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Biochimica et Biophysica Acta 1420 (1999) 252–265

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Effects of cannabinoids in membrane bilayers containing cholesterol

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Received 26 March 1999; received in revised form 21 June 1999; accepted 24 June 1999

Abstract

The thermotropic and dynamic properties of the biologically active Δ^8-tetrahydrocannabinol (Δ^8-THC) and its inactive congener *O*-methyl-Δ^8-tetrahydrocannabinol (Me-Δ^8-THC) in DPPC/cholesterol (CHOL) bilayers have been studied using a combination of DSC and solid-state NMR spectroscopy. The obtained results showed differential effects of the two cannabinoids under study. These are summarized as follows: (a) the presence of the active compound fluidizes more significantly the DPPC/CHOL bilayers than the inactive analog as it is revealed by DSC and NMR spectroscopy results; (b) cholesterol seems to play a significant role in the way cannabinoids act in membrane bilayers; (c) the observed additional peaks in ^{13}C/MAS-NMR spectra which were cannabinoid specific offer an evidence of their different dynamic properties in membranes. In particular, the aromatic part of the inactive cannabinoid appears more mobile than that of the active one. This finding is in agreement with previously obtained X-ray data which locate the inactive cannabinoid in the hydrophobic core of the bilayer while the active one in the polar region; and (d) the observed downfield shift of C-1 carbon in the preparation containing the active cannabinoid is a strong evidence that Δ^8-THC resides nearby the polar region where also cholesterol is well known to locate itself. Such downfield shift is absent when Me-Δ^8-THC is resided in the membrane bilayer. These differential effects of the two cannabinoids propose that the phospholipid/cholesterol core of the membrane may play an important role in the mode of cannabinoid action by regulating their thermotropic and dynamic properties.

Keywords: Δ^8-Tetrahydrocannabinol; *O*-Methyl-Δ^8-tetrahydrocannabinol; Dipalmitoylphosphatidyl choline; Model membrane; Phospholipid

1. Introduction

It has been argued in the literature that lipophilic compounds are energetically favored to act on their receptor through membrane bilayers. In particular, theoretical diffusion limited rates of drugs approach to the receptor through membrane pathway are approximately three orders of magnitude greater than aqueous diffusion through the bulk solvent [1,2]. The following experimental observations support a membrane bilayer pathway for 1,4-dihydropyridine derivatives (DHP). Patch-clamp studies with neonatal rat ventricular cells show that DHP derivatives added to the medium outside of the patch are still capable of binding and blocking single calcium channels within the patch [3]. In a study, drug diffusion within the membrane was demonstrated using Ca^{2+} channels in lipid planar bilayers. Thus, DHP, Bay K 8644 and phenylalkylamine D-600 must cross the lipid bilayer

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0005-2736/99/$ – see front matter
PII: S0005-2736(99)00106-6

in order to bind with specific sites in asymmetrically arranged Ca^{2+} receptors [4].

Cannabinoids are also highly lipophilic molecules and therefore may reach their specific receptor site through a two-step mechanism involving lipid interaction and lateral diffusion.

The above hypothesis is enhanced by the fact that not all physiological effects of cannabinoids can be attributed to the existence of receptors. Instead, some are clearly shown to be modulated through their interactions on cellular membranes [5–8].

Further evidence for the proposed mechanism can be provided by studying drug: membrane interactions. Thus, if these interactions are specific and account for the biological activity of drugs under study then the above mechanism of action gains a biological significance.

DPPC bilayers is a partially successful system in establishing structure activity relationships between biological activity and thermodynamic parameters as shown in our previous publication using DSC and X-ray diffraction [9]. Hitherto, DPPC bilayers were used in our studies for mainly two reasons: (a) DPPC bilayers which are spontaneously formed when the phospholipid is hydrated were studied extensively using various biophysical methods; and (b) DPPC bilayers consist a simple model that allows to study the physical chemical parameters that govern drug–membrane interactions. However, DPPC/cholesterol system is a more distinctive system and can account for more subtle differential dynamic or thermotropic changes caused by the additives [10].

It has been chosen to first study in detail the effects of the biologically active Δ^8-tetrahydrocannabinol (Δ^8-THC) and its inactive congener *O*-methyl-Δ^8-tetrahydrocannabinol (Me-Δ^8-THC) (Fig. 1) in model DPPC membrane using a combination of differential scanning calorimetry (DSC), ^{13}C/MAS-NMR and ^{13}C/CP/MAS-NMR as well as ^{31}P-NMR spectroscopy [11]. The rationale for choosing this pair of cannabinoid analogs is that it offers a system in which the role of amphipathicity of a molecule can be studied. For example, Δ^8-THC possess a phenolic hydroxyl group which gives it the possibility for amphoteric interactions. However, the inactive *O*-methyl analog lacks of this capability. The importance of the C-1 substituent in classical cannabinoids to CB_2 receptor selectivity has been recently studied [12]. We

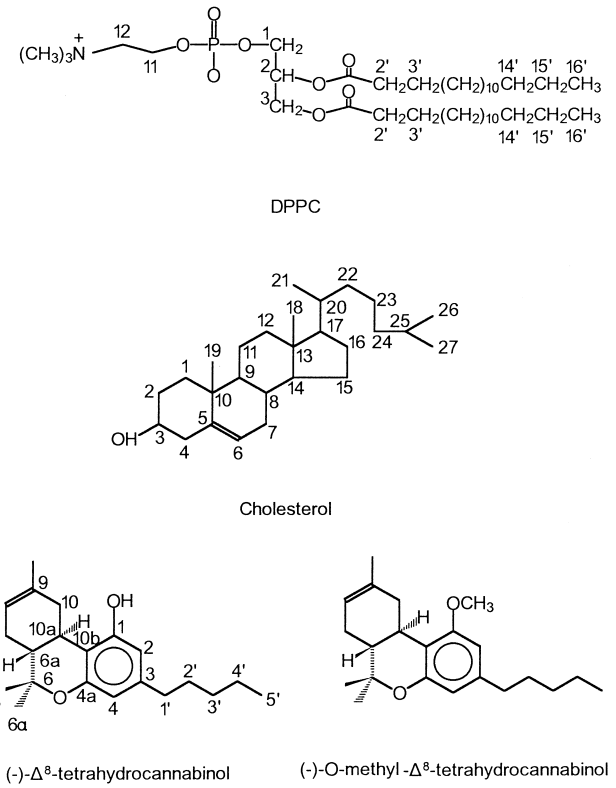

Fig. 1. Chemical structures of DPPC, Δ^8-THC, Me-Δ^8-THC and cholesterol.

believe that such a system is ideal for studying drug–membrane interactions in general. This is because it is known for other drug classes that derivatization of the phenolic hydroxyl groups has tremendous consequences in drug activity [13,14].

