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Chemical characterisation of oxidative degradation products of Δ^9-THC

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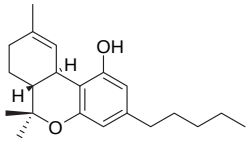
ABSTRACT

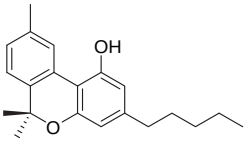
The chemical analysis of a sample of Δ^9-THC, which had been stored in an ethanol/propylene glycol solution for 5 years, resulted in the isolation of several hydroxylated Δ^9-THC derivatives, the main of which were *trans*-cannabitriol monoethyl ether (**4**) and *trans*-propanediol ethers **7** and **8**. *cis*-Cannabitriol monoethyl ether (**5**) and the oxidised derivatives **3** and **6** were detected in lesser amounts. The structure elucidation of the unprecedented cannabinoids **3**, **5**, **7** and **8** was achieved mainly by NMR techniques. Full NMR assignment of compounds **4** and **6** were also made. The detection of cannabitriol (**6**) and the corresponding solvent-adduct analogues (compounds **4**–**8**) was in agreement with the decomposition mechanisms previously proposed for Δ^9-THC. The isolation of the endoperoxide **3** represents indirect evidence of the existence of unstable precursors that were suspected to be intermediates in the non-enzymatic oxidation pathway of Δ^9-THC. Both isomers of cannabitriol monoethyl ether exhibited weak affinity at either CB_1 (K_i=2.25, 6.30 μM) or CB_2 cannabinoid receptors (K_i=1.97, 3.13 μM), the *trans* isomer always being more potent than the *cis* isomer.

1. Introduction

It has been well established that *Cannabis* based preparations lose potency under storage because of a decrease in the content of Δ^9-tetrahydrocannabinol (Δ^9-THC) (**1**), the major psychoactive principle of marijuana (*Cannabis sativa*).[1–4] This metabolite decomposes upon storage to a less psychoactive compound, cannabinol (CBN) (**2**), which is the final product of a degradation process that seems to be accomplished primarily by heat and light.[1,2,4,5] It has been suggested that the conversion of Δ^9-THC to CBN should proceed through the formation of more or less stable hydroxylated intermediates,[2,4] some of which have never been detected. An alternative free radical oxidation mechanism involving the formation of dienes and dienylic radicals has been also suggested.[6] In any case, even though the conversion of Δ^9-THC (**1**) to CBN (**2**) is considered the main pathway contributing to the deactivation of *Cannabis* samples, the possibility of the occurrence of additional oxidation mechanisms could not be ruled out.



Δ^9-THC (**1**)



CBN (**2**)

With the aim of identifying the minor hydroxylated Δ^9-THC derivatives formed along with the expected CBN in aged samples of the drug, we have analysed a Δ^9-THC extract sample, which had been stored in an ethanol/propylene glycol (50:50) solution for five years. In particular, some selected fractions containing THC-related molecules were obtained by flash chromatographic purification of the aged sample.

Six hydroxylated cannabinoids (compounds **3**–**8**), including the previously reported *trans*-cannabitriol monoethyl ether (**4**)[7] and *trans*-cannabitriol (**6**)[7–9] were purified and fully characterised. *trans*-Cannabitriol monoethyl ether (**4**) was recovered as the main hydroxylated metabolite formed under the storage conditions used, but significant amounts of the corresponding propanediol ethers **7** and **8** were also found. On the other hand, *cis*-cannabitriol monoethyl ether (**5**), *trans*-cannabitriol (**6**) and peroxide **3** were present in small amounts. In this paper, we describe the structure elucidation of the

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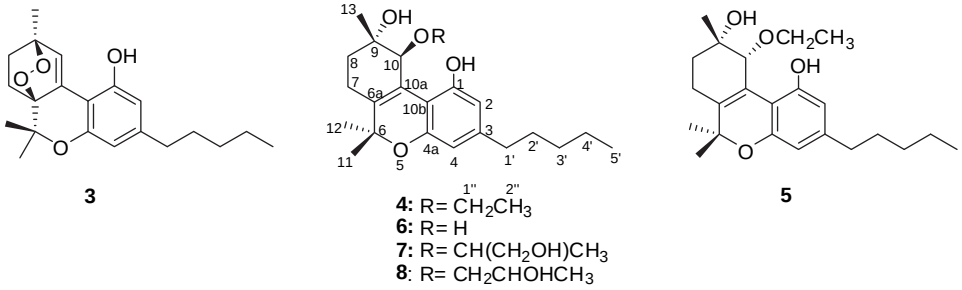
unprecedented cannabinoids **3**, **5**, **7** and **8** and the full NMR assignment of derivatives **4** and **6**, the chemical characterisation of which has been reported only partially in the literature.[7,8] The oxidation pattern of THC (**1**) when stored in alcoholic solutions is also discussed in the light of the oxidised derivatives identified in this work. Finally, we evaluated the binding affinity of both *trans* and *cis* isomers of cannabitriol monoethyl ether at cannabinoid CB_1 and CB_2 receptors.



