

## Biologically Active Cannabinoids from High-Potency *Cannabis sativa*

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Nine new cannabinoids (**1–9**) were isolated from a high-potency variety of *Cannabis sativa*. Their structures were identified as (±)-4-acetoxycannabichromene (**1**), (±)-3'-hydroxy- $\Delta^{4(5)}$ -cannabichromene (**2**), (-)-7-hydroxycannabichromane (**3**), (-)-7*R*-cannabicumaronic acid A (**4**), 5-acetyl-4-hydroxycannabigerol (**5**), 4-acetoxy-2-geranyl-5-hydroxy-3-*n*-pentylphenol (**6**), 8-hydroxycannabinol (**7**), 8-hydroxycannabinolic acid A (**8**), and 2-geranyl-5-hydroxy-3-*n*-pentyl-1,4-benzoquinone (**9**) through 1D and 2D NMR spectroscopy, GC-MS, and HRESIMS. The known sterol  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranosyl-6'-acetate was isolated for the first time from cannabis. Compounds **6** and **7** displayed significant antibacterial and antifungal activities, respectively, while **5** displayed strong antileishmanial activity.

More than 525 constituents have been identified from *Cannabis sativa* L. (Cannabaceae).<sup>1–7</sup> The best-known and most specific class of cannabis constituents are the C<sub>21</sub> terpenophenolic cannabinoids. Other phenolic cannabis constituents include flavonoids, spiroindans, dihydrostilbenes, phenanthrenes, and dihydrophenanthrenes.<sup>1–6,8,9</sup> As part of our program aimed at the discovery of new cannabinoids and other metabolites with significant biological activity from high-potency cannabis ( $\Delta^9$ -THC > 10%, w/w), we have reported 25 new metabolites.<sup>2–5</sup> In this paper, we report the isolation and identification of nine additional new cannabinoids (**1–9**), including three cannabichromene derivatives (**1–3**), (-)-7*R*-cannabicumaronic acid A (**4**), two cannabigerol derivatives (**5** and **6**), two cannabinol derivatives (**7** and **8**), and a C<sub>21</sub> benzoquinone derivative (**9**). The known sterol  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranosyl-6'-acetate was also isolated and identified for the first time from cannabis. The antifungal, antibacterial, antimalarial, antileishmanial, and cytotoxic activities of the isolates are also presented.

### Results and Discussion

Compound **1** was isolated as an optically inactive yellow oil. Its molecular formula was determined to be C<sub>23</sub>H<sub>32</sub>O<sub>4</sub> from GC-MS (*m/z* 372, [M]<sup>+</sup>) and HRESIMS (*m/z* 373.2409, [M + H]<sup>+</sup>), indicating eight degrees of unsaturation. The <sup>1</sup>H NMR spectrum of **1** (Table 1) displayed an AB olefinic spin system [ $\delta_{\text{H}}$  5.48 (d, *J* = 10.0 Hz, H-7), 6.57 (d, *J* = 10.0 Hz, H-8)], an isolated olefinic proton [ $\delta_{\text{H}}$  5.10 (t, *J* = 7.2 Hz, H-3'')], a sharp aromatic singlet [ $\delta_{\text{H}}$  6.07 (s, H-2)], six methylenes ( $\delta_{\text{H}}$  1.30–2.35), two olefinic methyls [ $\delta_{\text{H}}$  1.58 (s, H<sub>3</sub>-5''), 1.66 (s, H<sub>3</sub>-6'')], a tertiary methyl [ $\delta_{\text{H}}$  1.33 (s, H<sub>3</sub>-9)], and an acetoxy methyl resonance [ $\delta_{\text{H}}$  2.29 (s, OCOCH<sub>3</sub>)]. The small coupling constant between vicinal protons H-7 and H-8 (10.0 Hz) indicated a *cis* double bond.<sup>11</sup> The <sup>13</sup>C and APT NMR experiments (Table 1) revealed 23 carbons, including five methyl, six methylene, four methine, and eight quaternary carbon resonances. The quaternary carbons included one ester carbonyl ( $\delta_{\text{C}}$  169.7), three oxyaryl ( $\delta_{\text{C}}$  131.3, 145.3, 148.8), and one oxygenated sp<sup>3</sup> carbon ( $\delta_{\text{C}}$  79.1, C-6). The <sup>1</sup>H and <sup>13</sup>C NMR, IR, and UV spectroscopic data were similar to those reported for cannabichromene,<sup>12–14</sup> except for the substitution of an aromatic

proton by an acetoxy group at C-4. The location of the acetoxy group was established by the observed deshielding of C-4 and the shielding of C-4a and C-3 relative to cannabichromene.<sup>14</sup> Thus, the structure of **1** was determined to be (±)-4-acetoxycannabichromene.

