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Topography of tetrahydrocannabinol in model membranes using neutron diffraction

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Small-angle neutron scattering was used to determine the intralamellar location of (−)-Δ^8-tetrahydrocannabinol (Δ^8-THC) in hydrated dipalmitoylphosphatidylcholine (DPPC) bilayers. Nuclear scattering density profiles were calculated from measurements on deuterium and non-deuterium-labelled inclusions (8.3% (w/w)) of Δ^8-THC in DPPC multilayer samples. By comparing pairs of such nuclear density profiles, the locations of the deuterium labels were determined. Present results on the topography of Δ^8-THC in membranes are compared with earlier X-ray measurements using iodine labelling. Whereas the position of the phenolic hydroxy group is similar in both types of measurement, a difference is found in the conformation of the terminal methyl groups of the cannabinoid side-chains. The X-ray measurements on dimyristoylphosphatidylcholine (DMPC) indicated that the iodine-labelled cannabinoid side-chains assume an all-*trans* orientation with the terminal iodine atom pointing inward into the membrane away from the tricyclic region while the neutron measurements indicate that the terminal CH_3 group of Δ^8-THC aligns itself at the level of the tricyclic ring system implying that the side chain exists in a more compact conformation perpendicular to the DPPC hydrocarbons. A Gaussian function analysis of the data indicates that the Δ^8-THC molecule is significantly delocalized in the DPPC membrane in the liquid crystal phase. The mean location of Δ^8-THC suggests that the active site on a membrane-embedded receptor protein will lie near the polar interface at the base of the phospholipid headgroups.

Introduction

There is increasing interest in the biophysical and biochemical basis of cannabinoid activity in the central nervous system. Some of this interest stems from recent work [1] which suggests that cannabinoids produce their pharmacological properties, at least in part, by interacting with a cannabinoid receptor recently discovered in mammalian brains. Interest also arises from pharmacological applications of these molecules and as a result over one hundred derivatives have been investigated to determine structure-activity relationships. One impetus for this pharmacological research is the remarkably low toxicity of cannabinoids in animals and humans. So far tetrahydrocannabinol derivatives and analogs have shown potential as antiglaucoma, antinausea, analgesic, anticonvulsant and antihypersensitive agents. In their review on the subject Makriyannis and Rapaka [2] have noted that of all the known derivatives that have been investigated, the ones that retain potent activity invariably have an OH-group attached at the C-1 position on the phenolic ring of the molecule. The importance of the present neutron and past X-ray measurements [3] is that they serve to locate the position of this hydroxyl group relative to the headgroup region of phospholipid membranes.

This paper describes the results of neutron diffraction measurements carried out on multilayers of DPPC laid down on glass microscope slides. These multilayers all had 8.3% (w/w) of (−)Δ^8-tetrahydrocannbinol (Δ^8-THC) intercalated into each bilayer. Various diffraction orders were then observed and difference Fourier profiles of pairs of specimens, with and without deuterium labelling, revealed the locations of labelled sites on the Δ^8-THC with respect to the known bilayer structure. Because of pairwise similarity in the chemistry of the samples changes in Bragg repeat spacings

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Abbreviations: DPPC, dipalmitoylphosphatidylcholine; Rh, relative humidity; Δ^8-THC, (−)-Δ^8-tetrahydrocannabinol; DMPC, dimyristoylphosphatidylcholine; HTAB, hexadecyltrimethylammonium bromide.

due to heavy atom substitution [3] were avoided and most of the problems related to differing mesomorphic states were minimised.

Materials and Methods

Materials

Δ^8-THC was labelled by methods described earlier [4]. One of the deuterium labels involved changing the ^1H atom at the H10a position (labelled Ha in the upper diagram of Fig. 1) to ^2H. Another labelled molecule was produced by exchanging the CH_3 group at the 5′ position with a deuterated methyl. The lower diagram illustrates the (−)-5′-iodo-Δ^8-tetrahydrocannabinol molecule utilized in previous X-ray diffraction measuements on DMPC multilayers [3]. The DPPC used in the present experiment was obtained from Avanti Polar Lipids (Birmingham, AL, USA).

The selection of Δ^8-THC was predicated by its earlier use in X-ray diffraction experiments [3]. There has been some discussion in the literature (see Ref. 2 and references therein) as to why Δ^9-THC appears to be more active than Δ^8-THC in spite of the similarity of the diffraction results for both (see below). However recent findings (Mechoulam, R., personal communication) tend to indicate that in general Δ^8-THC is only approx. 30% less active than the Δ^9-isomer in most in vivo and in vitro experiments.

