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Studies on the thermotropic effects of cannabinoids on phosphatidylcholine bilayers using differential scanning calorimetry and small angle X-ray diffraction

T. Mavromoustakos [a,*], E. Theodoropoulou [a], D. Papahatjis [a], T. Kourouli [a], De-Ping Yang [b], M. Trumbore [c], A. Makriyannis [d]

[a] National Hellenic Research Foundation, Institute of Organic and Pharmaceutical Chemistry, Athens 116 35, Greece
[b] College of the Holy Cross, Worcester, MA 01610, USA
[c] University of Connecticut, Health Center, Biomolecular Structure Analysis Center, 263 Farmington Avenue, Farmington, CT 06032, USA
[d] Departments of Pharmaceutical Sciences, Molecular and Cell Biology and Institute of Materials Science, Storrs, CT 06269, USA

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Abstract

We have studied the thermotropic properties of a wide variety of cannabinoids in DPPC bilayers. The molecules under study were divided into four classes: (a) classical cannabinoids possessing a phenolic hydroxyl group; (b) Δ^9-THC metabolites with an additional hydroxyl group on the C ring; (c) non-classical cannabinoids, and (d) cannabinoids with a protected phenolic hydroxyl group. The results showed that the first three groups have similar effects on the thermotropic properties of DPPC bilayers up to $x = 0.05$ (molar ratio) and that these effects do not parallel their biological activity. For concentrations less than $x = 0.01$, cannabinoids affect mainly the pretransition temperature in a progressive manner until its final abolishment. At $x = 0.05$, they further affect the main phase transition by lowering its phase transition temperature and broadening its half width. At high concentrations the thermograms have multiple components, indicating that membranes are no longer homogeneous but rather consist of different domains. At these concentrations cannabinoids with more hydroxyl groups give simpler thermograms. Low concentrations of cannabinoids in group d affect significantly the pretransition temperature, while high concentrations affect only marginally the main phase transition by slightly lowering its temperature and broadening its half width. These results point out the importance of the phenolic hydroxyl group in inducing membrane perturbations. The d-spacing data from our small angle X-ray diffraction experiments show that Δ^8-THC produces significant structural changes in the lipid bilayer, including the gel-phase tilting angle, the intermolecular cooperativity and the *gauche:trans* conformer ratio. Conversely, the inactive analog Me-Δ^8-THC does not cause drastic changes to the bilayer structure.

Keywords: Differential scanning calorimetry; X-ray diffraction; Drug–membrane interaction; Cannabinoid

1. Introduction

Existing evidence suggests that not all physiological effects of cannabinoids can be attributed to the existence of cannabinoid receptors but some can be related to their effects on cellular membranes [1,2]. More specifically, cannabinoids produce biochemical changes in a variety of membrane preparations at concentrations which are arguably 'physiological' [3,4] and affect numerous membrane-related functions. They also exert non-receptor mediated effects on membrane-bound enzymes such as: Na^+-K^+-Mg^{2+}- and Ca^{2+}-ATPases [5–7], NADH dehydrogenase [8], lysophosphatidylcholine acyl transferase [9], phospholipase A_2 [10], monoamine oxidase [11] and adenylate cyclase [12]. Additionally, cannabinoids affect the function of neurotransmitter uptake systems [6], blood platelets [13], human lung fibroblast [14], neutrophils [15] and certain non-cannabinoid receptors [1,2] The cannabinoids are lipophilic molecules and are believed to interact firstly on the lipid microenvironment surrounding the membrane-associated enzymes [16,17]. The cannabinoid-membrane interactions presumably induce changes in the

Abbreviations: DSC, differential scanning calorimetry ; DPPC, dipalmitoylphosphatidylcholine; Δ^8-THC, $(-)$-Δ^8-tetrahydrocannabinol; Me-Δ^8-THC, *O*-methyl-Δ^8-tetrahydrocannabinol.

* Corresponding author. Fax: +30 17247913 or +30 17253821.