Compound **2** was obtained as an optically inactive brown oil. The HRESIMS exhibited an ion at *m/z* 331.2193 [M + H]<sup>+</sup> corresponding to the molecular formula C<sub>21</sub>H<sub>30</sub>O<sub>3</sub> (seven degrees of unsaturation). The UV and IR spectra of **2** exhibited patterns similar to those of cannabichromene.<sup>12–14</sup> The <sup>1</sup>H NMR spectrum of **2** (Table 1) included an AB olefinic spin system [ $\delta_{\text{H}}$  5.46 (d, *J* = 10.0 Hz, H-7), 6.62 (d, *J* = 10.0 Hz, H-8)], two aromatic protons [ $\delta_{\text{H}}$  6.12 (s, H-2), 6.23 (s, H-4)], and six methylene resonances ( $\delta_{\text{H}}$  1.35–2.57), confirming the cannabichromene skeleton.<sup>12–14</sup> The <sup>1</sup>H, <sup>13</sup>C, and DEPT NMR spectra displayed additional hydroxymethine [ $\delta_{\text{H}}$  4.07 (t, *J* = 6.0 Hz),  $\delta_{\text{C}}$  76.2] and exomethylene [ $\delta_{\text{H}}$  4.83 (bs), 4.92 (bs),  $\delta_{\text{C}}$  110.0] functionalities, which, in conjunction with the absence of the C-3''/C-4'' double bond, indicated a migration of the double bond to C-4''/C-5''. This was confirmed by HMBC correlations (H<sub>2</sub>-5''/C-6'', C-4'', C-3''; H<sub>3</sub>-6''/C-5'', C-3'') (Figure 1). The oxymethine proton was assigned at C-3'' on the basis of its downfield chemical shift and HMBC correlations with C-5'', C-1'', and C-6'' (Figure 1). Accordingly, **2** was identified as (±)-3''-hydroxy- $\Delta^{4(5)}$ -cannabichromene.

Compound **3** was obtained as an optically active pale yellow oil. The molecular formula was determined to be C<sub>21</sub>H<sub>32</sub>O<sub>3</sub> from its HRESIMS [M – H]<sup>−</sup> ion at *m/z* 331.2254, indicating six degrees of unsaturation. The <sup>13</sup>C, DEPT, and HMQC NMR spectra revealed 21 carbons (Table 1), including four methyl, seven methylene, four methine, and six quaternary resonances. The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of **3** (Table 1) were similar to those of cannabichromene,<sup>12–14</sup> except for the absence of the olefinic protons at C-7 and C-8 and the presence of a hydroxy group at C-7 [ $\delta_{\text{H}}$  4.68 (t, *J* = 6.8 Hz, H-7),  $\delta_{\text{C}}$  89.5], which was established by a COSY correlation between H-7 and H-8 and confirmed by HMBC correlations (H-7/C-9, C-1'', C-8a; H<sub>3</sub>-9/C-7, C-1'') (Figure 1). The GC-MS analysis of the trimethylsilyl derivative of **3** displayed a molecular ion at *m/z* 476, confirming the HRESIMS result as well as the presence of two hydroxy groups. The relative configuration at C-7 could not be determined due to insufficient material. Therefore, the structure of **3** was assigned as (-)-7-hydroxycannabichromane.

Compound **4** was isolated as a brown oil. Its molecular formula was found to be C<sub>22</sub>H<sub>28</sub>O<sub>5</sub> by HRESIMS (*m/z* 395.1847, [M + Na]<sup>+</sup>) and GC-MS (*m/z* 372, [M]<sup>+</sup>). The IR spectrum of **4** indicated the presence of two carbonyl groups ( $\nu_{\text{max}}$  1716, 1700 cm<sup>−1</sup>). The <sup>1</sup>H,

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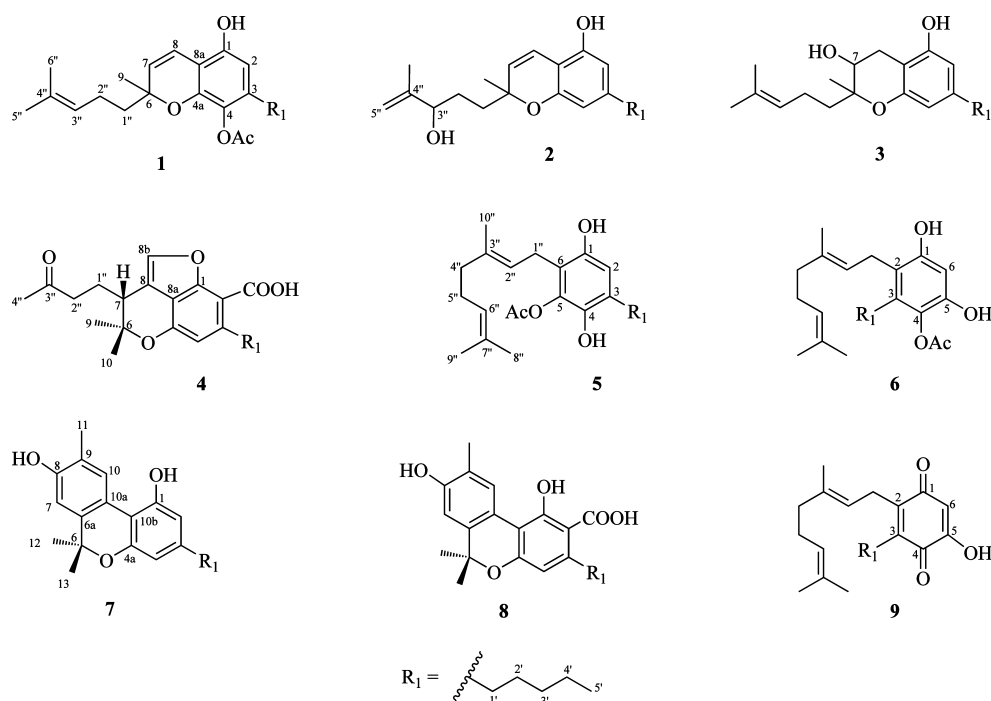
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## Chart 1

