2-METHYL- AND 4-METHYL-Δ^8-TETRAHYDROCANNABINOL: CORRELATION OF SPATIAL DISTINCTION WITH CANNABINOID RECEPTOR BINDING‡

Robert Glaser* and Itay Adin

Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

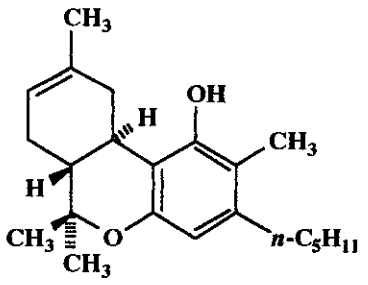
Raphael Mechoulam* and Lumir Hanuš

Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel

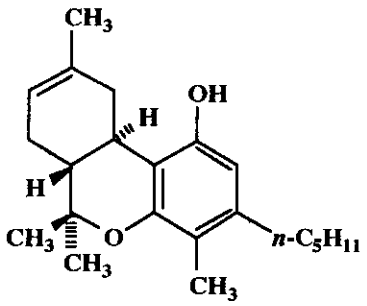
Abstract– A high level of binding to the brain cannabinoid receptor was found for 2-methyl-Δ^8-tetrahydrocannabinol (THC) while no binding was observed for 4-methyl-Δ^8-THC. Four energy minima were found by molecular mechanics for ethyl side-chain models of Δ^8-THC [syn- and antiperiplanar and (±)-orthogonal values for torsion angle C(4)–C(3)–C(α)–C(β)]. The active Δ^8-THC and 2-methyl-Δ^8-THC molecules share a common structural feature [a relatively low energy *synperiplanar* value C(4)–C(3)–C(α)–C(β) torsion angle conformation for the *n*-pentyl side chain] that is not exhibited by the inactive 4-methyl-Δ^8-THC analogue. This spatial distinction may represent the basis for the difference in biological activity between the two isomers examined.

‡ *This paper is dedicated to Dr. Arnold Brossi on the occasion of his 70th birthday*

INTRODUCTION



(1)



(2)

We have reported earlier[1] that 2-methyl-Δ^8-tetrahydrocannabinol (alternate nomenclature: 4'-methyl-Δ^6-THC) (**1**) and 4-methyl-Δ^8-tetrahydrocannabinol (6'-methyl-Δ^6-THC) (**2**) which structurally differ only in the position of a single methyl group, differ very significantly in their potency as cannabimimetics in monkeys. This distinction could be intrinsic, due to differences in binding to the receptor, presumably for steric reasons. However as the only test in the past was an *in vivo* one, a possibility that had to be excluded, was that the difference was not intrinsic, but was due to metabolic or pharmacokinetic reasons. Hence, it seemed of interest to confirm the structures of these constitutional isomers by modern techniques, to compare their level of direct binding to the brain cannabinoid receptor and to establish their spatial configurations. These data could throw light on the structural requirements of the cannabinoid receptor and compliment some of our earlier efforts[2,3] in this area.

This paper reports the structure determination of **1** and **2** based upon ^1H and ^{13}C nmr techniques including the nuclear Overhauser effect, the binding affinity of these methylated cannabinoid derivatives to the brain cannabinoid receptor and the effect of the 2- and 4-methyl substituents on the conformation about the C(3)–C(α) bond of the side chain. The latter effect was investigated by molecular mechanics.

RESULTS AND DISCUSSION

Compounds (**1**) and (**2**) were prepared, as previously described (see Figure 1 below),[1] from cannabidiol (**3**), readily available from Cannabis resin in an enantiomerically pure form, $[\alpha]_D$ −125°, in ethanol. The original assignments for **1** and **2**, made more than two decades ago on the basis of nmr measurements with 60 MHz instruments, had first to be confirmed.

