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Biologically Active Cannabinoids from High-Potency *Cannabis sativa*

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Abstract

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*-cannabicoumarononic 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 C_{21} 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*-cannabicoumarononic acid A (**4**), two cannabigerol derivatives (**5** and **6**), two cannabinol derivatives (**7** and **8**), and a C_{21} 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.

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

Results and Discussion

Compound **1** was isolated as an optically inactive yellow oil. Its molecular formula was determined to be $C_{23}H_{32}O_4$ from GC-MS (*m*/*z* 372, $[M]^+$) and HRESIMS (*m*/*z* 373.2409, $[M + H]^+$), indicating eight degrees of unsaturation. The ^{1}H 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, H_3-5″), 1.66 (s, H_3-6″)], a tertiary methyl [δ_H 1.33 (s, H_3-9)], and an acetoxy methyl resonance [δ_H 2.29 (s, OCO*CH*$_3$)]. The small coupling constant between vicinal protons H-7 and H-8 (10.0 Hz) indicated a *cis* double bond.[11] The ^{13}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 (δ_C 169.7), three oxyaryl (δ_C 131.3, 145.3, 148.8), and one oxygenated sp^3 carbon (δ_C 79.1, C-6). The ^{1}H and ^{13}C 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 $C_{21}H_{30}O_3$ (seven degrees of unsaturation). The UV and IR spectra of **2** exhibited patterns similar to those of cannabichromene.[12–14] The ^{1}H 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 ^{1}H, ^{13}C, 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 (H_2-5″/C-6″, C-4″, C-3″; H_3-6″/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 $C_{21}H_{32}O_3$ from its HRESIMS $[M - H]^-$ ion at *m*/*z* 331.2254, indicating six degrees of unsaturation. The ^{13}C, DEPT, and HMQC NMR spectra revealed 21 carbons (Table 1), including four methyl, seven methylene, four methine, and six quaternary resonances. The ^{1}H and ^{13}C 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; H_3-9/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 $C_{22}H_{28}O_5$ 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, ^{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 cannabicoumaronone,[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, $[M]^+$), and by the ^{13}C NMR carbonyl resonance at δ_C 170.6. The ROESY correlation between H-7 (δ_H 2.89) and pseudoequatorial H_3-10 (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 cannabichromanone 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 cannabichromanone derivatives that have H-7β configurations.[5a] Thus, the structure of **4** was established as (−)-7*R*-cannabicoumarononic acid A.

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; OCO*CH*$_3$/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).

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 F_{254} (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_{18} 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) 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 C_{18} 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_{18} 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 μA. 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 × 60 L), CH_2Cl_2 (48 L), EtOAc (40 L), EtOH (37.5 L), EtOH/H_2O (36 L, 1:1), and H_2O (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/H_2O (0.77 kg), and H_2O (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 C_6 (565 mg) was further chromatographed over a C_{18} SPE column (10 g), eluting with MeOH/H_2O (75:25), to afford **4** (170 mg), **9** (13.1 mg), and **7** (6.6 mg). Subfraction C_9 (3.2 g) was chromatographed over Sephadex LH-20 eluting with MeOH followed by C_{18} HPLC purification using MeCN/H_2O (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 μg) were treated with pyridine (5 μL, silylation grade, Pierce) and BSTFA [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide] (100 μL, 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-Acetoxycannabichromene (1): yellow oil; UV (MeOH) λ_{max} 227, 280 nm; IR (neat) ν_{max} 3415, 2930, 1735 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 372 $[M]^+$ (11), 357 (9), 331 (90), 289 (100), 247 (85), 190 (17), 69 (8), 43 (8); HRESIMS *m*/*z* 373.2409 $[M + H]^+$ (calcd for $C_{23}H_{33}O_4$, 373.2380).

(±)-3″-Hydroxy-Δ$^{(4″,5″)}$-cannabichromene (2): brown oil; UV (MeOH) λ_{max} 227, 280 nm; IR (neat) ν_{max} 3405, 3310, 2920, 1590 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 330 $[M]^+$ (3), 312 (5), 231 (100), 187 (5), 174 (16); HRESIMS *m*/*z* 331.2193 $[M + H]^+$ (calcd for $C_{21}H_{31}O_3$, 331.2273).

