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Fischedick, J.

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Author: Fischedick, Justin

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Chapter 3

Cannabinoid Receptor 1 Binding Activity and Quantitative Analysis of *Cannabis sativa* L. Smoke and Vapor

Jusin T. Fischedick, Frank van der Kooy, Robert Verpoorte

Natural Products Laboratory, Institute of Biology, Leiden University, 2300 RA Leiden, The Netherlands

Abstract

Cannabis sativa L. (cannabis) extracts, vapor produced by the Volcano vaporizer, and smoke made from burning cannabis joints were analyzed by GC-hydrogen flame ionization detector (FID), GC-MS and HPLC. Three different medicinal cannabis varieties were investigated Bedrocan®, Bedrobinol®, and Bediol®. Cannabinoids plus other components such as terpenoids and pyrolytic by-products were identified and quantified in all samples. Cannabis vapor and smoke was tested for cannabinoid receptor 1 (CB1) binding activity and compared to pure Δ^9 -tetrahydrocannabinol (Δ^9 -THC). The top five major compounds in Bedrocan extracts were Δ^9 -THC, cannabigerol (CBG), terpinolene, myrcene, and *cis*-ocimene in Bedrobinol Δ^9 -THC, myrcene, CBG, cannabichromene (CBC), and camphene in Bediol cannabidiol (CBD), Δ^9 -THC, myrcene, CBC, and CBG. The major components in Bedrocan vapor (>1.0 mg/g) were Δ^9 -THC, terpinolene, myrcene, CBG, *cis*-ocimene and CBD in Bedrobinol Δ^9 -THC, myrcene and CBD in Bediol CBD, Δ^9 -THC, myrcene, CBC and terpinolene. The major components in Bedrocan smoke (>1.0 mg/g) were Δ^9 -THC, cannabinol (CBN), terpinolene, CBG, myrcene and *cis*-ocimene in Bedrobinol Δ^9 -THC, CBN and myrcene in Bediol CBD, Δ^9 -THC, CBN, myrcene, CBC and terpinolene. There was no statistically significant difference between CB1 binding of pure Δ^9 -THC compared to cannabis smoke and vapor at an equivalent concentration of Δ^9 -THC.

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Introduction

More than 400 chemicals have been identified in *Cannabis sativa* L. (cannabis), of which 70 are a group of terpenophenolic compounds known as cannabinoids (Turner et al., 1980; ElSohly and Slade, 2005). Δ^9 -Tetrahydrocannabinol (Δ^9 -THC) is the main cannabinoid and is primarily responsible for the psychoactive and medicinal effects of cannabis. Δ^9 -THC exhibits many of its effects by interacting with two G-protein coupled receptors known as the cannabinoid receptor 1 (CB1) and the cannabinoid receptor 2 (CB2) (Costa, 2007). A variety of compounds both endogenous to the human body and synthetic, can interact with the CB receptors including fatty acid amides, fatty acid esters, aminoalkylindoles and diarylpyrazoles (Howlett, 1995).

Despite the illegality of cannabis in most nations a renewed interest in the medicinal properties of cannabis has resulted in the development of a number of cannabinoid based medicines. Oral Δ^9 -THC (Marinol[®]) and nabilone (Cesamet[®]) a synthetic analogue of Δ^9 -THC have been available since the 1980's as prescription medicine for treatment of nausea and appetite stimulation for patients undergoing chemotherapy or for AIDS wasting syndrome. More recently Sativex[®] a cannabinoid based oral mucosal spray containing Δ^9 -THC and cannabidiol (CBD) has become available in some countries for relief of neuropathic pain in multiple sclerosis (Pertwee, 2009). In the Netherlands cannabis can be legally prescribed by medical doctors for treatment of nausea (caused by chemotherapy and radiotherapy), for chronic pain, Tourette's syndrome and multiple sclerosis. Since March 2005, Bedrocan BV (The Netherlands) has been contracted by the Dutch Ministry of Health, Welfare and Sport for the growth and production of medicinal cannabis.

Cannabis is traditionally consumed by smoking, eating, or drinking in the form of a tea preparation. Heating the plant material plays an important role as this decarboxylates the naturally occurring non-psycho-active tetrahydrocannabinolic acid (THCA) into the psycho-active neutral cannabinoid Δ^9 -THC (Russo, 2007). A relatively new method of administration is to heat cannabis plant material at a temperature high enough to volatilize the active compounds without reaching temperatures which could cause combustion of the plant material. This technique is known as vaporizing and shows promise as a safe alternative to smoking while maintaining pharmacokinetic advantages of pulmonary administration (Abrams et al., 2007).

The identification of components in cannabis smoke condensate has been extensively studied (Fentiman et al., 1973; Adams and Jones, 1973; Jones and Foote, 1975; Lee et al., 1976; Maskarinec et al., 1976; Kettenes-Van Den Bosch and Saleminck, 1977; Novotný et al., 1982; Hiller et al., 1984; Van der Kooy et al., 2008; Van der Kooy et al., 2009). An excellent review on cannabis smoke condensate, its constituents and some biological effects is available (ElSohly, 2006). Recently, research has been undertaken to determine the safety and effectiveness of vaporization for the administration of cannabis and cannabinoids. Effectiveness in human subjects has been demonstrated (Abrams et al., 2007), the suppression of pyrolytic by-products has been shown (Gieringer et al., 2004), vaporization parameters of pure Δ^9 -THC have been optimized (Hazekamp et al., 2006), and the effect of different samples sizes and

temperatures on Δ^9 -THC levels has been studied (Pomahacova et al., 2009). However one shortcoming of the above studies is that other components delivered by cannabis smoke or vapor such as terpenoids were not investigated.