The obtained results showed differential effects of the two cannabinoids under study in the DPPC model membrane which may in part explain their different biological activity. These results coupled with previous results using X-ray and neutron diffraction as well as solid state ^{2}H-NMR spectroscopy build a model for the interactions of this pair of cannabinoids into the membrane bilayers consisting of the following characteristics: (a) Δ^8-THC is located near the membrane interface with its phenolic hydroxyl group anchored near the esterified carbonyl groups of phosphatidylcholines, while the more lipophilic Me-Δ^8-THC is located deeper in the membrane bilayer [15–17]; (b) solid state ^{2}H-NMR data have shown that Δ^8-THC orients itself with the long axis of the tricyclic structure perpendicular to the bilayer chains while 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 [18]; (c) ^{13}C/MAS-NMR and ^{13}C/CP/MAS-NMR spectroscopy gave direct evidence of the intercalation of both molecules into the membrane bilayers due to the specific additional peaks appeared in the DPPC/Δ^8-THC or DPPC/Me-Δ^8-THC preparations attributed solely to the added cannabinoid. The different peaks appeared in the ^{13}C/MAS-NMR and ^{13}C/CP/MAS-NMR spectra due to the presence of either cannabinoid showed their different mobility in DPPC bilayers [11]; and (d) ^{31}P-NMR spectroscopy revealed that the presence of the active analog causes significant conformational changes in the vicinity of the headgroup while the inactive analog only minor changes.

This article is an extension of our research activity focusing on the interactions of this pair of cannabinoids in membranes containing phospholipid with cholesterol using a combination of DSC and solid state NMR spectroscopy. The significance of this manuscript can be summarized as follows: (a) the membrane under study resembles closer to the biological one. Therefore, any observed differential effects between the two analogs under study in DPPC/cholesterol (DPPC/CHOL) bilayers would enhance the hypothesis that cannabinoids, a highly lipophilic class of molecules, may reach the receptor site probably by interacting productively with phospholipid core containing cholesterol. This phospholipid core may in fact mediate and control their biological action; (b) this study provides key information about the role of cholesterol in the cannabinoid molecular mechanism of action; and (c) it provides physical chemical information about the dynamic and thermotropic properties of a hydrated ternary system.

2. Materials and methods

2.1. Materials

Dipalmitoyl-glycero-*sn*-3-phosphorylcholine (DPPC) was obtained from Avanti Polar Lipids, Inc., AL, USA and cholesterol from Sigma. Δ^8-THC and Me-Δ^8-THC were kindly donated by Prof. A. Makriyannis laboratory.

2.2. Methods

2.2.1. DSC

Sample preparation procedures were identical for DSC and NMR spectroscopy. Appropriate amounts of the phospholipid/CHOL with or without cannabinoid were dissolved in spectroscopic grade chloroform. The solvent was then evaporated by passing a stream of O_2-free nitrogen over the solution at 50°C and the residue was placed under vacuum (0.1 mm Hg) for 12 h. For measurements this dry residue was dispersed in appropriate amounts of bidistilled water by vortexing. After dispersion in water (50% w/w), portions of the samples (ca. 5 mg) were sealed in stainless steel capsules obtained from Perkin–Elmer. Thermograms were obtained on a Perkin–Elmer DSC-7 calorimeter. Prior to scanning, the samples were held above their phase transition temperature for 1–2 min to ensure 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 indium (T_m = 156.6°C) as standard sample. Thermograms from samples stored at freezer temperatures (−15°C) for a few days were identical if run immediately after sample preparation. This signifies that thermal history of the sample does not affect the thermograms in this particular class of drug molecules [19].

2.2.2. NMR spectroscopy

Stationary and high resolution NMR spectra were obtained at 100.63 MHz on a Bruker MSL 400 NMR spectrometer capable of high-power ^{1}H-decoupling and equipped with magic angle spinning unit. The spinning rate for MAS NMR experiments used was 2.5 kHz, and was kept constant for the two temperatures (25 and 37°C). Each spectrum was an accumulation of 2000 scans for the stationary spectra and 20 000 scans for the high resolution spectra. The lipid content for the three samples used in the experiments was the same (∼50 mg). The delay time was 4 s, the 90° pulse width was 5 μs and the acquisition time was 40 ms. Chemical shifts values of DPPC bilayers were obtained from literature [20]. Peak assignments attributed to the drugs are based on their ^{13}C high resolution data in solution [11].

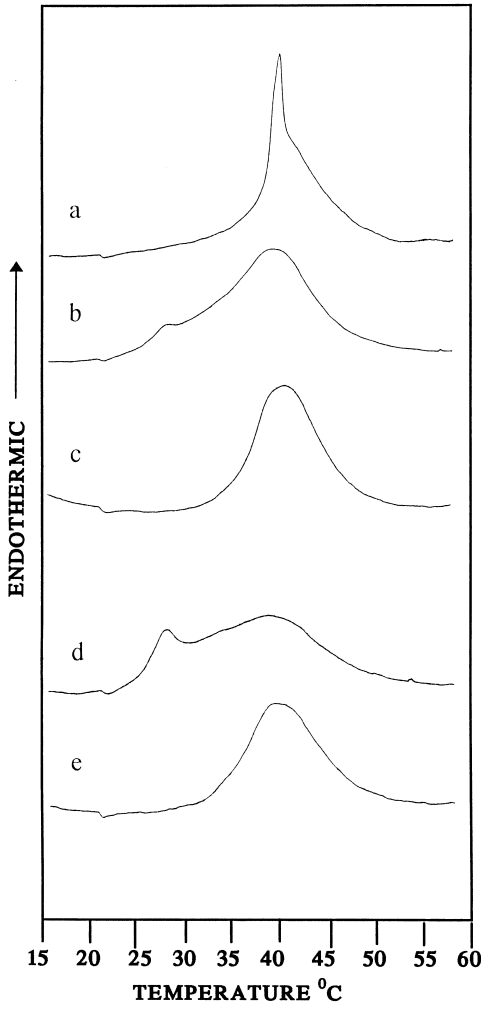

Fig. 2. DSC scans of (a) DPPC/CHOL ($x=0.20$) and the addition of (b) Δ^8-THC ($x=0.05$), (c) Me-Δ^8-THC ($x=0.05$), (d) Δ^8-THC ($x=0.10$) and (e) Me-Δ^8-THC ($x=0.10$).

