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# Small angle X-ray diffraction and differential scanning calorimetric studies on *O*-methyl-(−)-$\Delta^8$-tetrahydrocannabinol and its 5′ iodinated derivative in membrane bilayers

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**Abstract**

We have previously studied and compared the location of (−)-$\Delta^8$-tetrahydrocannabinol ($\Delta^8$-THC) with that of *O*-methyl-$\Delta^8$-THC (Me-$\Delta^8$-THC) in the membrane using partially hydrated dimyristoylphosphatidylcholine (DMPC) bilayers ((Mavromoustakos et al. (1990) Biophys. Acta 1024, 336–344; Yang et al. (1993) Life Sci. 53, 117–122). $\Delta^8$-THC was found to be located near the membrane interface with its phenolic hydroxyl group anchored near the carbonyl groups of DMPC while the more lipophilic Me-$\Delta^8$-THC is located deeper in the membrane bilayer. Parallel experiments using Me-$\Delta^8$-THC and its 5′-iodo analog (5′-I-Me-$\Delta^8$-THC) allowed us to determine the topography of these two molecules in the bilayer. Our results from small angle X-ray diffraction and differential scanning calorimetry (DSC) combined with previous data on the orientation of Me-$\Delta^8$-THC in model membranes, led us to the conclusion that these molecules intercalate between contiguous acyl chains in the lipophilic moiety of the membrane bilayer. The terminal iodo group in 5′-I-Me-$\Delta^8$-THC was found to reside in a region extending approx. ± 5 Å from the center of the bilayers. The location of Me-$\Delta^8$-THC in the membranes as well as its orientation may explain its inability to effectively perturb the bilayer lipid chains.

*Keywords:* Small angle X-ray diffraction; DSC; Drug–membrane interaction; Cannabinoid; (−)-*O*-Methyl-$\Delta^8$-tetrahydrocannabinol; 5′-Iodo-*O*-methyl-$\Delta^8$-tetrahydrocannabinol

## 1. Introduction

It is well known that the phenolic hydroxyl group of a cannabinoid is a strict requirement for cannabimimetic activity [1] and methylation of this group to give the *O*-methyl ether results in an analog devoid of biological activity [2].

Earlier work [3–5] compared the effects of a pharmacologically active cannabinoid possessing a phenolic hydroxyl group ($\Delta^8$-THC) with its inactive *O*-methyl analog (Me-$\Delta^8$-THC) (Fig. 1). In these studies we showed, using DSC and solid state $^2$H-NMR, that the phenolic -OH in $\Delta^8$-THC allows it to interact with the membrane at the bilayer interface, while Me-$\Delta^8$-THC fails to do so. Solid state $^2$H-NMR data have also shown that $\Delta^8$-THC orients with the long axis of its tricyclic structure perpendicular to the bilayer chains. Conversely, Me-$\Delta^8$-THC was shown to assume an orientation in which the long axis of the tricyclic structure, is parallel to the lipid chains [6].

In a previous publication, small-angle X-ray diffraction was used to determine the location of $\Delta^8$-THC in partially hydrated DMPC bilayers [7]. The work, which involved the use of $\Delta^8$-THC and its 5′-iodo analog in parallel experiments, allowed us to determine the location of the iodine atom in the bilayer. Based on these results and previous data on the orientation of the cannabinoid in model membranes, we concluded that the phenolic hydroxyl of the drug molecule is located near the carbonyl groups of DMPC and the average position of the iodine atom is approx. 5.5 Å from the center of the DMPC bilayer (terminal methyl region). This requires the cannabi-

Abbreviations: DSC, differential scanning calorimetry; DMPC, dimyristoylphosphatidylcholine; RH, relative humidity; $\Delta^8$-THC, (−)-$\Delta^8$-tetrahydrocannabinol; Me-$\Delta^8$-THC, *O*-methyl-$\Delta^8$-tetrahydrocannabinol; 5′-I-Me-$\Delta^8$-THC, 5′-iodo-*O*-methyl-$\Delta^8$-tetrahydrocannabinol.