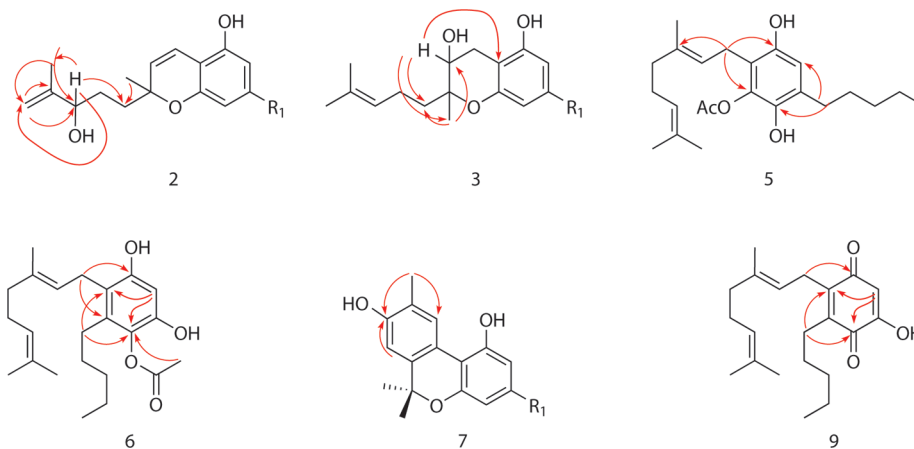
Table 1.  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) Spectroscopic Data of 1–4 ( $\text{CDCl}_3$ )<sup>a</sup>

carbon	1		2		3		4	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ , mult. ( $J$ in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ , mult. ( $J$ in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ , mult. ( $J$ in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ , mult. ( $J$ in Hz)
1	145.3		154.1		161.4		154.8	
2	107.6	6.07, s	108.0	6.12, s	102.3	6.21, s	106.7	
3	135.9		145.1		145.1		148.6	
4	131.3		109.2	6.23, s	108.2	6.14, s	111.3	6.55, s
4a	148.8		151.3		152.3		153.2	
6	79.1		78.3		74.1		83.5	
7	127.7	5.48, d (10.0)	127.1	5.46, d (10.0)	89.5	4.68, t (6.8)	41.4	2.89, dd (3.6, 10.8)
8	117.1	6.57, d (10.0)	117.3	6.62, d (10.0)	27.5	3.03, d (6.8)	115.3	
8a	108.4		108.0		110.1		115.4	
8b							138.5	7.37, s
9	26.3	1.33, s	17.9	1.37, s	23.1	1.28	25.0	1.48, s
10							27.2	1.29, s
11								
1'	30.4	2.35, t (7.2)	36.9	2.49, t (7.2)	36.1	2.53, t (7.2)	35.5	3.01 t (7.2)
2'	29.7	1.54, m	31.4	1.59, m	31.3	1.54, m	32.4	1.63 m
3'	31.8	1.30, m	32.1	1.35, m	31.7	1.28, m	32.1	1.34, m
4'	22.6	1.31, m	22.7	1.35, m	22.7	1.28, m	22.8	1.34, m
5'	14.2	0.87, t (6.8)	14.2	0.87, t (7.2)	14.3	0.89, t (7.2)	14.3	0.88, t (7.2)
1''	41.4	1.65, m	37.2	2.57, m	37.1	2.62, m	23.8	2.15, m
2''	22.8	2.06, m	29.6	1.68, m	22.8	2.05, m	41.3	2.55, m
3''	124.4	5.10, t (7.2)	76.2	4.07, t (6.0)	124.3	5.08, t (7.2)	208.6	
4''	131.9		147.5		132.2		30.8	2.08, s
5''	17.8	1.58, s	110.0	4.83, bs/4.92, bs	17.8	1.58, s		
6''	25.9	1.66, s	26.7	1.70	25.9	1.66, s		
OCOCH <sub>3</sub>	20.7	2.29, s						
OCOCH <sub>3</sub>	169.7							
COOH							170.6	

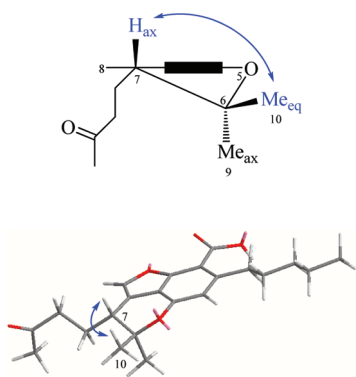
<sup>a</sup> Assignments confirmed by DEPT-135, HMQC, COSY, and HMBC NMR experiments.