(−)-7-Hydroxycannabichromane (3): pale yellow oil; $[\alpha]^{25}_{D}$ −66.2 (*c* 0.15, MeOH); UV (MeOH) λ_{max} 227, 252 nm; IR (neat) ν_{max} 3410, 3310, 2920, 1590 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 332 $[M]^+$ (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 $[M - H]^-$ (calcd for $C_{21}H_{31}O_3$, 331.2273).

(−)-7*R*-Cannabicoumarononic acid A (4): brown oil; $[\alpha]^{25}_{D}$ −15.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} 225, 280 nm; IR (neat) ν_{max} 2910, 1716, 1700, 1640, 1570 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 372 $[M]^+$ (15), 354 (8), 329 (10), 311 (100), 297 (8), 284 (14), 258 (20), 213 (9); HRESIMS *m*/*z* 395.1847 $[M + Na]^+$ (calcd for $C_{22}H_{28}O_5Na$, 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}; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m*/*z* 374 $[M]^+$ (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 $[M + H]^+$ (calcd for $C_{23}H_{35}O_4$, 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}; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m*/*z* 374 $[M]^+$ (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 $[M + H]^+$ (calcd for $C_{23}H_{35}O_4$, 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}; ^{1}H and ^{13}C NMR, see Table 3; EIMS *m*/*z* 326 $[M]^+$ (25), 311 (100), 254 (20), 239 (18); HRESIMS *m*/*z* 349.1781 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_3Na$, 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}; ^{1}H and ^{13}C NMR, see Table 3; EIMS (decarboxylated compound) *m*/*z* 326 $[M]^+$ (25), 311 (100), 254 (20), 239 (18); HRESIMS *m*/*z* 369.1731 $[M - H]^-$ (calcd for $C_{22}H_{25}O_5$, 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}; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m*/*z* 330 $[M]^+$ (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 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_3Na$, 353.2092).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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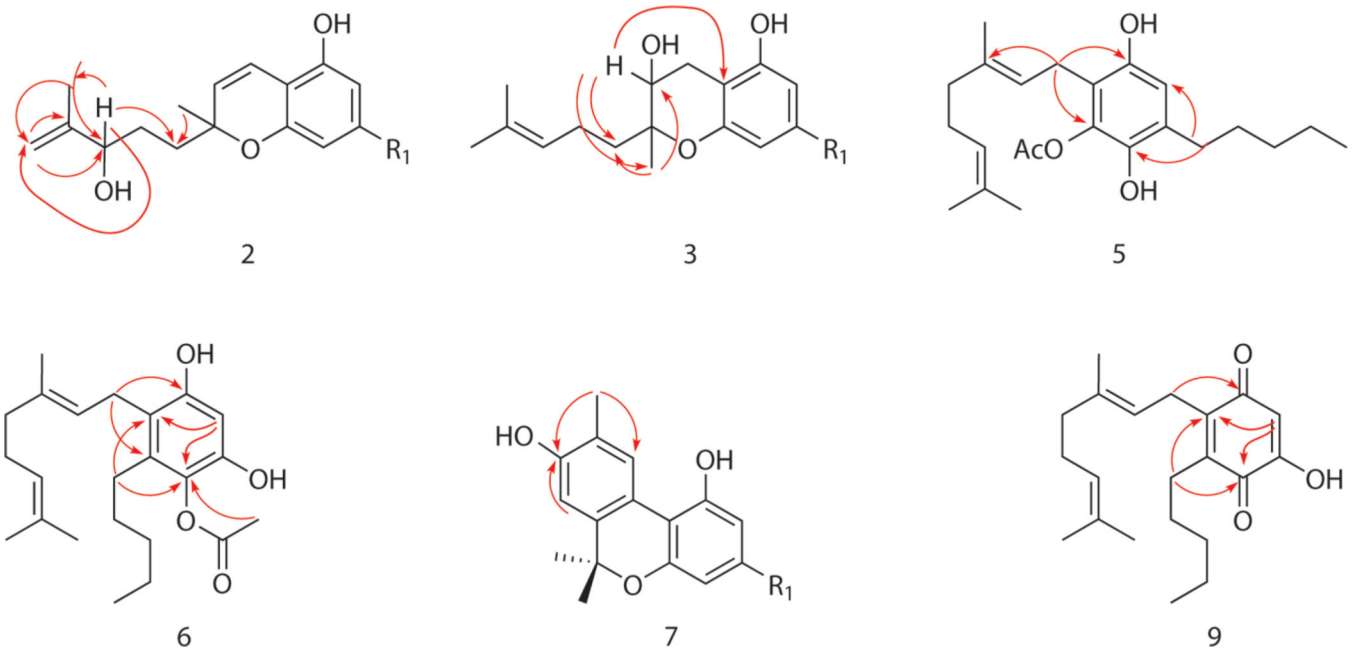