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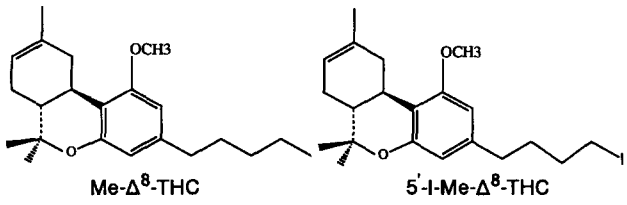


Fig. 1. Chemical structures of Me-$\Delta^8$-THC and 5'-I-Me-$\Delta^8$-THC.

noid side-chain to assume an orientation parallel to the bilayer chains. Recently, the location of Me-$\Delta^8$-THC in the membrane was also studied using small angle X-ray diffraction. We found that this highly hydrophobic molecule distributes deeper in the bilayer away from the interface [8].

In the present study 5'-I-Me-$\Delta^8$-THC was used to determine the location of the iodine atom in the bilayer and thus obtain information on the conformation of the alkyl chain. The analysis of the small angle X-ray diffraction results involved the determination of the signs of the structure factors using the swelling approach [9,10] and obtaining the electron density profile using a Fourier transform algorithm [11]. The electron density profiles were then placed on an absolute scale using the step-function model method [12–14]. The location of the drug molecules in the bilayer was obtained by comparing the electron density profiles of the hydrated DMPC preparations without and with the cannabinoids. Finally, DSC experiments were performed to study the mesomorphic phases of DMPC bilayers in the absence and presence of Me-$\Delta^8$-THC and its iodinated derivative.

## 2. Materials and methods

### 2.1. Materials

Me-$\Delta^8$-THC and 5'-I-Me-$\Delta^8$-THC were synthesized in our laboratory using methods reported elsewhere [15]. DMPC was obtained from Avanti Polar Lipids, Birmingham, AL.

### 2.2. Sample preparation

DMPC alone or with the appropriate amount of cannabinoid (lipid to drug molar ratio 85:15, or $x = 0.15$) were dissolved in chloroform (99 + % pure, Aldrich, Milwaukee, WI). The solvent was then removed using an $O_2$-free $N_2$ stream and the samples were dried under vacuum for 6 h. After adding distilled water, each sample was vortexed and sonicated in a water bath (50°C) for 15 min until the suspension was translucent. For the X-ray diffraction experiments, the preparations were deposited on an aluminum foil, mounted on a curved glass and dried at 35°C. This procedure took place in a chamber filled and continuously purged with nitrogen. For the DSC experiments, the vesicles were deposited on a glass surface, dried at 35°C and scraped into stainless steel DSC pans. Prior to the experiments, samples were suspended in closed vials with regulated relative humidities (RH) using appropriate saturated salt solutions for 24 h and 48 h, respectively. This produces partially hydrated multilamellar vesicles. The salts (Sigma, St. Louis, MO) used for the various relative humidities were LiCl for RH 13%, $NaNO_2$ for RH 66% and $K_2SO_4$ for RH 98%. For the X-ray experiments, the preparations were transferred into RH-regulated containers 3–5 h before the experiments. To keep the partial hydration of the DSC samples constant, the samples were sealed hermetically in stainless steel capsules. The partially hydrated samples prepared in this manner gave identical thermograms when tested repeatedly over a 7-day period.

### 2.3. Differential scanning calorimetry

Thermograms were obtained on a Perkin-Elmer DSC-7 instrument and the temperature scale of the calorimeter was calibrated using fully hydrated DMPC and indium as standard samples. Prior to scanning the samples were held above their phase transition temperature for 1–2 min to ensure complete equilibration. All samples were then scanned at least twice until identical thermograms were obtained using a scanning rate of 1.5 C°/min. We found that samples gave the same thermograms if allowed to equilibrate for more than 48 h.

### 2.4. X-ray diffraction

X-ray diffraction experiments were carried out with an Elliot GX18 rotating anode generator (Marconi Avionics), equipped with a camera utilizing a single vertical Franks' mirror [16]. Small angle X-ray diffraction patterns were collected using a Braun position sensitive proportional counting gas flow detector (Innovative Technology, Newburyport, MA). The sample temperature was kept constant with a Neslab 4B circulating water bath (Neslab Instruments, Newington, NH) and monitored with a thermister probe placed next to the canister. During the experiment, a helium path was used to reduce background scatter for a specimen-to-detector distance of 130 mm. The diffraction data were then collected with total accumulations of $1 \cdot 10^6$ to $2 \cdot 10^6$ counts to improve the signal-to-noise ratio. Data was then transferred to a VAX computer system and the intensities were integrated directly from the computer plots by calculating the area under the diffraction peaks.

