

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-hydroxycannabichromene (**3**), (-)-7*R*-cannabicoumaronic 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 β -sitosterol-3-*O*- β -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).^{1–7} The best-known and most specific class of cannabis constituents are the C₂₁ terpenophenolic cannabinoids. Other phenolic cannabis constituents include flavonoids, spiroindans, dihydrostilbenes, phenanthrenes, and dihydrophenanthrenes.^{1–6,8,9} As part of our program aimed at the discovery of new cannabinoids and other metabolites with significant biological activity from high-potency cannabis (Δ^9 -THC > 10%, w/w), we have reported 25 new metabolites.^{2–5} In this paper, we report the isolation and identification of nine additional new cannabinoids (**1–9**), including three cannabichromene derivatives (**1–3**), (-)-7*R*-cannabicoumaronic acid A (**4**), two cannabigerol derivatives (**5** and **6**), two cannabinol derivatives (**7** and **8**), and a C₂₁ benzoquinone derivative (**9**). The known sterol β -sitosterol-3-*O*- β -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 C₂₃H₃₂O₄ from GC-MS (*m/z* 372, [M]⁺) and HRESIMS (*m/z* 373.2409, [M + H]⁺), indicating eight degrees of unsaturation. The ¹H NMR spectrum of **1** (Table 1) displayed an AB olefinic spin system [δ_{H} 5.48 (d, *J* = 10.0 Hz, H-7), 6.57 (d, *J* = 10.0 Hz, H-8)], an isolated olefinic proton [δ_{H} 5.10 (t, *J* = 7.2 Hz, H-3'')], a sharp aromatic singlet [δ_{H} 6.07 (s, H-2)], six methylenes (δ_{H} 1.30–2.35), two olefinic methyls [δ_{H} 1.58 (s, H₃-5''), 1.66 (s, H₃-6'')], a tertiary methyl [δ_{H} 1.33 (s, H₃-9)], and an acetoxy methyl resonance [δ_{H} 2.29 (s, OCOCH₃)]. The small coupling constant between vicinal protons H-7 and H-8 (10.0 Hz) indicated a *cis* double bond.¹¹ The ¹³C 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 (δ_{C} 169.7), three oxyaryl (δ_{C} 131.3, 145.3, 148.8), and one oxygenated sp³ carbon (δ_{C} 79.1, C-6). The ¹H and ¹³C NMR, IR, and UV spectroscopic data were similar to those reported for cannabichromene,^{12–14} 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.¹⁴ 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]⁺ corresponding to the molecular formula C₂₁H₃₀O₃ (seven degrees of unsaturation). The UV and IR spectra of **2** exhibited patterns similar to those of cannabichromene.^{12–14} The ¹H NMR spectrum of **2** (Table 1) included an AB olefinic spin system [δ_{H} 5.46 (d, *J* = 10.0 Hz, H-7), 6.62 (d, *J* = 10.0 Hz, H-8)], two aromatic protons [δ_{H} 6.12 (s, H-2), 6.23 (s, H-4)], and six methylene resonances (δ_{H} 1.35–2.57), confirming the cannabichromene skeleton.^{12–14} The ¹H, ¹³C, and DEPT NMR spectra displayed additional hydroxymethine [δ_{H} 4.07 (t, *J* = 6.0 Hz), δ_{C} 76.2] and exomethylene [δ_{H} 4.83 (bs), 4.92 (bs), δ_{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 (H₂-5''/C-6'', C-4'', C-3''; H₃-6''/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 C₂₁H₃₂O₃ from its HRESIMS [M – H][–] ion at *m/z* 331.2254, indicating six degrees of unsaturation. The ¹³C, DEPT, and HMQC NMR spectra revealed 21 carbons (Table 1), including four methyl, seven methylene, four methine, and six quaternary resonances. The ¹H and ¹³C NMR spectroscopic data of **3** (Table 1) were similar to those of cannabichromene,^{12–14} except for the absence of the olefinic protons at C-7 and C-8 and the presence of a hydroxy group at C-7 [δ_{H} 4.68 (t, *J* = 6.8 Hz, H-7), δ_{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; H₃-9/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-hydroxycannabichromene.