Therefore in order to continue to evaluate the effectiveness of vaporization versus smoking our research focused on the identification and quantification of the components of cannabis smoke and vapor as well as CB1 binding activity of the collected samples. The goal of the CB activity test was to observe whether or not levels of Δ^9 -THC in cannabis smoke and vapor was equivalent to CB1 binding activity of pure Δ^9 -THC.

Materials and Methods

Plant Material

The plant material was obtained from Bedrocan BV (Groningen, The Netherlands) under the opium regulation register number 105815 CO/w. It consisted of mature flower tops of three cannabis varieties Bedrocan (dried), Bedrobinol (dried) and Bediol (granular, dried). According to the producer Bedrocan contains 18% Δ^9 -THC and <1% CBD, Bedrobinol contains 11% Δ^9 -THC and <1% CBD, and Bediol contains 6% Δ^9 -THC and 7% CBD. Upon receiving the plant material it was stored at 4° C in the dark until use.

Chemicals

All reference terpenoids were purchased from Sigma-Aldrich (Steinheim, Germany), Fluka (Steinheim, Germany) or Chromadex (California, USA) and included α -thujene, camphene, sabine, 1-8-cineol, terpinene-4-ol, 1-4-cineol, α -humulene, camphor, α -bisabolol, β -pinene, linalool, myrcene, terpineol, α -pinene, γ -terpineol, limonene, caryophyllene-oxide, (-)-carvacrol, Δ^3 -carene, p-cymene, terpinolene, citronellal, geranyl acetate, pulegone, citral, α -terpinene, α -fenchyl alcohol, calamene, γ -cadinene, bornyl acetate, cis-trans-ocimene, α -cedrene, α -phellandrene, nerol, β -phellandrene, nerolol, piperitonoxide, β -caryophyllene and geraniol. The cannabinoid references for Δ^9 -THC, THCA, Δ^8 -tetrahydrocannabinol (Δ^8 -THC), CBD, cannabigerol (CBG), cannabichromene (CBC), tetrahydrocannabivarin (THCV), and cannabinol (CBN) were purified and quantified as previously described (Hazekamp et al., 2004a; Hazekamp et al., 2004b) by PRISNA BV (Leiden, The Netherlands). All cannabinoids references were >98% pure. Organic solvents used for extraction and sample preparation were of analytical reagent (AR) grade. Solvents used for HPLC were of HPLC grade.

Sample Preparation

Cannabis plant material was extracted using previous validated methodology (Hazekamp, 2007). Extracts from each cannabis variety were prepared in triplicate. One gram of plant material was transferred to 50 ml falcon tubes for extraction. The amount

of ethanol was brought to 40 ml and the falcon tubes were placed on a shaker for 15 min at 300 rpm. After shaking the samples were centrifuged at 2500 rpm for 5 min and the supernatant was collected in a 100 ml volumetric flask. The same procedure was repeated two more times with 25 ml ethanol. The final volume of ethanol was made up to 100 ml and samples were filtered through a 25 mm PTFE membrane syringe filter (0.45 μ m).

For the smoke experiments the procedure described by Van der Kooy et al., (2009) was followed. Each cannabis joint was separately weighed (1 g/joint) and numbered. For each sample 2 joints were prepared. The puff frequency was one puff (lasting 3 sec) every 30 sec while the puff volume was 35 ml. The smoke was collected in two gas traps connected in series containing each 50 ml of a 1:1 mixture of ethanol and hexane. The final volume for each sample was 100 ml. A total of 3 samples were collected for each variety.

For the vapor collection the procedures described by Pomahacova et al., 2009 were followed. The Volcano® was obtained from Storz & Bickel GmbH & Co. (Tuttlingen, Germany) and was used according to the manual as provided by the manufacturer. The volume of the plastic bag used was 8 L. For each vaporization 250 mg of plant material was used. This process was repeated with 5 (total) separate 250 mg portions per sample (1.25 g cannabis material/sample). Samples were prepared in triplicate for each of the cannabis varieties. At the start of each experiment the Volcano was preheated until the indicator light showed that the target temperature of 200 °C was reached. The bag, connected to the filling chamber, was then immediately placed onto the Volcano and the ventilation was started. When the bag was completely inflated, ventilation was stopped and the bag was removed and reattached to a tube connected to the solvent trap (ethanol: *n*-hexane 1:1, 100 ml). Using a pump connected to the solvent system *via* a tube, the smoke was collected into the solvents. All resulting samples were analyzed with GC-FID, GC-MS, and HPLC.

GC-FID Analysis

An Agilent GC 6890 series equipped with a 7683 autosampler and injector was used for quantification. The column used for separation was a VA5ms (0.25 mm x 30 m, film thickness 0.25 μ m, Varian, Walnut Creek, CA, USA). The injector temperature was set to 230 °C with an injection volume of 4 μ l, a split ratio of 10 and a N₂ flow of 2 ml/min. The oven temperature program began at 60 °C with a ramp rate of 3 °C/min. The final temperature was 240 °C which was held for 5 min making a total run time of 65 min/sample. The FID detector temperature was 250 °C. Five point standard curves of myrcene, α -humulene and Δ^9 -THC (0.01-1.0 mg/ml) diluted in ethanol were measured for quantification. All samples were analyzed undiluted and reference compounds were run at a concentration of 1 mg/ml.

GC-MS Analysis

The GC-MS analyses for compound identification were performed on a Varian 3800 GC, Varian Saturn 2000 GC ms/ms with a Varian 8200 autosampler and injector.