Figure 1.
Key HMBC correlations for **2**, **3**, **5**, **6**, **7**, and **9**.

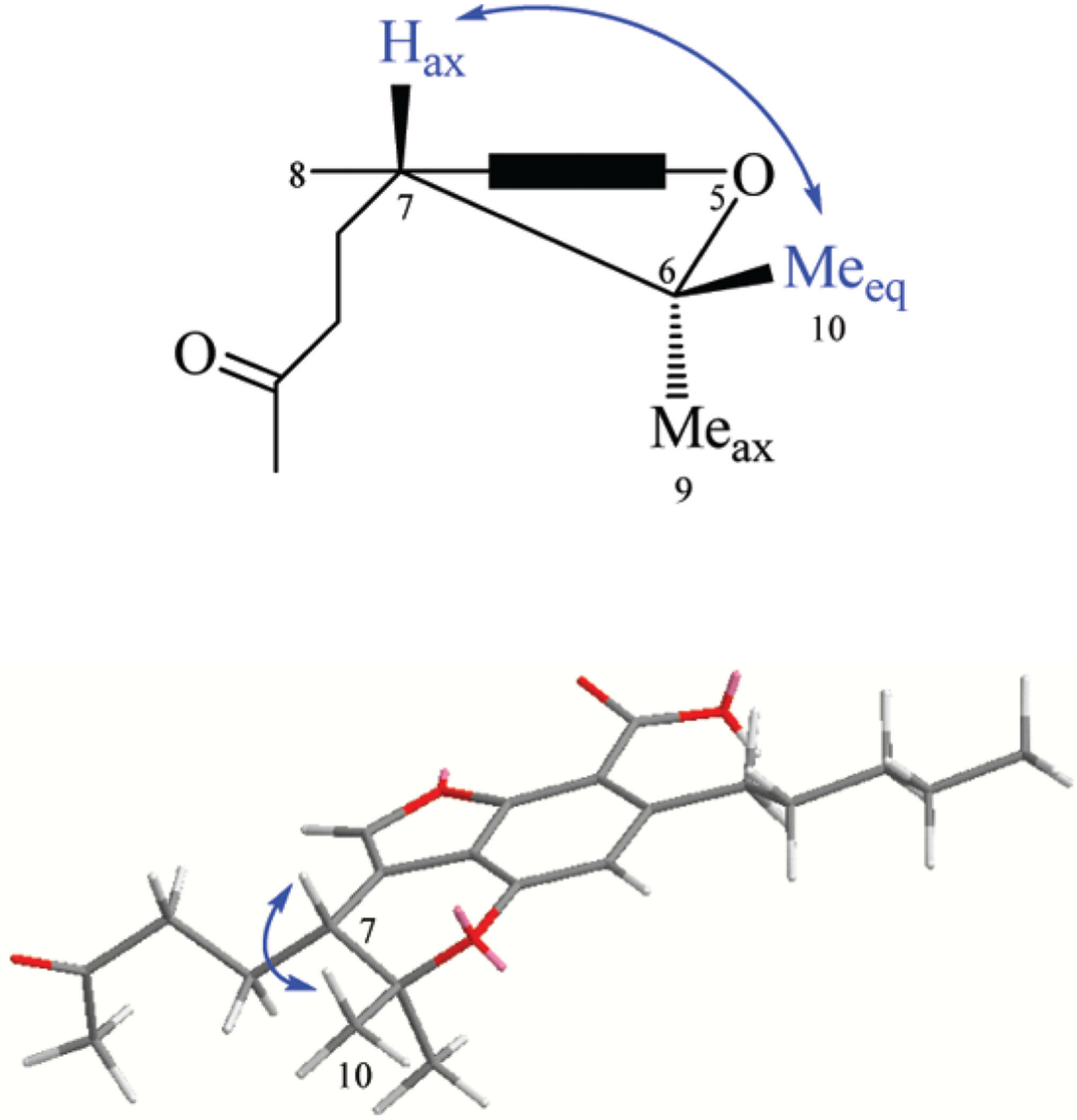
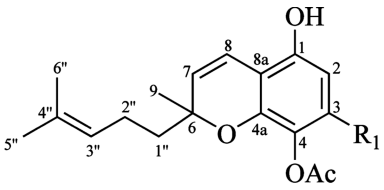