Compound **4** was isolated as a brown oil. Its molecular formula was found to be C₂₂H₂₈O₅ by HRESIMS (*m/z* 395.1847, [M + Na]⁺) and GC-MS (*m/z* 372, [M]⁺). The IR spectrum of **4** indicated the presence of two carbonyl groups (ν_{max} 1716, 1700 cm^{–1}). The ¹H,

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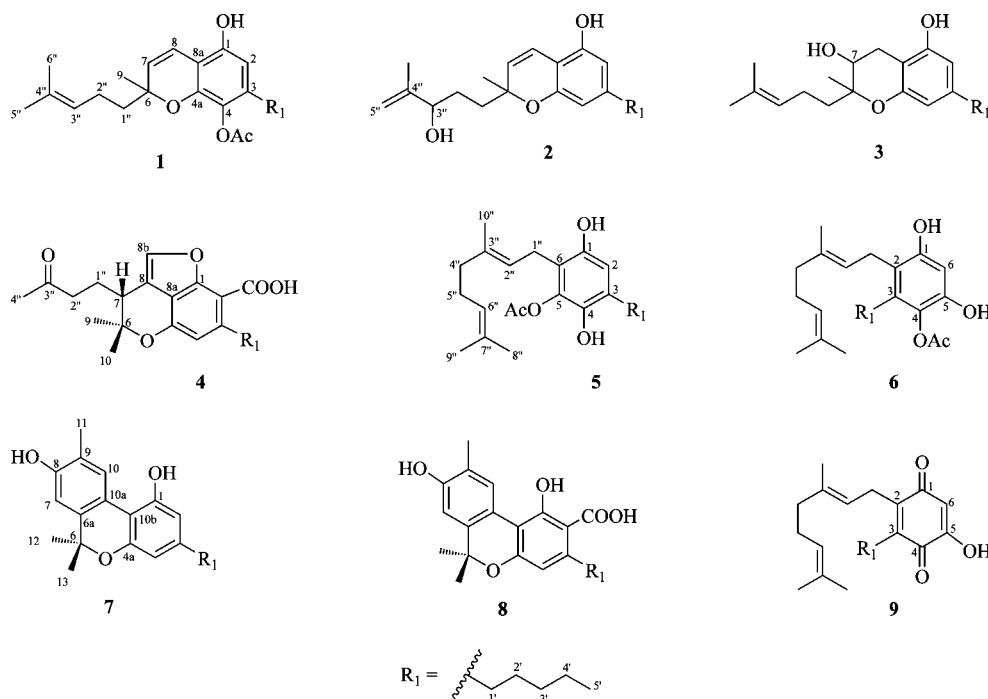
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Chart 1

**Table 1.** ^1H (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data of **1–4** (CDCl_3)^a

carbon	1		2		3		4	
	δ_{C}	δ_{H} , mult. (J in Hz)	δ_{C}	δ_{H} , mult. (J in Hz)	δ_{C}	δ_{H} , mult. (J in Hz)	δ_{C}	δ_{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 ₃	20.7	2.29, s						
OCOCH ₃	169.7							
COOH							170.6	

^a Assignments confirmed by DEPT-135, HMQC, COSY, and HMBC NMR experiments.

^{13}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 ^1H and ^{13}C NMR data of **4** were in good agreement with those reported for cannabichromone,¹⁵ 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}C NMR carbonyl resonance at δ_{C} 170.6. The ROESY correlation between H-7 (δ_{H} 2.89) and pseudoaxial H₃-10 (δ_{H} 1.29, δ_{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 (–)- Δ^9 -THC, (–)- Δ^9 -THC acid A, (–)- Δ^8 -THC, (–)-hexahydrocannabinol, and a series of cannabichromane derivatives.⁵ The ^{13}C NMR chemical shift of the β -pseudoequatorial C-6 methyl is downfield from the α -pseudoaxial C-6 methyl for these compounds.⁵ 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 ^1H NMR chemical shift of H-7 of **4** were in agreement with the cannabichromane derivatives that have H-7 β configurations.^{5a} Thus, the structure of **4** was established as (–)-7*R*-cannabichromone acid A.