The injection volume was 3 μ l with a split ratio of 20. The column used for separation was a DB5ms. (0.25 mm x 30 m, film thickness 0.25 μ m, J&W Scientific, Folsom, CA, USA). The oven temperature program was the same as GC-FID. The transfer line temperature was 275 $^{\circ}$ C, manifold temperature 60 $^{\circ}$ C, and ion trap temperature 220 $^{\circ}$ C. Electron impact was used at an ionization mode of 70 eV and a scan range of 41-500 amu. All samples were analyzed undiluted and reference compounds were analyzed at a concentration of 1 mg/ml. The NIST library (Standard Reference Data Program of the National Institute of Standards and Technology) was used to aid in compound identification when no reference standard was available.

HPLC Analysis

The quantification of acidic and neutral cannabinoids was performed on an Agilent 1200 HPLC system equipped with an autosampler and injector and a photodiode array detector. The column used for separation was a GraceVydac (Deerfield, IL, USA) (250 x 4.6 mm 5 μ M C₁₈) equipped with a guard column containing the same material as the column (All-guard 7.5 x 4.6 mm 5 μ M C₁₈). The mobile phase consisted of solvent A (50% MeOH and 0.1% formic acid) and solvent B (100% MeOH and 0.1% formic acid). The gradient employed started with 70% solvent A at time 0 and increased to 100% solvent B in 25 min. At 26 min the system was returned to 70% solvent A and 4 min was allowed for re-equilibration. The total run time was 30 min/sample. The flow rate was 1.5 ml/min and the detection wave length was 228 nm. Quantitative HPLC analysis of all samples was performed based previously validated methodology (Hazekamp, 2007).

CB1 Radioactive Displacement Assay

The CB1 receptor containing membranes (0.63 pmol/mg membrane protein; 16.4 mg/ml protein concentration) from Sf9 cells coexpressed with $\text{G}\alpha_{i3}\beta_1\gamma_2$ were purchased from PerkinElmer (Boston, MA, USA). The radioactive ligand CP-55,940, [Side chain-2,3,4(N)-³H] was purchased from PerkinElmer. The CB1 containing membranes were diluted at a ratio of 1:200 with assay buffer (20 mM Hepes, 5 nM MgCl₂, 1 mM ethylene-diamine-tetra-acetic acid (EDTA), 0.3% bovine serum albumin (BSA), pH 7.4). Receptor solutions were used on the same day and all buffers were freshly prepared. The total assay volume was 550 μ l of which 500 μ l was the receptor solution, 25 μ l the radioactive ligand (0.5 nM final concentration) and 25 μ l the sample. All vapor and smoke samples were diluted to a final concentration of 10 nM Δ^9 -THC in the final assay solution and were assayed in triplicate. Samples containing 10 nM of pure Δ^9 -THC were also assayed (n=6). To determine non-specific binding CP-55,940 was assayed at final concentration of 10 μ M (n=6). Blank samples were assayed to determine total binding of the radioactive ligand (n=6). All samples including controls Δ^9 -THC, CP-55,940, and blanks contained $\leq 0.3\%$ ethanol in the final assay solution.

The radioactive displacement assay was performed according to the recommended assay conditions of PerkinElmer with an incubation time of 1 hour at 30 $^{\circ}$ C. After incubation samples were filtered with a Brandel harvester (Gaithersburg, MD,

USA) over GF/C filters. The harvester can handle 24 filters at a time. After filtration the filters were collected in plastic scintillation vials to which 3 ml scintillation fluid was added. The scintillation fluid (brand: 'emulsifier safe') contained ethoxylated phenol. After adding the scintillation fluid and a brief vortex the samples were counted in a PerkinElmer scintillation counter (Tri-carb 2900TR). A student t-test (two tailed; two sample unequal variance) was performed in order to compare statistical significance between pure Δ^9 -THC and group of samples (variety and smoke or vapor). A p-value <0.05 was considered significant.

Results and Discussion

HPLC quantification

The results of HPLC quantification of THCA and Δ^9 -THC are shown in Table 1. The amount of THCA in the extracted cannabis plant material was used to calculate the total theoretical amount of Δ^9 -THC in the ethanol extracts taking into account the difference in molecular weight (Δ^9 -THC% = THCA% x (314.47 / 358.48)). Δ^9 -THC levels for Bedrocan were higher than claimed by the producer (21.7%). This difference could be due to the fact that Bedrocan material was supplied as intact dried flower buds rather than granulated as it is normally supplied to pharmacies. Granulating the plant material causes some trichomes which contain the most cannabinoids to fall off. As expected the amount of Δ^9 -THC in the vapor and smoke declined with the original content of Δ^9 -THC in the plant varieties. The smoke and vapor samples showed an inverse relationship between Δ^9 -THC volatilization efficiency compared to original Δ^9 -THC content with the Bediol variety having the highest efficiency. Δ^9 -THC volatilization efficiency was higher for each variety when vaporized compared to smoked. The absolute quantities of Δ^9 -THC in the smoke samples of the Bedrocan variety confirms earlier reports (Pomahacova et al., 2009) which found Δ^9 -THC levels of around 40 mg/g in the smoke samples. Δ^9 -THC levels in vaporized samples cannot be directly compared with previous research as differences in sample weights vaporized causes differences in Δ^9 -THC levels (Pomahacova et al., 2009).