2. Results and discussion

Five selected fractions (AS2, AS3, AS4, AS6 and AS7) obtained from a flash chromatographic purification of an aged sample of Δ^9-THC extract in ethanol/propylene glycol (50:50) were submitted to preliminary ^{1}H NMR analysis that revealed the presence in all fractions of hydroxylated cannabinoid derivatives. With the exception of fraction AS3, which was constituted of pure *trans*-cannabitriol monoethyl ether (**4**), the other four fractions were further purified by reverse-phase HPLC, as reported in the Experimental section, to give cannabinoid derivatives **3** and **5**–**8**.

The ^{1}H NMR spectrum of fraction AS2 (see Fig. 1a) suggested that it was constituted of a main THC-related component that was highly unstable. In fact, a complete transformation of this molecule rapidly occurred in the NMR tube (see Fig. 1b), thus preventing the measurement of further NMR experiments. Two different ^{1}H NMR spectra were recorded from the same sample at t_0 and t_1 strongly indicating that the Δ^9-THC-related compound underwent a very rapid chemical conversion. We decided to purify the transformed product **3** by HPLC and subsequently to fully characterize it with the aim of tracing the possible precursor.

Compound **3** had the molecular formula $C_{21}H_{28}O_4$ as deduced from the HRESIMS sodiated molecular peak at *m*/*z* 367.1892 $[M+Na]^+$, thus implying two additional oxygen atoms and one more degree of unsaturation with respect to Δ^9-THC (**1**). Analysis of the ^{1}H and ^{13}C NMR data of **3** revealed the presence of structural features the same as Δ^9-THC such as the phenolic ring C bearing at C-3 the 5-carbon alkyl chain that was fused to an oxirane cycle at C-4a/C-10b, whereas in the A ring the trisubstituted double bond was shifted at C-10/C-10a. In addition, in the terpene ring A of **3**, oxygen atoms linked to both C-9 and C-6a were present. Accordingly, the ^{1}H NMR spectrum of **3** displayed a 1H singlet at δ 7.03 correlating with a carbon at δ 128.4 in the HSQC spectrum, which was attributed to the isolated olefinic proton H-10, while the 3H singlet at δ 1.45 (H_3-13) was assigned to a tertiary methyl linked to an oxygenated carbon. The HMBC spectrum of **3** revealed the presence of signals at δ 73.2 (C-9) and δ 77.6 (C-6a), which were due to two additional oxygenated quaternary carbons with respect to Δ^9-THC, in addition to the expected resonance of C-6 (δ 75.5).

Thus, taking into account the required additional unsaturation, a peroxide moiety was introduced between C-9 and C-6a to satisfy the molecular formula of compound **3**.

The proposed structure was consistent with all NMR data. The interpretation of 2D NMR experiments and, in particular, of the HMBC spectrum of **3** allowed the complete assignment as reported in Tables 1 and 2. The absolute stereochemistry of the peroxide bridge was, however, undetermined, and thus the configuration depicted in formula **3** is arbitrary.

Having assessed the structure of **3**, we re-analysed the ^{1}H NMR spectrum (Fig. 1a) of the unstable precursor **9** detected in the original fraction AS2 in comparison with that of compound **3**. A diagnostic shift was observed for two selected signals: the broad 3H singlet at δ 1.91, attributed to vinyl methyl at C-9, and the 1H singlet at δ 6.42 due to the olefinic proton H-10. This suggested that the unstable metabolite **9** could have a structure exhibiting a $\Delta^{9,10a}$ diene system, which easily undergoes a Diels–Alder cycloaddition by atmospheric O_2 to give the peroxide adduct **3** (Scheme 1). The intermediate **9** could in turn derive by dehydration from a hypothetical hydroxyl derivative **10** formed by allylic oxidation of Δ^9-THC (**1**) (Scheme 1). The existence of the precursor **10** was suggested by the LC-MS profile of the original fraction AS2 showing a peak at *m*/*z* 330, which was consistent with the molecular weight of compound **10**.