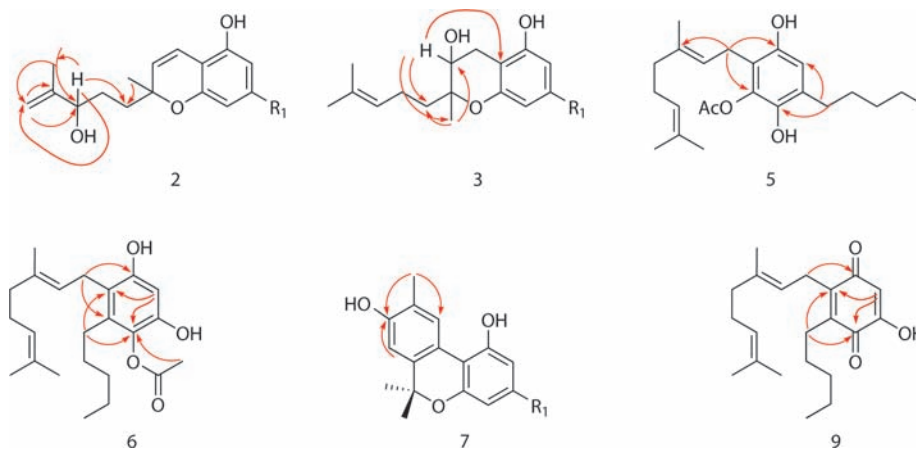


Figure 1. Key HMBC correlations for 2, 3, 5, 6, 7, and 9.

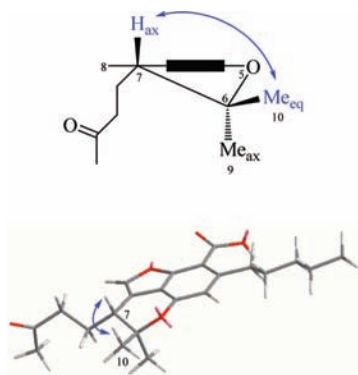


Figure 2. Key ROESY correlation between H-7 and pseudoequatorial H₃-10 of 4.

The molecular formula of 5 ($C_{23}H_{34}O_4$) was established from HRESIMS (m/z 375.2530, $[M + H]^+$) and ^{13}C NMR data. The 1H , ^{13}C , and DEPT NMR spectroscopic data (Table 2) showed the presence of one aromatic methine, a geranyl moiety,² an *n*-pentyl group,² and an acetoxy group [δ_H 2.33 (s), δ_C 20.8, 170.1]. The presence of the acetoxy group was supported by the IR absorption band at ν_{max} 1735 cm^{-1} . The spectroscopic data of 5 were similar to those reported for cannabigerol,¹⁶ 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 ($H_2-1''/C-1$, C-5; $H_2-1'/C-4$, C-2) (Figure 1). Thus, 5 was established as 5-acetyl-4-hydroxycannabigerol.

Compound 6 was isolated as a yellow oil with molecular formula $C_{23}H_{34}O_4$ (HRESIMS: m/z 375.2528, $[M + H]^+$; GC-MS: m/z 374, $[M]^+$). The ^{13}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 ($H_2-1''/C-3$, C-1; $H_2-1'/C-2$, C-4), the acetoxy group at C-4, and the second hydroxy group at C-5 ($H-6/C-4$, C-2; $OCOCH_3/C-4$) (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 $C_{21}H_{26}O_3$ from its HRESIMS (m/z 349.1781, $[M + Na]^+$) and ^{13}C NMR data. 1H NMR data showed three methyl singlets, a primary methyl group, and four aromatic and four methylene protons (Table 3). The ^{13}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, $[M]^+$) suggested 7 to be a hydroxylated cannabinol derivative,¹⁶ while HMBC correlations ($H_3-11/C-8$, 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.¹⁷

The molecular formula of 8 was found to be $C_{22}H_{26}O_5$ by HRESIMS (m/z 369.1731, $[M - H]^-$), and its IR spectrum showed hydroxy and carbonyl absorption bands at ν_{max} 3400 and 1650 cm^{-1} , respectively. The ^{13}C NMR spectroscopic data of 8 (Table 3) were similar to those of 7, with the addition of a carboxylic group (δ_C 176.0) located at C-2, as confirmed in the 1H NMR spectrum by the presence of a downfield shifted hydrogen-bonded hydroxy proton (δ_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 $C_{21}H_{30}O_3$ was established by HRESIMS (m/z 353.2066, $[M + Na]^+$). The IR spectrum of 9 indicated the presence of an α,β -unsaturated ketone moiety (ν_{max} 1663 cm^{-1}). The ^{13}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 δ_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.^{3,18} 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.³