Membrane sample preparation

Glass microscope slides were used for sample substrates. These were first cleaned with ethanol, wiped dry with tissue and then cleaned again with acetone. Subsequently, they were soaked in a sulphuric-chromic acid mixture (known commercially as Chromerge) at 120°C for 5 min and then rinsed with distilled water. Next, a film of a 1% solution of HTAB in dichloromethane was drawn across the slide with wetted lens paper.

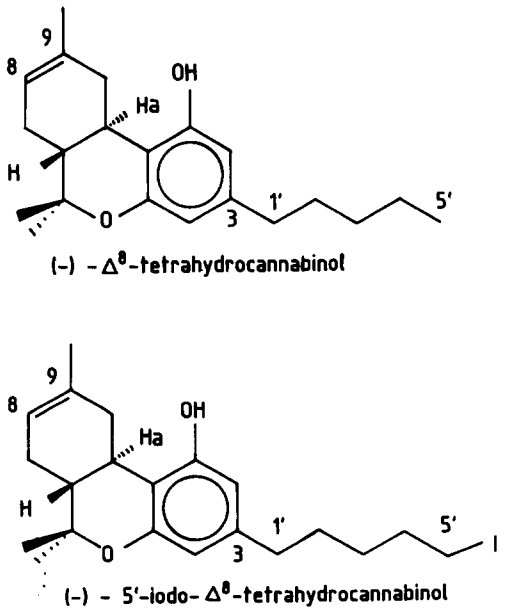

Fig. 1. Skeletal models of (−)-Δ^8-THC and (−)-5′-I-Δ^8-THC. Deuteron labelling was carried out at the Ha (H10a) and 5′ positions of (−)-Δ^8-THC.

Because Δ^8-THC is oxidizable, specimen preparation was carried out in a nitrogen atmosphere. Stock solutions of DPPC and Δ^8-THC (8.3% (w/w)) in ethanol were prepared under nitrogen. Appropriate quantities of solution sufficient to deposit approx. 10000 bilayers were deposited on the slides under nitrogen in a humidity chamber placed in an oven oscillating in temperature between 37 and 42°C for a period of approx. 3 h. Dry and wetted N_2 gas was alternately passed through the chamber for three days, with the specimen either wet or dry for 12 h at a stretch. This annealing process produced high quality samples with mosaic widths typically 0.15°.

Neutron diffraction

The diffraction measurements were carried out with the large planar surfaces of the slides vertical in a small heated compartment contained within a larger aluminum container. For measurements with a relative humidity (Rh) of 37% at 45°C, the humidity was controlled by regulating the flow of argon through a water reservoir connected to the sample chamber. For measurements with Rh = 96% at 53°C a small dish containing saturated K_2SO_4 solution was placed close to the bottom of the sample inside the inner sample chamber.

Neutrons with a wavelength of 1.54 Å were employed for measurements at the T3 small angle scattering spectrometer and the E3 spectrometer at the NRU reactor of AECL Research (Chalk River, Ontario, Canada). For the low temperature measurements four orders of diffraction were observed and for the high temperature five. The corrected intensities, $I_{cor}(n)$ were obtained using the relation [5]

$$I_{cor}(n) = I_{obs}(n)/\{L(\theta_B)\cdot As(\theta_B)\cdot B(\theta_B)\} \quad (1)$$

where θ_B is the Bragg angle, $As(\theta_B)$ (a function of the total scattering cross section) is the absorption correction for the specimen, $B(\theta_B)$ is the correction for vertical divergence [6] of the diffracted beam, $L(\theta_B) = 1/\sin(2\theta_B)$ is the Lorentz factor and $I_{obs}(n)$ the integrated intensity observed for the nth order peak. Eqn. 1 was applied directly to the data obtained with the T3 spectrometer which has an open 60 cm long linear position-sensitive detector. When a diffractometer with a small detector was employed an additional factor [7] due to finite beam width before the detector was also necessary.