3. Results

3.1. Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a fast and relatively inexpensive thermodynamic technique which allows the study of the thermotropic properties of the membranes in the absence and presence of bioactive molecules. Therefore, it is used in our laboratory as a diagnostic technique to investigate differential effects that may be caused by the incorporation of additives under study. When such differential effects are observed, then techniques which offer complementary and more detailed information on the thermal and dynamic properties of membranes, with or without the presence of additives are applied. Fig. 2 shows DSC scans of DPPC/CHOL without or with the presence of either cannabinoid under study. The DPPC/CHOL ($x=0.20$ or 80:20 molar ratio) scan shows a phase transition which consists of a narrow low temperature and a broad high temperature components. The narrow component consists mainly of a pool containing DPPC and the broad component of a pool containing mainly cholesterol [21]. The addition of $x=0.05$ Δ^8-THC relative to DPPC in DPPC/CHOL bilayers affects its narrow component by significantly broadening it (Fig. 2) while the addition of Me-Δ^8-THC has a smaller effect. The quantitative results for these preparations are shown in Table 1. A progressive addition of cannabinoid ($x=0.10$) relative to DPPC signifies the differential effects between the two cannabinoids. The active cannabinoid broadens further

Table 1
Values of onset temperature (T_{onset}), half-width temperature ($T_{m1/2}$), peak temperature (T_m) and enthalpy change (ΔH) of DPPC/CHOL, DPPC/CHOL/Δ^8-THC and DPPC/CHOL/Me-Δ^8-THC preparations

Samples	T_{onset} (°C)	ΔH (kcal/mol)	$T_{m1/2}$ (°C)	T_m (°C)	
DPPC+CHOL ($x=0.20$)	39.0	14.8	3.1		40.5
DPPC+CHOL+Δ^8-THC ($x=0.05$)	33.0	20.6	11.2		40.2
DPPC+CHOL+Me-Δ^8-THC ($x=0.05$)	36.5	15.9	7.6		40.9
DPPC+CHOL+Δ^8-THC ($x=0.1$)	25.7	18.2	19.0	28.5	39.3
DPPC+CHOL+Me-Δ^8-THC ($x=0.1$)	35.6	15.1	8.7		40.1
DPPC+CHOL ($x=0.15$)	40.1	15.8	3.2		41.8
DPPC+CHOL+Δ^8-THC ($x=0.2$)	25.4	25.4	13.5	27.9	35.3
DPPC+CHOL+Me-Δ^8-THC ($x=0.2$)	33.1	26.1	8.0	37.8	39.5
DPPC+CHOL ($x=0.30$)	37.0	7.0	13.7		42.8
DPPC+CHOL+Δ^8-THC ($x=0.2$)	33.7	10.9	12.2		39.3
DPPC+CHOL+Me-Δ^8-THC ($x=0.2$)	28.9	7.6	14.0		42.8

the phase transition of DPPC/CHOL giving a scan with a sharp and broad maxima centered at 28.5 and 39.3°C, respectively, while the inactive one shows a saturation effect (Table 1). Thus, the DSC scan containing the inactive analog resembles the preparation which contains only $x = 0.05$ of cannabinoid.

DPPC bilayers containing different concentration of cholesterol ($x = 0.15$ and $x = 0.30$) and constant concentration of cannabinoid ($x = 0.20$) relative to DPPC were examined in an attempt to study the effects of cholesterol in these bilayers (Fig. 3). At DPPC/CHOL ($x = 0.15$) preparation the presence of the active cannabinoid broadens more significantly the phase transition of DPPC/CHOL in comparison to the inactive one and produces a more complex thermogram [22–24]. The low temperature maximum is split into two distinct maxima (27.9 and 35.3°C, 37.8°C and 39.5°C) in the presence of an active and inactive cannabinoid correspondingly.

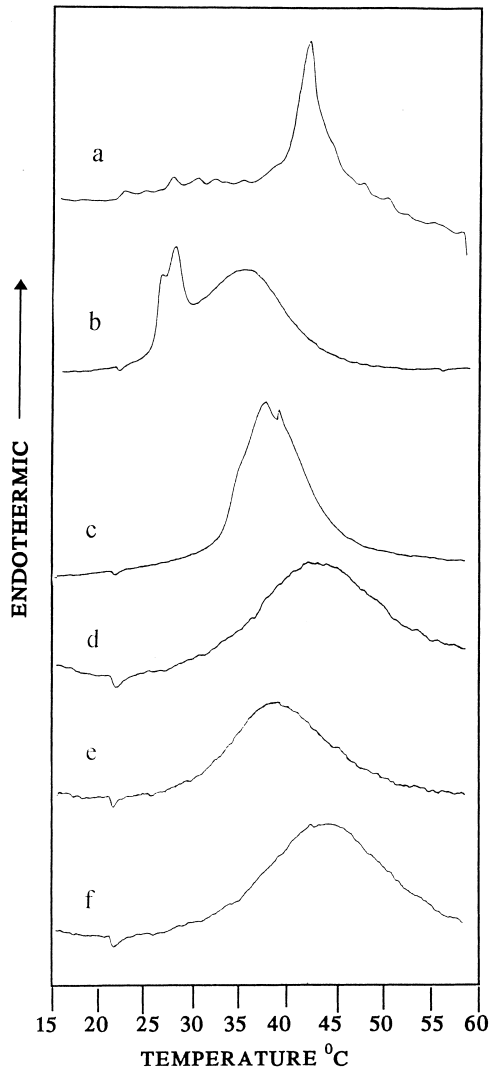

Fig. 3. DSC scans of (a) DPPC/CHOL ($x = 0.15$) and the addition of (b) Δ^8-THC ($x = 0.20$), (c) Me-Δ^8-THC ($x = 0.20$), (d) DPPC/CHOL($x = 0.30$) and the addition of (e) Δ^8-THC ($x = 0.20$) and (f) Me-Δ^8-THC ($x = 0.20$).

When a higher concentration of cholesterol is used ($x = 0.3$) only Δ^8-THC affects the phase transition of DPPC/CHOL. The thermograms consist of only one very broad peak. This is a well known effect of cholesterol which at increasing concentrations causes significant broadening and finally abolishes the phase transition of DPPC bilayers [21].

3.2. ^{13}C-NMR spectroscopy

To gain more information about the structure of the membrane bilayers and the effects of cannabinoids when inserted into DPPC bilayers, solid state ^{13}C-NMR spectroscopy was applied. In particular, stationary ^{13}C-NMR spectroscopy was utilized to study the structure of membrane bilayers, while high resolution ^{13}C-NMR spectroscopy was performed to explore the dynamic properties of drug molecules in the membrane bilayer.

Solid-state ^{13}C-NMR experiments were run at temperatures of 25–43°C for the three preparations under study (Figs. 4 and 5). Each spectrum consists of three regions, namely, carbon atoms in the hydrophobic region (10–40 ppm), those in the glycerol backbone region (40–80 ppm), and the esterified carbonyls (near 170 ppm).

When a molecule is present in the phospholipid bilayer it can cause the following changes to the ^{13}C-NMR spectrum. (a) Changes in peak intensity and line-width due to modified membrane fluidity. (b) Changes in chemical shift values of individual carbon nuclei of the membrane lipid due to modified phase transition profiles. (c) Appearance of a specific subset of peaks from the carbon nuclei of the incorporated cannabinoid molecules. These spectral features will be analyzed both qualitatively and quantitatively, and the results will be combined with DSC experiments to gain better understanding of the membrane's thermotropic properties induced by each of the cannabinoids.