GC Identification and Quantification

All components identified and quantified by GC-FID and GC-MS are shown in tables 2-4. A representative chromatogram for a Bedrocan extract, smoke and vapor sample is shown in figure 1. Compound identification was based on mass spectra, retention times compared with authentic standards and retention indexes reported in literature (Ross and ElSohly, 1996; Adams, 1989). Mono-terpenoids were quantified using a linear calibration curve for myrcene ($y=6945.1x$; $r^2=0.997$), sesquiterpenoids with α -humulene ($y=7529.5x$; $r^2=0.998$), and cannabinoids with Δ^9 -THC ($y=5873.4x$; $r^2=0.999$). The % difference in response coefficients between the above three compounds classes was 12.4%. Putative identification of pyrolytic by-products using a NIST library (2005) is reported in smoke samples. These compounds did not fit into the above 3 compound groups therefore they were quantified using the standard compound that was most similar in mass as response coefficients in FID detectors are mass sensitive. Standard curves were not generated for every compound quantified so the

data represents a normalized quantitation. A number of compounds had fragmentation patterns that were typical of cannabinoids or sesquiterpenoids but identification could not be confirmed based on available data. For such compounds mass ions were reported and they were labeled as unknown sesquiterpenoids or cannabinoids.

Table 1. HPLC Quantification of THC and THCA in the three cannabis varieties

Sample	Varieties	THC mg/g	%RSD n=3	Efficiency of THC volatilization	THCA mg/g	%RSD n=3
Extract	Bedrocan	217.0 ^{a)}	2.4	-	240.9	2.5
	Bedrobinol	103.0 ^{a)}	3.5	-	114.8	3.5
	Bediol	62.0 ^{a)}	1.4	-	66.9	1.3
Vapor	Bedrocan	47.7	5.7	22.0%	2.3	17.4
	Bedrobinol	36.3	10.9	35.2%	2.2	6.3
	Bediol	24.5	22.2	39.5%	1.2	19.9
Smoke	Bedrocan	34.6	33.4	15.9%	ND ^{b)}	--
	Bedrobinol	26.3	6.4	25.5%	ND	--
	Bediol	18.5	12.4	29.8%	ND	--

a) THC equivalents based on the amount of THCA in the samples. b) ND = not detected.

Table 2 lists all the components which were identified and quantified in the cannabis extracts. No acidic cannabinoids were observed as expected because the high temperature used in GC decarboxylates them into their neutral forms. The concentration of Δ^9 -THC determined by GC confirms the results obtained by HPLC. No CBN, a Δ^9 -THC degradation product, was detected in any of the initial sample extracts. The top five major compounds in Bedrocan extracts were Δ^9 -THC, CBG, terpinolene, myrcene, and *cis*-ocimene. In Bedrobinol Δ^9 -THC, myrcene, CBG, CBC, and camphene were major components and in Bediol CBD, Δ^9 -THC, myrcene, CBC, and CBG.

Table 3 lists the components identified and quantified in the vapor samples. Most of the components identified in the initial extracts can also be seen in the vapor samples. The major components of Bedrocan vapor (>1.0 mg/g) were Δ^9 -THC, terpinolene, myrcene, CBG, *cis*-ocimene, and CBD. Bedrobinol contained mostly Δ^9 -THC, myrcene, and CBD. Note that the levels of CBD were higher in Bedrocan and Bedrobinol vapor samples than they were in the original extracts. We suspect this observation is a result of the degradation of another cannabinoid, perhaps Δ^9 -THC, into CBD. Since the %RSD was also very high (>50%) and the effect was not observed in cannabis smoke (Table 4) we suspect that such degradation is not reproducible. In Bediol vapor the major components (>1.0 mg/g) were CBD, Δ^9 -THC, myrcene, CBC, and terpinolene. Only a small amount of CBN (<0.1 mg/g) was formed in vapor samples. No new compounds that were not observed in the cannabis extracts were detected in cannabis vapor.

In contrast to vapor samples smoked cannabis contained many compounds not observed in extracts or vapor (Table 4). In total 23 unknown cannabinoids, various

hydrocarbons, phenolic compounds, nitrogen containing compounds, Δ^8 -THC, 1-oxo-cannabinol and significant amounts of CBN (>2.0 mg/g) were observed in cannabis smoke. These results suggest a much higher degree of pyrolytic degradation in cannabis smoke when compared to cannabis vapor and is consistent with previous literature (Gieringer et al., 2004). The major compounds in Bedrocan smoke (>1.0 mg/g) were Δ^9 -THC, CBN, terpinolene, CBG, myrcene and *cis*-ocimene. In Bedrobinol Δ^9 -THC, CBN and myrcene were the major compounds. In Bediol CBD, Δ^9 -THC, CBN, myrcene, CBC and terpinolene were the major compounds.

