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THE CONFORMATION OF (-) 8α AND (-) 8β-HYDROXY-Δ^9-TETRAHYDROCANNABINOLS AND THEIR INTERACTIONS WITH MODEL MEMBRANES

Cornelis J. Van der Schyf+, Thomas Mavromoustakos and Alexandros Makriyannis*

Department of Medicinal Chemistry and Pharmacognosy, and Institute of Materials Science, The University of Connecticut, Storrs, CT 06268 and the Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139.

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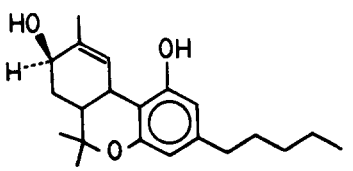
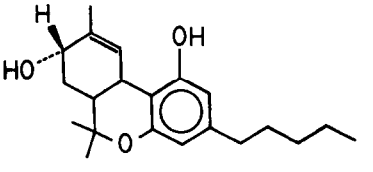
Summary

8α- and 8β-Hydroxy-Δ^9-tetrahydrocannabinols (THC's), two metabolites of the naturally occurring Δ^9-THC have been shown to possess differences in pharmacological activity. We have studied the conformations of these two compounds, as well as their interactions with model membrane systems and compared them with Δ^9-THC. The conformational study, carried out in solution and using high resolution NMR indicated that differences in the ring conformations of these two compounds were negligible but that the 8-hydroxy group of the 8β-OH compound extended approximately 1.4Å higher above the plane of the aromatic ring than in the 8α-OH isomer. This difference could prove significant in the interaction of these molecules with lipid bilayers. We found that both 8α- and 8β-OH analogs affected the melting behavior of hydrated DPPC bilayers including a lowering of the main transition temperature (T_c), a broadening of that transition and the abolishment of the pretransition of DPPC. The effects of the more active compound, 8β-OH-Δ^9-THC on the model membrane approximated closely those of Δ^9-THC, while the less active 8α-OH epimer produced different thermotropic changes.

Since the first modern systematic study of the structure-activity relationships of cannabinoids was carried out (1,2), it became evident that small structural changes in these compounds, with no significant effect on their partitioning properties, may lead to large differences in pharmacological activity. Some aspects of this observation was addressed in our earlier work on two tetrahydrocannabinols (THC's), Δ^9-THC and $\Delta^{9,11}$-THC (3). These two analogs differ structurally only in the position of the double bond in ring C, but show very different psychotropic activities; Δ^9-THC being a potent psychoactive agent, while $\Delta^{9,11}$THC is practically inactive. We have demonstrated



8ß-OH-Δ^9-THC 1



8α-OH-Δ^9-THC 2

+On Sabbatical Leave from the Department of Pharmaceutical Chemistry, Potchefstroom University for CHE, Potchefstroom 2520, South Africa.

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that the shift in position of the double bond introduces a change in the geometry of the C ring, leading it to assume a conformation in which all three rings are coplanar in the case of $\Delta^{9,11}$-THC, while Δ^{9}-THC has the C-ring protruding out of the plane of the other two rings. We have also shown that Δ^{9}-THC perturbed model membranes more effectively than its inactive $\Delta^{9,11}$-isomer (3). It has been suggested that these conformational differences can be used to provide an explanation for the observed differences in the manner in which the active and inactive analogs interact with model and biological membranes (3). Recently we provided evidence for this hypothesis from ^{2}H solid-state NMR experiments where we showed that (-)-Δ^{9}-THC perturbed a DPPC model membrane preparation more effectively than its inactive (-)-$\Delta^{9,11}$-THC (4). Similarly, we found (-)-Δ^{9}-THC to perturb the same membrane preparation more than its much less active (+)-enantiomer. Differential scanning calorimetry (DSC) (5) and solid state ^{2}H NMR spectroscopy (3) have indicated a broadening of the phase transition and a lowering of the main phase transition temperature (T_c) in hydrated dispersions of dipalmitoylphosphatidylcholine (DPPC) in the presence of Δ^{9}-THC. These changes are probably caused by an increase in the number of gauche: trans segments in the chains, apparently as a result of conformational alterations ("perturbations") brought about by the insertion of Δ^{9}-THC in the bilayer (5).