## 3. Results and discussion

### 3.1. Differential scanning calorimetry

To ensure that comparisons were made between the membrane preparations at the same mesomorphic state, the

phase properties were studied using DSC. Thermograms from DMPC preparations equilibrated at RH 98% without and with cannabinoid are shown in Fig. 2. The partially hydrated DMPC bilayer (top) shows a phase transition at 29°C. The presence of the inactive cannabinoid Me-$\Delta^8$-THC lowers only marginally the main phase transition temperature while it broadens it (middle) and a small pretransition peak appears at 15° C. Similar results were observed with the preparation containing 5′-I-Me-$\Delta^8$-THC (bottom). Thus, these results indicate that both Me-$\Delta^8$-THC and 5′-I-Me-$\Delta^8$-THC do not affect significantly the thermotropic properties of DMPC bilayers. The fact that the pretransition was only observed in the preparations containing cannabinoid is not surprising. We are aware that this small transition preceding the main one is very sensitive to the water content as it is well known that fully hydrated DMPC bilayers show a pretransition. Therefore, we believe that its appearance only reflects the different water content between the preparations with and without the cannabinoid.

### 3.2. Small angle X-ray diffraction

*Temperature dependence of d-spacing*

The small angle X-ray diffraction patterns of the partially hydrated DMPC preparations consisted of six diffraction orders when the temperature was below the phase transition and four diffraction orders when above the phase transition. The total period repeat distance ($d$-spacing) was

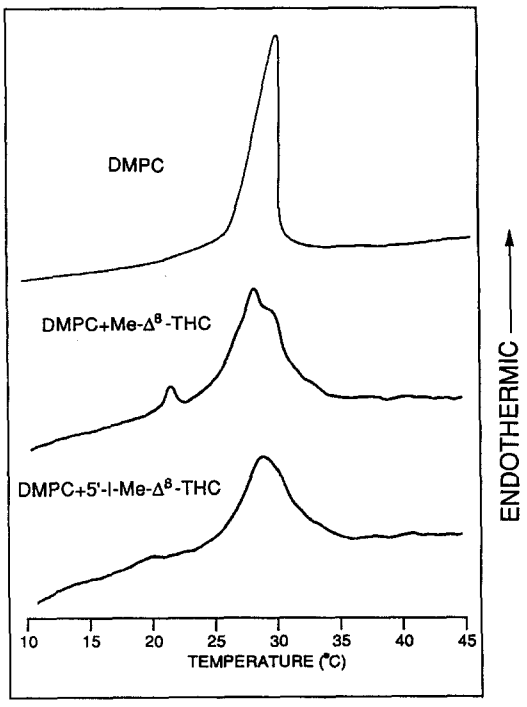


Fig. 2. Differential scanning calorimetry thermograms of partially hydrated bilayers at RH 98%. DMPC alone (top), DMPC + Me-$\Delta^8$-THC ($x = 0.15$) (middle) and DMPC + 5′-I-Me-$\Delta^8$-THC ($x = 0.15$) (bottom).

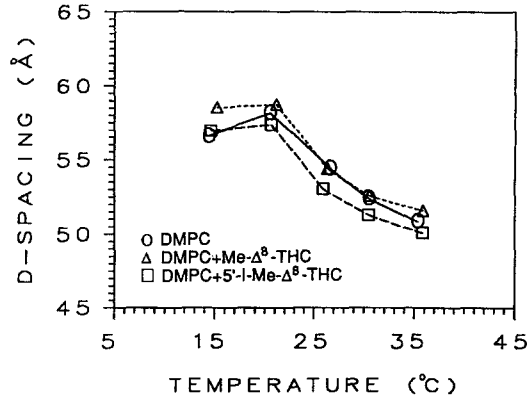


Fig. 3. $d$-spacing versus temperature for bilayer preparations of DMPC (circles and solid lines), DMPC + Me-$\Delta^8$-THC ($x = 0.15$, triangles and dotted lines) and DMPC + 5′-I-Me-$\Delta^8$-THC ($x = 0.15$, squares and dashed lines) equilibrated at RH 98%.