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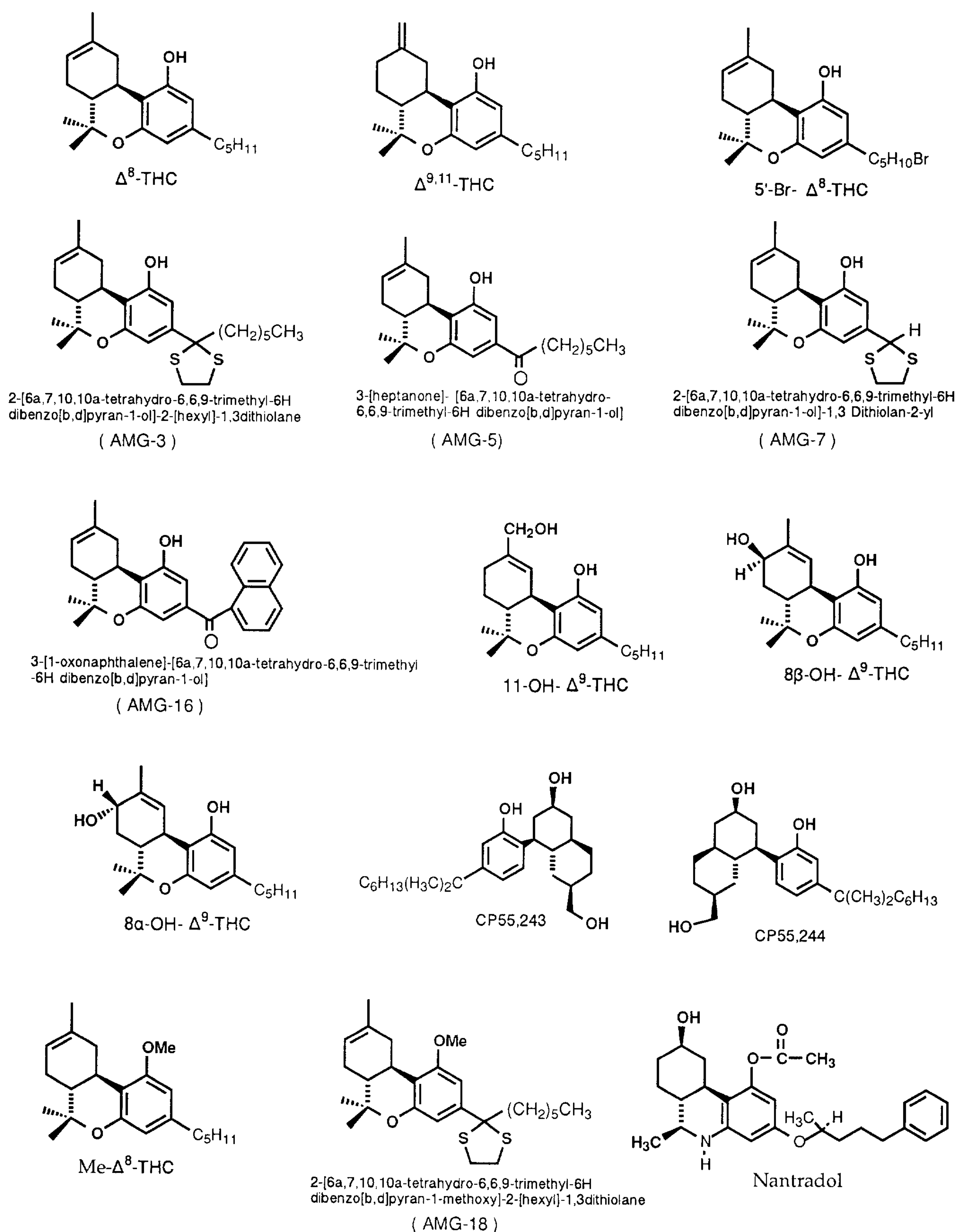

Fig. 1. Chemical structures of the cannabinoids under study.

functions of a number of these proteins and thus alter a variety of physiological functions. Experimental evidence for such a mechanism of action involving cannabinoid-phospholipid interactions came from calorimetric and fluorescence measurements [18–20], electron spin resonance experiments [21], ^{1}H-NMR [22] and solid state ^{2}H-NMR [23–26].

Differential scanning calorimetry (DSC) is a relatively inexpensive thermodynamic technique which allows us to study the thermotropic properties of membranes in the absence and presence of biologically active molecules. DSC was used by Bach and his collaborators [18] to investigate the interactions of Δ^9-tetrahydrocannabinol (Δ^9-THC) the principal active ingredient of cannabis and cannabidiol in DPPC and calf brain lipid bilayers. They found that both drugs affected the transition of DPPC from gel to liquid crystal and the enthalpy of this transition in the same manner. The same authors presented evidence from DSC and enzymatic activity measurements that Δ^9-THC also affected the thermotropic properties of liver microsomal membranes and microsomal lipids.

DSC was used by Burstein et al. to study the differences between the inactive (+)-Δ^9-THC and the psychoactive (−)-Δ^9-THC [20]. Both enantiomers induce some general perturbation of the melting behavior of the DPPC bilayer but at different concentrations. For the biologically active enantiomer the enthalpy of transition (ΔH) never decreased, in contrast to the inactive one where the enthalpy of transition diminished for THC concentrations above the lipid/drug molar ratio of 80:20 ($x = 0.20$).

Bruggeman and Melchior [19] used DSC to study the interactions of Δ^9-THC with various phosphatidylcholines. The most significant results of this study are as follows: (a) Addition of THC broadens the main transition and gradually lowers its temperature. (b) The addition of THC creates a sharp, distinct peak several degrees below that of the original main transition. This is interpreted as the formation of a phospholipid:THC complex. (c) The enthalpy (ΔH) of the entire transition is equal to or less than that of the main transition of pure PC. (d) Above a certain characteristic molar fraction ($x = 0.23$) of THC, the appearance of the transition does not change significantly. This observation is due to the limit of incorporation of the cannabinoid in the phospholipid bilayer. (e) The presence of cholesterol below $x = 0.20$ in DPPC bilayers enhanced THC:DPPC complex formation while above $x = 0.20$ this was not evident.

Earlier DSC work from our laboratory using high concentrations of Δ^8-THC and Me-Δ^8-THC showed that only Δ^8-THC complexes with DPPC [27]. Other DSC work from our laboratory also showed that 8α and 8β-hydroxy-Δ^9-tetrahydrocannabinols (two metabolites of the naturally occurring Δ^9-THC strongly affected the melting behavior of hydrated DPPC bilayers, inducing a lowering of the main transition temperature, a broadening of that transition and the abolishment of the pretransition [28].

In this study we examined the thermotropic properties of a wide variety of cannabinoids possessing a wide range of pharmacological potencies (Fig. 1) on DPPC membrane bilayers. The cannabinoids used were classified into four groups: (a) classical cannabinoids possessing a phenolic hydroxyl group; (b) classical cannabinoids with an additional hydroxyl group on the C-ring (Δ^9-THC metabolites); (c) tricyclic analogs with three hydroxyl groups (non-classical cannabinoids); (d) classical cannabinoids with a protected phenolic hydroxyl group.