8α-and 8β-OH-Δ^{9}-THC are two epimeric Δ^{9}-THC metabolites exhibiting differences in their psychotropic activity. The 8β-hydroxy epimer (1) closely mimics the types of effects typical to Δ^{9}-THC, although it is much less active, while the 8α-hydroxy analog (2) lacks such activity (2,6,7).

We felt that differences between the two analogs in molecular geometry and/or their effects on cellular membranes may explain the observed differences in pharmacological activity.

The present study examines the conformations of 8α- and 8β-OH-Δ^{9}-THC in solution, using ^{1}H NMR spectroscopy. Vicinal coupling constants were used to calculate proton-proton dihedral angles in the B and C ring systems of the two molecules in order to determine the ring geometry, while NOE values were used to establish the spatial proximity of pairs of protons in the same molecule. We have further studied, by using DSC, the effects of different concentrations of these two compounds on model membranes of hydrated DPPC with and without addition of cholesterol. These effects were compared with those produced by Δ^{9}-THC under identical experimental conditions.

Experimental

^{1}H high resolution NMR spectra were obtained on a home-built spectrometer at the Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA, operating at 500 MHz. A sweep width of 4000 Hz and 8192 data points were used. Sample concentrations were 0.01M in $CDCl_3$, and were fully degassed to remove all oxygen.

^{1}H-^{1}H nuclear Overhauser enhancement (NOE) (8) measurements were obtained by irradiating the required frequency with a low power for a duration of 0.5 sec prior to accumulation of the free induction decay. A recycle delay of 10 sec between decays was allowed. The power requirements for irradiation were chosed to optimize the NOE effect while still maintaining selectivity for a specified frequency and were generally in the region of 25-27 dB. To obtain a reference spectrum with no NOE, the oscillator frequency was moved to a frequency greater than 100 Hz from any proton absorption. Difference spectra (NOEDS) were obtained by subtracting the FID's of the ^{1}H-irradiated spectra from the FID of the reference spectrum. Percentage NOE values were obtained from peak height measurements on the difference spectra.

^1H-^1H coupling constants were extracted from the corresponding ^1H spectra with the help of spectral simulations where second order effects disallowed first order analysis (9-11). We used the ITRCAL program developed by the Nicolet Instrument Corporation which uses the method reported by Castellano and Bothner-By (10). A 12K Nicolet 1080 computer system and an NIC-294 Disk Memory were used to perform the calculations (12). The spectral simulations were useful in determining accurate values of coupling constants used in the conformational analysis of the compounds (Table II). ^1H NMR assignments were made from an analysis of the scalar couplings and extensive homonuclear decoupling experiments.

Differential scanning calorimetric (DSC) traces were obtained on a Perkin-Elmer DSC-2 instrument. The samples were prepared by dissolving 5-10 mg of DPPC (Sigma) and the appropriate amounts of cholesterol and/or 8α-OH-Δ^9-THC (2) and 8β-OH-Δ^9-THC (1), in chloroform. After removing the solvent using an O_2-free N_2 stream, the samples were dried under vacuum for 6 h. The preparations were scraped into weighed stainless steel pans, distilled and deionized water (50% w/w) was added and the pans were sealed. In order to ensure full hydration of the bilayers, the samples were cycled several times through the transition (up to 50°C, which is above the phase transition temperature for pure DPPC) until identical scans were obtained. The samples were then left to equilibrate in a refrigerator overnight. The samples were scanned at a heating rate of 2.5K/min. The temperature used was from 7° to 57°C. Concentrations of the two compounds (1 and 2), Δ^9-THC and cholesterol in the DPPC bilayers, are expressed as mole fractions against DPPC and represented by X_{THC1} X_{THC2}, X_{THC3} and X_{CHOL}, respectively. These concentrations are dependable to within ±5%.

