}$  –8.4° (*c* 0.5, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>)  $\nu_{max}$  3398 (OH), 2928, 1377, 1266, 1005, and 978 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  3.91 (1H, d, *J* = 5.2 Hz, H-9), 3.80 (1H, ddd, *J* = 4.8, 4.4, 3.2 Hz, H-2), 2.15 (1H, dd, *J* = 12.8, 4.8 Hz, H-1), 1.98 (1H, ddd, *J* = 12.8, 9.2, 4.0 Hz, H-5), 1.68 (1H, m, H-8a), 1.66 (1H, dh, *J* = 6.8, 2.4 Hz, H-11), 1.63 (1H, m, H-7), 1.53 (1H, dd, *J* = 9.2, 5.2 Hz, H-6b), 1.49 (3H, s, H-14), 1.41 (1H, m, H-3b), 1.30 (1H, m, H-6a), 1.24 (1H, m, H-8b), 1.17 (3H, s, H-15), 0.99 (3H, d, *J* = 6.8 Hz, H-13), 0.98 (3H, d, *J* = 6.8 Hz, H-12), 0.98 (1H, m, H-3a); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  87.4 (C, C-4), 86.8 (CH, C-9), 77.4 (C, C-10), 68.7 (CH, C-2), 61.8 (CH, C-5), 47.5 (CH, C-7), 41.6 (CH, C-1), 32.1 (CH<sub>3</sub>, C-15), 31.5 (CH<sub>2</sub>, C-6), 30.2 (CH<sub>2</sub>, C-3), 28.5 (CH, C-11), 26.6 (CH<sub>2</sub>, C-8), 21.5 (CH<sub>3</sub>, C-14), 21.4 (CH<sub>3</sub>, C-12), 21.3 (CH<sub>3</sub>, C-13); EIMS *m/z* 238 [M<sup>+</sup> – O<sub>2</sub>, C<sub>15</sub>H<sub>26</sub>O<sub>2</sub>] (9), 220 [M<sup>+</sup> – O<sub>2</sub> – H<sub>2</sub>O] (23), 205 [M<sup>+</sup> – O<sub>2</sub> – H<sub>2</sub>O – CH<sub>3</sub>] (9), 195 [M<sup>+</sup> – O<sub>2</sub> – C<sub>3</sub>H<sub>7</sub>] (50), 181 [M<sup>+</sup> – O<sub>2</sub> – C<sub>3</sub>H<sub>6</sub> – CH<sub>3</sub>] (39), 177 (38), 162 (45), 152 (55), 140 (37), 123 (81), 109 (83), 94 (100), 81 (53), 43 (67); HREIMS *m/z* 238.1933 (calcd for C<sub>15</sub>H<sub>26</sub>O<sub>2</sub>, M<sup>+</sup> – O<sub>2</sub>, 238.3702).

**Anti-inflammatory Assay.** The anti-inflammatory activity of extracts, fractions, and pure compounds at a dose of 0.5 mg/ear was evaluated against the ear edema in mice produced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), according to the previously described procedure,<sup>1,2</sup> using indomethacin (61.2% inhibition, 0.5 mg/ear) as positive control. The *n*-hexane and acetone extracts showed activity (48.6 and 54.1% of inhibition, respectively) and were combined for fractionation. Fractions G-2, G-3, and G-5 were active (32.9, 22.7, and 29.3% inhibition), which after purification afforded the active natural products 5 $\alpha$ ,10 $\beta$ -4(15)-eudesmen-1 $\beta$ ,6 $\beta$ -diol (4),<sup>3</sup> spathulenol (5),<sup>4</sup> 5 $\alpha$ ,10 $\beta$ -3-eudesmen-1 $\beta$ ,6 $\alpha$ -diol (6),<sup>5</sup> and junceic acid (7),<sup>6</sup> which showed 29.9, 31.5, 28.0, and 38.4% inhibition, respectively.

**Acknowledgment.** We are indebted to E. Salazar Leyva and R. Patiño Maya for technical assistance. This work was financially supported by CONACyT (Project No. 40405).

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NP030485F