The known compound β -sitosterol-3-*O*- β -D-glucopyranosyl-6'-acetate was identified by comparison of its spectroscopic data with literature values.¹⁹

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_{50} 4.6 μM), while 2, 6, and 8 showed weak anticandidal activity. Compounds 2 and 6 possessed mild anti-MRSA activity (IC_{50} 24.4 and 6.7 μM , respectively), and 8 showed good anti-*Staphylococcus aureus* activity (IC_{50} 3.5 μM). Compound 7 exhibited moderate antibacterial activity against *Mycobacterium intracellulare* (IC_{50} 30.6 μM) (Table 4). Compound 5 showed strong antileishmanial activity (IC_{50} 10.7, IC_{90} 18.7 μM), 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. ^1H (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data of **5**, **6**, and **9** (CDCl_3)^a

carbon	5		6		9	
	δ_{C}	δ_{H} , mult. (J in Hz)	δ_{C}	δ_{H} , mult. (J in Hz)	δ_{C}	δ_{H} , mult. (J in Hz)
1	152.6		152.9		187.7	
2	108.6		118.6		141.2	
3	133.8	6.27, s	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 ₃	20.8	2.33, s	20.8	2.28, s		
OCOCH ₃	170.1		170.0			

^a Assignments confirmed by DEPT-135, HMQC, COSY, and HMBC NMR experiments.**Table 3.** ^1H (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data of **7** and **8** (CDCl_3)^a

carbon	7		8	
	δ_{C}	δ_{H} , mult. (J in Hz)	δ_{C}	δ_{H} , 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

^a 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_3 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 F₂₅₄ (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 C₁₈ 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_{50} in μM)^a

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>
2	60.5	60.5	24.4	29.6	na	na
5	na	nt	53.4	na	na	na
6	na	53.4	6.7	12.2	na	na
7	4.6	nt	nt	nt	na	30.6
8	na	54.0	nt	3.5	54.0	na
amphotericin B	0.3	0.7				
ciprofloxacin			0.4	0.4	0.0	1.5

^a IC_{50} = 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_{50} and IC_{90} in μM)^a

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

^a IC_{50} = the test concentration that kills 50% cells compared to the solvent controls. IC_{90} = 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_2 , 50 °C) using a Phenomenex Luna C₁₈ 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 C₁₈ 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 μ 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)].^{2,21–23}

Extraction and Isolation. The plant material (9.0 kg) was sequentially extracted with hexanes (2 $\times$ 60 L), CH_2Cl_2 (48 L), EtOAc (40 L), EtOH (37.5 L), EtOH/H₂O (36 L, 1:1), and H₂O (40 L) at room temperature. The extracts were evaporated under reduced pressure at 40 °C to afford hexanes (1.48 kg), CH_2Cl_2 (0.15 kg), EtOAc (0.13 kg), EtOH (0.09 kg), EtOH/H₂O (0.77 kg), and H₂O (0.54 kg) extracts for a total extract of 3.16 kg (35.1%). Portions of the CH_2Cl_2 , 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_{17–20} (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 C₆ (565 mg) was further chromatographed over a C₁₈ SPE column (10 g), eluting with MeOH/H₂O (75:25), to afford **4** (170 mg), **9** (13.1 mg), and **7** (6.6 mg). Subfraction C₉ (3.2 g) was chromatographed over Sephadex LH-20 eluting with MeOH followed by C₁₈ HPLC purification using MeCN/H₂O (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 β -sitosterol-3-*O*- β -D-glucopyranosyl-6'-acetate (208 mg).

Trimethylsilyl Derivatization. Dried samples (ca. 100 μ g) were treated with pyridine (5 μ L, silylation grade, Pierce) and BSTFA [N,O-bis(trimethylsilyl)trifluoroacetamide] (100 μ 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.

($\pm$)-4-Acetoxycannabichromene (1): yellow oil; UV (MeOH) λ_{max} 227, 280 nm; IR (neat) ν_{max} 3415, 2930, 1735 cm^{-1} ; ¹H and ¹³C NMR, see Table 1; EIMS m/z 372 [$\text{M}]^+$ (11), 357 (9), 331 (90), 289 (100), 247 (85), 190 (17), 69 (8), 43 (80); HRESIMS m/z 373.2409 [$\text{M} + \text{H}]^+$ (calcd for C₂₃H₃₃O₄, 373.2380).