$^{13}\text{C}$ , and DEPT NMR spectroscopic data (Table 1) showed the presence of four methyl, six methylene, three methine, and nine quaternary carbons. The IR, UV, GC-MS, and  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **4** were in good agreement with those reported for cannabicumaronone,<sup>15</sup> except for the substitution of the aromatic proton at C-2 by a carboxylic acid group, which was confirmed by the additional 44 amu in the GC-MS and HRESIMS analyses, by the GC-MS analysis of the trimethylsilyl derivative of **4** ( $m/z$  444,  $[\text{M}]^+$ ), and by the  $^{13}\text{C}$  NMR carbonyl resonance at  $\delta_{\text{C}}$  170.6. The ROESY correlation between H-7 ( $\delta_{\text{H}}$  2.89) and pseudoaxial H<sub>3</sub>-10 ( $\delta_{\text{H}}$  1.29,  $\delta_{\text{C}}$  27.2) indicated a 7*R* absolute configuration (Figure 2). The conformation of the C-6 methyl substituents is based

on published NMR values for (–)- $\Delta^9$ -THC, (–)- $\Delta^9$ -THC acid A, (–)- $\Delta^8$ -THC, (–)-hexahydrocannabinol, and a series of cannabichromanone derivatives.<sup>5</sup> The  $^{13}\text{C}$  NMR chemical shift of the  $\beta$ -pseudoequatorial C-6 methyl is downfield from the  $\alpha$ -pseudoaxial C-6 methyl for these compounds.<sup>5</sup> The CD spectrum of **4** (0.1 mg/mL, MeOH) displayed a positive CE at 246 nm ( $\pi \rightarrow \pi^*$ ) and a negative CE at 295 nm ( $n \rightarrow \pi^*$ ), indicating a 7*R* absolute configuration. Also, the negative specific rotation and the  $^1\text{H}$  NMR chemical shift of H-7 of **4** were in agreement with the cannabichromanone derivatives that have H-7 $\beta$  configurations.<sup>5a</sup> Thus, the structure of **4** was established as (–)-7*R*-cannabicumarononic acid A.



**Figure 1.** Key HMBC correlations for **2**, **3**, **5**, **6**, **7**, and **9**.



**Figure 2.** Key ROESY correlation between H-7 and pseudoequatorial H<sub>3</sub>-10 of **4**.

The molecular formula of **5** (C<sub>23</sub>H<sub>34</sub>O<sub>4</sub>) was established from HRESIMS ( $m/z$  375.2530, [M + H]<sup>+</sup>) and <sup>13</sup>C NMR data. The <sup>1</sup>H, <sup>13</sup>C, and DEPT NMR spectroscopic data (Table 2) showed the presence of one aromatic methine, a geranyl moiety,<sup>2</sup> an *n*-pentyl group,<sup>2</sup> and an acetoxy group [ $\delta_{\text{H}}$  2.33 (s),  $\delta_{\text{C}}$  20.8, 170.1]. The presence of the acetoxy group was supported by the IR absorption band at  $\nu_{\text{max}}$  1735 cm<sup>-1</sup>. The spectroscopic data of **5** were similar to those reported for cannabigerol,<sup>16</sup> except for the presence of the acetyl and hydroxy groups at C-5 and C-4, respectively, based on their chemical shifts and HMBC correlations (H<sub>2</sub>-1''/C-1, C-5; H<sub>2</sub>-1'/C-4, C-2) (Figure 1). Thus, **5** was established as 5-acetyl-4-hydroxycannabigerol.

Compound **6** was isolated as a yellow oil with molecular formula C<sub>23</sub>H<sub>34</sub>O<sub>4</sub> (HRESIMS:  $m/z$  375.2528, [M + H]<sup>+</sup>; GC-MS:  $m/z$  374, [M]<sup>+</sup>). The <sup>13</sup>C, DEPT, and HMQC NMR spectra (Table 2) revealed 23 carbons, including five methyl, seven methylene, three methine, and eight quaternary resonances. The spectroscopic data of **6** (Table 2) resembled those of **5**, except for the chemical shifts of the aromatic carbons, indicating a different substitution pattern of the functional groups. HMBC correlations fixed the *n*-pentyl moiety at C-3 (H<sub>2</sub>-1''/C-3, C-1; H<sub>2</sub>-1'/C-2, C-4), the acetoxy group at C-4, and the second hydroxy group at C-5 (H-6/C-4, C-2; OCOCH<sub>3</sub>/C-4) (Figure 1). Thus, the structure of **6** was established as 4-acetoxy-2-geranyl-5-hydroxy-3-*n*-pentylphenol.

Compound **7** was assigned the molecular formula C<sub>21</sub>H<sub>26</sub>O<sub>3</sub> from its HRESIMS ( $m/z$  349.1781, [M + Na]<sup>+</sup>) and <sup>13</sup>C NMR data. <sup>1</sup>H NMR data showed three methyl singlets, a primary methyl group, and four aromatic and four methylene protons (Table 3). The <sup>13</sup>C and DEPT NMR data revealed four methyl, four methylene, four methine, and nine quaternary carbons. The NMR and GC-MS data ( $m/z$  326, [M]<sup>+</sup>) suggested **7** to be a hydroxylated cannabinol derivative,<sup>16</sup> while HMBC correlations (H<sub>3</sub>-11/C-8, C-10; H-7/C-

8) (Figure 1) fixed the structure as 8-hydroxycannabinol. This is the first report of **7** from a natural source; however, it has been prepared synthetically.<sup>17</sup>

The molecular formula of **8** was found to be C<sub>22</sub>H<sub>26</sub>O<sub>5</sub> by HRESIMS ( $m/z$  369.1731, [M - H]<sup>-</sup>), and its IR spectrum showed hydroxy and carbonyl absorption bands at  $\nu_{\text{max}}$  3400 and 1650 cm<sup>-1</sup>, respectively. The <sup>13</sup>C NMR spectroscopic data of **8** (Table 3) were similar to those of **7**, with the addition of a carboxylic group ( $\delta_{\text{C}}$  176.0) located at C-2, as confirmed in the <sup>1</sup>H NMR spectrum by the presence of a downfield shifted hydrogen-bonded hydroxy proton ( $\delta_{\text{H}}$  12.6) and the absence of the H-2 proton resonance observed in **7**. The GC-MS data of **8** and **7** were identical due to the *in situ* decarboxylation of **8** that occurs upon injection at 250 °C. On the basis of the above, **8** was elucidated as 8-hydroxycannabinolic acid A.

