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Minor oxygenated cannabinoids from high potency *Cannabis sativa* L.

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ABSTRACT

Nine oxygenated cannabinoids were isolated from a high potency *Cannabis sativa* L. variety. Structure elucidation was achieved using spectroscopic techniques, including 1D and 2D NMR, HRMS and GC–MS. These minor compounds include four hexahydrocannabinols, four tetrahydrocannabinols, and one hydroxylated cannabinol, namely 9α-hydroxyhexahydrocannabinol, 7-oxo-9α-hydroxyhexa-hydrocannabinol, 10α-hydroxyhexahydrocannabinol, 10aR-hydroxyhexahydrocannabinol, Δ^9-THC aldehyde A, 8-oxo-Δ^9-THC, 10aα-hydroxy-10-oxo-Δ^8-THC, 9α-hydroxy-10-oxo-$\Delta^{6a,10a}$-THC, and 1′*S*-hydroxy-cannabinol, respectively. The latter compound showed moderate anti-MRSa (IC_{50} 10.0 μg/mL), moderate antileishmanial (IC_{50} 14.0 μg/mL) and mild antimalarial activity against *Plasmodium falciparum* (D6 clone) and *P. falciparum* (W2 clone) with IC_{50} values of 3.4 and 2.3 μg/mL, respectively.

1. Introduction

Cannabinoids are the most distinctive and specific class of compounds known to exist only in the cannabis plant, which are responsible for the majority of the biological activities of the cannabis plant. The best-known and the most specific class of cannabis constituents is the C21 terpenophenolic cannabinoids, with (−)-Δ^9-trans-(6a*R*, 10a*R*)-tetrahydrocannabinol (Δ^9-THC) being the most psychologically active constituent (Mechoulam and Gaoni, 1967a,b). Although several subclasses of cannabinoids have been identified, the skeletons of these subclasses do not differ greatly from one another. Modification of the structures are limited to changes in the side-chain and the terpenoid portion of the molecule (ElSohly and Slade, 2005). The total number of natural cannabinoids identified in *C. sativa* L. was 66 in 1995, 70 in 2005 and 105 in 2014 (Ahmed et al., 2008a,b; Appendino et al., 2008; Radwan et al., 2008a,b, 2009; ElSohly and Slade, 2005; ElSohly and Gul, 2014; Ross and ElSohly, 1995).

In efforts to study the chemistry of high potency cannabis, a variety of new constituents were isolated (Radwan et al., 2008a,b, 2009; Ahmed et al., 2008a,b). Herein reported are the isolation and structure elucidation of nine new oxygenated cannabinoids (**1**–**9**) namely, 9α-hydroxyhexahydrocannabinol (**1**), 7-oxo-9α-hydroxy-hexahydrocannabinol (**2**), 10α-hydroxyhexa-hydrocannabinol (**3**), 10a*R*-hydroxyhexa-hydrocannabinol (**4**), Δ^9-THC aldehyde A (**5**), 8-oxo-Δ^9-THC (**6**), 10aR-hydroxy-10-oxo-Δ^8-THC (**7**), 9α-hydroxy-10-oxo-$\Delta^{6a,10a}$-THC (**8**), and 1′*S*-hydroxycannabinol (**9**) along with other previously identified constituents.

2. Results and discussions

Compound **1** was obtained as a yellow oil and its molecular formula was determined to be $C_{21}H_{32}O_3$ from GC–MS (*m*/*z* 332 at Rt 12.23 min) and HRESIMS (*m*/*z* 333.2495 $[M+H]^+$), representing six degrees of unsaturation The ^{13}C NMR spectrum showed signals indicating four methyl, seven methylene, four methine and six quaternary carbons [two oxyaryl (C-1, C-4a), two oxygenated sp^3 (C-6, C-9) and two aryl sp^2 (C-3, C-10b) carbons]. Comparing the ^1H and ^{13}C NMR spectroscopic data of **1** (Tables 1 and 2) with

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Table 1
^{1}H NMR spectroscopic data (400 MHz, $CDCl_3$) for compounds (**1**–**9**).