When the bilayer is below the phase transition (gel

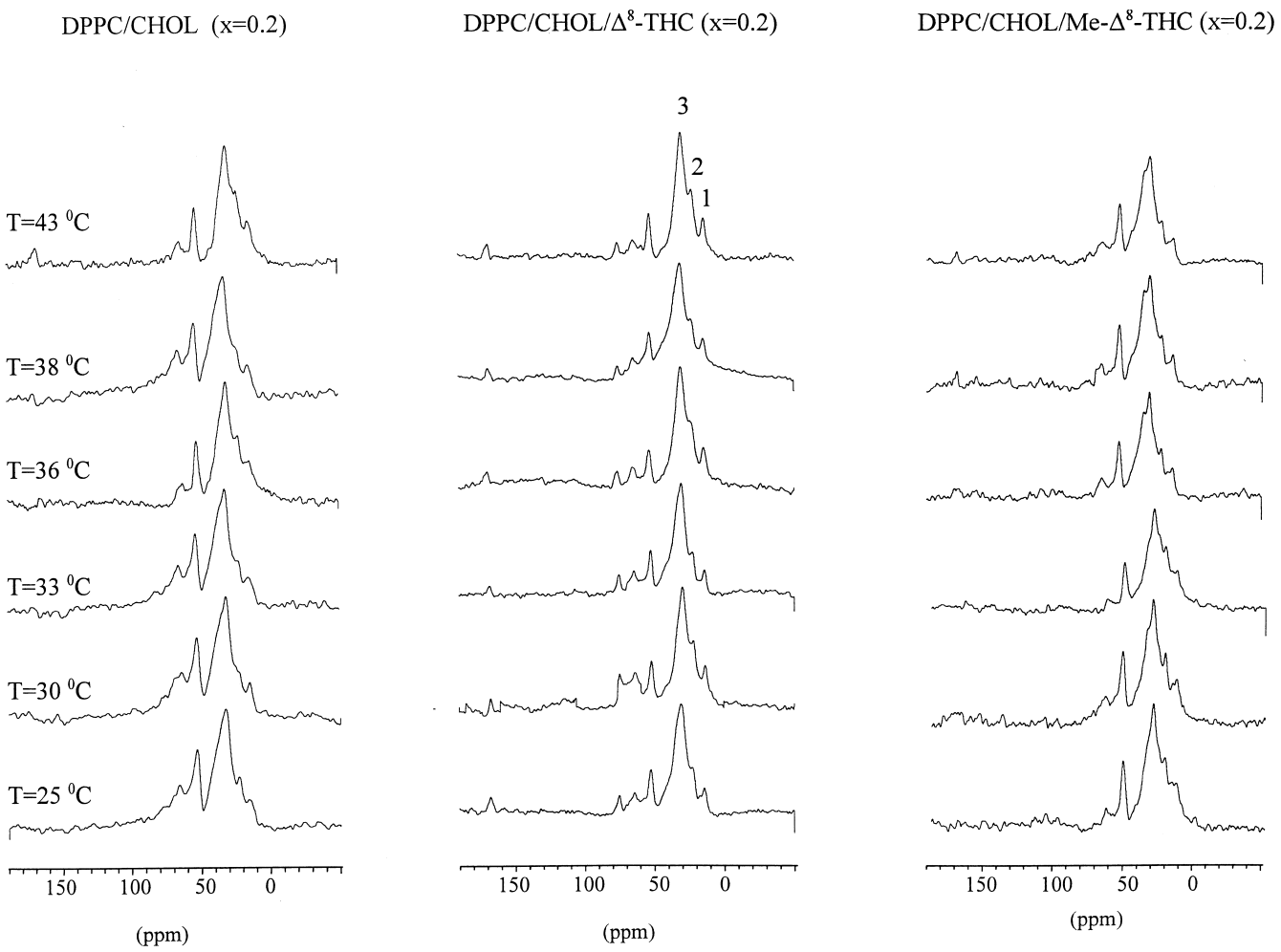

Fig. 4. Stationary ^{13}C-NMR spectra of DPPC/CHOL ($x=0.20$) without or with either ($x=0.20$) active cannabinoid Δ^8-THC and ($x=0.20$) inactive cannabinoid Me-Δ^8-THC at temperature range of 25–43°C.

phase) the carbonyl signal is broad and asymmetric. Above the phase transition, where the fluidity of the membrane is increased this peak becomes narrower. Other peaks also above the phase transition become narrower and additional peaks are resolved. Above the main phase transition temperature several changes are observed not only in the lineshape of the peaks but also in their chemical shift. For example, a large upfield shift of the $(CH_2)_n$ peaks is observed which reflects an increase in the acyl chains population of gauche conformations [25].

3.3. Stationary ^{13}C-NMR spectroscopy

3.3.1. Carbonyl region

At 43°C and ca. 172 ppm a peak is observed attributed to the carbonyls (C-1′) signal from the phospholipids in DPPC/CHOL ($x=0.20$) bilayer (Fig. 4). The presence of carbonyl group is already apparent from the lower temperature of 25°C when the active cannabinoid is incorporated in DPPC/CHOL preparation. This, as it is explained above, indicates fluidization effect in DPPC/CHOL membrane by Δ^8-THC analog. The inactive analog exerts also a fluidizing effect but to a lesser degree because the carbonyl peak appears only at 5°C lower temperature vis à vis DPPC/CHOL preparation.

3.3.2. Hydrophobic and headgroup regions

The total halfwidth of the complex $(CH_2)_n$ region containing the various methylene resonances in the three preparations is simulated for the representative

temperatures (25, 36 and 43°C) using the LINESIM program provided by Bruker company and incorporated into the NMR spectrometer. The complex $(CH_2)_n$ region was simulated considering that it consists of three individual peaks. These are designated in a representative peak of DPPC/CHOL/Δ^8-THC preparation at 43°C as 1, 2 and 3 (Fig. 4). Analytically, the simulation results are shown in Table 2. The major conclusions derived from this table are: (a) using the total half width of the simulated peaks as a comparison parameter it was found that this was narrower in the active cannabinoid containing preparation. This result indicates that the active cannabinoid causes the most fluidization in the DPPC/CHOL bilayers; (b) the intensities of the simulated peaks in the three preparations varied depicting once more the distinct dynamic properties of the three preparations; (c) the total halfwidth of the three preparations was temperature dependent. The general trend was that increase of temperature caused reduction of the total halfwidth in the three preparations; and (d) the active cannabinoid containing preparation contained more resolved peaks, signifying again that this preparation was the most fluid.