The absorption correction is large and the value

taken for the total cross section must be accurate. This cross-section varies with incident neutron energy particularily for light atoms such as hydrogen. For 1.54-Å neutrons it was found experimentally that the total scattering cross section of the samples was half that at 4 Å. This is the change expected for liquid water. Because the neutron cross-section for carbon is similar to that for oxygen this indicates that the degree to which the hydrogens in the CH_2 groups of the hydrocarbon chains are bound is much the same on average as those in H_2O molecules in the liquid state, when viewed on a time-scale of picoseconds (typical of the neutron-nucleus interaction). This is in accord with the notion that phospholipid membranes are fluid-like. Precise quasielastic neutron scattering measurements would be necessary to verify these conclusions.

The corrected integrated intensities are proportional to the squares of the structure factors, $F(n)$, occurring in the scattering amplitude profiles, $\rho(x)$. For a centrosymmetric membrane system:

$$\rho(x) = C + (2/d) \sum_{n=1}^{N} F(n) \cos(2\pi nx/d) \qquad (2)$$

In Eqn. 2, d is the Bragg repeat distance, N the number of orders observed, and C a constant ($= F(0)/d$) which depends on the unobservable structure factor for the zero angle peak and effectively sets the reference level for $\rho(x)$. Since the $F(n)$ values are proportional to the square roots of the intensities the problem of determining the signs of the $F(n)$ values arises. This is conveniently solved by varying the percentage of D_2O from zero to 100% in the multilayer samples. Simple rules [8] than permit the sign of $F(n)$ in centrosymmetric multilayer samples to be determined since the slopes of all terms with n odd must be negative as a function of increasing D_2O percentage assuming the origin, $x = 0$ to be at the center of the bilayer. Terms with n even have positive slopes. Fig. 2 shows typical results for a sample having 8.3% (w/w) of undeuterated Δ^8-THC. In this case the phase sequence in light water is easily seen to be $-$, $-$, $+$, $-$, $-$ for $F(1)$, $F(2)$, $F(3)$, $F(4)$ and $F(5)$, respectively.

Results

Step function analysis

Fig. 3 shows the results of analysis for samples with Rh = 37% at 45°C. The solid wavy line in the lower part of the figure represents a difference Fourier plot. This plot results from subtracting the $\rho(x)$ profile for the unlabelled sample from the one with the label at Ha (cf., Fig. 1). In order to normalize the two profiles prior to subtraction, a multiplicative factor and an additive constant were utilized to bring the profiles into congruence at the centers of the water and bilayer

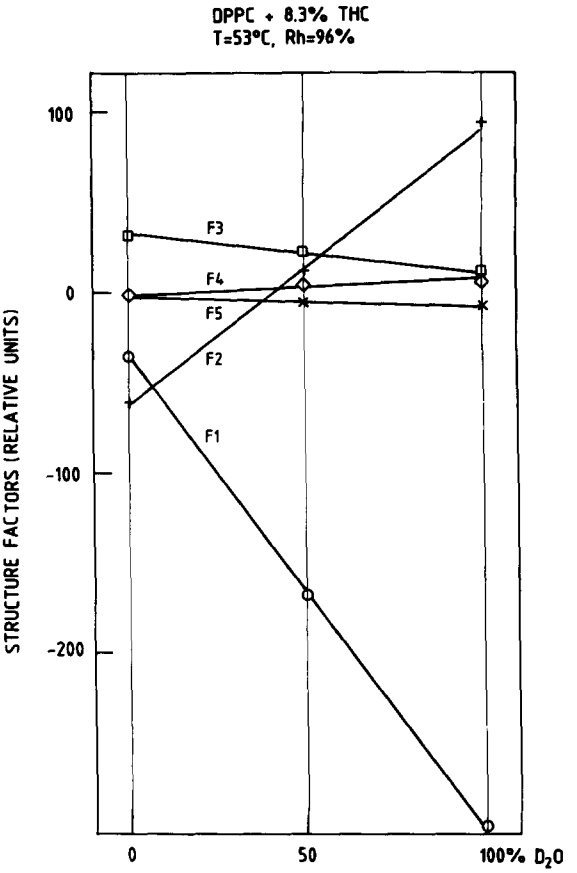

Fig. 2. Typical structure factors as a function of D_2O percentage. The DPPC bilayers contained 8.3% (w/w) of unlabelled Δ^8-THC.

regions. This normalization procedure was assumed to be reasonable, since previous X-ray [3] and neutron diffraction [9] measurements indicated the carbonyl region below the headgroup to be near the site of