The lipophilic properties of 8α-OH and 8β-OH Δ^9-THC were assessed using reversed phase TLC on silica gel G plate impregnated with high temperature silicone oil (Aldrich) (13). R_m values were determined using MeOH/H_2O system (60:40 v/v). The mean of 3 R_f values was used to determine the R_m value from the formula: $R_m = \log\left(\frac{1}{R_f} - 1\right)$.

Results

Conformational analyses

The chemical shift assignments for 8β-(1) and 8α-OH-Δ^9-THC (2), are given in Table I. These are in agreement with previously reported work by Wall (12) and Ben-Zvi, et al. (15), and provide the first comprehensive study on the 8α-OH epimer. Dihedral angles were calculated using a modified Karplus (16,17) equation, the details of which are given in Table II. Of critical importance to our conformational analysis are the dihedral angles between the 7β-8α and 7β-8β protons in 1 and 2 which were found to be 57 and 48 degrees, respectively. These indicate that the substituents on C-7 and C-8 are not perfectly staggered, and that the cyclohexene (C) ring exists in a distorted half-chair conformation with C-8 and C-9 above the plane of the aromatic and B-rings. These findings are in good agreement with the crystallographic data reported for the 8β-OH-Δ^9-THC/DMF complex by Ottersen and Rosenqvist (18) and further indicate that the ring conformations of the two epimers do not differ significantly. This similarity in conformation between the two epimers is further confirmed by the NOE values (Table III). The very similar NOE's for the 8α and 8β protons in 1 and 2 respectively, upon irradiation of the 9 methyl group, indicate that the $9CH_3$ orients approximately halfway between the 8H and 8OH when viewed along the C8-C9 bond. Using molecular graphics (CHEMX, developed and distributed by Chemical Design Ltd., Oxford, England), we found that the oxygen atom of the 8β hydroxyl is situated approximately 3.2Å above

TABLE I

^{1}H NMR (500 MHz) Chemical Shift Assignments for 8β-OH-Δ^9-THC and 8α-OH-Δ^9-THC in $CDCl_3$ Solution at 25°C. Chemical Shift (ppm)[a]

Proton	8β-OH-Δ^9-THC[c]		8α-OH-Δ^9-THC[d]	
2	6.1	(6.11)	6.11	
4	6.26	(6.24)	6.24	
6a	1.79		1.76	
6α-CH	1.08	(1.04)	1.1	
6β-CH	1.4	(1.35)	1.39	
7α	b		1.35	
7β	2.01		2.32	
8	4.07	(4.05)	4.28	(4.32)
9-CH_3	1.81	(1.76)	1.76	
10	6.64	(6.69)	6.51	
10a	3.1	(3.06)	3.28	
1'	2.41	(2.41)	2.41	
2'	1.53	1.53	1.54	
3'	1.28		1.28	
4'	1.28		1.28	
5'	0.85	(0.88)	0.85	
1-OH	4.83		4.84	

a. Relative to TMS at 0 ppm
b. Not observed due to peak overlap
c. Values in parentheses (at 100 MHz) are from (14)
d. Value in parentheses is from (15)

TABLE II

^{1}H NMR Geminal and Vicinal Coupling Constants and Dihedral Angles Determined for 8β-OH-Δ^9-THC and 8α-OH-Δ^9-THC