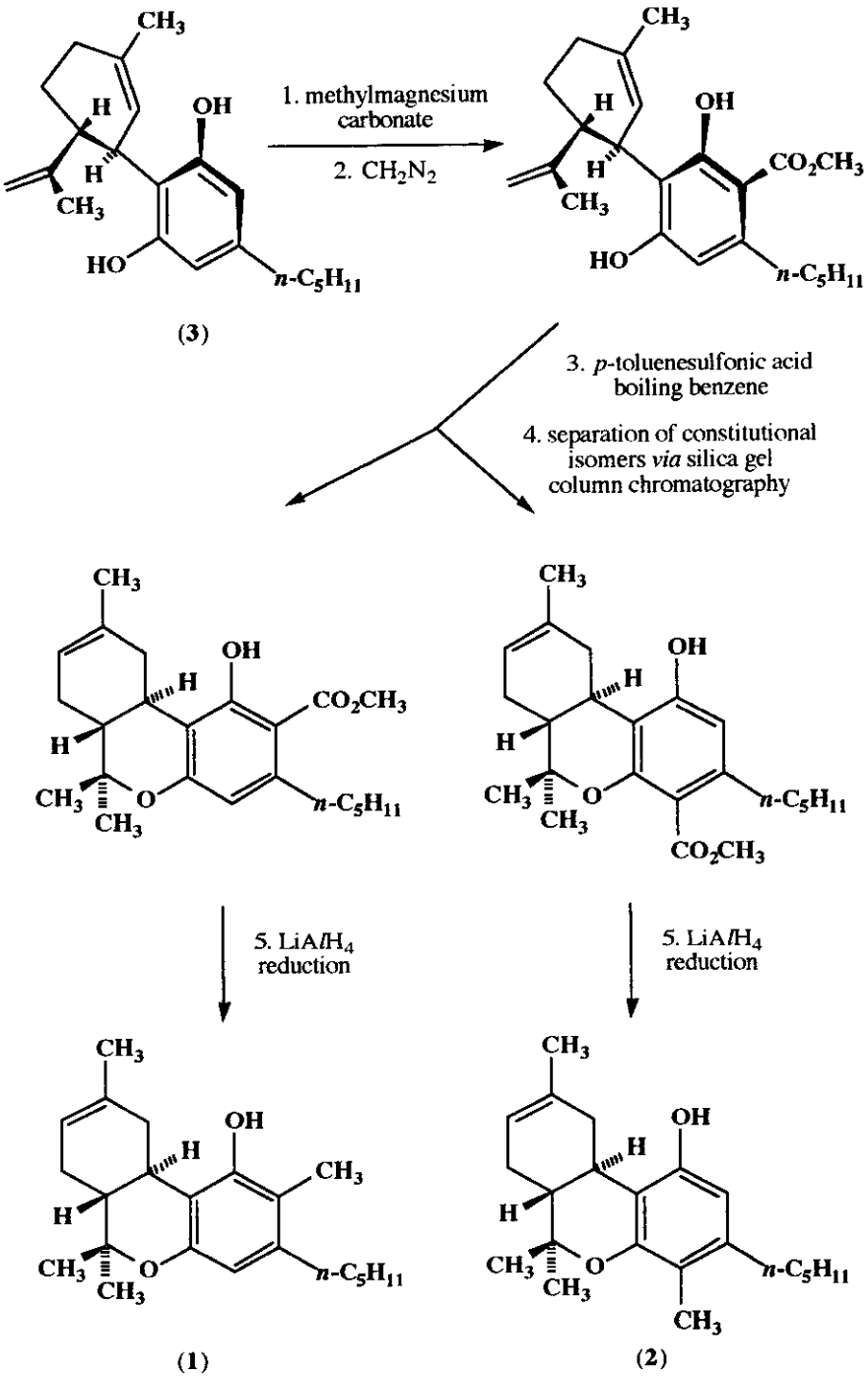
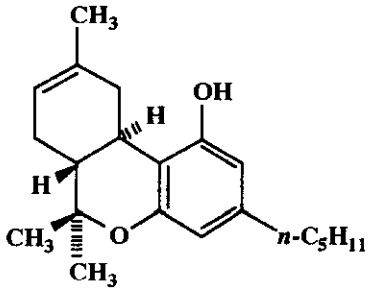
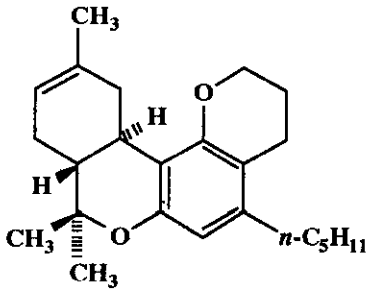


Figure 1. Scheme for the synthesis of 2-methyl- and 4-methyl-Δ^8-tetrahydrocannabinol (**1**) and (**2**) from cannabidiol (**3**) as starting material.