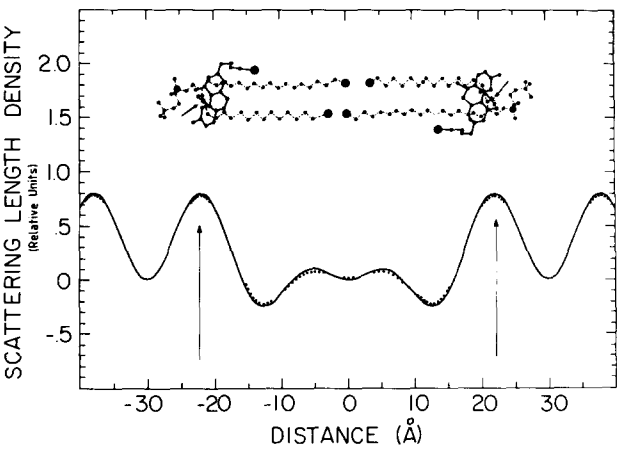

Fig. 3. Neutron-scattering length density profile differences obtained by Fourier transformation of diffracted intensities from DPPC with and without the deuteron label at the 10a position. The relative humidity was 37% and the temperature 45°C. A schematic representation of the DPPC bilayer with Δ^8-THC superimposed is shown above the profile. The arrows indicate the location of the label. The dotted line represents a strip-model calculation (see text).

intercalation. Since the scattering length of the deuteron is positive and that of the proton is negative subtraction of the $\rho(x)$ profiles results in a positive peak in the difference Fourier at the labelled site. The small positive peaks near ± 5 Å are thought to be due to the fact that only four terms were used in the Fourier series. According to this analysis the H10a label is located 8 ± 2 Å below the center of the water layer at 45°C (Rh = 37%).

The dotted line in Fig. 3 represents the result of a least squares fit using a strip-model calculation of the type described by King and White [10]. In this calculation the widths of the water, headgroup, acyl and methyl regions were found to be 9.5, 7.0, 13.0 and 10.5 Å, respectively, and the label was assumed to be in the center of the headgroup strip. According to this calculation the label lies $(9.5 + 7.0)/2$ or approx. 8 Å in from the center of the water strip in good agreement with our previously derived value. The locations of the Δ^8-THC molecules in the schematic have been adjusted by translating the superimposed molecules so as to have the deuterons (starred species) at the center of the peak in the difference profile. The orientations of the cyclic portions of the THC molecule have been assumed to be the same as those found previously for DMPC [3]. This assumption is in accord with previous NMR measurements on THC in DPPC above 45°C [11]. The sharp turn in the cannabinoid side-chain has been assumed to be the same as in earlier X-ray diffraction measurements on DMPC [3]. Our only neutron measurements of side-chain orientation were made at 53°C and these are reported below.

In order to obtain a bilayer conformation more like the liquid crystalline phase, and hence more biologically relevant, measurements at higher temperature and humidity were deemed necessary. For this purpose a saturated solution of K_2SO_4 was utilized to obtain Rh $\geqq$ 96%. The minimum in the Bragg repeat was then determined by varying the temperature of the sample. Normally when this minimum is achieved the hydrocarbon chains of pure DPPC become disordered in a manner characteristic of the liquid crystal phase and this results in a shorter hydrocarbon region.

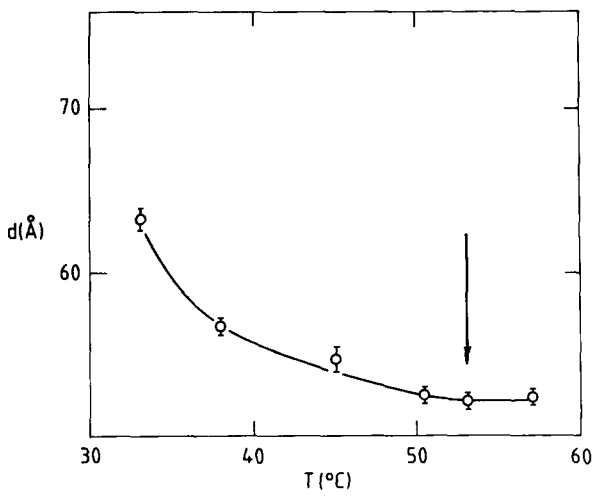

Fig. 4. Variation in the interlamellar Bragg repeat as a function of temperature for relative humidity $\geqq$ 96% in a DPPC multilayer sample with 8.3% (w/w) of Δ^8-THC present. The arrow indicates the temperature (53°C) at which our high temperature neutron diffraction measurements were made.