Compound **4** was identified as *trans*-cannabitriol monoethyl ether by interpretation of the spectroscopic data. This cannabinoid derivative was previously isolated from ethanolic extracts of *Cannabis*[7] and its structure was deduced by X-ray analysis of the corresponding hydroxyl derivative *trans*-cannabitriol.[9] However, compound **4** has never been characterized by NMR spectroscopy and no spectroscopic data have been published. The main differences with Δ^9-THC were in the substitution pattern in the terpene ring A, containing a tetrasubstituted double bond and two oxygenated functions. In the present work, a detailed 1D and 2D NMR

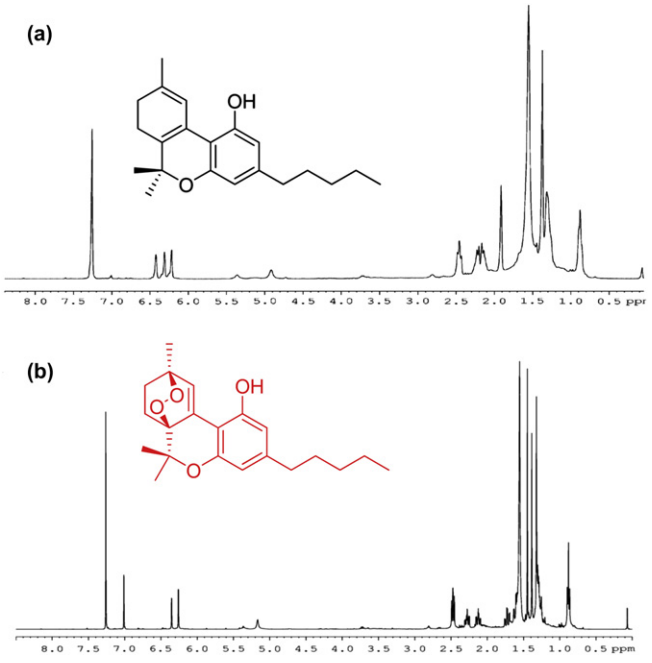


Fig. 1. (a) ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of fraction AS2 recorded at t_0. (b) ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of fraction AS2 recorded at t_1.

Table 1
^{1}H NMR data[a,b] for compounds **3**–**8**

C	**3** δ_H (m, *J* Hz)	**4** δ_H (m, *J* Hz)	**5** δ_H (m, *J* Hz)	**6** δ_H (m, *J* Hz)	**7** δ_H (m, *J* Hz)	**8** δ_H (m, *J* Hz)
2	6.42 (s)	6.35 (s)	6.36 (d, l)	6.35 (s)	6.32 (s)	6.32 (s)
4	6.31 (s)	6.28 (s)	6.28 (d, l)	6.30 (s)	6.30(s)	6.30(s)
7	2.25 (m)	2.47 (m)	2.31 (m)	2.43 (m)	2.40 (m)	2.40 (m)
	1.64 (m)	2.19 (dd, 19,7)		2.17 (m)	2.25 (dd, 19,7)	2.30 (m)
8	2.16 (m)	1.89 (dd, 14,7)	1.82 (m)	1.78 (m)	1.90 (m)	1.90 (m)
	1.74 (m)	1.74 (m)			1.83 (m)	1.85 (m)
10	7.03 (s)	4.29 (s)	4.31 (s)	4.20 (b s)	4.67 (s)	4.46 (s)
11	1.31 (s)	1.29 (s)	1.29 (s)	1.24 (s)	1.35 (s)	1.32 (s)
12	1.37 (s)	1.50 (s)	1.45 (s)	1.46 (s)	1.43 (s)	1.43 (s)
13	1.45 (s)	1.42 (s)	1.19 (s)	1.41 (s)	1.41 (s)	1.41 (s)
1′	2.47 (q, 8)	2.46 (app.t, 7)	2.47 (app.t, 7)	2.47 (q, 8)	2.46 (t, 8)	2.46 (t, 8)
2′	1.58 (m)	1.58 (m)	1.56 (m)	1.60 (m)	1.60 (m)	1.60 (m)
3′	1.31 (m)	1.31 (m)	1.31 (m)	1.31 (m)	1.32 (m)	1.30 (m)
4′	1.28 (m)	1.31 (m)	1.31 (m)	1.28 (m)	1.30 (m)	1.32 (m)
5′	0.88 (t, 7)	0.88 (t, 7)	0.88 (t, 7)	0.88 (t, 7)	0.88 (t, 7)	0.88 (t, 7)
1″	—	3.56 (m)	3.74 (m)		3.57 (dd, 11,4)	3.58 (dd, 9,3)
					3.50 (dd, 11,6)	3.38 (app. t, 9)
2″	—	1.22 (t, 7)	1.27 (t, 7)		3.98 (m)	3.99 (m)
3″	—				1.13 (d, 6)	1.12 (d, 6)
−OH		9.60 (s)				