derived from the diffraction patterns using Bragg's Law $2d\sin\Theta = h\lambda$; where $\Theta$ is the diffraction angle and h is the diffraction order. The $d$-spacing change versus temperature was studied to identify equivalent temperatures at which appropriate comparisons could be made. At these temperatures, the drug-free and drug-containing DMPC preparations have similar $d$-spacings and are in the same mesomorphic phase. By comparing the data obtained at equivalent temperatures we then ensured that any electron density differences observed between preparations with and without the cannabinoid are solely due to the electron density contribution of the drug and not the different phase properties of the bilayers and/or any perturbation resulting from the presence of the cannabinoid.

From the various relative humidities used in the experiments, the RH of 98% was chosen for our comparisons. Previous work had shown that optimal results are obtained when DMPC, DMPC + $\Delta^8$-THC and DMPC + Me-$\Delta^8$-THC were compared under such conditions [8]. As can be seen in Fig. 3, preparations of DMPC, DMPC + Me-$\Delta^8$-THC and DMPC + 5′-I-Me-$\Delta^8$-THC are all in the liquid

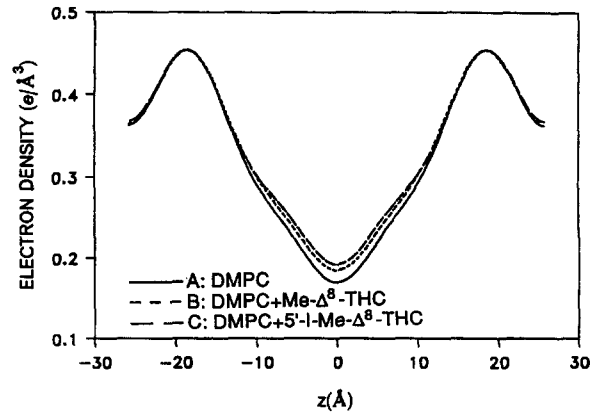


Fig. 4. Comparison of electron density profiles of DMPC (solid line), DMPC + Me-$\Delta^8$-THC (short line) and DMPC + 5′-I-Me-$\Delta^8$-THC (long dashed line) at 36°C equilibrated at RH 98%.

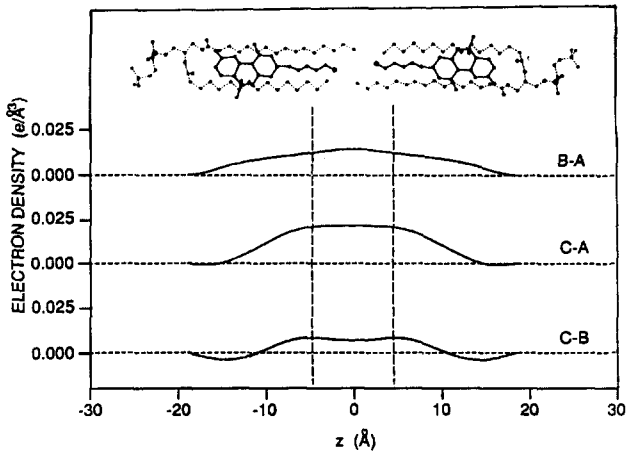


Fig. 5. Electron density profile differences inside the bilayer: Curve B-A is the difference between profiles of DMPC + Me-$\Delta^8$-THC and DMPC, curve C-A is the difference between those of DMPC + 5′-I-Me-$\Delta^8$-THC and DMPC and curve C-B is the difference between those of DMPC + 5′-I-Me-$\Delta^8$-THC and DMPC + Me-$\Delta^8$-THC. The vertical dashed lines indicate the peaks in curve C-B. They represent the positions of the center of $\Delta^8$-THC and the iodine atom of 5′-I-Me-$\Delta^8$-THC in the bilayer.

crystalline phase (see also thermograms in Fig. 2) and have similar *d*-spacings of 50.84 Å, 51.60 Å and 50.59 Å, respectively. On this basis, comparisons were made between the electron density profiles of these three preparations at the equivalent temperature of 36°C.