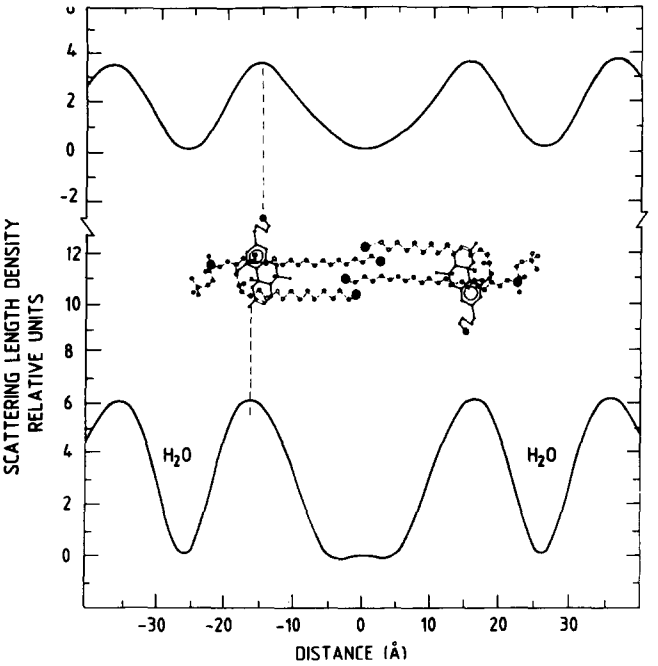

Fig. 5. Difference Fourier profiles obtained with the deuterium label at H10a (below) and with the label near 5′ (above). The dashed lines indicate how the peaks in the difference Fouriers relate to the embedded Δ^8-THC molecule, shown schematically in the center of the figure.

Fig. 4 shows the results of our measurements of the Bragg repeat as a function of temperature. These indicate a minimal value (52 Å) at approx. 53°C. Fig. 5 is a highly schematic representation of the thinner membrane which occurs in the liquid crystal phase. The hydrocarbon tails are not actually ordered as shown, since narrowing of the hydrocarbon region results from *trans-gauche* isomerisation of the chain methylene groups.

The lower wavy line in Fig. 5 represents a difference Fourier profile: DPPC with 8.3% (w/w) of labelled Δ^8-THC minus DPPC with the same amount of undeuterated cannabinoid. The label was at the Ha position shown in Fig. 1. The Rh was 96% and the temperature 53°C. As in Fig. 3 the origin of the distance coordinate is at the center of the bilayer. The 'valleys' where interlamellar water is located are marked 'H_2O'. A dashed line marks the positive maximum due to the label which has a positive neutron-scattering length. The dashed line indicates the most likely position of

the H10a atom. It is 9 ± 2 Å from the center of the water layer. This value is close to that derived from the measurements at 45°C and Rh = 37%.

The upper difference profile also refers to measurements made with Rh = 96% at 53°C. As before the same amounts of Δ^8-THC (8.3% (w/w)) had been inserted into the DPPC but in this case the label was the CH_3 group converted to CD_3 at the end of the cannabinoid side-chain. Here the maximum in the difference profile is located at 11 ± 2 Å in from the center of the interlamellar water layer. This result is different from previous X-ray measurements on DMPC with (−)-5′-iodo-Δ^8-tetrahydrocannabinol intercalated in bilayers. These latter measurments indicated the twisted all-*trans* side-chain conformation shown in Fig. 3. The distance of the iodine label was then found to be approx. 20 Å in from the the center of the interlamellar water region. The *gauche-trans-gauche* conformation of the cannabinoid tails illustrated in Fig. 5 is the same as that suggested by computer modelling for Δ^8-THC in synaptosomal membranes [14] where the tricyclic and tail components were also found to lie at approximately the same level below the phospholipid headgroups.

Gaussian function analysis

It has recently been pointed out (Ref. 12 and references therein) that there are no sharp boundaries in a biological membrane at high temperatures and humidities. These authors have shown that Gaussian distributions are the only physically meaningful real-space representations of different regions lying along the direction normal to the bilayer planes.