The headgroup region of its spectrum contains clearly three peaks versus only two observed in DPPC/CHOL, or DPPC/CHOL/Me-Δ^8-THC bilayers. The headgroup region is also better resolved in the active cannabinoid containing membrane preparation. The obtained DPPC/CHOL/Me-Δ^8-THC spectra resemble those of DPPC/CHOL bilayers.

3.3. High-resolution ^{13}C-NMR spectroscopy

Representative ^{13}C/MAS-NMR spectra for the three preparations under study at temperatures of

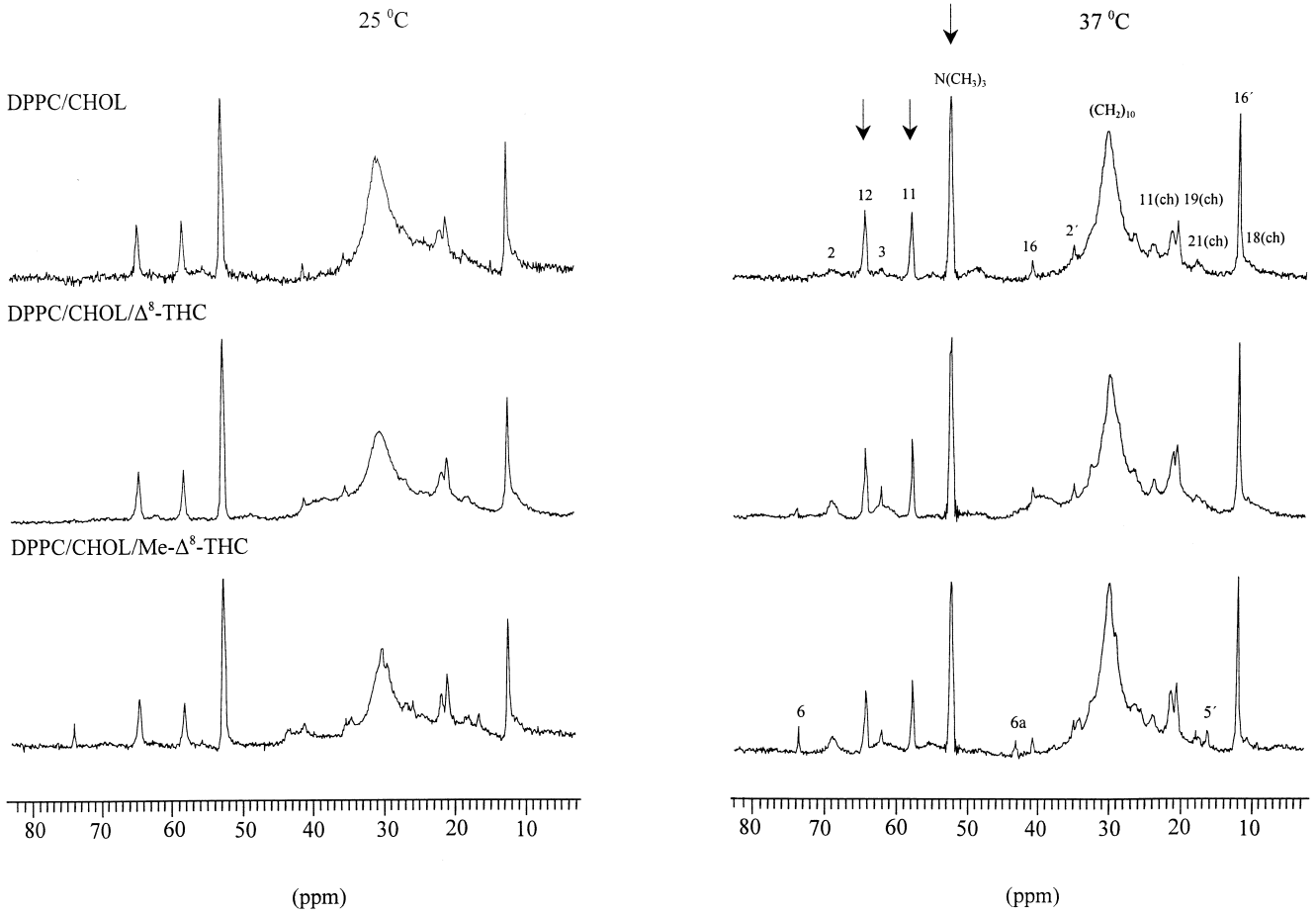

Fig. 5. ^{13}C/MAS-NMR spectra of hydrophobic and polar region of DPPC/CHOL ($x=0.20$) without or with either ($x=0.20$) active cannabinoid Δ^8-THC and ($x=0.20$) inactive cannabinoid Me-Δ^8-THC at temperatures of 25 and 37°C.

Table 2
Intensities, halfwidths ($\nu_{1/2}$) and total widths of the simulated methylene peak (10–50 ppm) at representative temperatures of 25, 36 and 43°C

Samples	Temperature (°C)	Intensity %			Half width ($\nu_{1/2}$)			Total width
		Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3	
DPPC/CHOL	25	15.9	14.3	69.8	907.81	397.53	1377.56	2682.9
	36	14.3	18.9	66.8	703.22	496.37	1101.52	2301.1
	43	16.6	24.2	59.2	591.24	631.23	1022.06	2245.5
DPPC/CHOL/Δ^8-THC	25	13.9	18.1	68.1	408.91	492.90	948.80	1850.6
	36	15.4	16.7	67.8	366.53	493.27	972.89	1832.7
	43	15.6	22.9	61.5	377.15	520.30	868.43	1765.6
DPPC/CHOL/Me-Δ^8-THC	25	16.5	19.3	64.2	680.53	455.33	1137.46	2273.3
	36	19.0	16.7	64.3	567.15	340.47	1309.18	2246.8
	43	10.3	11.1	78.6	387.04	175.58	1375.22	1937.8

Table 3
C-13 assignment of preparations DPPC/CHOL, DPPC/CHOL/Δ^8-THC and DPPC/CHOL/Me-Δ^8-THC

Assignment	Chemical shifts					
	25°C			37°C		
	DPPC/CHOL	DPPC/CHOL/Δ^8-THC	DPPC/CHOL/Me-Δ^8-THC	DPPC/CHOL	DPPC/CHOL/Δ^8-THC	DPPC/CHOL/Me-Δ^8-THC
C-16′	12.47	12.47	12.47	12.36	12.35	12.35
C-15′	24.05	24.15	24.60	24.50	24.27	24.38
C-3	26.80	26.80	26.91	26.91	27.02	26.80
$(CH_2)_{10}$	30.88	30.44	30.22	30.66	30.44	30.44
C-2′	35.41	35.41	35.30	35.41	35.30	35.30
$N(CH_3)_3$	52.73	52.61	52.72	52.72	52.61	52.72
C-11	58.24	58.13	58.13	58.13	58.13	58.13
C-12	64.53	64.53	64.53	64.64	64.64	64.64
C-18 (ch)	12.00	11.13	11.13	12.30	11.02	10.90
C-21 (ch)	18.53	18.42	18.53	18.42	18.42	18.31
C-19 (ch)	21.07	20.96	21.07	20.96	21.07	20.96
C-11 (ch)	22.06	21.62	21.95	21.84	21.62	21.84
C-16 (ch)	41.15	41.14	41.25	41.25	41.14	41.14
C-5′			16.55			16.66
C-6a			43.57			43.57
C-6			74.01			74.01
C-2			100.92			100.92
					108.64	108.97
C-4			109.85			109.85
C-8			117.79			117.57
C-9			132.79		133.67	132.79
C-3			139.96		140.07	139.96
C-4a			152.75			152.87
C-1			157.06		161.36	157.17
C=O				172.00	171.84	171.40