*Location of the cannabinoid in DMPC bilayers*

To compare the electron density profiles of bilayers with and without Me-$\Delta^8$-THC or 5′-I-Me-$\Delta^8$-THC we assumed that DMPC and the membrane containing cannabinoid preparations had identical electron densities at the level of the head groups and the terminal methyl groups [5]. On this basis, the profiles of the above three preparations were superimposed as shown in Fig. 4.

Earlier solid state $^2$H-NMR studies, had shown [7] that at $x = 0.15$ Me-$\Delta^8$-THC incorporates as readily as $\Delta^8$-THC into phospholipid bilayers. We, thus, expected Me-$\Delta^8$-THC to show a similar increase in the electron density profile as $\Delta^8$-THC (0.216 $e/\text{Å}^2$). The ratio of the number of electrons of Me-$\Delta^8$-THC versus that of $\Delta^8$-THC (180/172) should be equal to the ratio of the increase in electron density (0.216 $e/\text{Å}^2$). To fully account for such an increase in electron density due to the presence of Me-$\Delta^8$-THC in the DMPC bilayer, the trough of DMPC + Me-$\Delta^8$-THC profile was adjusted to a position 0.015 $e/\text{Å}^2$ above that of the DMPC while maintaining the same electron density at the head group. The long dashed line in Fig. 4 shows the result of this superimposition. The same rationale was used in the superimposition of 5′-I-Me-$\Delta^8$-THC whose total number of electrons is 232 and its electron density contribution is expected to be equal to 0.29 $e/\text{Å}^2$ (see Fig. 4).

Fig. 5 shows the differences between the three profiles with an expanded scale. B-A represents the electron density contribution due to Me-$\Delta^8$-THC in the DMPC bilayer. Its contribution has a maximum at the center of the bilayer and extends up to the interface with discernible shoulders at $z = \pm 10$ Å. This can be explained by assuming two preferred locations for Me-$\Delta^8$-THC, one at the center of the bilayer between the two membrane leaflets and a smaller portion residing between the contiguous lipid chains. The contribution due to the iodinated analog 5′-I-Me-$\Delta^8$-THC is depicted as C-A which shows an enhance-

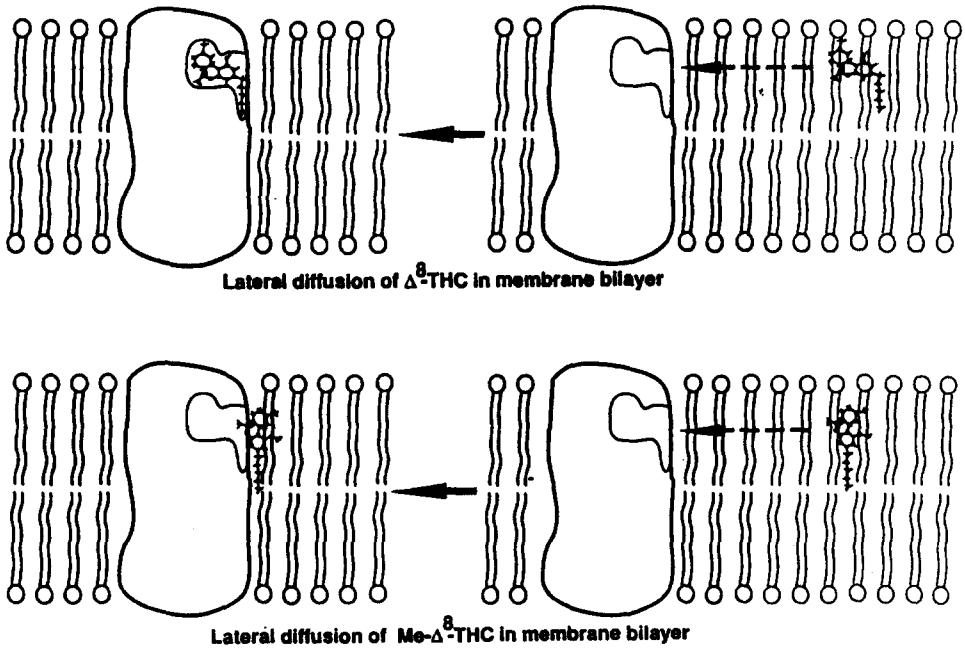


Fig. 6. A proposed model for the molecular mechanism of action of $\Delta^8$-THC and Me-$\Delta^8$-THC.