[a] The spectra were recorded in $CDCl_3$ at 400 MHz.
[b] Assignments made by ^{1}H-^{1}H COSY, HSQC and HMBC (*J*=10 Hz) experiments.

Table 2
^{13}C NMR data[a] for compounds **3**–**8**

C	**3** δ_C (*m*)[b]	**4** δ_C (*m*)[b]	**5** δ_C (*m*)[b]	**6** δ_C (*m*)[b]	**7** δ_C (*m*)[b]	**8** δ_C(*m*)[b]
1	153.3 (s)	153.3[c] (s)	153.2[c] (s)	153.2 (s)	n.a.	152.7(s)
2	111.1 (d)	111.2 (d)	111.1 (d)	110.2 (d)	110.8 (d)	111.2 (d)
3	144.9 (s)	145.0 (s)	145.1 (s)	144.8 (s)	144.9 (s)	144.9 (s)
4	109.7 (d)	108.5 (d)	108.7 (d)	107.3 (d)	109.5 (d)	109.2 (d)
4a	153.3 (s)	153.5[c] (s)	153.4[c] (s)	153.2 (s)	n.a.	152.7 (s)
6	75.5 (s)	76.3 (s)	76.6 (s)	77.2 (s)	76.8 (s)	76.6 (s)
6a	77.6 (s)	137.0 (s)	137.2 (s)	137.6 (s)	136.8 (s)	137.1 (s)
7	23.7 (t)	22.1 (t)	24.9 (t)	22.3 (t)	22.5 (t)	22.8 (t)
8	30.0 (t)	30.9 (t)	31.6 (t)	29.9 (t)	30.5 (t)	31.6 (t)
9	73.2 (s)	70.2 (s)	70.6 (s)	72.2 (s)	70.1 (s)	71.2 (s)
10	128.4 (d)	77.3 (d)	77.4 (d)	71.9 (d)	76.3 (d)	77.8 (d)
10a	134.2 (s)	118.3 (s)	119.5 (s)	120.2 (s)	118.6 (s)	118.9 (s)
10b	107.3 (s)	108.4 (s)	108.4 (s)	108.7 (s)	108.3 (s)	108.4 (s)
11	22.1 (q)	23.7 (q)	23.6 (q)	23.5 (q)	23.8 (q)	24.2 (q)
12	24.0 (q)	25.4 (q)	25.4 (q)	25.0 (q)	25.5 (q)	25.4 (q)
13	25.7 (q)	26.4 (q)	24.8 (q)	25.2 (q)	25.8 (q)	24.8 (q)
1′	35.6 (t)	35.6 (t)	35.6 (t)	35.5 (t)	35.6 (t)	35.5 (t)
2′	30.4(t)	30.4 (t)	30.4 (t)	30.5 (t)	30.4 (t)	30.4 (t)
3′	31.4 (t)	31.5 (t)	31.5 (t)	31.3 (t)	32.2 (t)	31.5 (t)
4′	21.8 (t)	22.5 (t)	22.5 (t)	23.7 (t)	22.3 (t)	22.4 (t)
5′	14.0 (q)	14.0 (q)	14.0 (q)	14.0 (q)	14.0 (q)	14.0 (q)
1″		60.0 (t)	62.3 (t)		67.2 (t)	71.6 (t)
2″		15.4 (s)	15.5 (s)		72.1 (d)	66.8 (d)
3″					18.5 (q)	18.7 (q)

[a] The spectra were recorded in $CDCl_3$ at 300 MHz.
[b] By DEPT sequence.
[c] May be interchanged.

analysis has been carried out with the aim of making a complete proton and carbon assignment as reported in Table 2.