25 and 37°C are shown in Fig. 5. The preparations containing cannabinoid show additional peaks due to its presence in the membrane bilayers. Peaks due to the presence of drug molecules are clearly shown and labeled on the spectra (Table 3). For example, the peak at 74 ppm which corresponds to C-6 of the cannabinoid at 25°C is only observed in the bilayer containing the inactive cannabinoid. This peak is also more pronounced in the bilayer containing the inactive cannabinoid Me-Δ^8-THC at 37°C. These results show that the tricyclic part of the inactive cannabinoid is more mobile. Simulation of the $\nu_{1/2}$ for the representative peaks (C-12, C-11 and C-16′) present at both temperatures of 25 and 37°C in the three preparations are shown in Table 4. The major conclusions derived from this table are the following: the $\nu_{1/2}$ of the simulated peaks is temperature dependent; all three simulated peaks become narrower with increase of temperature. The presence of active cannabinoid in DPPC/CHOL reduces considerably $\nu_{1/2}$ of peaks C-11 and C-12 at liquid crystalline phase. This is in additional evidence which shows that the active cannabinoid is placed at the vicinity of the polar group. The presence of the almost inactive cannabinoid at the liquid crystalline phase in DPPC/CHOL reduces considerably $\nu_{1/2}$ of peak C-16′ showing that is placed at the hydrophobic region. Interestingly, the presence of almost inactive analog reduces considerably $\nu_{1/2}$ of the simulated peaks at 25°C. In contrast, the presence of the active analog reduces $\nu_{1/2}$ of C-11 and increases $\nu_{1/2}$ of C-12 and C-16′.

The aromatic and carbonyl regions of the three preparations are shown in Fig. 6. The DPPC/CHOL bilayer contains a peak centered at 172 ppm due to the carbonyl groups of DPPC. The presence of either cannabinoid in DPPC/CHOL bilayers results in the sharpening and increasing of fluidization of this bilayer. Several other peaks due to the presence of either cannabinoid in the aromatic region are present (see also Table 3). These peaks are more pronounced in DPPC/CHOL/Me-Δ^8-THC bilayers showing once again the higher mobility of this region in the DPPC/CHOL bilayers. This observation confirms previous X-ray data which locate the inactive analog deep in the hydrophobic core of the membrane bilayer where it is anticipated to have a high mobility. In contrast, the active one is found to anchor in the mesophase or headgroup of the DPPC bilayers with a restricted motion. Peaks corresponding to C-4, C-10b, C-8, C-4a and C-2 are evident only in DPPC/CHOL/Me-Δ^8-THC bilayer. This is a nice application of ^{13}C/MAS-NMR spectroscopy which clearly shows a different molecular motion of chemically similar drug molecules that have different biological activity in cholesterol-containing membrane bilayers.

Another striking evidence which positions the active cannabinoid in the polar region is the downfield shift of C-1 (161.36 ppm) observed in DPPC/CHOL/Δ^8-THC preparation at temperature of 37°C. The peak is clearly attributed to the cannabinoid because in both DPPC bilayers alone and DPPC/CHOL bilayers the carbonyl peak resonates at 172 ppm [11]. This carbon is adjacent to phenolic hydroxyl group and is expected to be sensitive to the local environment. This downfield shift was observed neither in DPPC/CHOL preparation nor in DPPC/CHOL/Me-Δ^8-THC. Using aprotic or not solvents or preparations without cholesterol, this downfield shift was not observed. It appears that the presence of cholesterol contributes to the cause of this downfield shift. It is probable that the presence of cholesterol shifts the topography of the active cannabinoid upper in the

Table 4
Simulated $\nu_{1/2}$ values of the representative peaks C-11, C-12 and C-16′ at 25 and 37°C of the preparations DPPC/CHOL, DPPC/CHOL/Δ^8-THC and DPPC/CHOL/Me-Δ^8-THC

Samples	Half width ($\nu_{1/2}$)					
	C-12		C-11		C-16′	
	25°C	37°C	25°C	37°C	25°C	37°C
DPPC/CHOL	47.59	42.35	42.82	41.76	34.13	29.88
DPPC/CHOL/Δ^8-THC	51.16	37.38	40.20	26.72	37.58	34.15
DPPC/CHOL/Me-Δ^8-THC	49.22	37.66	39.06	32.31	33.48	29.44