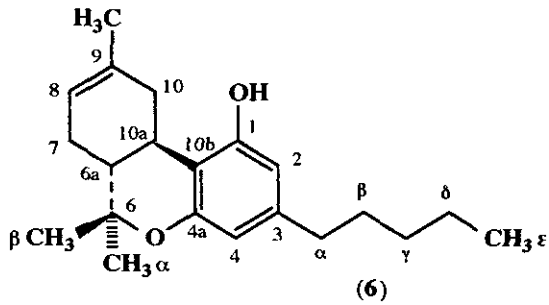
Determination of the position of the aromatic methyl substituent in the two isomers was unequivocally made by nmr proton-proton nuclear Overhauser effect difference experiments (NOEDIFF). A 1.9% intensity enhancement of the 4.77 ppm O*H* signal in **1** was observed {C(2)-C*H*$_3$, 2.10 ppm}, while no significant NOE was found upon irradiation of the externally heterotopic aromatic methyl protons in (**2**) {C(4)-C*H*$_3$, 2.04 ppm}. The internally heterotopic pair of H(2) and H(4) protons in the parent Δ^8-THC (**4**) resonate at 6.11 and 6.30 ppm,[4,5] respectively, while an external comparison of the signals from this same pair of nuclei in **2** and **1** show them to appear at 6.09 and 6.31 ppm, respectively. The 2-substituted Δ^8-THC derivative [*O*,2-propano-Δ^8-tetrahydrocannabinol] (**5**) shows a similar 6.28 ppm value for H(4),[6] which is consistent with the above-mentioned comparisons. Compounds (**1**, **2** and **5**) have only one protonated aromatic carbon nucleus each, and thus the appearance of its resonance in the ^{13}C {^1H} nmr spectrum is readily recognized in terms of multiplicity and in its relatively high intensity (vis-à-vis quaternary aromatic carbons). The internally heterotopic pair of C(2) and C(4) carbons in **4** resonate at 107.6 and 109.8 ppm,[5] respectively, while an external comparison of the signals from this same pair of nuclei in **2** and **1** show them to appear at 107.6 and 110.4 ppm, respectively. The C(4) nucleus in the *O*,2-propano model (**5**) shows a similar 109.6 ppm value.[6] The ^{13}C and selected ^1H nmr spectral parameters for compounds (**1**) and (**2**) (recorded at 50 and 200 MHz respectively) and (**4**) and (**5**) (literature data)[4-6] are presented in Table 1 [see structure (**6**) for the numbering scheme]. Inspection of Table 1 shows that methylation at either C(2) or C(4) positions did not result in a marked change in the ^{13}C chemical shifts for these two carbon nuclei [δC(2) is 108.1 for **2** and δC(4) is 110.5(1) for **4**]. These nmr results clearly confirm the original stereochemical assignment of isomers (**1**) and (**2**).



(**4**)



(**5**)



(6)

Table 1. ^{13}C and selected ^{1}H nmr chemical shift values for 2-methyl-Δ^8-tetrahydrocannabinol (**1**), 4-methyl-Δ^8-tetrahydrocannabinol (**2**), Δ^8-tetrahydrocannabinol (**4**), and *O*,2-propano-Δ^8-tetrahydrocannabinol (**5**).[a]