The ^{13}C NMR spectrum contained signals assigned to two quaternary sp^2 carbons at δ 137.0 (C-6a) and 118.3 (C-10a), and to a tertiary and a quaternary sp^3 carbons both bearing oxygen, which resonated at δ 77.3 (C-10) and 70.2 (C-9), respectively. The ^{1}H NMR spectrum displayed a 3H triplet at δ 1.22 (H_3-2″) coupled with a methylene at δ 3.56 (H_2-1″), which was easily attributed to an ethoxy group, a 1H singlet at δ 4.29 (H-10) attributable to an isolated oxygenated methine, and a 3H singlet at δ 1.42 (H_3-13) assigned to a tertiary methyl linked to a carbon bearing oxygen.

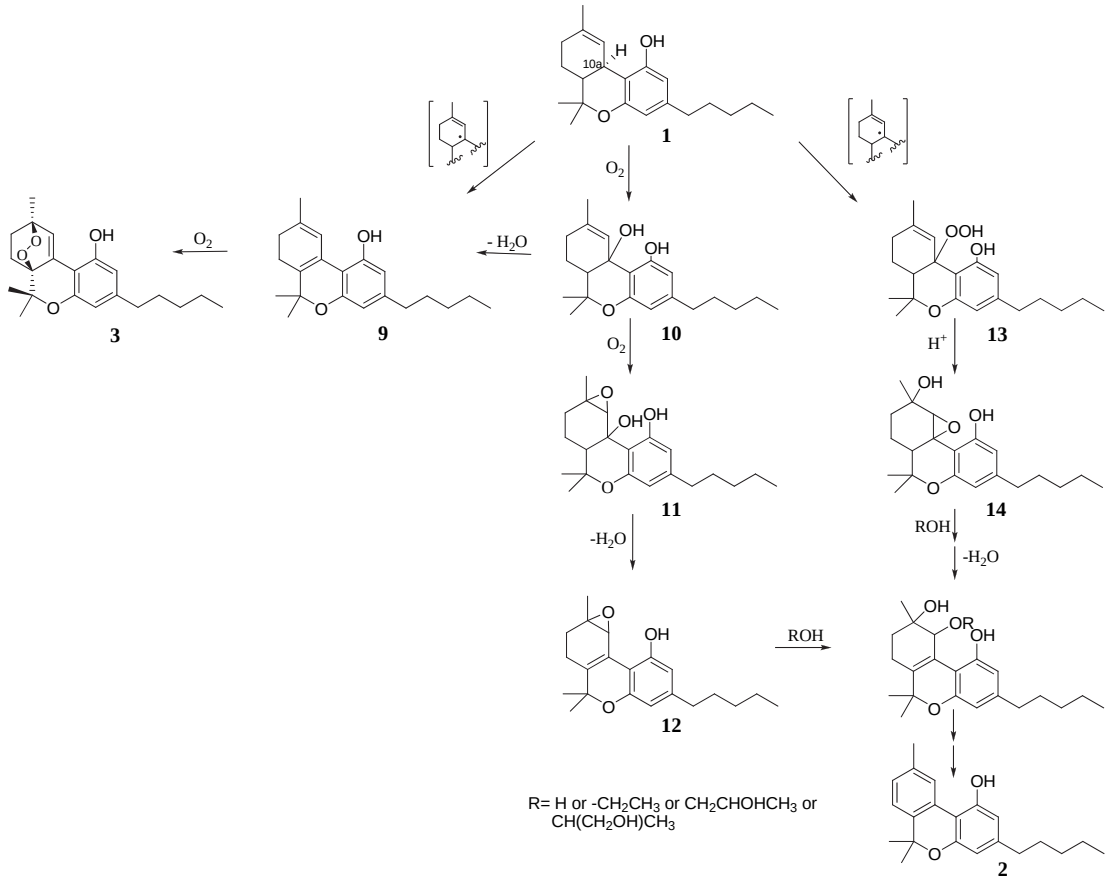
Analysis of the ^{1}H–^{1}H COSY experiment indicated the presence in the terpene ring A of a diastereotopic methylene group (H_2-8) at δ 1.89 (1H, dd, *J*=14 and 7 Hz) and δ 1.74 (1H, m), which was coupled to another methylene (H_2-7) at δ 2.19 (1H, dd, *J*=19 and 7 Hz) and δ 2.47 (1H, m). On these bases, the structural arrangement of ring A was defined by locating the double bond at C-4a and C-6a and assigning an ethoxy group and an −OH function at C-10 and C-9, respectively.