The aim of this study was two-fold: First, to examine the role of phospholipids in the interactions of cannabinoids with the membrane bilayers, and second, to investigate any structure activity relationships using thermogram parameters (ΔH, T_m, $T_{c,1/2}$).

2. Materials and methods

2.1. Materials

Cannabinoids coded as AMGs (Fig. 1) were synthesized in our laboratory. Details of their synthesis will be discussed elsewhere. The rest of the cannabinoids were kindly provided by NIH. DPPC was purchased from Avanti Polar Lipids, Birmingham, AL.

2.2. Sample preparation

DPPC alone or with the appropriate amount of cannabinoid were dissolved in chloroform (99 + % pure, Aldrich Chemical Co., Milwaukee, WI). The solvent was then removed using an O_2-free N_2 stream and the samples were dried under high vacuum for 6 h.

2.3. Differential scanning calorimetry

After adding distilled water (50% w/w), a portion of the sample (ca. 5 mg) was sealed in a stainless steel capsule. Thermograms were obtained on a Perkin-Elmer DSC 2 and DSC 7 instruments. Prior to scanning, the samples were held above their phase transition temperature for 1–2 min to ensure complete equilibration. All samples were scanned at least twice until identical thermograms were obtained using a scanning rate of 2.5°C/min. The temperature scale of the calorimeter was calibrated using fully hydrated DPPC and indium as standard samples. We found that the thermograms from samples stored at freezer temperatures (−15°C) for a few days were identical if run immediately after sample preparation.

2.4. Small angle X-ray diffraction

Another smaller portion (ca. 2 mg) of each hydrated sample was drawn into a thin-walled capillary tube (inner diameter 0.5 mm) which was then sealed at both ends.

X-ray diffraction experiments were carried out with an Elliott GX18 generator (Marconi Avionics), equipped with a camera utilizing a single vertical Franks' mirror [29]. Small angle X-ray diffraction patterns were collected using a Braun one-dimensional, position-sensitive proportional counter (PSPC) gas flow detector (Innovative Technology, Inc., Newburyport, MA). During the experiment, we used a helium path for a specimen-to-detector distance of 210 mm and collected the diffraction data with digital accumulations of 1×10^6 to 2×10^6 counts to improve the signal-to-noise ratio. Data was transferred to a VAX 8200 (Digital Equipment Corporation) computer system where the d-spacings were calculated.

3. Results and discussion

3.1. Classical cannabinoids possessing a phenolic hydroxyl group

Δ^8-THC an analog equipotent with Δ^9-THC but chemically more stable was used as a parent compound in this group [30]. Normalized thermograms of DPPC preparations containing increasing concentrations of Δ^8-THC are shown in Fig. 2. The thermogram of pure DPPC indicates a pretransition centered at 35°C while the onset and the peak maximum (T_c) for the main transition occurs at 41.2°C, respectively. At a low concentration (molar ratio $x = 0.001$), Δ^8-THC produces a slight broadening of the pretransition and shifting to a lower temperature while the main transition remains unaffected. At $x = 0.01$ the broadening of the pretransition becomes more pronounced and almost coalesces with main transition. At $x = 0.05$, Δ^8-THC produces an elimination of the pretransition and a shift of T_c to 39.6°C. At $x = 0.10$ the main transition temperature is further lowered to 38°C while two new broad peaks appear in the thermogram at lower temperatures. At $x = 0.20$, the middle peak intensifies and becomes dominant in the thermogram. The appearance of this latter peak is interpreted to be due to the formation of a THC:DPPC complex [19,23,28]. Δ^8-THC/DPPC bilayer samples show identical thermograms to those obtained from Δ^9-THC/DPPC bilayers at corresponding molar ratios [23].

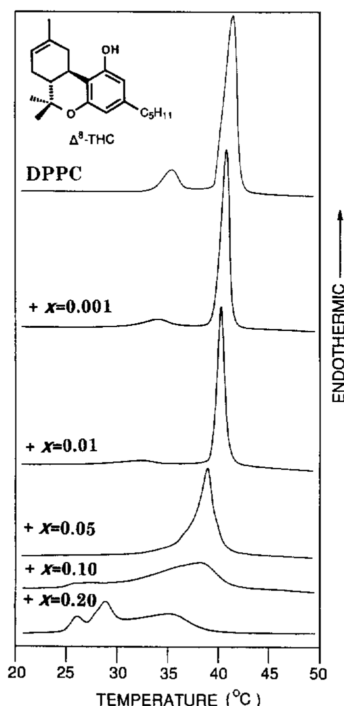

Fig. 2. Normalized differential scanning calorimetry thermograms of fully hydrated bilayers of DPPC with or without Δ^8-THC.

We then examined and compared the effects of structurally related analogs possessing a wide range of biological potencies. Normalized thermograms of DPPC preparations containing increasing concentrations of the pharmacologically inactive analog $\Delta^{9,11}$-THC (left) and the highly potent 5′-Br-Δ^8-THC (right) are shown in Fig. 3. The results are similar to those observed with the Δ^8-THC/DPPC bilayers. These results lead to the conclusion that the pharmacological potencies of cannabinoids cannot be directly correlated with their membrane-perturbing effects.