δ_C	(1)	(2)	(4) [b]	(5) [c]
C(1)	152.9	152.3	154.6	154.1
C(2)	108.1	107.6	107.6	111.6
C(3)	140.6	140.2	142.4	140.3
C(4)	110.4	110.4 [d]	109.8	109.6
C(4a)	152.2	152.7	154.4	152.1
C(6)	76.3	76.2	76.3	76.1
C(6a)	45.3	44.9	44.8	45.2
C(7)	28.0	28.0	27.8	28.0
C(8)	119.5	119.4	119.1	119.1
C(9)	134.7	134.9	134.5	135.0
C(10)	36.2	35.8	35.9	36.2
C(10a)	31.9	32.0	31.5	31.9
C(10b)	112.1	110.6 [d]	110.4	111.8
C(2)-$\underline{C}H_3$	10.7	[e]	[e]	[e]
C(4)-$\underline{C}H_3$	[e]	10.8	[e]	[e]
C(6)-$\underline{C}H_3$ (α)	18.4	18.7	18.4	18.3
C(6)-$\underline{C}H_3$ (β)	27.6	27.6	27.5	27.5
C(9)-$\underline{C}H_3$	23.5	23.4	23.4	23.4
C(α)	33.8	33.4	35.4	32.2
C(β)	30.1	30.0	30.5	29.3
C(γ)	31.9	32.0	31.5	31.9
C(δ)	22.6	22.6	22.5	22.5
C(ε)	14.1	14.0	14.0	13.9

Table 1 Continued

δ_H	**1**	**2**	**4** [b]	**5**
H(2)	[e]	6.09 [f]	6.11	[e]
H(4)	6.31 [f]	[e]	6.30	6.28
H(8)	5.45 [g]	5.42 [g]	5.45	5.42
H(10a)	2.72 [h]	2.70 [h]	[i]	2.64
H(10α)	3.21 [j]	3.20 [k]	3.22	3.17
C(2)-C$\underline{H}_3$	2.10 [f]	[e]	[e]	[e]
C(4)-C$\underline{H}_3$	[e]	2.04 [f]	[e]	[e]
C(6)-C$\underline{H}_3$ (α)	1.10 [f]	1.06 [f]	1.09	1.09
C(6)-C$\underline{H}_3$ (β)	1.38 [f]	1.39 [f]	1.37	1.36
C(9)-C$\underline{H}_3$	1.71 [f]	1.69 [f]	1.70	1.70
C(α)$\underline{H}_2$	2.50 [h]	2.46 [h]	2.45	2.43
C(ε)$\underline{H}_3$	0.76 [l]	0.92 [l]	0.87	0.90
O$\underline{H}$	4.77 [f]	4.91 [f]	4.81	[e]

[a] ^{13}C (50.3 MHz) and ^{1}H (200.1 MHz) nmr chemical shifts are in ppm downfield from tetramethylsilane, $CDCl_3$ solvent, ^{13}C multiplicities were ascertained for **1** and **2** from the ^{13}C {^{1}H} and DEPT (*D*istortionless *E*nhancement by *P*olarization *T*ransfer, 90° and 135° pulse widths) spectra. ^{13}C and ^{1}H resonances for **1** and **2** were assigned based upon corresponding values for **4** (ref. 4,5) and **5** (ref. 6). [b] ^{13}C nmr data from ref. 5, ^{1}H nmr data from ref. 4. [c] ^{13}C and ^{1}H nmr data from ref. 6. [d] C(4) and C(10b) assignments may be interchanged. [e] Not applicable. [f] Singlet. [g] Doublet, 4.2 Hz. [h] Multiplet. [i] Not given. [j] Doublet of doublets, 4.1 and –16.3 Hz. [k] Doublet of doublets, 4.2 and –16.3 Hz. [l] Triplet, 7 Hz.

The ability of **1** and **2** to bind to the cannabinoid receptor was assessed in a synaptosomal membrane preparation derived from rat whole brain, without brain stem, using a centrifugal assay, [^{3}H]HU-243 being the labeled probe, as previously described.[7-10] In this competition binding assay 2-methyl-Δ^8-THC (**1**) inhibited the binding of the probe with a K_i of 135.9±12.4 nM (see Figure 2 below), while the 4-methyl isomer (**2**) was inactive in concentrations up to 10 μM.