Compound **9** was isolated as an orange, amorphous powder. The molecular formula C<sub>21</sub>H<sub>30</sub>O<sub>3</sub> was established by HRESIMS ( $m/z$  353.2066, [M + Na]<sup>+</sup>). The IR spectrum of **9** indicated the presence of an  $\alpha,\beta$ -unsaturated ketone moiety ( $\nu_{\text{max}}$  1663 cm<sup>-1</sup>). The <sup>13</sup>C NMR, DEPT, and HMQC spectra of **9** revealed 21 resonances, including four methyl, seven methylene, three olefinic methine, and seven quaternary carbons (Table 2). The two carbonyl carbons resonating at  $\delta_{\text{C}}$  187.7 and 184.7 (Table 2) are characteristic for a benzoquinone skeleton, while NMR analysis suggested geranyl, *n*-pentyl, and hydroxy substituents, indicating a trisubstituted-1,4-benzoquinone derivative.<sup>3,18</sup> The HMBC correlations placed the geranyl moiety at C-2 (H-1''/C-1), the *n*-pentyl moiety at C-3 (H-1'/C-2, C-4), and the hydroxy group at C-5 (H-6/C-2, C-4) (Figure 1), confirming **9** to be 2-geranyl-5-hydroxy-3-*n*-pentyl-1,4-benzoquinone. Compound **9** is the second reported 1,4-benzoquinone derivative isolated from cannabis.<sup>3</sup>

The known compound  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranosyl-6'-acetate was identified by comparison of its spectroscopic data with literature values.<sup>19</sup>

**Biological Activity.** The isolated compounds were evaluated for their antimicrobial (Table 4), antiprotozoal (Table 5), and cytotoxic activities. Compound **7** exhibited good antifungal activity against *Candida albicans* (IC<sub>50</sub> 4.6  $\mu$ M), while **2**, **6**, and **8** showed weak anticandidal activity. Compounds **2** and **6** possessed mild anti-MRSA activity (IC<sub>50</sub> 24.4 and 6.7  $\mu$ M, respectively), and **8** showed good anti-*Staphylococcus aureus* activity (IC<sub>50</sub> 3.5  $\mu$ M). Compound **7** exhibited moderate antibacterial activity against *Mycobacterium intracellulare* (IC<sub>50</sub> 30.6  $\mu$ M) (Table 4). Compound **5** showed strong antileishmanial activity (IC<sub>50</sub> 10.7, IC<sub>90</sub> 18.7  $\mu$ M), while **1**, **2**, and **6** possessed moderate antileishmanial activity. Compounds **1** and **5** had mild antimalarial activities (Table 5). All the isolates lacked cytotoxicity against Vero cells (African green monkey kidney fibroblast).

**Table 2.** <sup>1</sup>H (400 MHz) and <sup>13</sup>C NMR (100 MHz) Spectroscopic Data of **5**, **6**, and **9** (CDCl<sub>3</sub>)<sup>a</sup>

carbon	<b>5</b>		<b>6</b>		<b>9</b>	
	δ <sub>C</sub>	δ <sub>H</sub> , mult. (J in Hz)	δ <sub>C</sub>	δ <sub>H</sub> , mult. (J in Hz)	δ <sub>C</sub>	δ <sub>H</sub> , mult. (J in Hz)
1	152.6		152.9		187.7	
2	108.6	6.27, s	118.6		141.2	
3	133.8		135.1		146.3	
4	131.4		131.2		184.7	
5	146.4		146.1		154.3	
6	113.0		102.8	6.28, s	107.8	6.04, s
1'	30.4	2.40, t (7.6)	27.7	2.42, t (7.2)	26.7	2.48, t (7.8)
2'	29.7	1.52, m	30.0	1.40, m	29.0	1.49, m
3'	31.8	1.30, m	32.4	1.31, m	32.4	1.33, m
4'	22.6	1.30, m	22.6	1.31, m	22.6	1.33, m
5'	14.2	0.88, t (6.8)	14.3	0.88, t (6.4)	14.1	0.89, t (6.8)
1''	23.1	3.40, d (7.6)	25.3	3.26, d (6.0)	25.8	3.21, d (6.8)
2''	123.9	5.04, t (7.6)	123.3	5.09, t (6.0)	119.9	4.93, t (6.8)
3''	139.5		136.5		137.5	
4''	39.9	2.05, m	39.9	1.98, m	40.0	1.97, m
5''	26.5	2.10, m	26.7	2.06, m	26.5	2.05, m
6''	121.6	5.27, t (6.4)	124.3	5.04, t (6.4)	124.2	5.03, t (6.8)
7''	132.4		131.8		131.7	
8''	17.9	1.59, s	17.9	1.57, s	17.9	1.57, s
9''	25.9	1.67, s	25.9	1.65, s	25.8	1.65, s
10''	16.4	1.79, s	16.4	1.75, s	16.6	1.73, s
OCOCH <sub>3</sub>	20.8	2.33, s	20.8	2.28, s		
OCOCH <sub>3</sub>	170.1		170.0			