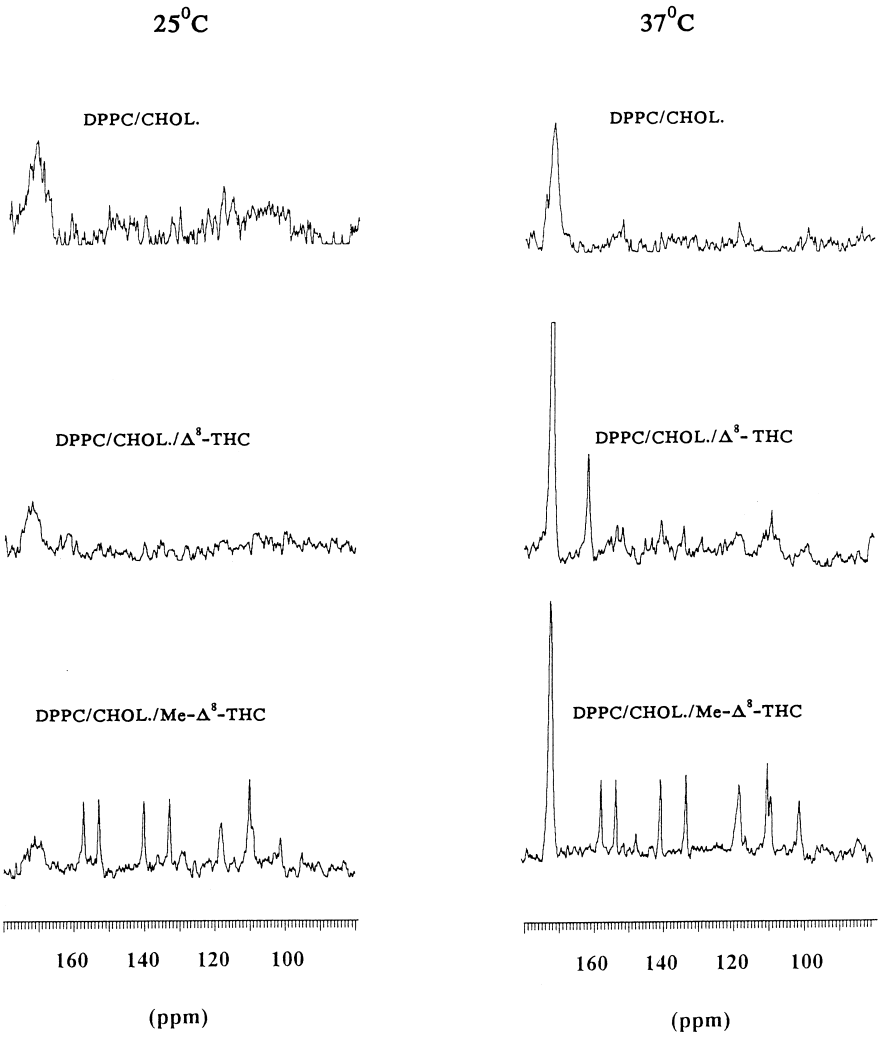

Fig. 6. ^{13}C/MAS-NMR spectra of aromatic and carbonyl regions of membrane bilayers DPPC/CHOL, DPPC/CHOL/Δ^8-THC and DPPC/CHOL/Me-Δ^8-THC.

headgroup region of the membrane bilayers. This shift may due to hydrogen bonding of the phenolic hydroxyl group with the headgroups of the phospholipid. The topography of the inactive Me-Δ^8-THC does not seem to be affected by the presence of cholesterol. A graphical representations of the obtained results are shown in Figs. 7 and 8.

4. Discussion

We have applied a combination of DSC and solid-state NMR spectroscopy to study the effects of a pair of cannabinoids in membrane bilayers. This pair of cannabinoids is characterized by a distinct different biological action. Thus, Δ^8-THC is an active psychotropic cannabinoid drug molecule while its methylated analog Me-Δ^8-THC is almost devoid of any biological activity.

Hitherto, our biophysical studies were focused in a simple phospholipid bilayer system. In this study, we extended the complexity of the membrane bilayers by incorporating cholesterol, a well-known vital constituent of membrane bilayers.

The present study reveals differential effects between the pair of cannabinoids under study. The active cannabinoid when it is incorporated into the membrane causes more drastic changes. In particular, it affects more the phase properties of DPPC/CHOL bilayers vis à vis the inactive analog. For

example, the broadening of the phase transition is more significant when the active Δ^8-THC is incorporated in the DPPC/CHOL meaning that it decreases more the cooperativity of DPPC/CHOL membrane bilayer. The DSC data show different effects of the pair of cannabinoids under study on the thermotropic properties of DPPC/CHOL bilayers that depend both on cholesterol and drug concentration. In both cases, the effects are more pronounced in membrane bilayers containing the active cannabinoid than the membrane bilayers containing the inactive one.

An important feature observed solely in bilayers containing cholesterol and the active cannabinoid Δ^8-THC was the appearance of a peak centered at 28.5°C. This peak was observed also by Bruggemann et al. [22] using similar preparations of PC/Δ^9-THC without and with cholesterol samples. The authors explained this peak as a not simple association or complex between PC/Δ^9-THC. When a membrane bilayer is without cholesterol this peak is evident only when Δ^8-THC is incorporated at $x \geq 0.10$ [9]. However, in the presence of cholesterol this peak is evident even when Δ^8-THC is added at $x = 0.05$. This means that cholesterol enhances the association of Δ^8-THC with DPPC. The thermogram of DPPC/CHOL ($x = 0.20$)/Δ^8-THC ($x = 0.10$) confirms the above observation because it resembles with that of DPPC/Δ^8-THC ($x = 0.20$). At higher cholesterol content ($x = 0.30$), this peak is absent in preparations