This conclusion is further supported by the results obtained with the potent cannabinoid AMG-5 (Fig. 4, right) and the almost inactive AMG-16 (Fig. 4, left), both of which are Δ^8-THC analogs with side chains modified respectively to an α-ketohexyl or an α-keto-naphthyl substituent. Both molecules exert similar thermotropic effects on DPPC bilayers as Δ^8-THC (Fig. 2).

We have also examined the effects of AMG-3 (Fig. 5, right) and AMG-7 (Fig. 5, left) two side chain analogs of Δ^8-THC on DPPC bilayers and found only small differences with those of Δ^8-THC. AMG-3 which incorporates a dithiolane ring in the 1′ position of an n-heptyl side chain is a potent cannabinoid while AMG-7 in which the six carbon chain of AMG-3 has been removed is almost devoid of cannabimimetic activity. Small differences were observed only with preparations of $x = 0.01$ and $x = 0.05$ when compared to the corresponding DPPC/Δ^8-THC samples. Both AMG-3 or AMG-7 produced more complex thermograms as manifested by the shoulder to the right of the main peak. In the case of Δ^8-THC, only a broadening is observed at these drugs concentrations but no separate shoulder.

It appears from the above results that the alkyl side chain of the cannabinoids does not play an important role in the DPPC:cannabinoid interactions because its substitution with other hydrophilic or hydrophobic moieties or even its absence in the cannabinoid structure does not alter significantly the thermotropic effects of cannabinoids on DPPC bilayers.

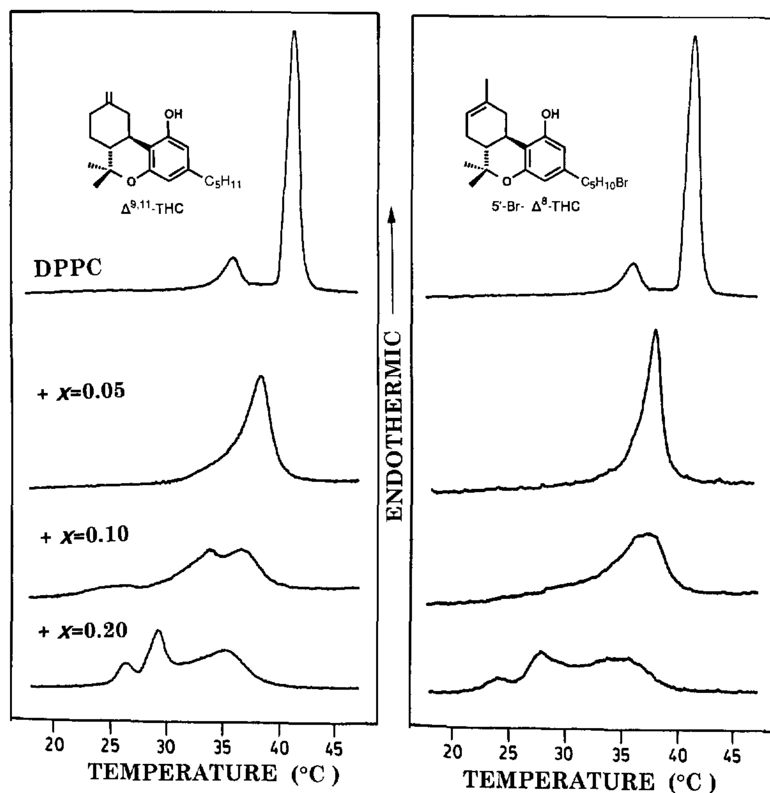

Fig. 3. Normalized differential scanning calorimetry thermograms of fully hydrated bilayers of DPPC with or without $\Delta^{9,11}$-THC (left) and DPPC with or without 5′-Br-Δ^8-THC (right).

3.2. Metabolites of THC

We have compared the effects of three metabolites of Δ^9-THC, namely 11-OH-, 8α-OH- and 8β-OH-Δ^9-THC on DPPC bilayers. 11-OH-Δ^9-THC is a biologically active metabolite of Δ^9-THC which is more potent than the parent compound. 8β-OH-Δ^9-THC is pharmacologically active but less potent than Δ^9-THC, whereas 8α-OH-Δ^9-

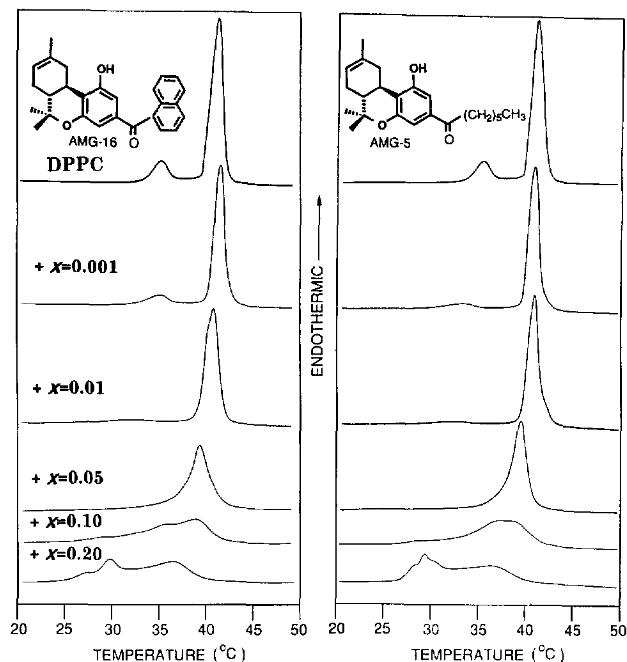

Fig. 4. Normalized differential scanning calorimetry thermograms of fully hydrated bilayers of DPPC with or without AMG-16 (left) and DPPC with or without AMG-5 (right).