<sup>a</sup> Assignments confirmed by DEPT-135, HMQC, COSY, and HMBC NMR experiments.**Table 3.** <sup>1</sup>H (400 MHz) and <sup>13</sup>C NMR (100 MHz) Spectroscopic Data of **7** and **8** (CDCl<sub>3</sub>)<sup>a</sup>

carbon	<b>7</b>		<b>8</b>	
	δ <sub>C</sub>	δ <sub>H</sub> , mult. (J in Hz)	δ <sub>C</sub>	δ <sub>H</sub> , mult. (J in Hz)
1	153.9		162.9	
2	110.1	6.27, s	104.3	
3	143.7		148.1	
4	111.0	6.42, s	113.2	6.42, s
4a	152.6		153.3	
6	77.2		78.3	
6a	120.7		119.8	
7	109.8	6.68, s	109.6	6.68, s
8	152.9		158.7	
9	139.6		138.6	
10	129.0	8.14, s	129.8	8.41, s
10a	122.5		122.6	
10b	110.0		109.1	
11	16.0	2.23, s	15.9	2.29, s
12	27.3	1.60, s	27.6	1.59, s
13	27.3	1.60, s	27.6	1.59, s
1'	35.8	2.48, t (7.6)	36.9	2.93, t (7.2)
2'	30.8	1.60, m	31.4	1.59, m
3'	31.7	1.30, m	32.1	1.35, m
4'	22.8	1.31, m	22.7	1.35, m
5'	14.3	0.88, t (7.2)	14.2	0.87, t (7.2)
COOH			176.0	
1-OH				12.6

<sup>a</sup> Assignments confirmed by DEPT-135, HMQC, COSY, and HMBC NMR experiments.

### Experimental Section

**General Experimental Procedures.** 1D and 2D NMR spectra were recorded in CDCl<sub>3</sub> on a Varian AS 400 spectrometer. IR spectra were recorded on a Bruker Tensor 27 spectrophotometer. UV spectra were obtained on a Varian Cary 50 Bio UV–visible spectrophotometer. Specific rotations were measured at ambient temperature using a Rudolph Research Analytical Autopol IV automatic polarimeter. HRESIMS were obtained using a Bruker Bioapex FTMS in ESI mode. TLC was carried out on aluminum-backed plates precoated with silica gel F<sub>254</sub> (20 × 20 cm, 200 μm, 60 Å, Merck). Visualization was accomplished by spraying with Fast Blue B salt (0.5% w/w in water) or *p*-anisaldehyde [0.5 mL in glacial acetic acid (50 mL) and sulfuric acid (97%, 1 mL)] spray reagent followed by heating. Flash silica gel (40–63 μm, 60 Å, SiliCycle) and SiliaBond C<sub>18</sub> silica gel (40–63 μm, 60 Å, 17% carbon loading, SiliCycle) were used for column chromatography. Analytical HPLC was performed on a Waters 2695 separations module connected to a Waters 2996 photodiode array (PDA)

**Table 4.** *In Vitro* Antimicrobial Activities of **2**, **5**, **6**, **7**, and **8** (IC<sub>50</sub> in μM)<sup>a</sup>

compound	antifungal		antibacterial			
	<i>C. albicans</i>	<i>C. krusei</i>	<i>MRSa</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>M. intracellulare</i>
<b>2</b>	60.5	60.5	24.4	29.6	na	na
<b>5</b>	na	nt	53.4	na	na	na
<b>6</b>	na	53.4	6.7	12.2	na	na
<b>7</b>	4.6	nt	nt	nt	na	30.6
<b>8</b>	na	54.0	nt	3.5	54.0	na
amphotericin B	0.3	0.7				
ciprofloxacin			0.4	0.4	0.0	1.5

<sup>a</sup> IC<sub>50</sub> = the test concentration that affords 50% inhibition of growth. *MRSa* = methicillin-resistant *Staphylococcus aureus*. na = not active. nt = not tested.**Table 5.** *In Vitro* Antiprotozoal Activities of **1**, **2**, **5**, and **6** (IC<sub>50</sub> and IC<sub>90</sub> in μM)<sup>a</sup>

compound	antileishmanial		antimalarial	
	<i>L. donovani</i>		<i>P. falciparum</i>	
	IC <sub>50</sub>	IC <sub>90</sub>	D6	W2
<b>1</b>	40.3	91.3	7.2	4.0
<b>2</b>	57.5	96.8	na	na
<b>5</b>	10.7	18.7	7.2	6.7
<b>6</b>	42.7	85.4	na	na
pentamidine	3.8	19.1		
chloroquine			0.1	0.5

<sup>a</sup> IC<sub>50</sub> = the test concentration that kills 50% cells compared to the solvent controls. IC<sub>90</sub> = the test concentration that kills 90% cells compared to the solvent controls.