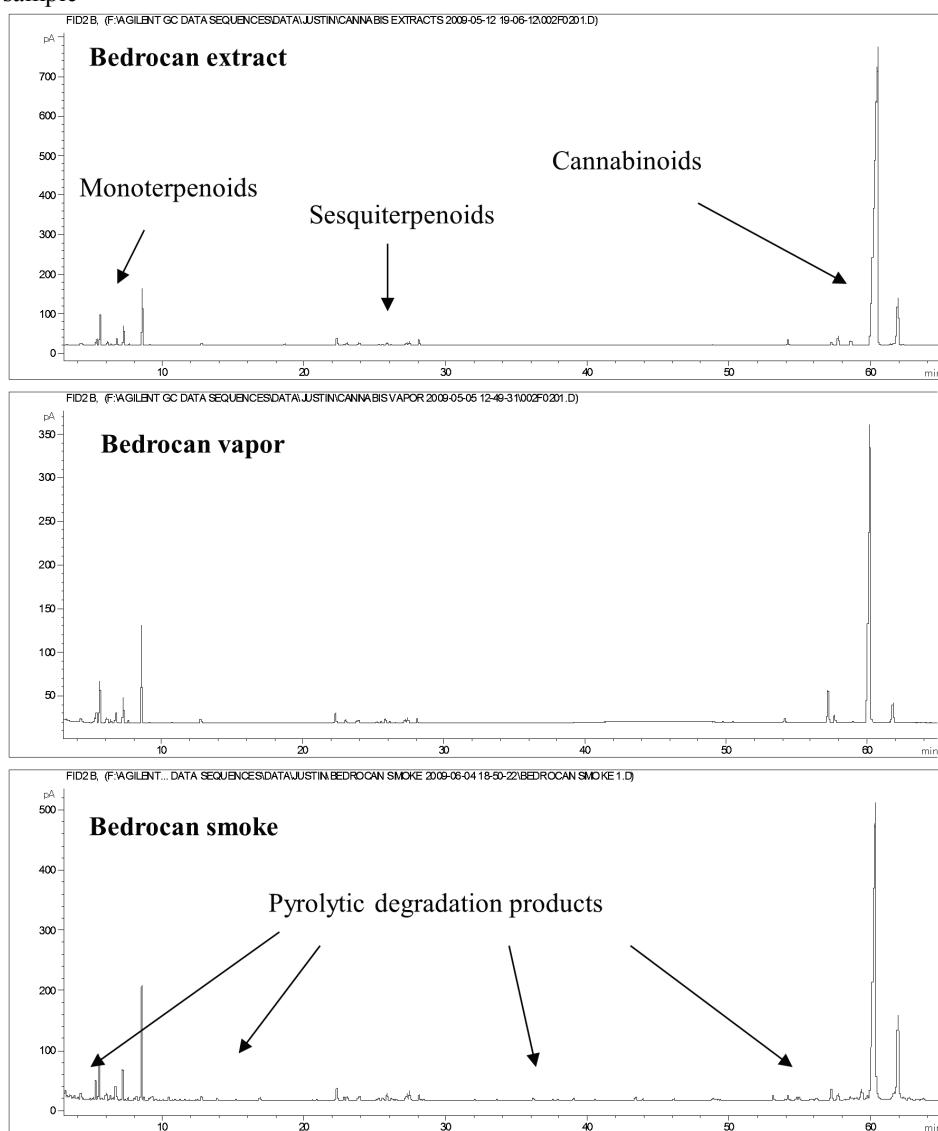
CB1 binding activity

Cannabis smoke and vapor samples were diluted to a concentration of 10 nM which is very near the EC₅₀ of Δ^9 -THC. This was done to maximize the ability of the assay to show an increase or decrease in binding. The EC₅₀ of Δ^9 -THC was determined to be 9.9 nM with a K_i of 3.8 nM from a dose response curve performed under the same assay conditions using the same batches of ligands and receptors (data not shown). The K_i and EC₅₀ for Δ^9 -THC is comparable with literature reports (Pertwee, 2008). Figure 2 shows the % displacement of CP-55,940, [Side chain-2,3,4(N)-³H] caused by binding to the CB1 receptor. No significant difference was found between smoke and vapor samples when compared with pure Δ^9 -THC (Figure 2). This suggests that no additional CB1 binding is taking place in cannabis smoke or vapor samples when compared with pure Δ^9 -THC.

Conclusions

Our CB1 binding results verify previous reports in humans which showed that the subjective psychoactive effects of cannabis are primarily due to Δ^9 -THC content (Wachtel et al., 2002; Ilan et al., 2005). Our results demonstrate that any non- Δ^9 -THC components in cannabis smoke and vapor are too diluted to have any significant effects *in vitro* on CB1 binding. However there still exists evidence that other components in cannabis extracts play a role in the plant's overall therapeutic effects (Pickens, 1981; Fairbairn and Pickens, 1981; Zuardi et al., 1982; Wilkinson et al., 2003; Whalley et al., 2004; Ryan et al., 2006). There has even been considerable controversy over this issue (ElSohly et al., 2003; Russo and McPartland, 2003). We propose that any additional beneficial effects observed by patients using cannabis are due to effects other than CB1 agonism. Such benefits could come from other components in cannabis that interact with the CB2 receptors or new potential cannabinoid receptors such as the transient receptor potential vanilloid 1 (Begg et al., 2005).

Figure 1. Typical GC-FID chromatograms of a Bedrocan extract, vapor and smoke sample



Quantitative comparison of cannabis smoke and vapor shows that vaporizing cannabis with the Volcano is a more reliable and safer administration form for the delivery of Δ^9 -THC due to the lack of pyrolytic degradation and more efficient Δ^9 -THC volatilization. Analysis of cannabis smoke and vapor showed for the first time in a quantitative manner that terpenoids are major components of the smoke and vapor of 3 medicinal cannabis varieties. Myrcene has analgesic and anti-inflammatory properties which may contribute to the medical benefits of cannabis. Other compounds identified

in our samples terpineol, terpinene-4-ol, γ -terpinene, limonene and α -pinene are acetylcholine esterase inhibitors that may act by reducing acetylcholine deficits in the hippocampus induced by Δ^9 -THC (McPartland and Russo, 2001). Further research should be done to determine whether or not terpenoids and other non- Δ^9 -THC components of cannabis are contributing to the overall medical benefits of herbal cannabis.