ment in the region near the center of the bilayer. The net contribution due to the iodine atom can be obtained from the electron density difference between the Me-$\Delta^8$-THC and 5'-I-Me-$\Delta^8$-THC containing samples (C-B). There is an almost uniform increase from $z = -5$ Å to $z = 5$ Å indicating that the iodine atom does not occupy a specific location within the limits of resolution. Instead it has an almost equal probability of being located in a region confined within 5 Å from the center of the bilayer.

## 4. Conclusions

In this study, small angle X-ray diffraction and DSC were used to obtain information on the location and the thermotropic properties of Me-$\Delta^8$-THC and 5'-I-Me-$\Delta^8$-THC in DMPC bilayers. The present X-ray diffraction experiments showed substantial incorporation of Me-$\Delta^8$-THC in the bilayer. We found that the electron density increase due to Me-$\Delta^8$-THC is distributed over a relatively wide region across the membrane bilayer near its center. This allowed us to draw the conclusion that the interaction of Me-$\Delta^8$-THC in the membrane bilayer is dictated exclusively by lipophilic considerations and that the molecule does not anchor itself at the polar interface of the bilayer as does the psychoactive $\Delta^8$-THC [17]. Such interactions allow Me-$\Delta^8$-THC to distribute itself within the inner core of the bilayer without being confined to a fixed location. The wide distribution of 5'-I-Me-$\Delta^8$-THC within the inner layer of the membrane is also evidenced by the enhancement in electron density due to the presence of the iodine within a 10 Å site from the center of the membrane bilayer. The X-ray diffraction results here are congruent with the solid state $^2$H-NMR experiments where $^2$H-labelled Me-$\Delta^8$-THC was used in order to determine its preferred orientation in the bilayer. These results will be discussed in details elsewhere.

It earlier work we showed that Me-$\Delta^8$-THC does not increase the *gauche:trans* conformer ratio in DMPC bilayers as opposed to the known disordering effects produced by $\Delta^8$-THC [7]. This supports reported DSC and solid state $^2$H-NMR data which showed that Me-$\Delta^8$-THC perturbs the membrane bilayer only marginally [3]. This lack of membrane perturbing ability may be largely due to the fact that only a small fraction of Me-$\Delta^8$-THC can be accommodated between the acyl chains of the contiguous lipid molecules in the bilayer while the remaining Me-$\Delta^8$-THC residing in the center of the bilayer perturbs the membrane minimally.

Cannabinoids are highly lipophilic [18–22] molecules and are known to produce their pharmacological effects by interacting with cellular membranes. These effects can thus be induced by one of the following two mechanisms [23]: (a) cannabinoids alter the conformational properties of lipids or other membrane components and thus alter membrane function; (b) cannabinoids may penetrate into the membrane, assume a suitable orientation and location, and then through lateral diffusion reach the specific site(s) of action on a membrane associated protein. The amphipathic interactions of these molecules with the membrane bilayers are accompanied by reduced chain cooperativity and induce significant membrane perturbation. $\Delta^8$-THC and other biologically active amphipathic cannabinoids localize near the membrane interface. Furthermore, they assume the proper location and orientation within the bilayer allowing them to reach the receptor through lateral diffusion and engage in a productive interaction with the active site (Fig. 6, top). Conversely, the pharmacologically inactive Me-$\Delta^8$-THC fails to exert significant perturbation of the membrane bilayers. It may also assume an improper location and orientation in the membrane bilayer and is, thus, unable to productively interact with the receptor active site as shown in Fig. 6 (bottom). The above model [23] may provide a molecular interpretation of the differences in pharmacologically activity between the cannabinoids discussed in this study.

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