Figure 2.
Key ROESY correlation between H-7 and pseudoequatorial H_3-10 of **4**.



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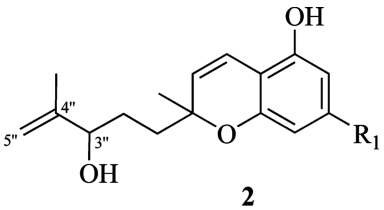

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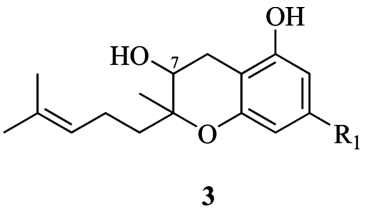

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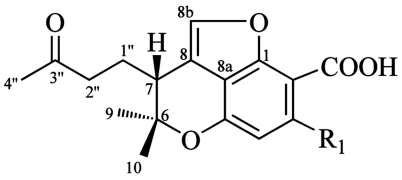

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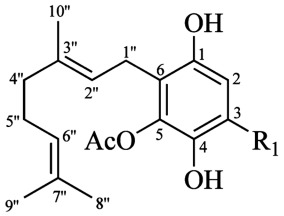

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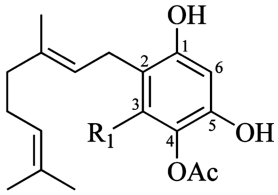

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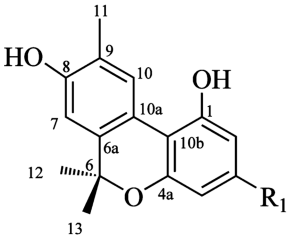

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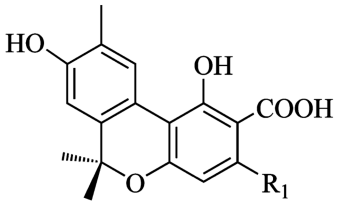

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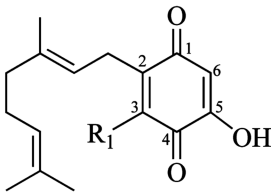

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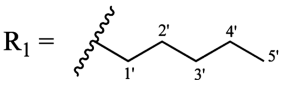

Chart 1.

Table 1

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

	1		**2**		**3**		**4**	
carbon	**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		
OCO*CH*$_3$	20.7	2.29, s						
O*C*OCH$_3$	169.7							

	1		2		3		4	
carbon	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)
*C*OOH							170.6	

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

Table 2

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data of **5, 6**, and **9** ($CDCl_3$)[a]

	5		**6**		**9**	
carbon	**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	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
OCO*C*H$_3$	20.8	2.33, s	20.8	2.28, s		
O*C*OCH$_3$	170.1		170.0			

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

Table 3

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data of **7** and **8** ($CDCl_3$)[a]

	7		8	
carbon	**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)
*C*OOH			176.0	
1-OH				12.6

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

Table 4

In Vitro Antimicrobial Activities of **2, 5, 6, 7**, and **8** (IC_{50} in μM)[a]

	antifungal		antibacterial			
compound	***C. albicans***	***C. krusei***	***MRSa***	***S. aureus***	***E. coli***	***M. intracellulare***
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]

	antileishmanial		antimalarial	
	L. donovani		*P. falciparum*	
compound	**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.