			8β-OH-Δ^9-THC		8α-OH-Δ^9-THC	
Type of coupling	H':H	Designation	n_J[a] (Hz)	Dihedral angle[b] (degrees)	n_J[a] (Hz)	Dihedral angle[b] (degrees)
Geminal, 2 bond	7α-7β	a-e	-13.2	c	-12.0	c
Vicinal 3 bond	6a-7α	a-a	12.2	171	12.3	173
	6a-7β	a-e	2.2	67	1.7	70
	6a-10a	a-a	9.2	149	10.8	158
	7α-8α	a-e	2.9	63	---	---
	7α-8β	a-a	---	---	9.1	149
	7β-8α	e-e	.91	75	---	---
	7β-8β	e-a	---	---	5.9	50
	10a-10a	---	2.1	112.5[d]	2	112[d]

a. Determined from spectra obtained at 500 MHz and spectral simulation using ITRCAL; n is the number of bonds through which coupling occurs; gem., n=2, vic., n=3.
b. Calculated using the equation $\varphi = \cos^{-1}\sqrt{n_J / K}$; where K_{a-a} 12.5 Hz, $K_{a-e} = K_{e-a}$ = 14.3 Hz and K_{e-e} = 12.9 Hz, φ is the dihedral angle, K values calculated from 1,3,5-trimethyl-cyclohexane (20).
c. Vicinal bond angles cannot be quantitatively determined.
d. This vicinal coupling involves an sp^2 hybridized carbon but still obeys the Karplus relationship. The small magnitude of ^3J indicates an angle near 90°.

the plane of the aromatic ring, while the 8α hydroxyl is only about 1.8Å above that plane. This positional difference in the 8-hydroxy groups thus present the only significant conformational difference between the two molecules (see Fig. 1).

TABLE III

NOE's Observed for 8β-OH-Δ^9-THC and 8α-OH-Δ^9-THC. The Numbers Represent % Enhancement Compared to a Control Spectrum.

Compound	Proton irradiated	Proton observed	% NOE
8β-OH-Δ^9-THC	6α	10	9.4
	6β	7β	7.4
	11Me	8α	3.5
	11Me	10	5.6
8α-OH-Δ^9-THC	6α	10a	10.8
	6β	7β	7.8
	11Me	8β	3
	11Me	10	5.4

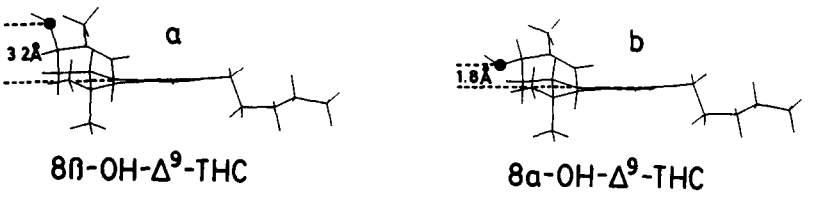

FIG. 1

Position of the 8-hydroxy group of 8β-OH-Δ^9-THC (a) and 8α-OH-Δ^9-THC (b) above a plane through the aromatic ring.

Differential scanning calorimetry

Normalized thermograms of DPPC preparations containing increasing concentration of each of the two cannabinoids (X_{THC1}, X_{THC2} = 0-0.2) are shown in Fig. 2. The thermogram for pure DPPC indicate a pretransition centered at 34° C while the onset and the peak maximum (T_c) for the main transition occurs at 40.14°C and 41.2°C, respectively. At low concentrations of the compounds (X_{THC} =0.05), the pretransition is eliminated, while the main transition is broadened and both the onset temperature and T_c occur at lower temperatures. Both the broadening of the main transition, as well as the lowering of T_c, are more pronounced at X_{THC1}=0.05, than at X_{THC2}=0.05 (Table IV). As X_{THC} increases to 0.1, severe broadening of the main transition occurs, while a new peak appears as a shoulder at 32°C (1) and 31°C (2). At a further increase of X_{THC} to 0.2, two peaks appear clearly in the thermograms. The lower with maxima at 30°C (1) and 24°C (2) becoming more distinct than the higher temperature peaks, a feature which is also observed for Δ^9-THC/DPPC bilayers (4) (see Fig. 3).

In the concentration region studied (X_{THC}= 0 to 0.2) the enthalpy of phase transition (ΔH) for DPPC did not change significantly and was similar to the ΔH of pure DPPC within experimental error.