The relative stereochemistry at chiral centres C-9 and C-10 was deduced by a series of NOE (nuclear Overhauser effect) difference experiments. The irradiation of H-10 (δ 4.29) resulted in a strong enhancement of the signals at δ 9.60 (1-OH) and 1.42 (H_3-13) while the irradiation of H_2-1″ (δ 3.56) led to the increment of H-8 axial (δ 1.74) and H_3-13. These effects were in agreement with an axial orientation of the ethoxy group at C-10. On the other hand, the methyl H_3-13 showed steric interactions with both H-10 and H_2-1″ and with H-8 equatorial (δ 1.89), thus implying its equatorial orientation and the *trans*-arrangement of the two oxygenated groups at C-9 and C-10.

The spectral data of the related minor compound **5**, the structure of which was established as *cis*-cannabitriol monoethyl ether, were similar to those of **4**. The main differences were observed in the ^{13}C NMR values of the carbons of the ring A and of the substituents at C-9 and C-10 (Table 2). This clearly indicated that the two compounds only differed in the relative stereochemistry at C-9 and C-10, whereas the remaining part of the molecule was identical. In the ^{1}H NMR spectrum of compound **2** the methylene groups H_2-7 and H_2-8 resonated as unresolved 2H multiplets at δ 2.31 and 1.82, respectively, supporting a different influence of the oxygenated substituents at C-9 and C-10 due to a different relative orientation. Bearing in mind the *trans*-relative stereochemistry of the two oxygenated substituents in compound **4**, it is obvious that such groups should be *cis*-oriented in compound **5**. This relative stereochemistry was confirmed by careful analysis of a series of NOE difference experiments conducted on **5** by using the same NMR parameters as in compound **4**. No steric interaction was observed between H_3-13 and H_2-1″, thus implying a *trans*-diaxial stereochemistry of the methyl at C-9 and the ethoxy group at C-10, as expected. Extensive 2D NMR experiments allowed the assignment of all proton and carbon resonances as reported in Tables 1 and 2. The corresponding hydroxyl derivative *cis*-cannabitriol was only described from a sample of an Indian variant of *C. sativa* L.[9,10]

trans-Cannabitriol (**6**) was isolated from fraction AS6. The analysis of 2D NMR spectra led us to completely assign all proton and carbon resonances as reported in Tables 1 and 2.

Compounds **7** and **8**, which were purified from the more polar fraction, were isomers as it was evidenced by the HRESIMS spectra indicating the same molecular formula. The ^{1}H and ^{13}C NMR spectra revealed that compounds **7** and **8** were structurally similar,



Scheme 1. Proposed correlation between the oxidative derivatives of Δ^9-THC (**1**).

both being derivatives of *trans*-cannabitriol (**6**) with two different hydroxyl residues attached at the 10-OH position by an ether linkage. The analysis of proton and carbon NMR data allowed us to identify the ether residue in **7** and **8** as a 1,2-propanediol unit linked to the cannabinoid framework through the secondary hydroxyl group in **7** and the primary hydroxyl group in **8**. Accordingly, in the proton spectra of **7** and **8** the main differences were due to the signals assigned to the methylene protons H_2-1″ of the propanediol residue [δ 3.50 (1H, dd, J=11 and 6 Hz, H_2-1″a) and δ 3.57 (1H, dd, J=11 and 4 Hz, H_2-1″b) in **7**; δ 3.38 (1H, app. t, J=9 Hz, H_2-1″a) and δ 3.58 (1H, dd, J=9 and 3 Hz, H_2-1″b) in **8**] as well as to the carbinol proton H-10 (δ 4.67 in **7**; δ 4.46 in **8**). Diagnostic HMBC correlations were observed between H-10 and C-2″ in compound **7** and between H-10 and C-1″ in compound **8**, consistent with the different position of the hydroxyl group involved in the ether linkage. The interpretation of 2D NMR experiments confirmed the suggested structures and allowed the complete assignments of compounds **7** and **8** as reported in Tables 1 and 2.