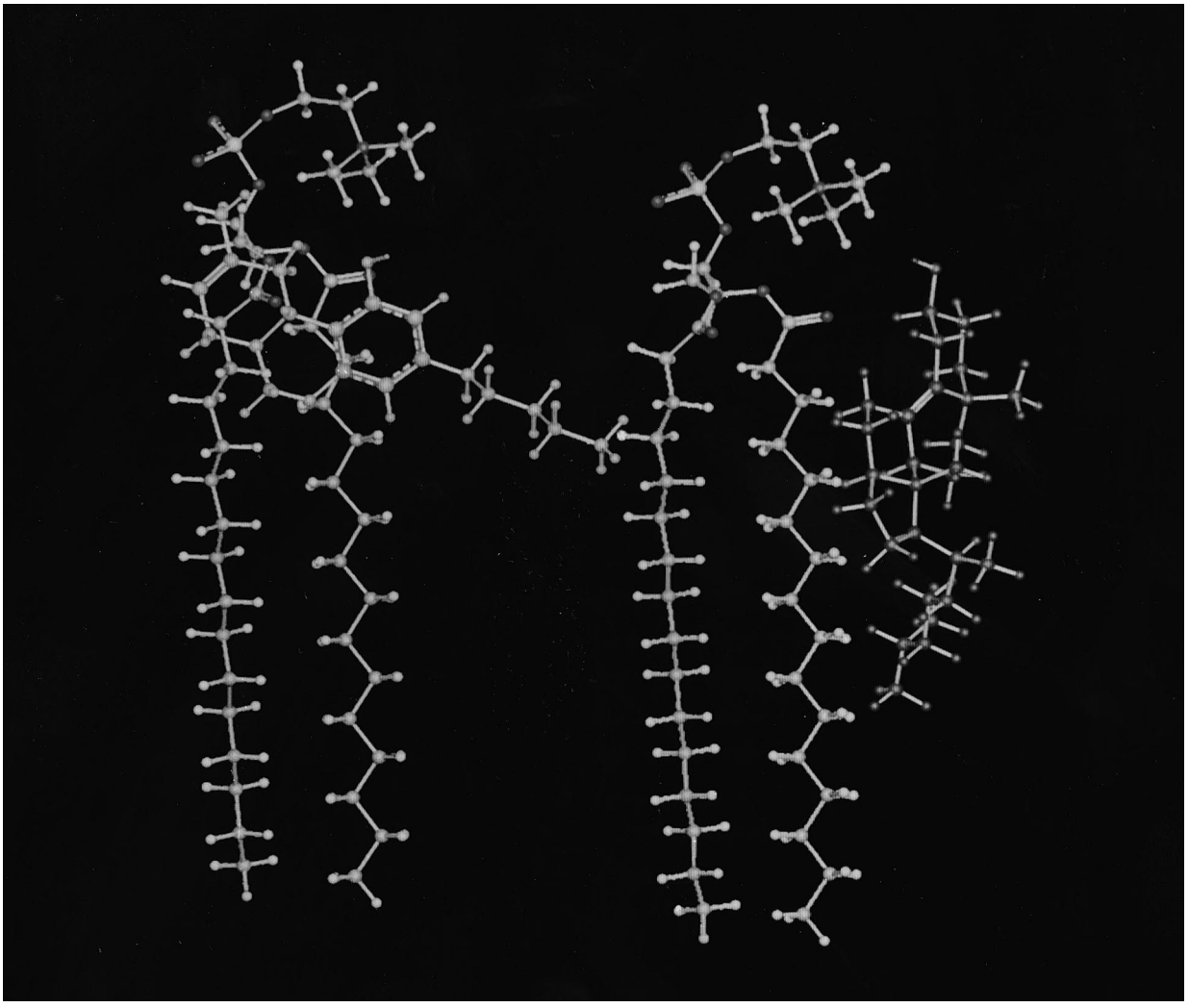

Fig. 7. Molecular graphics showing the topography of Δ^8-THC in DPPC/CHOL bilayers.

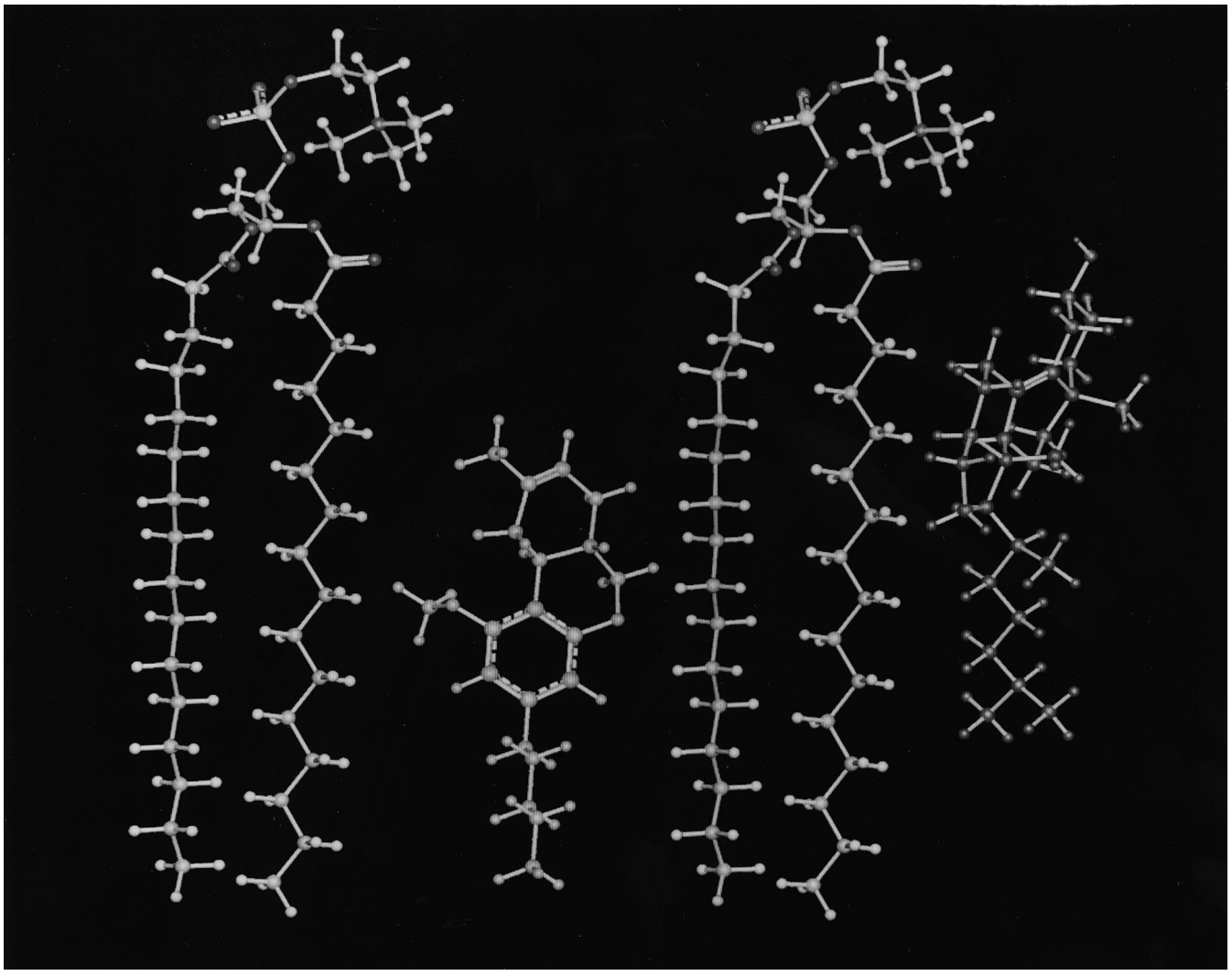

Fig. 8. Molecular graphics showing the topography of Me-Δ^8-THC in DPPC/CHOL bilayers.

containing either Δ^8-THC or Me-Δ^8-THC. It appears that cholesterol acts differently at high concentrations in a lipid environment containing cannabinoid. An explanation for this may be that cholesterol at high concentrations associates with DPPC. Thus, it prevents the association of cannabinoid with phospholipid.

^{13}C-NMR spectroscopy was widely used for the study of DPPC phospholipid bilayers with or without cholesterol and also other additives [26–37]. However, examples of the use of this technique for comparison of pairs of drugs possessing distinct different biological activity are not found in the literature.

We believe that such experiments are mainly important for two reasons: (a) they may provide insight on the molecular mechanism of action of a drug at membrane level; and (b) examine the sensitivity of MAS technique to provide information on the physicochemical parameters that govern drug–membrane interactions.

The ^{13}C-NMR experiments showed that the active analog positions itself in a different topography than the inactive analog. More specifically, the obtained data point out that the active analog is anchored in mesophase-polar region of membrane bilayer while the inactive analog in the hydrophobic region. This explains the less drastic effects caused by Me-Δ^8-THC on DPPC/CHOL bilayers. It also explains its high mobility in this region of the membrane. In

contrast, the active one which is anchored in the mesophase-polar region with its phenolic hydroxyl group probably hydrogen bonding with phosphate groups or water molecules has a limited flexibility.

In conclusion, the obtained results suggest that DPPC/CHOL bilayers can serve as a matrix to explain the differential effects of the pair of cannabinoids which have distinctly different activity. The obtained data suggest that the biological action of cannabinoids may first be screened in the core of the membrane bilayer before they laterally diffuse into the receptor site. It is apparent that if such postulation is true, the topography and orientation of cannabinoids into the membrane bilayers as revealed in our studies are important factors that govern their activities.

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