No.	1	2	3	4	5	6	7	8	9
2	6.20 s	6.20 s	6.20	6.24 s		6.23 s	6.34 s	6.32 s	6.55s
4	6.22 s	6.4s	6.22	6.25 s	6.18 s	6.27 s	6.47 s	6.47	6.46
6a	1.43	1.92 (d, *J* = 8.0)	1.66 m	1.81	1.88	2.2	2.38		
7	1.40		1.51 m	1.40	1.40	2.69	2.70	2.62	7.10 (d, *J* = 7.6)
	1.70		1.79 m	1.92	1.92		2.34		
8	1.90	2.10 s	2.75 m	1.77	2.15		6.89 br. S	2.13	7.13 (d, *J* = 7.6)
9			1.83 m	1.38					
10	1.90	1.85	3.42	2.01	6.41 s	7.83 s			8.27 s
10a	1.54	3.52	1.66 m		3.22	3.52			
11	1.28 s	1.41s	0.88 (d, *J* = 6.8)	0.87	1.67 s	1.81 s		1.86 s	2.36 s
12	1.35 s	1.40 s	1.21 s	1.31 s	1.10 s	1.14 s		1.33 s	1.56 s
13	1.00 s	1.50 s	1.21 s	1.35 s	1.43 s	1.36 s		1.40 s	1.59 s
1′	2.40 (t, *J* = 7.4)	2.42 (t, *J* = 7.4)	2.40 (t, *J* = 7.4)	2.45 (t, *J* = 7.4)	2.30 (t, *J* = 7.4)	2.43 (t, *J* = 7.4)	2.47 (t, *J* = 7.4)	2.47 (t, *J* = 7.4)	4.59 (t, *J* = 6.8)
2′	1.56	1.55	1.56	1.58	1.57	1.54	1.57	1.57	1.65
3′	1.30	1.30	1.29	1.31	1.33	1.29	1.29	1.27	1.29
4′	1.30	1.30	1.29	1.31	1.33	1.29	1.29	1.27	1.29
5′	0.86 (t, *J* = 6.8)	0.87 (t, *J* = 6.8)	0.85 (t, *J* = 6.8)	0.87 (t, *J* = 6.4)	0.87 (t, *J* = 6.4)	0.86 (t, *J* = 6.4)	0.87 (t, *J* = 6.6)	0.87 (t, *J* = 6.4)	0.87 (t, *J* = 6.4)
CHO					10.01 s				

Table 2
^{13}C NMR spectroscopic data (400 MHz, $CDCl_3$) for compounds (**1**–**9**).

No.	1	2	3	4	5	6	7	8	9
1	154.8	153.7	153.9	154.3	161.3	154.9	154.6	153.6	154.8
2	108.6	108.3	106.9	107.8	102.6	108.1	112.7	110.0	107.3
3	142.9	146.1	142.7	143.6	147.0	144.0	146.6	146.7	146.0
4	110.1	110.8	109.2	107.9	111.5	110.3	112.2	113.5	108.2
4a	155.1	154.7	154.2	157.1	158.2	154.6	155.4	153.5	154.5
6	77.0	77.4	76.2	74.5	79.6	76.2	77.3	77.5	77.3
6a	49.4	56.4	46.9	51.8	45.5	47.5	50.0	163.3	137.0
7	24.2	213.7	17.9	22.5	25.1	41.3	24.8	25.7	122.7
8	39.4	39.6	28.3	38.4	31.4	199.9	146.2	33.4	127.7
9	71.2	75.2	27.5	27.8	134.3	134.8	133.1	73.4	137.0
10	42.3	19.0	78.5	40.0	123.2	150.5	199.3	206.0	128.0
10a	30.6	35.3	46.9	74.3	33.3	35.1	72.2	124.6	127.2
10b	110.0	110.8	110.0	110.0	110.0	106.3	109.7	106.1	110.4
11	31.9	25.0	19.9	24.3	23.5	16.1	16.7	25.0	21.8
12	19.2	27.4	19.9	27.3	19.9	19.5	20.5	22.3	27.4
13	27.8	27.1	14.5	28.9	27.6	27.1	28.3	25.2	27.4
1′	35.5	36.0	36.0	36.0	36.3	35.8	35.8	35.7	74.7
2′	31.8	30.6	31.1	30.9	31.2	30.9	30.5	30.5	38.4
3′	30.9	31.7	31.7	31.9	32.1	31.7	31.7	31.7	28.1
4′	22.7	22.7	22.7	22.8	22.7	22.8	22.7	22.8	22.8
5′	14.2	14.2	14.3	14.3	14.2	14.3	14.2	14.3	14.3
CHO					193.0				