Figure 2. CB1 activity of Cannabis Smoke and Vapor

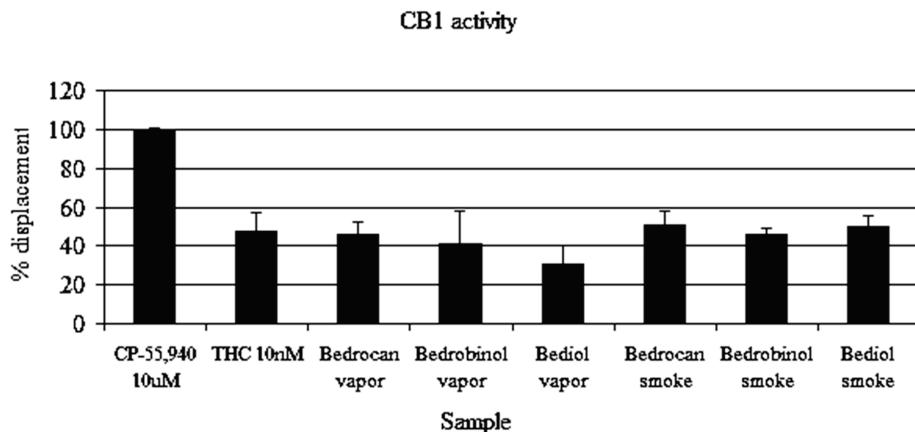


Table 2. GC Identification and Quantification of Components in Cannabis Extracts

RT min ^{a)}	Compound	Bedrocan mg/g	%RSD n=3	Bedrobinol mg/g	%RSD n=3	Bediol mg/g	%RSD n=3
4.20	α -pinene	0.4	5	0.9	8	0.6	6
4.34	camphene	0.4	4	1.1	8	0.7	7
5.23	sabinene	0.5	8	ND		0.2	10
5.35	β -pinene	0.9	1	0.3	4	0.4	9
5.60	myrcene	5.0	10	12.0	8	11.3	3
6.08	α -phellandrene	0.5	4	ND		ND	
6.14	Δ^3 -carene	0.3	6	ND		0.2	0.0
6.37	α -terpinene	0.2	7	ND		ND	
6.75	β -phellandrene	0.9	4	ND		0.2	2
6.77	limonene	0.7	1	ND		0.2	4
7.25	<i>cis</i> -ocimene	3.0	13	0.7	4	0.7	3
7.64	γ -terpineol	0.2	0.4	ND		ND	
8.56	terpinolene	8.9	4	ND		1.9	17
9.15	linalool	0.3	15	ND		0.3	0.0
10.70	camphor	ND		ND		0.2	18
12.49	terpinene-4-ol	0.2	10	ND		0.2	0.0
12.74	terpineol	0.7	8	ND		0.6	1
22.30	β -caryophyllene	1.7	13	0.6	8	0.8	2
22.82	<i>trans</i> - α -bergamotene	0.2	6	ND		ND	
23.03	α -guaiene	0.6	16	ND		0.5	3
23.82	α -humulene	0.6	13	0.4	33	0.3	23
23.95	<i>cis</i> - β -farnesene	0.5	23	ND		0.5	5
25.24	β -selinene	0.2	32	0.2		0.3	0.0
25.55	α -selinene	0.2	30	0.3	32	0.2	45
25.83	ST <i>m/z</i> : 204 (M+) 189, 107, 91, 77	0.5	14	ND		0.3	25
26.14	γ -cadinene	0.2	13	0.3	26	0.4	6
27.11	ST <i>m/z</i> : 204 (M+) 189, 161, 133	0.2	0.0	ND		ND	
27.24	ST <i>m/z</i> : 204 (M+) 161, 133, 105	0.5	15	0.2	20	0.2	16
27.42	ST <i>m/z</i> : 204 (M+) 161, 122, 102	0.7	16	0.2	10	0.2	5
28.10	γ -elemene	1.1	9	0.3	62	0.3	58
48.85	CB <i>m/z</i> : 258 (M+) 243, 215, 275	ND		ND		0.3	0.0
51.19	CB <i>m/z</i> : 286 (M+) 271, 243, 203	ND		ND		0.3	10
54.16	THCV	1.5	7	0.8	6	0.5	13
57.31	CBD	0.8	2	0.4	4	85.6	2
57.71	CBC	2.6	7	1.7	6	6.5	2

58.58	CB <i>m/z</i> : 313 (M ⁺) 297, 272, 244 CB <i>m/z</i> : 314 (M ⁺) 299, 272, 244	1.8	12	0.7	9	0.5	5
59.02	60.36	ND		ND		1.4	2
61.38	Δ ⁹ -THC	220.8	4	110.1	5	67.6	2
61.86	CB <i>m/z</i> : 314 (M ⁺) 297, 232	0.3	25	ND		ND	
63.91	CBG	16.0	11	2.7	22	3.1	3
	CB <i>m/z</i> : 314 (M ⁺) 294, 272, 232	ND		ND		0.5	14

ND= not detected. ST= unknown sesquiterpenoid. CB= unknown cannabinoid. a) Retention time in GC-FID.