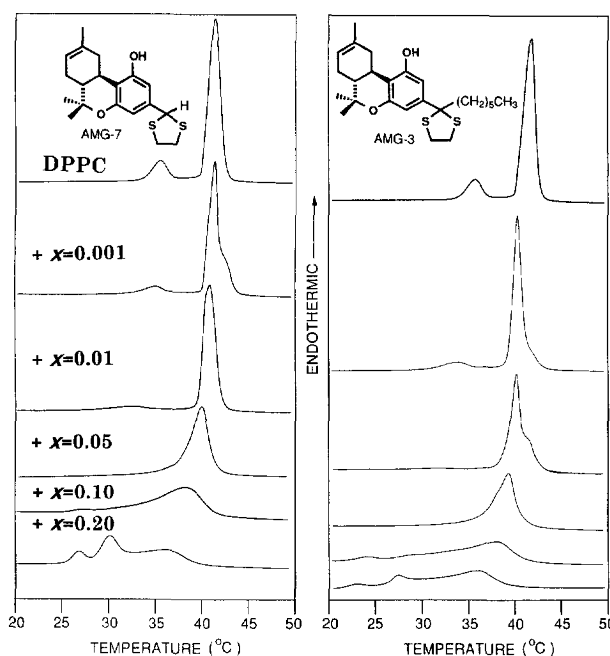

Fig. 5. Normalized differential scanning calorimetry thermograms of fully hydrated bilayers of DPPC with or without AMG-7 (left) and DPPC with or without AMG-3 (right).

THC is an almost inactive epimer. The choice of these three metabolites allowed us to examine the significance of a second hydroxyl group with regard to the effects of cannabinoid analogs on membranes. The thermotropic effects of 8β-OH-Δ^9-THC and 8α-OH-Δ^9-THC have already been reported in detail in a previous publication [28]. However, a brief description of these results is included here for the sake of completeness.

At $x = 0.05$ and $x = 0.10$ cannabinoid concentrations (Fig. 6), the two preparations showed similar thermotropic behavior to those containing Δ^8-THC (Fig. 2). However, at $x = 0.20$, the DPPC preparation containing the epimeric two compounds produced different thermograms from that of Δ^9-THC/DPPC. Of the two peaks observed, the low temperature one was more pronounced in the case of 8β-OH-Δ^9-THC than in 8α-OH-Δ^9-THC. The thermogram of Δ^9-THC/DPPC at $x = 0.20$ (identical with that of Δ^8-THC/DPPC) is similar to that of 8β-OH-Δ^9-THC. These results show that the position of the hydroxyl group on the C-ring plays an important role in the interaction of the two epimeric cannabinoids with the DPPC bilayers.

Normalized thermograms of DPPC preparations containing increasing concentrations of 11-OH-Δ^9-THC are shown in Fig. 7. The effects of this metabolite resemble those of 8α-OH-Δ^9-THC and differ from those of 8β-OH-Δ^9-THC. These results may reflect the similar orientation of the C-ring hydroxyl group of DPPC/8α-OH-Δ^9-THC and DPPC/11-OH-Δ^9-THC within the bilayer. They also point out that the position of the second hydroxyl group plays an important role in the thermotropic effects of the cannabinoids in the membrane bilayers.

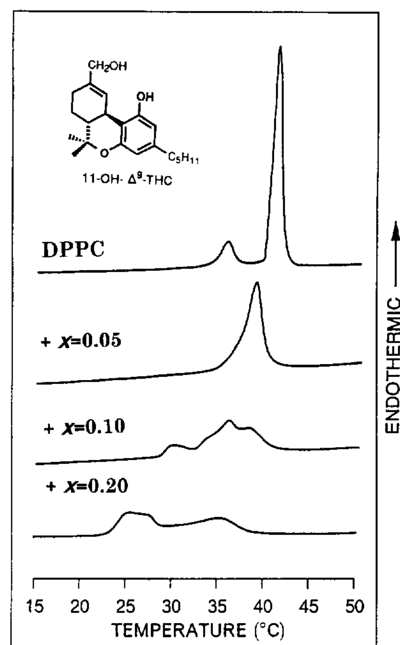

Fig. 7. Normalized differential scanning calorimetry thermograms of fully hydrated bilayers of DPPC with or without 11-OH-Δ^9-THC.

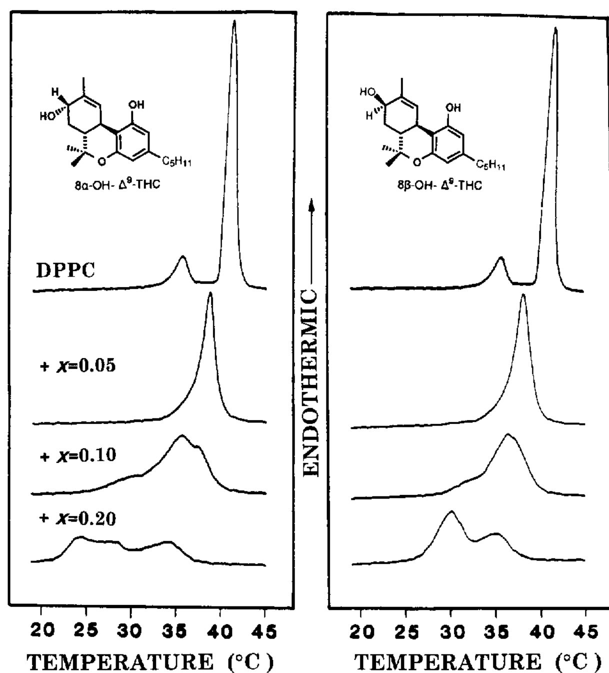

Fig. 6. Normalized differential scanning calorimetry thermograms of fully hydrated bilayers of DPPC with or without 8α-OH-Δ^9-THC (left) and DPPC with or without 8β-OH-Δ^9-THC (right).