Δ^9-THC indicated that **1** is a hexahydrocannabinol derivative. Significant differences between **1** and Δ^9-THC were observed in the NMR spectra. This included the absence of the olefinic carbon resonances at δ_C 134.6 (C-9), and δ_C 123.6 (C-10) in the carbon spectrum, the lack of a broad olefinic resonance at δ_H 6.41 (1H, s, H-10) in the proton spectrum, and the appearance of an oxygenated sp^3 carbon at δ_C 71.2 (C-9) and a methylene carbon at δ_C 42.3 (C-10) in the carbon spectrum of 1. Oxygenation of C-9 led to changes in the chemical shifts of the nearest methyl protons of carbon C-11 from δ_H 1.67 (3H, s) to δ_H 1.28 s (3H, s). This assumption was supported by the ^{1}H–^{1}H COSY correlations of H-9 with H-10 and H-10 with H-10a and HMBC correlations of H-10a with C-9 and H_3-11 with C-9 (Fig. 1). The molecular formula, degrees of unsaturation and 2D NMR spectroscopic analysis (Fig. 1), pointed towards a presence of free hydroxyl group at C-9, which was supported by the presence of hydroxyl absorption band in IR spectrum at ν_{max} 3460 cm^{-1}. The 9α-hydroxyhexahydrocannabinol configuration assignment was supported by ROESY correlations of H_3-13, H-6a and H_3-11 (Fig. 1).

Compound **2** was obtained as a yellow oil and its molecular formula was determined to be $C_{21}H_{30}O_4$ by HRESIMS (*m*/*z* 347.2235 [M+H]$^+$), representing seven degrees of unsaturation. The ^{13}C NMR spectrum showed signals for four methyl, six methylene, four methine and seven quaternary carbons [two oxyaryl (C-1, C-4a), two oxygenated sp^3 (C-6, C-9) and two aryl sp2 (C-3, C-10b) and one carbonyl (C-7)]. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) with **1** and Δ^9-THC indicated that compound **2** belongs to the hexahydrocannabinol series. Significant differences between **2** and **1** were observed in the NMR spectra in which a carbonyl carbon appears at δ_C 213.7 in the spectrum of **2** instead of a methylene carbon in **1**. HMBC correlations of H_2-8/C-7 ($^2J_{CH}$), H-10a/C-7 ($^3J_{CH}$), H-12/C-6a ($^3J_{CH}$) and H-13/C-6a ($^3J_{CH}$) support that the oxo substitution existed on C-7 (Fig. 1). The 9α-hydroxyhexahydrocannabinol configuration assignment was supported by the ROESY correlations of H_3-13, H-6a and H_3-11 (Fig. 1).

Compound **3** was obtained as a yellow oil and its molecular formula was determined to be $C_{21}H_{30}O_3$ by GC–MS (*m*/*z* 332 at Rt 13.46 min) and HRESIMS (*m*/*z* 333.2413 [M+H]$^+$), representing six degrees of unsaturation. The NMR spectra was similar to those of Δ^9-THC except for the disappearance of olefin carbon resonances at δ_C 134.6 (C-9) and δ_C 123.6 (C-10), as well as a broad olefinic resonance at δ_C 6.41 (1H, s, H-10) and the appearance of a sp3 methine and oxygenated methane at δ_C 28.3 (C-9) and δ_C 78.5