The relationships among the oxidised derivatives of Δ^9-THC that were identified in this work are depicted in Scheme 1. The isolation of peroxide **3** among the Δ^9-THC oxidation products is an extremely interesting result. In fact, **3** might derive from **10** through **9** by dehydration and subsequent addition of O_2. Consequently it might represent the indirect evidence of the existence of unstable precursors, such as the 10a-hydroxy derivative of Δ^9-THC, compound **10**, and diene **9** (Scheme 1). These latter molecules were suspected to be intermediates in the oxidation process of Δ^9-THC (**1**)[2,4–6] even though they have never been identified to date. The formation of **10** by allylic oxidation at C-10 of **1** was suggested to be the initial step in the decomposition pathway in either dried samples[2] or alcoholic solutions of Δ^9-THC (**1**).[4] In the proposed schemes,[2,4] the following steps were supposed to include the epoxidation of Δ^9-double bond to form **11** as well as the opening of epoxide **12** by either H_2O or an alcoholic solvent to give hydroxyl intermediates and the corresponding solvent-adduct analogues. Alternatively, according to the oxidation process via free radical mechanism,[6] the initial benzylic/allylic radical, which should be formed by removal of the H-10a proton in Δ^9-THC (**1**), could give either diene **9** by loss of an hydrogen atom,[6] or the corresponding 10-hydroperoxide derivative **13** by the addition of O_2. The subsequent rearrangement[11] of **13** would produce the hydroxyl-epoxide **14**, which could give rise to hydroxyl and solvent-adduct derivatives, analogous to **12**. The final oxidised product CBN (**2**) should derive from these intermediates by elimination of H_2O or the alcoholic residue. The identification of cannabitriol and the corresponding solvent-adduct analogues (compounds **4**–**8**) in the aged Δ^9-THC sample analysed in this work is in agreement with the previously proposed decomposition pathways.[2,4] In addition, the detection of diene **9** and the corresponding endoperoxide derivative **3** implied the existence of a side degradation pathway that starts from the initial intermediate 10a-hydroxyl Δ^9-THC (**10**) but does not lead to CBN (**2**).

Finally, when tested in displacement binding assays carried out with a radiolabelled high affinity ligand and membranes of cells over-expressing the human recombinant CB_1 or CB_2 receptors, both isomers of cannabitriol monoethyl ether exhibited appreciable, although weak, affinity for either receptor. The *trans* isomer was more potent a ligand than the *cis* isomer, with K_i=2.25 and 6.30 μM for CB_1 receptors, respectively, and K_i=1.97 and 3.13 μM for CB_2 receptors, respectively. Under the same experimental conditions, Δ^9-THC exhibited much lower K_i values (1.60 and 2.42 nm for CB_1 and CB_2 receptors, respectively). This finding is in agreement with the loss of activity in aged samples of *Cannabis* and Δ^9-THC.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Jasco DIP 370 digital polarimeter. HPLC separation was performed on a Shimazdu High-performance liquid chromatography using a Shimadzu liquid chromatograph LC-10AD equipped with an UV SPD-10A wavelength detector. NMR experiments were recorded at ICB-NMR Service Centre. 1D and 2D NMR spectra were acquired in $CDCl_3$ (shifts are referenced to the solvent signal) on a Bruker Avance-400 operating at 400 MHz, using an inverse probe fitted with a gradient along the *Z*-axis.[13]C NMR were recorded on a Bruker DPX-300 operating at 300 MHz using a dual probe. Quaternary carbon values of compounds **3, 6, 7,** and **8** were indirectly detected by HMBC experiments. Low and high resolution ESIMS were performed on a Micromass Q-TOF Micro™ coupled with an HPLC Waters Alliance 2695. The instrument was calibrated by using a PEG mixture from 200 to 1000 MW (resolution specification 5000 FWHM, deviation <5 ppm rms in the presence of a known lock mass).

3.2. Biological material

The sample consisted of a liquid CO_2 extract of flower and leaf material from a high Δ^9-THC chemotype of *C. sativa* dissolved in a 50:50 mix of ethanol and propylene glycol to produce a Δ^9-THC content of 25 mg/mL. This sample was stored at 25°C/60%RH for 5 years prior to the recovery process. When analysed this sample showed a Δ^9-THC content of 16 mg/mL (63.7% of initial).

3.3. Isolation of oxidised cannabinoid derivatives

An aliquot (ca. 25 mL) of the aged sample was recovered from the excipients by addition of water followed by liquid–liquid extraction with ethyl acetate. The organic phase (916.0 mg) was then submitted to RP-flash chromatography (CH_3OH/H_2O). The obtained cannabinoid mixture (338.5 mg) was further purified by flash chromatography on silica gel column (*n*-hexane/ethyl acetate) to give seven fractions containing minor cannabinoids (AS1–AS7) (see Supplementary data). These fractions were first analysed by LC-MS and ^{1}H NMR. Fractions AS2, AS3, AS4, AS6 and AS7 were considered.