3.3. Non-classical cannabinoids

We compared the thermotropic effects of the potent non-classical cannabinoid CP 55,243 and its inactive enantiomer CP 55,244 (Fig. 8) on the thermal properties of DPPC bilayers. These molecules which were synthesized at Pfizer as potential analgesic agents differ from the naturally occurring tetrahydrocannabinols and their metabolites by (a) the absence of tetrahydropyran ring, (b) having a longer 1,1-dimethylheptyl side chain, and (c) the presence of a hydroxyl group which is attached to a cyclohexyl component corresponding to the C-ring of the natural cannabinoids [31,32]. Although the non-classical cannabinoids were principally tested for analgesic activity [33], there is enough data indicating that they also exhibit animal behavioral effects similar to those of the 'classical' analogs [34].

The incorporation of the two enantiomers into DPPC bilayers resulted in identical thermograms. These results indicate that the phospholipid environment, although asymmetric, does not interact differently in any observable fashion with the two non-classical cannabinoid enantiomers.

It is interesting to note that all three groups of analogs so far studied show small differential effects up to $x = 0.05$. Above this concentration their differential effects are enhanced. A possible reason for this is that above this concentration the cannabinoids produce different domains within the bilayer as indicated by the appearance of new

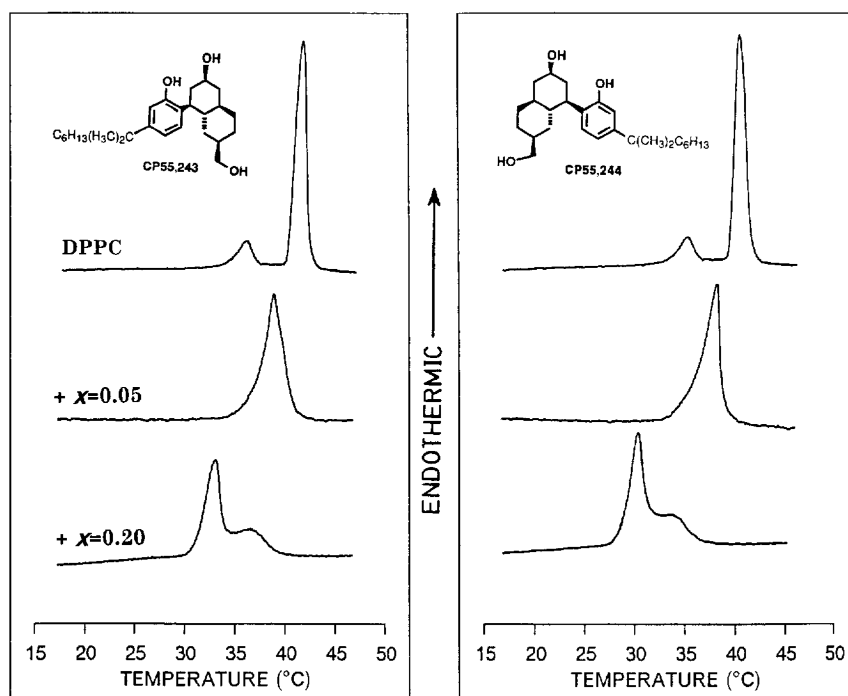

Fig. 8. Normalized differential scanning calorimetry thermograms of fully hydrated bilayers of DPPC with or without CP55,243 (left) and DPPC with or without CP55,244 (right).

peaks. The extent to which these domains are formed varies with the different analogs. We also observed a trend that suggests that the more hydrophilic the cannabinoid (more hydroxyl groups), the lesser its tendency for producing domains. These results also suggest that at low concentrations $\leq x = 0.05$ the cannabinoids are incorporated at the same location in membrane bilayer where they exert 'similar membrane-perturbing effects'. Our results may also reflect the experimental limitations in designing biophysical experiments for studying drug perturbations and relating these results to pharmacological potency. Apparently, the 'perturbations' induced by the cannabinoid molecules at very low concentrations are not significant enough to be differentiated by this method. Details on these experimental uncertainties are described elsewhere [35].

3.4. Classical cannabinoids with a protected phenolic hydroxyl group

It is well known that the phenolic hydroxyl group of a cannabinoid is a strict requirement for cannabimimetic activity and methylation of this group to give the *O*-methyl ether results in an analog devoid of biological activity [36]. Earlier work from our laboratory [24] compared the effects of the pharmacologically active Δ^8-THC possessing a phenolic hydroxyl group with its inactive O-methyl analog (Me-Δ^8-THC) at high concentrations (Fig. 1). In these studies we showed, using DSC and solid state ^{2}H-NMR, that the phenolic -OH in Δ^8-THC allows it to interact with the membrane at the bilayer interface, whereas this was not observed with Me-Δ^8-THC. Solid state ^{2}H-NMR data have also shown that Δ^8-THC orients with the long axis of its tricyclic structure perpendicular to the bilayer chains. Conversely, Me-Δ^8-THC was shown to assume an orientation in which the long axis of the tricyclic structure is parallel to the lipid chains.