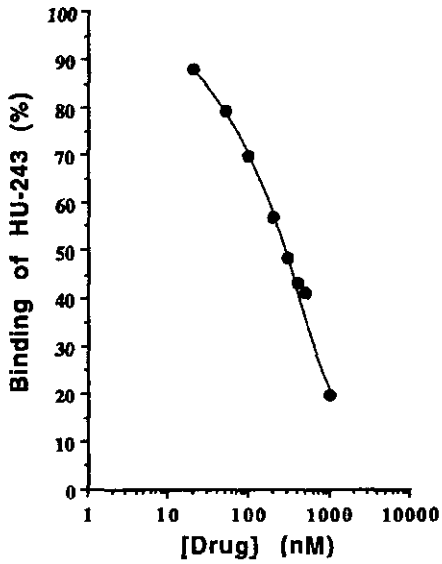
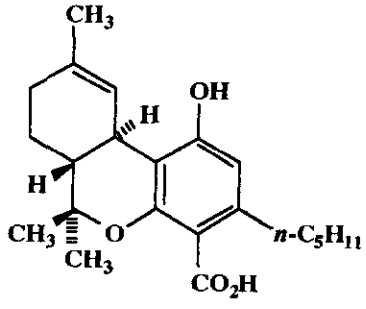


Figure 2. Competitive inhibition of [^{3}H]HU-243 binding by 2-methyl-Δ^8-THC (**1**).

Compound (**1**) is therefore about as active as Δ^9-THC, the natural active plant constituent, which has a K_i in the above test of 52±1.8 nM and anandamide, the major endogenous brain cannabinoid ligand (K_i 39±5 nM).[8] A similar pattern of binding to the cannabinoid receptor was recently observed with halogenated cannabinoids: the 2-iodo-Δ^8-THC had high binding potency, while the 2,4-diiodo and 4-bromo substituted derivatives were *inactive*. The 4-iodo-Δ^8-THC isomer was not prepared and assayed.[11] Our binding results parallel the *in vivo* results obtained with rhesus monkeys.[1] We have reported that **1**, though somewhat less active than Δ^9- or Δ^8-THC, is still quite potent. At 1.0 mg/kg dose of **1** administered intravenously the monkeys showed drowsiness, decreased motor activity, occasional partial ptosis and occasional head drop; at 5.0 mg/kg the animals had severe stupor, ataxia and full ptosis. By contrast **2** caused no effects in doses up to 10 mg/kg. These contrasting results, both *in vivo* and *in vitro*, led us to examine the stereochemical differences between the two compounds. Our assumption was that while substitution at C(2) causes only minor changes in the overall stereochemistry of the molecule (compared to Δ^8-THC), and hence no change in binding potency is observed, the C(4)-methyl group produces a significant change in the stereochemistry of the molecule thus effectively preventing its binding to the receptor. A number of rationalizations can be offered for these observations, *e.g.* a steric barrier in the region of the 4-methyl group within the receptor binding-site, while the

2-methyl substituent is unhindered in the bound state. The *bioactive conformation* of the side-chain C(3)–C(α) bond might also be effected by the presence or absence of nearby 2- and 4-substituents on the aromatic ring. Modeling studies using the MMX[12] molecular mechanics force field were undertaken to investigate this possibility. MMX is an enhanced version of Allinger's MM2 program[13] with MMP1 π-subroutines[14] incorporated for localized π-electron systems. An ethyl rather than *n*-pentyl side-chain was utilized in the calculated models since it was reasoned that the effect of 2- or 4-methyl substituents on the side-chain would be primarily observed as torsional constraints involving the C(3)–C(α) bond. The *X*-ray crystallographically determined structure of (–)-Δ^9-THC acid B (**7**)[15] was used as the cannabinoid input structure, and then modified into a Δ^8-analogue having an ethyl side-chain. Torsion angle C(4)–C(3)–C(α)–C(β) was utilized as a reference angle for comparison of C(3)–C(α) bond conformations. Since the torsion angle C(4)–C(3)–C(a)–C(β) involves an C_{sp^2}–C_{sp^3} bond, it was varied in 60° increments starting from 0° to 300° and used as the input angle. The final values for the reference torsion angle and the calculated relative energies for models (**1**, **2** and **4**) are listed in Table 2 below.