detector (190–500 nm) and a Sedere Sedex 75 evaporative light scattering (ELS) detector (3.5 psi N<sub>2</sub>, 50 °C) using a Phenomenex Luna C<sub>18</sub> HPLC column (150 × 4.6 mm, 5 μm, 100 Å). Semipreparative HPLC was performed on a Waters Delta Prep 4000 preparative chromatography system connected to a Waters 486 tunable absorbance detector (206 nm) using Phenomenex Luna Silica and C<sub>18</sub> HPLC columns (250 × 21.2 mm, 5 μm, 100 Å). GC-MS analysis was carried out on a HP 6890 series GC, equipped with a split/splitless capillary injector, a HP 6890 Series injector autosampler, and an Agilent DB-5 ms column (30 m × 0.25 mm × 0.25 μm). The GC was interfaced to a HP 5973 quadrupole mass selective detector through a transfer line set at 280 °C. The injector temperature was 250 °C, and 1 μL injections were performed in the split (1:10) mode. Column flow was set at a

constant pressure of 20 psi, giving an initial flow of 2.2 mL/min, using helium as carrier gas. The oven temperature was raised from 70 to 300 °C (hold 8.5 min) at a rate of 20 °C/min, for a total run time of 20 min. The filament was operated at 70 eV, with an emission current of 35  $\mu$ A. The multiplier voltage was automatically set to 2247 V. The ion source and quadrupole temperatures were 230 and 150 °C, respectively. The acquisition range was  $m/z$  30–800 at 1.95 scans per second, starting 3.5 min after injection.

**Plant Material.** Plants were grown from high-potency Mexican *C. sativa* seeds (variety code CHPF-01). The seeds and plants were authenticated by Dr. Suman Chandra, The University of Mississippi, and a specimen (S1310V1) was deposited at the Coy Waller Complex, The University of Mississippi. Whole buds of mature female plants were harvested, air-dried, packed in barrels, and stored at low temperature (–24 °C).

**Biological Assays.** The isolated compounds were evaluated for *in vitro* antifungal (*Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, and *Aspergillus fumigatus* ATCC 90906), antibacterial (methicillin-resistant *Staphylococcus aureus* ATCC 33591, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068), antileishmanial (culture of *Leishmania donovani*), antimalarial [*Plasmodium falciparum* (D6 clone) and *P. falciparum* (W2 clone)], and cytotoxic activity [Vero cells (African green monkey kidney fibroblast)].<sup>2,21–23</sup>

**Extraction and Isolation.** The plant material (9.0 kg) was sequentially extracted with hexanes (2  $\times$  60 L), CH<sub>2</sub>Cl<sub>2</sub> (48 L), EtOAc (40 L), EtOH (37.5 L), EtOH/H<sub>2</sub>O (36 L, 1:1), and H<sub>2</sub>O (40 L) at room temperature. The extracts were evaporated under reduced pressure at 40 °C to afford hexanes (1.48 kg), CH<sub>2</sub>Cl<sub>2</sub> (0.15 kg), EtOAc (0.13 kg), EtOH (0.09 kg), EtOH/H<sub>2</sub>O (0.77 kg), and H<sub>2</sub>O (0.54 kg) extracts for a total extract of 3.16 kg (35.1%). Portions of the CH<sub>2</sub>Cl<sub>2</sub>, EtOAc, and EtOH extracts were combined (191.0 g) based on similar TLC profiles (EtOAc/*n*-hexane, 4:6) and were subjected to silica gel VLC, eluting with EtOAc/*n*-hexane [0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 75:25, 100:0 (2 L of each mixture)] followed by EtOH (4 L), yielding nine fractions (A–I). Fraction A (13.1 g) was fractionated over a silica gel column eluted with EtOAc/*n*-hexane (0:100 to 5:95, 5% stepwise) to afford 22 subfractions. Subfraction A<sub>17–20</sub> (106 mg) was purified on silica gel HPLC eluting with EtOH/*n*-hexane (5:95) to yield **1** (2.8 mg), **3** (0.8 mg), **5** (8.9 mg), and **6** (4.0 mg). Fraction C (16.7 g) was applied to a silica gel column using EtOAc/*n*-hexane (0:100 to 20:80) to give 10 subfractions. Subfraction C<sub>6</sub> (565 mg) was further chromatographed over a C<sub>18</sub> SPE column (10 g), eluting with MeOH/H<sub>2</sub>O (75:25), to afford **4** (170 mg), **9** (13.1 mg), and **7** (6.6 mg). Subfraction C<sub>9</sub> (3.2 g) was chromatographed over Sephadex LH-20 eluting with MeOH followed by C<sub>18</sub> HPLC purification using MeCN/H<sub>2</sub>O (55:45), yielding **2** (2.4 mg) and **8** (6 mg). Fraction E (5.7 g) was chromatographed on a silica gel column using EtOAc/*n*-hexane (20:80) as a mobile phase to afford  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranosyl-6'-acetate (208 mg).

**Trimethylsilyl Derivatization.** Dried samples (ca. 100  $\mu$ g) were treated with pyridine (5  $\mu$ L, silylation grade, Pierce) and BSTFA [*N,O*-bis(trimethylsilyl)trifluoroacetamide] (100  $\mu$ L, 98+%, Acros Organics), followed by heating at 75 °C for 1 h. After cooling to room temperature, methylene chloride (0.9 mL) was added to the reaction mixture and the solution analyzed by GC-MS.

(**±**)-**4-Acetoxy-cannabichromene (1)**: yellow oil; UV (MeOH)  $\lambda_{\max}$  227, 280 nm; IR (neat)  $\nu_{\max}$  3415, 2930, 1735 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; EIMS  $m/z$  372 [M]<sup>+</sup> (11), 357 (9), 331 (90), 289 (100), 247 (85), 190 (17), 69 (8), 43 (8); HRESIMS  $m/z$  373.2409 [M + H]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>33</sub>O<sub>4</sub>, 373.2380).