($\pm$)-3''-Hydroxy- $\Delta^{(4'',5'')}$ -cannabichromene (2): brown oil; UV (MeOH) λ_{max} 227, 280 nm; IR (neat) ν_{max} 3405, 3310, 2920, 1590 cm^{-1} ; ¹H and ¹³C NMR, see Table 1; EIMS m/z 330 [$\text{M}]^+$ (3), 312 (5), 231 (100), 187 (5), 174 (16); HRESIMS m/z 331.2193 [$\text{M} + \text{H}]^+$ (calcd for C₂₁H₃₁O₃, 331.2273).

(−)-7-Hydroxycannabichromene (3): pale yellow oil; [α]_D²⁵ −66.2 (c 0.15, MeOH); UV (MeOH) λ_{max} 227, 252 nm; IR (neat) ν_{max} 3410, 3310, 2920, 1590 cm^{-1} ; ¹H and ¹³C NMR, see Table 1; EIMS m/z 332 [$\text{M}]^+$ (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 [$\text{M} - \text{H}]^-$ (calcd for C₂₁H₃₁O₃, 331.2273).

(−)-7R-Cannabichromenonic acid A (4): brown oil; [α]_D²⁵ −15.0 (c 0.10, MeOH); UV (MeOH) λ_{max} 225, 280 nm; IR (neat) ν_{max} 2910,

1716, 1700, 1640, 1570 cm^{-1} ; ¹H and ¹³C NMR, see Table 1; EIMS m/z 372 [$\text{M}]^+$ (15), 354 (8), 329 (10), 311 (100), 297 (8), 284 (14), 258 (20), 213 (9); HRESIMS m/z 395.1847 [$\text{M} + \text{Na}]^+$ (calcd for C₂₂H₂₈O₅Na, 395.1835).

5-Acetyl-4-hydroxycannabigerol (5): brown oil; UV (MeOH) λ_{max} 215, 255, 300 nm; IR (neat) ν_{max} 3402, 1735, 1610 cm^{-1} ; ¹H and ¹³C NMR, see Table 2; EIMS m/z 374 [$\text{M}]^+$ (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 [$\text{M} + \text{H}]^+$ (calcd for C₂₃H₃₅O₄, 375.2535).

4-Acetoxy-2-geranyl-5-hydroxy-3-n-pentylphenol (6): yellow oil; UV (MeOH) λ_{max} 215, 255, 300 nm; IR (neat) ν_{max} 3402, 1735, 1610 cm^{-1} ; ¹H and ¹³C NMR, see Table 2; EIMS m/z 374 [$\text{M}]^+$ (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 [$\text{M} + \text{H}]^+$ (calcd for C₂₃H₃₅O₄, 375.2535).

8-Hydroxycannabinol (7): brown, amorphous powder; UV (MeOH) λ_{max} 220, 267, 330 nm; IR (neat) ν_{max} 3400, 1641, 1610, 873 cm^{-1} ; ¹H and ¹³C NMR, see Table 3; EIMS m/z 326 [$\text{M}]^+$ (25), 311 (100), 254 (20), 239 (18); HRESIMS m/z 349.1781 [$\text{M} + \text{Na}]^+$ (calcd for C₂₁H₂₆O₃Na, 349.1780).

8-Hydroxycannabinolic acid A (8): brown oil; UV (MeOH) λ_{max} 220, 267, 330 nm; IR (neat) ν_{max} 3400, 1650, 1610, 873 cm^{-1} ; ¹H and ¹³C NMR, see Table 3; EIMS (decarboxylated compound) m/z 326 [$\text{M}]^+$ (25), 311 (100), 254 (20), 239 (18); HRESIMS m/z 369.1731 [$\text{M} - \text{H}]^-$ (calcd for C₂₂H₂₅O₅, 369.1702).

2-Geranyl-5-hydroxy-3-n-pentyl-1,4-benzoquinone (9): orange, amorphous powder; UV (MeOH) λ_{max} 205, 270, 385 nm; IR (neat) ν_{max} 1663, 1613 cm^{-1} ; ¹H and ¹³C NMR, see Table 2; EIMS m/z 330 [$\text{M}]^+$ (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 [$\text{M} + \text{Na}]^+$ (calcd for C₂₁H₃₀O₃Na, 353.2092).

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Supporting Information Available: ¹H and ¹³C 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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