Thermograms for Me-Δ^8-THC/DPPC preparations at low and high concentrations are shown in Fig. 9. The effects of this molecule on the DPPC bilayers differ sharply from those of classical and non-classical cannabinoids which contain a free phenolic hydroxyl group. At $x = 0.001$, Me-Δ^8-THC broadens the pretransition but does not affect the main transition and at $x = 0.01$, the pretransition is further broadened. The main transition has the same half-width as that of the pure DPPC with only a small decrease in Tc. At higher drug concentrations ($x = 0.05$, $x = 0.10$ and $x = 0.20$), no major change in the DPPC thermograms was observed. There is no appreciable change in the onset temperature of the main transition; only a progressive broadening of the transition occurs and a shoulder appears at the low temperature end when $x = 0.20$. The respective O-methyl dithiolane analog (AMG-18) exerts similar thermotropic effects when incorporated in DPPC bilayer preparations as those with Me-Δ^8-THC (Fig. 9, right).

A similar effect on the thermotropic properties of DPPC bilayers is exhibited by the analgesic nantradol [22] in which the phenolic hydroxyl is acetylated. It is generally believed that during metabolism the acetyl group is hydro-

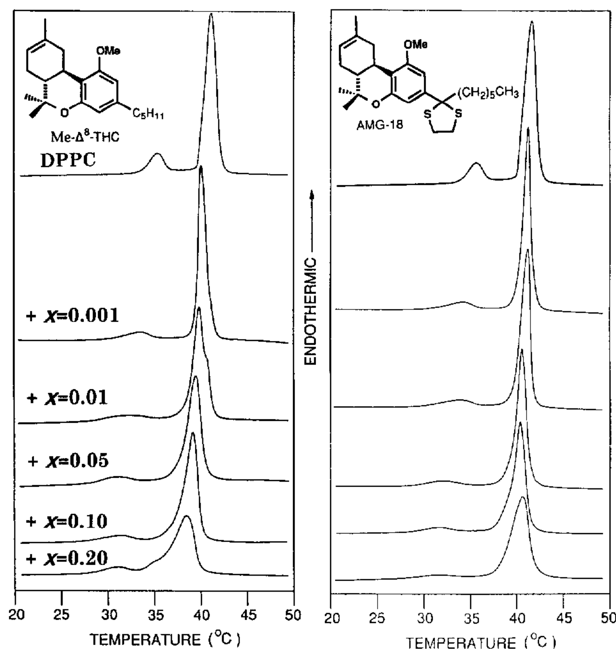

Fig. 9. Normalized differential scanning calorimetry thermograms of fully hydrated bilayers of DPPC with or without Me-Δ^8-THC (left) and DPPC with or without AMG-18 (right).

lyzed to an analog with a free phenolic hydroxyl which is also responsible for its biological effects. The thermograms of the DPPC/nantradol preparations again resemble those of the Me-Δ^8-THC samples (see Fig. 10). At a very high drug concentration ($x = 0.20$) this preparation did not

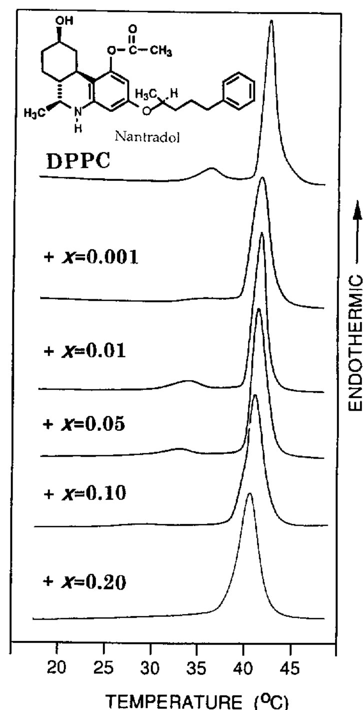

Fig. 10. Normalized differential scanning calorimetry thermograms of fully hydrated bilayers of DPPC with or without Nantradol.

produce any inhomogeneity in the model membrane probably because of the high lipophilic character of the drug molecule.

For all cannabinoid/DPPC concentrations studied, the enthalpy for DPPC did not change significantly and was similar to that observed for pure DPPC within experimental error.

The above results do not allow any correlations between the effects of cannabinoids on the thermotropic properties of membranes and their pharmacological potencies. This leads to the conclusion that the membrane-perturbing effect of cannabinoids cannot fully account for their biological activities.

4. X-ray diffraction

In an effort to better understand the observed different thermotropic effects between classical cannabinoids lacking the phenolic hydroxyl group with the rest of the groups, we used small angle X-ray diffraction on DPPC preparations without or with Δ^8-THC and Me-Δ^8-THC.