Fraction AS2 (5.5 mg) was purified by RP-HPLC [Supelco: Discovery® 5 μ C18, 250×10 mm, 30 min gradient from 80 to 100% CH_3OH in H_2O, flow 2 mL/min, UV detector (λ 254 nm)] to give pure compound **3** (1.5 mg).

Fraction AS3 (16.5 mg) did not require further purification being composed by pure compound **4**.

Fraction AS4 (5.4 mg) was submitted to RP-HPLC [Supelco: Discovery® 5 μ C18, 250×4.6 mm, 20 min CH_3OH/H_2O 85:15, flow 1.5 mL/min, UV detector (λ 254 nm)] to obtain pure compound **5** (1.3 mg).

Fraction AS6 (9.8 mg) was purified by RP-HPLC [Supelco: Discovery® 5 μ C18, 250×4.6 mm, 40 min CH_3CN/H_2O 60:40, flow 1.2 mL/min, UV detector (λ 254 nm)] to yield pure compound **5** (3.0 mg).

Fraction AS7 (21 mg) was submitted to RP-HPLC [Supelco: Discovery® 5 μ C18, 250×10 mm, 40 min gradient from 60 to 100% CH_3OH in H_2O, flow 3 mL/min, UV detector (λ 254 nm)] to give pure compounds **7** (6.8 mg) and **8** (8.5 mg).

*3.3.1. Compound **3**.* Colourless oil; $[\alpha]_D^{25}$ −15.3 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (300 MHz, $CDCl_3$) see Table 2; ESIMS positive mode: *m*/*z* 367 $[M+Na]^+$; HRESIMS: *m*/*z* 367.1892 (calcd for $C_{21}H_{28}O_4Na$, 367.1885).

*3.3.2. trans-Cannabitriol monoethyl ether (**4**).* Colourless oil; $[\alpha]_D^{25}$ −8.0 (*c* 0.51, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (300 MHz, $CDCl_3$) see Table 2; ESIMS positive mode: *m*/*z* 397 $[M+Na]^+$; HRESIMS: *m*/*z* 397.2362 (calcd for $C_{23}H_{34}O_4Na$, 397.2355).

*3.3.3. cis-Cannabitriol monoethyl ether (**5**).* Colourless oil; $[\alpha]_D^{25}$ −35.0 (*c* 0.12, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (300 MHz, $CDCl_3$) see Table 2; ESIMS positive mode: *m*/*z* 397 $[M+Na]^+$; HRESIMS: *m*/*z* 397.3626 (calcd for $C_{23}H_{34}O_4Na$, 397.2355).

*3.3.4. trans-Cannabitriol (**6**).* Colourless oil; $[\alpha]_D^{25}$ −25.4 (*c* 0.09, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (300 MHz, $CDCl_3$) see Table 2; ESIMS positive mode: *m*/*z* 369 $[M+Na]^+$; HRESIMS: *m*/*z* 369.2044 (calcd for $C_{21}H_{30}O_4Na$, 369.2042).

*3.3.5. Compound **7**.* Colourless oil; $[\alpha]_D^{25}$ −21.1 (*c* 0.08, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (300 MHz, $CDCl_3$) see Table 2; ESIMS positive mode: *m*/*z* 427 $[M+Na]^+$; HRESIMS: *m*/*z* 427.2457 (calcd for $C_{24}H_{36}O_5Na$, 427.2460).

*3.3.6. Compound **8**.* Colourless oil; $[\alpha]_D^{25}$ −15.4 (*c* 0.11, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (300 MHz, $CDCl_3$) see Table 2; ESIMS positive mode: *m*/*z* 427 $[M+Na]^+$; HRESIMS: *m*/*z* 427.2451 (calcd for $C_{24}H_{36}O_5Na$, 427.2460).

3.4. Binding assays

For both receptor binding assays, the compounds were tested using membranes from HEK-293 cells transfected with either the human recombinant CB1 or CB2 receptor, and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)-cyclohexanol ([^{3}H]CP-55,940) as the high affinity ligand. *K*i: 'Equilibrium dissociation constant', that is, the concentration of the competing ligand that will bind to half the binding sites at equilibrium, in the absence of radioligand or other competitors. CB1 and CB2 receptor binding assays were performed exactly as described previously.[12]

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.10.025. These data include MOL files and InChiKeys of the most important compounds described in this article.

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