(**7**)

Only four energy minima were found by molecular mechanics for ethyl side-chain models of Δ^8-THC (**4**) [syn- and antiperiplanar and (±)-orthogonal values for torsion angle C(4)–C(3)–C(α)–C(β)] when the initial values of 0, +60, +120, and 180° were used. These minima were observed to be rather broad (*ca.* ±10°). Inspection of Table 2 shows two sets of approximately isoenergetic C(3)–C(α) bond conformations [a lower energy (+)-orthogonal value set, and a slightly higher set containing the syn- and antiperiplanar value conformations]. We

note that the *X*-ray crystallographically determined structure of (–)-Δ^9-THC acid B (**7**) has a (+)-orthogonal value [90.1°] for the C(4)–C(3)–C(α)–C(β) torsion angle.[15] Non-bonded interactions resulting from substitution of a 2- or 4-methyl group effectively remove the side-chain conformation which places C(β) synperiplanar relative to its methyl neighbor. Initial conformations having values of 180° for the reference torsion angle C(4)–C(3)–C(α)–C(β) in model (**1**) and 0° for the corresponding angle in model (**2**) afforded high energy structures which were converted to orthogonal ethyl side-chain conformations upon energy minimization. The active Δ^8-THC (**4**) and 2-methyl- Δ^8-THC (**1**) molecules share a common structural feature [a relatively low energy *synperiplanar* value torsion angle C(4)–C(3)–C(α)–C(β) conformation for the *n*-pentyl side chain] that is not exhibited by the inactive 4-methyl-Δ^8-THC (**2**) analogue. This spatial distinction may represent the structural basis for the difference in biological activity between the two isomers examined. However, the relevance of this observation to the cannabinoid SAR remains to be proven.

Table 2. Molecular mechanics calculated relative energies for ethyl side-chain modified models of Δ^8-THC (**4**), 2-methyl-Δ^8-THC (**1**), and 4-methyl-Δ^8-THC (**2**) versus torsion angle C(4)–C(3)–C(α)–C(β).[a]

C(4)–C(3)–C(α)–C(β) reference torsion angle descriptor	relative calcd. energy for model of Δ^8-THC (**4**)	relative calcd. energy for model of 2-methyl-Δ^8-THC (**1**)	relative calcd. energy for model of 4-methyl-Δ^8-THC (**2**)
synperiplanar	4.18 [–7.1°]	5.06 [3.4°]	*unstable*
(+)-orthogonal	0.00 [88.7°]	0.00 [82.4°]	0.00 [96.2°]
antiperiplanar	4.26 [–175.7°]	*unstable*	4.47 [–162.8°]
(–)-orthogonal	0.17 [–88.9°]	0.54 [–87.3°]	0.63 [–95.1°]

[a] Calculated energies relative to the (+)-orthogonal conformation for models of (–)-(6a*R*,10a*R*)-(**1**, **2**, and **4**) cannabinoids are given in kJoule/mol, final values of torsion angle C(4)–C(3)–C(α)–C(β) are presented in square brackets.

EXPERIMENTAL

2-Methyl-Δ^8-THC (**1**) and 4-methyl-Δ^8-THC (**2**) were prepared by the methods described in the literature[1] (see synthesis scheme summarized in Figure 1). ^1H and ^{13}C nmr spectra ($CDCl_3$) were recorded at 200.1 and 50.3 MHz, respectively, on a Bruker WP-200-SY Fourier transform spectrometer (4.7 T). The deuterated solvent was used as an internal lock, and tetramethylsilane was used as an internal reference. Carbon multiplicities and proton-proton NOE experiments were performed using the respective DEPT (90° and 135° pulse widths) and NOEDIFF pulse sequences in the Bruker microprogram library. The minimized-energy geometry of the molecular mechanics model compounds were determined by the PCMODEL/MMX 4.51 program[12] running on a Macintosh Quadra-950 computer.

Ligand binding assay. The synaptosomal membrane preparation from rat brains and the ligand-binding assay with a [^{3}H]HU-243 probe as used in our laboratory have been described.[9,10] The [^{3}H]HU-243 probe (45 to 55 pM) was incubated with synaptosomal membranes (3 to 4 μg) for 90 min at 30 °C with the indicated concentrations of anandamide or with the vehicle alone (fatty-acid-free bovine serum albumin at a final concentration of 0.5 mg/ml). Bound and free radioligand were separated by centrifugation. The data were normalized to 100% of specific binding, which was determined with 50 nM unlabeled HU-243. Specific binding accounted for 77 to 82% of the total radioactivity bound to the membranes. The binding curve for compound (**1**) is presented in Figure 2 above.

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