Small angle X-ray diffraction patterns from fully hydrated bilayer preparations of both DPPC with and without Δ^8-THC and Me-Δ^8-THC at various temperatures were obtained and analyzed. Two diffraction peaks were observed on each side of the main incident beam which was kept from reaching the detector by a small beam-stop. These peaks were indexed to be $h = 1$ and $h = 2$ on the left and $h = -1$ and $h = -2$ on the right according to Bragg's law $2d\sin\theta = h\lambda$. By measuring the exact locations of the peaks in the diffraction pattern, we calculated the unit-cell repeat distance (d-spacing). Values of d-spacing for fully hydrated preparations of DPPC alone, DPPC/($x = 0.20$) Δ^8-THC and DPPC/($x = 0.20$) Me-Δ^8-THC are shown in Fig. 11. For the DPPC preparation, our measured d-spacing values and their temperature dependence were consistent with results reported previously [37]. For the DPPC bilayer, the d-spacing increases as the temperature rises, reaches a maximum near the gel-to-liquid-crystal phase transition, and then decreases after passing the chain melting temperature. Therefore, by moni-

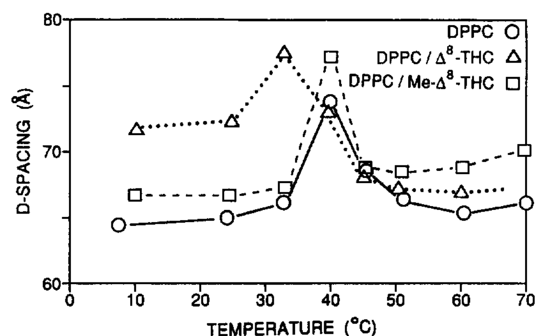

Fig. 11. d-spacing of fully hydrated DPPC bilayer preparations in the absence and presence of Δ^8-THC ($x = 0.20$) and Me-Δ^8-THC ($x = 0.20$).

toring the d-spacing variation with temperature, we can observe the maximum d-spacing and the corresponding temperature at which the bilayer preparation is on the verge of the phase transition. The temperature dependence of the d-spacings provides information on the phase transition properties complementary to that obtained from DSC thermograms.

When the biologically active Δ^8-THC ($x = 0.20$) is incorporated into DPPC bilayers, the d-spacing is 8 Å larger than that of pure DPPC in the temperature range below the phase transition. Also, its temperature dependence diagram exhibits a broader peak with the maximum point occurring at 32°C, i.e., 8°C below the corresponding temperature for pure DPPC. Conversely, the d-spacing of the DPPC preparation containing the inactive Me-Δ^8-THC shows a temperature dependence almost identical to that of pure DPPC. The location of the maximum and the width of the peak are exactly the same as observed from pure DPPC. The only noticeable difference is that the d-spacing values of DPPC + Me-Δ^8-THC at all temperatures are consistently higher than those of DPPC by 2 to 3 Å as we have shown in previous publications [38–40]

The d-spacing data from our small angle X-ray diffraction experiments show that Δ^8-THC produces significant structural changes in the lipid bilayer, including the gel-phase tilting angle, the intermolecular cooperativity and the *gauche / trans* conformer ratio. On the other hand, the inactive analog does not seem to change the bilayer structure except that it increases the d-spacing values by about 2 to 3 Å. This effect may be caused by the presence of Me-Δ^8-THC in the center (terminal methyl region) of the bilayer [38,39]. This observation is congruent with our previous X-ray results in partially hydrated bilayers showing that this analog resides in the center of the bilayer. In contrast, the active analog did not show such an increase. This again agrees with our previous results showing this cannabinoid to intercalate at the interface of the bilayer [40].

5. Conclusions

We were able to show that cannabinoids possessing a phenolic hydroxyl group induce distinct changes in the thermotropic properties of a model membrane preparation as compared to cannabinoids without a free phenolic hydroxyl which produce only marginal thermotropic effects. This points out the important role of the phenolic hydroxyl for perturbing a membrane bilayer.

We observed some differences among the various classes of cannabinoids in their effects on model membrane preparations. These differences became more pronounced with increasing drug concentrations. As a general trend, the more hydroxylated cannabinoids showed fewer endotherms in their thermograms, an observation which may be attributed to the increased polar character of these molecules. The comparison between the two non-classical enantiomeric cannabinoids showed that they exert similar effects on the thermotropic properties of DPPC bilayers which, although asymmetric, appear not to discriminate between two enantiomers.

We are currently using DSC to study the differences between the effects of Δ^8-THC and Me-Δ^8-THC on the thermotropic properties of other lipid bilayers. Such experiments will provide evidence of any specific interactions between the drug molecules and their membrane effects. We are also studying the effects of the above molecules on membranes containing cholesterol. These preparations resemble more closely the biological membranes. Such data combined with those obtained here would give us more detailed information on perturbing effects of these molecules on membranes. Our inability to detect any significant differences between several cannabinoids using DSC may be due to that these differences are too small to be detected by this technique. It remains to be examined if insertion of cholesterol magnifies these differences. Details with these results will be published elsewhere.

The small angle X-ray diffraction experiments show that the incorporation of Δ^8-THC in hydrated DPPC preparations produces significant structural changes on the bilayer structure. Conversely, the inactive analogs in which the phenolic OH is substituted do not seem to significantly affect the properties of the bilayer except that they lead to an increase in the d-spacing values. This result validates earlier data from our laboratory showing that Me-Δ^8-THC resides in the center of the bilayer in partially hydrated DMPC bilayers and is responsible for the increase of the d-spacing. Unlike Δ^8-THC that anchors in the interface of the bilayer and does not increase the d-spacing.

It appears that the biological activity of the cannabinoids cannot simply be explained by their interactions with phospholipid bilayers. However, their important role is established since significant differences between the groups with and without phenolic hydroxyl group were observed. Their different perturbation may at least in part explain their inability to exert any biological activity. However, for the other classes the cannabinoid:receptor interaction may be the decisive factor for cannabinoid activity.

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