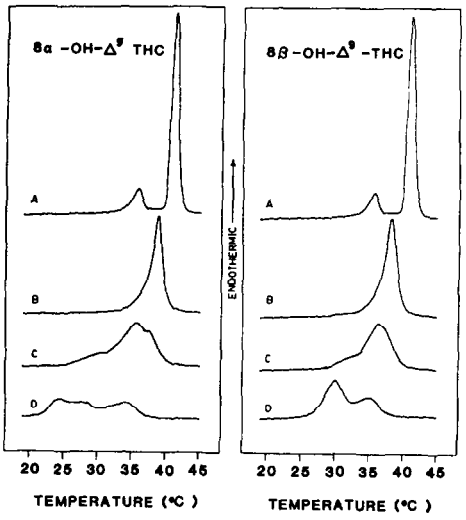

FIG. 2

Thermograms of DPPC with 8α-OH-Δ^9-THC (left): (A) DPPC alone (B) X_{THC2}=0.05 (C) X_{THC2}=0.1 (D) X_{THC2}=0.2, and 8β-OH-Δ^9-THC (right): (A) DPPC alone (B) X_{THC1}=0.05 (C) X_{THC1}=0.1 (D) X_{THC2}=0.2.

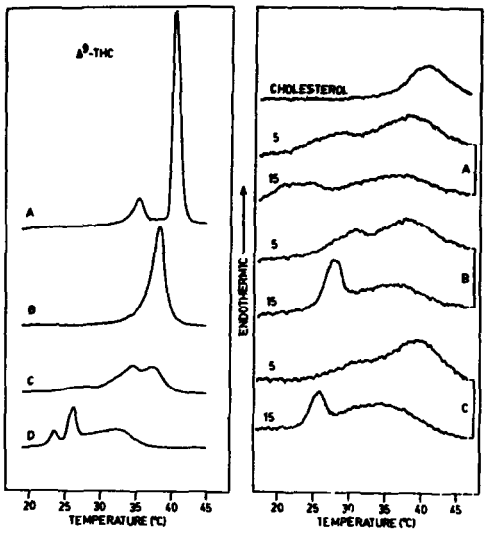

FIG. 3

Left: Thermograms of DPPC with Δ^9-THC (A) DPPC alone (B) X_{THC3}=0.05 (C) X_{THC3}=0.1 (D) X_{THC3}=0.2. Right: Thermogram of DPPC with 15% cholesterol (top tracing) (A) 8α-OH-Δ^9-THC (2) (B) 8β-OH-Δ^9-THC (1) (C) Δ^9-THC. Top tracings depict drug concentrations at X_{THC}=0.05. Bottom tracings, X_{THC}=0.15.

The addition of cholesterol to the DPPC bilayers containing the two analogs significantly affected the melting properties of the bilayers. Fig. 3 shows the effect of X_{CHOL}=0.15 on the behavior of THC/DPPC bilayers at X_{THC} =0.05 and 0.15 respectively. Addition of cholesterol greatly accentuates the effect of the two compounds on the model membrane. At X_{THC1} and X_{THC2}=0.05, a very pronounced broadening, flattening and lowering of the transitions occur. The scans obtained resemble those obtained from the X_{THC}=0.2 (without cholesterol, Figs. 2 and 3) experiments, but the transitions are much flatter, while a significant decrease in Δ H also occurs (see Table IV). An increase in THC concentration to X_{THC}=0.15 results in a further lowering of T_c, while the thermogram of DPPC and 1 shows a sharpening of the lower temperature peak and virtual elimination of the other, a situation virtually identical to that produced by Δ^9-THC (Fig. 3). The trace obtained from DPPC and 2 shows two very broad, similar phases.

TABLE IV

Changes in Midpoint of the Transition Temperature, T_c and Enthalpy Change of the Gel to Liquid Crystalline Transition, ΔH in DPPC Bilayers Containing Different Molar Fractions of 8β-OH-Δ^9-THC, 8α-OH-Δ^9-THC and Δ^9-THC, Obtained by Differential Scanning Calorimetry.