Table 3. GC Identification and Quantification of Components in Cannabis Vapor

RT min ^{a)}	Compound	Bedrocan mg/g	% RSD n=3	Bedrobinol mg/g	% RSD n=3	Bediol mg/g	% RSD n=3
4.26	α-pinene	0.2	34	0.7	12	0.3	3
4.33	camphene	0.2	7	0.9	10	0.4	9
5.26	Sabinene	0.3	3	0.2	3	0.1	4
5.35	β-pinene	0.6	6	0.3	5	0.2	10
5.60	Myrcene	2.8	10	7.1	4	5.6	6
6.08	α-phellandrene	0.3	13	ND		ND	
6.13	Δ ³ -carene	0.2	7	ND		ND	
6.37	α-terpinene	0.2	8	ND		ND	
6.74	β-phellandrene	0.7	35	0.1	6	ND	
6.77	limonene	0.4	8	ND		0.2	11
7.25	cis-ocimene	1.7	13	0.6	5	0.4	5
7.64	γ-terpinene	0.2	7	ND		ND	
8.55	terpinolene	6.5	10	0.6	83	1.9	3
12.74	terpineol	0.3	24	0.2	2	0.3	31
22.30	β-caryophyllene	0.9	16	0.6	13	0.6	12
23.02	α-guaiane	0.2	20	0.2	12	0.3	19
23.83	α-humulene	0.3	16	0.2	10	0.2	11
23.95	cis-β-farnesene	0.2	19	0.1	18	0.2	20
25.55	α-selinene	0.1	11	ND		0.1	0.0
25.83	ST <i>m/z</i> : 204 (M ⁺) 189, 107, 91, 77	0.4	19	0.2	24	0.4	17
26.13	γ-cadinene	0.1	11	0.1	0.0	0.1	15
27.24	ST <i>m/z</i> : 204 (M ⁺) 161, 133, 105	0.2	19	0.2	18	0.2	33
27.42	ST <i>m/z</i> : 204 (M ⁺) 161, 122, 102, 91	0.4	17	0.3	14	0.3	15
28.10	γ-elemene	0.4	23	0.2	43	0.2	26
51.19	CB <i>m/z</i> : 286 (M ⁺) 271, 243, 203	ND		ND		0.2	0.0
54.16	THCV	0.4	8	0.3	8	0.1	3
57.27	CBD	1.5	109	1.6	70	28.0	20
57.69	CBC	0.6	8	0.7	8	1.9	22

	CB <i>m/z</i> : 314 (M ⁺) 299, 272, 244	ND		ND	0.4	21
60.18	Δ^9 -THC	46.5	6	35.4	10	23.5
61.81	CBG ^{b)}	2.3	11	0.7	20	0.9
61.81	CBN ^{b)}	0.1	5	0.1	8	<0.1

ND= not detected. ST= unknown sesquiterpenoid. CB= unknown cannabinoid. a) Retention time in GC-FID. b) Values determined by HPLC due to overlap in GC-FID.

Table 4. GC Identification and Quantification of Components in Cannabis smoke

RT min ^{a)}	Compound	Bedrocan mg/g	%RSD n=3	Bedrobinol mg/g	%RSD n=3	Bediol mg/g	%RSD n=3
3.10	ethyl benzene ^{b)}	0.2	30	0.1	19	0.2	16
3.14	<i>ortho</i> -xylene ^{b)}	0.2	12	0.1	27	0.2	22
3.47	1, 3, 5, 7-cyclooctatetraene ^{b)}	0.1	12	0.1	36	0.2	8
3.75	Ethanone, 1-(2-furanyl) ^{b)}	ND		0.1	17	0.1	10
3.84	1,3-benzenediamine ^{b)}	ND		0.1	9	0.1	13
	unknown <i>m/z</i> : 110 (M ⁺) 95, 58	0.1	22	ND		ND	
4.08	α -thujene	0.1	8	ND		ND	
4.23	α -pinene	0.4	7	0.7	5	0.3	8
4.92	1,3,5-trimethylbenzene ^{b)}	ND		ND		0.1	7
5.13	sabinene	0.1		ND		ND	
5.27	β -pinene	0.8	9	0.6	14	0.5	13
5.53	β -myrcene	2.1	8	1.9	4	1.9	8
5.99	α -phellandrene	0.3	10	ND		0.5	14
6.14	Δ^3 -carene	0.2	7	ND		0.1	4
6.30	α -terpinene	0.2	21	ND		ND	
6.53	cymene	0.1	18	0.1	40	0.9	8
6.68	β -phellandrene	0.5	12	0.1	4	0.3	7
6.70	limonene	0.4	21	ND		ND	
7.20	<i>cis</i> -ocimene	1.4	7	0.2	3	0.2	3
7.41	phenol, 3-methyl ^{b)}	ND		0.1	15	0.1	17
7.58	γ -terpineol	0.1	1	ND		ND	
8.17	phenol, 4-methyl ^{b)}	0.2	24	0.3	37	0.2	0.4
8.51	terpinolene	5.4	11	0.2	24	1.3	9
8.69	<i>para</i> -cymene	0.1	25	0.1		0.4	10
9.06	linalool	0.1	16	ND		ND	
9.28	4-pyridinol ^{b)}	0.4	21	0.4	36	0.4	19
9.51	1,3,8- <i>p</i> -menthatriene ^{b)}	ND		ND		0.9	18
10.32	cycloheptane, 1,3,5-tris(methylene) ^{b)}	ND		ND		0.1	11
10.44	benzene, 1-isocyano-2-methyl ^{b)}	0.1	28	0.1	13	0.2	23
11.68	phenyl, 4-ethyl ^{b)}	ND		0.1	6	ND	