(**±**)-**3''-Hydroxy- $\Delta$ (<sup>4''</sup>,<sup>5''</sup>)-cannabichromene (2)**: brown oil; UV (MeOH)  $\lambda_{\max}$  227, 280 nm; IR (neat)  $\nu_{\max}$  3405, 3310, 2920, 1590 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; EIMS  $m/z$  330 [M]<sup>+</sup> (3), 312 (5), 231 (100), 187 (5), 174 (16); HRESIMS  $m/z$  331.2193 [M + H]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>31</sub>O<sub>3</sub>, 331.2273).

(**–**)-**7-Hydroxycannabichromane (3)**: pale yellow oil; [ $\alpha$ ]<sub>D</sub><sup>25</sup> –66.2 (c 0.15, MeOH); UV (MeOH)  $\lambda_{\max}$  227, 252 nm; IR (neat)  $\nu_{\max}$  3410, 3310, 2920, 1590 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; EIMS  $m/z$  332 [M]<sup>+</sup> (30), 314 (5), 299 (7), 271 (5), 247 (30), 231 (24), 206 (65), 193 (20), 164 (20), 150 (100), 135 (62), 109 (60), 69 (35), 43 (33); HRESIMS  $m/z$  331.2254 [M – H]<sup>–</sup> (calcd for C<sub>21</sub>H<sub>31</sub>O<sub>3</sub>, 331.2273).

(**–**)-**7R-Cannabicoarmonic acid A (4)**: brown oil; [ $\alpha$ ]<sub>D</sub><sup>25</sup> –15.0 (c 0.10, MeOH); UV (MeOH)  $\lambda_{\max}$  225, 280 nm; IR (neat)  $\nu_{\max}$  2910,

1716, 1700, 1640, 1570 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; EIMS  $m/z$  372 [M]<sup>+</sup> (15), 354 (8), 329 (10), 311 (100), 297 (8), 284 (14), 258 (20), 213 (9); HRESIMS  $m/z$  395.1847 [M + Na]<sup>+</sup> (calcd for C<sub>22</sub>H<sub>28</sub>O<sub>5</sub>Na, 395.1835).

**5-Acetyl-4-hydroxycannabigerol (5)**: brown oil; UV (MeOH)  $\lambda_{\max}$  215, 255, 300 nm; IR (neat)  $\nu_{\max}$  3402, 1735, 1610 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2; EIMS  $m/z$  374 [M]<sup>+</sup> (14), 332 (87), 289 (10), 263 (10), 247 (50), 209 (100), 190 (10), 152 (35), 123 (22), 69 (26), 43 (20); HRESIMS  $m/z$  375.2530 [M + H]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>35</sub>O<sub>4</sub>, 375.2535).

**4-Acetoxy-2-geranyl-5-hydroxy-3-n-pentylphenol (6)**: yellow oil; UV (MeOH)  $\lambda_{\max}$  215, 255, 300 nm; IR (neat)  $\nu_{\max}$  3402, 1735, 1610 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2; EIMS  $m/z$  374 [M]<sup>+</sup> (11), 332 (57), 317 (4), 263 (6), 247 (75), 209 (60), 191 (37), 153 (100), 123 (14), 91 (10), 69 (35), 43 (30); HRESIMS  $m/z$  375.2528 [M + H]<sup>+</sup> (calcd for C<sub>23</sub>H<sub>35</sub>O<sub>4</sub>, 375.2535).

**8-Hydroxycannabinol (7)**: brown, amorphous powder; UV (MeOH)  $\lambda_{\max}$  220, 267, 330 nm; IR (neat)  $\nu_{\max}$  3400, 1641, 1610, 873 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 3; EIMS  $m/z$  326 [M]<sup>+</sup> (25), 311 (100), 254 (20), 239 (18); HRESIMS  $m/z$  349.1781 [M + Na]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>26</sub>O<sub>3</sub>Na, 349.1780).

**8-Hydroxycannabinolic acid A (8)**: brown oil; UV (MeOH)  $\lambda_{\max}$  220, 267, 330 nm; IR (neat)  $\nu_{\max}$  3400, 1650, 1610, 873 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 3; EIMS (decarboxylated compound)  $m/z$  326 [M]<sup>+</sup> (25), 311 (100), 254 (20), 239 (18); HRESIMS  $m/z$  369.1731 [M – H]<sup>–</sup> (calcd for C<sub>22</sub>H<sub>25</sub>O<sub>5</sub>, 369.1702).

**2-Geranyl-5-hydroxy-3-n-pentyl-1,4-benzoquinone (9)**: orange, amorphous powder; UV (MeOH)  $\lambda_{\max}$  205, 270, 385 nm; IR (neat)  $\nu_{\max}$  1663, 1613 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2; EIMS  $m/z$  330 [M]<sup>+</sup> (3), 274 (5), 261 (14), 247 (25), 231 (5), 191 (14), 163 (14), 119 (16), 91 (16), 69 (100), 41 (65); HRESIMS  $m/z$  353.2066 [M + Na]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>30</sub>O<sub>3</sub>Na, 353.2092).

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**Supporting Information Available:** <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data for compounds **1–9**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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