Compound	X_{THC}	T (°C)	Δ H (cal/g)
DPPC alone	0	41.2	9.76
8α-OH-Δ^9-THC	.05	37.96	11.12
	.1	36.45	10.4
	.2	35.0	10.08
8α-OH-Δ^9-THC	.05	38.7	10.41
	.1	35.51	10.48
	.2	34.95	11.25
Δ^9-THC	.04	38.44	10.33
	.1	36.89	10.77
	.2	32.21	10.09
8β-OH-Δ^9-THC+	.05	37.28	6.38
15% cholesterol	.15	27.22(lower peak)	7.62
8α-OH-Δ^9-THC+	.05	37.16	6.96
15% cholesterol	.15	34.28	5.68
Δ^9-THC+	.05	39.28	6.62
15% cholesterol	.15	25.21(lower peak)	7.5
15% cholesterol	0	39.71	5.38

Discussion

The NMR data show a clear similarity between the conformations of 1 and 2. It is tempting to speculate that the orientation of the 8OH, being the only substantial difference between the two compounds, holds the key to the difference in *in vivo* pharmacological activity of these epimers. As had already been suggested by Bruggemann and Melchior (5), cannabinols tend to locate at or near the membrane interface, presumably because of the phenol

group, which orients itself at the bilayer interphase. It is well known (19) that an hydroxyl group at the C_1 position is a requirement for activity in THC's, while the introduction of hydroxyl groups in the C-ring modifies the activity dramatically; some, like 11-OH-Δ^9-THC, being more active than Δ^9-THC, while others, such as 1 and 2, are less active. Subtle stereochemical modification of some derivatives, such as transposing the -OH from an axial to an equatorial position, leads to dramatic modification in activity. This very phenomenon is exemplified by the two compounds under study and lends support to the hypothesis that cell membrane phospholipids can act as selective cannabinoid recognition sites and that perturbation of membrane phospholipid ordering is one mechanism of action of cannabinoids.

Our thermograms of the three cannabinoids and DPPC agree well with earlier work done on Δ^9-THC/DPPC bilayers (5). Although there is a significant difference in the thermograms of DPPC with 1 and 2 at X_{THC}=0.05, the differences become more obvious at X_{THC}=0.2 where the appearance of a second peak at lower temperature than T_c is apparent in both thermograms. The shapes of the thermograms at this stage indicate some degree of solid-solid immiscibility, with the first component being DPPC and the second due to some association or complex formation between the cannabinoids and DPPC (4) which contributes to the newly observed peak. This second peak is much sharper and more distinct in the thermogram of DPPC + 1 than in the thermogram of DPPC + 2 (Fig. 2) which may indicate a greater tendency for 1 (active) to form such association or complex with the phospholipids in the bilayer than the less active epimer 2. The behavior of 1 in the model membrane resembles that of Δ^9-THC much more closely than 2. The similarity of the behavior of 1 and Δ^9-THC becomes even more apparent in the thermograms of DPPC from the cholesterol model membranes. Interestingly, we found the active 8β-OH (1) analog to have smaller chromatographic value (R_m = 0.31 ± 0.01) than its inactive isomer 8α-OH (2) (R_m = 0.48 ± 0.01).

This finding argues against the possibility that the difference in pharmacological activities between the two isomers is due to a difference in their respective solubilities in the membrane since this would require the most active analog to be also more lipophilic and have larger partition coefficient in the membrane.

Although the exact mechanism of action of cannabinoids is still subject to debate (4), the results presented here clearly indicate that interactions of active compounds with model membranes are very different from those produced by inactive ones. The fact that the mere transposition of an hydroxyl in the molecule, without any significant change in molecular geometry can produce such distinct changes in their interactions with phospholipids, suggests that polar-hydrophobic interactions with membranes are an important part of the mechanism of cannabinoid action.

Acknowledgments

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