	unknown <i>m/z</i> : 134 (M ⁺) 89,						
12.72	71, 56	0.2	4	ND	0.4	6	
12.76	terpineol	0.2	11	ND	ND		
13.88	benzaldehyde, 2-methyl ^{b)}	ND		0.2	21	0.2	21
16.88	indole ^{b)}	0.1	26	0.2	17	0.2	7
20.88	1H-indole, 3-methyl ^{b)}	ND		0.1		0.1	16
22.30	β-caryophyllene	0.8	14	0.5	14	0.5	5
22.81	<i>trans</i> -α-bergamotene	0.2	16	ND		ND	
23.03	α-guaiene	0.3	4	ND		0.4	5
23.82	α-humulene	0.3	8	0.2	17	0.2	1
23.95	<i>cis</i> -β-farnesene	0.2	11	ND		0.3	5
25.24	β-selinene	0.1	36	0.1	11	0.1	9
25.55	α-selinene	0.1	39	0.1	21	0.1	4
	ST <i>m/z</i> : 204 (M ⁺) 189, 107,						
25.84	91, 77	0.4	15	ND		0.4	5
26.14	γ-cadinene	0.1		0.1	20	0.2	13
26.52	β-gurjunene ^{b)}	0.1		ND		ND	
	ST <i>m/z</i> : 204 (M ⁺) 189, 161,						
27.10	133, 105	0.1		0.1	17	ND	
	ST <i>m/z</i> : 204 (M ⁺) 161, 133,						
27.24	105, 91	0.3	19	0.2	17	0.2	2
	ST <i>m/z</i> : 204 (M ⁺) 161, 122,						
27.42	102, 91	0.5	18	0.3	16	0.3	3
28.10	γ-elemene	0.3	21	0.1	15	0.1	6
	ST <i>m/z</i> : 204 (M ⁺) 161, 107,						
28.47	91, 69	0.1		ND		0.1	9
32.02	Δ-selinene ^{b)}	ND		ND		ND	
36.21	olivitol ^{b)}	0.1	34	0.1		0.6	6
37.57	1-(3-methylbutyl)-2,3,5,6-tetramethylbenzene ^{b)}	0.1		ND		0.1	
39.04	7-octadecyne, 2-methyl ^{b)}	0.2	7	0.2	11	0.3	3
40.54	3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol ^{b)}	0.1	3	0.1	19	0.1	4
43.42	CB <i>m/z</i> : 232 (M ⁺) 231, 174	0.2	36	0.3	33	0.3	17
	CB <i>m/z</i> : 246 (M ⁺) 232, 231,						
43.94	190, 175	0.1	23	ND		0.4	6
	CB <i>m/z</i> : 258 (M ⁺) 244, 243,						
47.95	215, 175	0.1	2	0.2	24	0.1	
	CB <i>m/z</i> : 248 (M ⁺) 206, 193.						
48.90	136	ND		ND		0.2	13
	CB <i>m/z</i> : 258 (M ⁺) 243, 215,						
49.07	175	ND		ND		0.2	10
	CB <i>m/z</i> : 286 (M ⁺) 271, 243,						
51.23	203	ND		ND		0.1	5
	CB <i>m/z</i> : 314 (M ⁺) 299, 271,						
53.12	258, 232	0.3	29	0.3	16	0.8	3
54.24	THCV	0.3	34	0.2	6	0.1	7
	CB <i>m/z</i> : 314 (M ⁺) 299, 258,						
54.79	243, 232	0.2	11	0.1	22	0.3	9
	CB <i>m/z</i> : 312 (M ⁺) 270, 256,						
54.99	257, 214	0.2	4	ND		0.1	6

55.87	CB <i>m/z</i> : 310 (M+) 295, 238, 223	0.1	8	0.1	ND	0.2
56.25	CB <i>m/z</i> : 316 (M+) 274, 260, 232	0.1	ND	0.1	35	0.2
56.73	CB <i>m/z</i> : 314 (M+) 246, 231, 175	ND	ND	ND	0.4	11
57.35	CBD	0.5	80	0.1	ND	21.1
57.82	CBC	0.4	34	0.3	36	1.3
58.48	CB <i>m/z</i> : 313 (M+) 297, 272, 244, 231	0.2	ND	0.2	59	0.1
58.68	CB <i>m/z</i> : 314 (M+) 299, 272, 244, 232	0.1	ND	ND	ND	ND
59.02	CB <i>m/z</i> : 314 (M+) 299, 272, 243, 232	0.2	ND	ND	ND	ND
58.99	Δ^8 -THC	1.0	17	0.2	12	0.5
59.39	CB <i>m/z</i> : 352 (M+) 314, 282, 259, 232	ND	ND	ND	0.5	4
59.67	CB <i>m/z</i> : 299 (M+) 300	0.4	18	0.3	41	0.1
60.23	Δ^9 -THC	36.2	39	26.7	9	17.6
60.80	CB <i>m/z</i> : 314 (M+) 299, 272, 256, 243	ND	ND	ND	0.3	ND
61.71	CB <i>m/z</i> : 312 (M+) 298, 270, 257, 232	0.4	97	0.2	41	0.2
61.94	CBG ^{c)}	2.5	16	0.9	25	1.0
61.94	CBN ^{c)}	6.9	2	3.5	25	2.9
62.33	CB <i>m/z</i> : 312 (M+) 296, 272, 270, 257	0.1	17	ND	ND	ND
62.70	CB <i>m/z</i> : 337 (M+) 312, 298, 282	0.2	18	ND	ND	ND
63.22	1'-oxo-cannabinol ^{b)}	0.1	12	ND	ND	ND
63.56	CB <i>m/z</i> : 334 (M+) 319, 300, 263	0.1	13	ND	ND	ND
63.74	CB <i>m/z</i> : 352 (M+) 338, 310, 270	0.2	38	ND	ND	ND

ND= not detected. ST= unknown sesquiterpenoid. CB= unknown cannabinoid. a) Retention time in GC-FID. b) Compounds putatively identified on NIST (2005) library search >80% match. c) Values determined by HPLC due to overlap in GC-FID.

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