The ρ values for the labelled and unlabelled samples were normalized in the strip function calculations described above but in applying the Gaussian function analysis the $F(n)$ are normalized. The scaling of the $F(n)$ to one another can be most conveniently done by noting that the percentage of water in our sample pairs should be the same. After the $F(n)$ have been adjusted to yield the same ρ values at $x = 0$ (the membrane center) the assumption that all the water is centered at $x = d/2$ and manipulation of Eqn. 2 yields the scaling factor K as follows:

$$K = \sum_m F_P(m) \Big/ \sum_m F_D(m) \tag{3}$$

where the summation is for terms with m even and $m \neq 0$, and F_P and F_D represent F values for the samples with undeuterated and deuterated labels, respectively. Multiplication by K of the F values from samples with deuterium labels then puts them on a similar scale to their unlabelled counterparts. It may be noted that with our samples K was always close to unity.

After scaling by K the differences in the $\rho(x)$ values yielded the double-peak structure (solid line) shown in Fig. 6 where the origin for the abscissa is now at the membrane center. For these samples the label was at the H10a position. As noted before these positive peaks result from the fact that the deuteron has a positive neutron scattering length while that for the proton is negative. The dashed line represents a Gaussian fit to the difference Fourier transforms and suggests that the location of the label does indeed have a

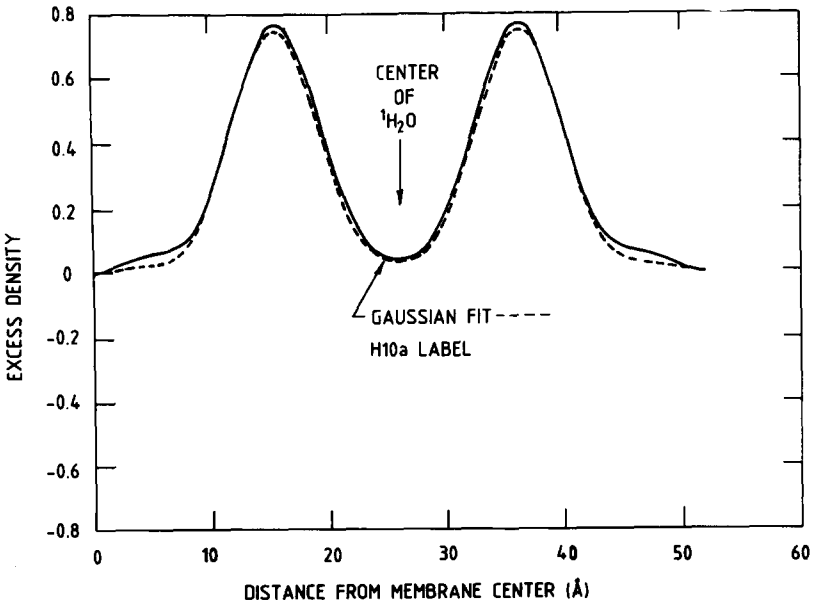

Fig. 6. Plot of the excess neutron scattering amplitude density (solid line) due to the deuterium label at H10a found from profiles obtained by scaling as described in the text. T = 53°C and Rh = 96%. The dashed line represents a Gaussian fit to the data.

Gaussian distribution centered at $X_i = 15.6 \pm 0.4$ Å with a half-width A_i of approx. 5.2 Å. A value of $X_i = 15.6$ Å translates into a distance from the center of the water layer of 26–15.6 = 10.4 Å in fair agreement with the value of 9 ± 2 Å obtained from our strip function analysis.

The method of Wiener and White [12] involves a protocol for 'parsing' the various regions perpendicular to the bilayer planes and in the simplest case the five regions, illustrated in Fig. 7, involve methyl, methylene, glycerol plus carbonyl, phosphate plus choline, and water layers (The results of our analysis place the Δ^8-THC molecule in the third sector and this is denoted in Fig. 7 by adding THC to the region denoted as 'glycerol plus carbonyl'). Since there are seldom more than half a dozen orders to the scattering at high temperatures more divisions cannot be distinguished unambiguously [12]. The resulting groupings give rise to what is known as the 'quasimolecular' model. In this model the total neutron scattering amplitude density, B_i, of each region is evaluated from its chemical composition and a variational approach is employed to evaluate the half-widths, A_i and centroids, X_i, of each region. The intercalated species can be taken into account by evaluating its B_i and assigning a Gaussian to it. Alternatively, the location of the deuterium label can be found by inserting the intercalating species in

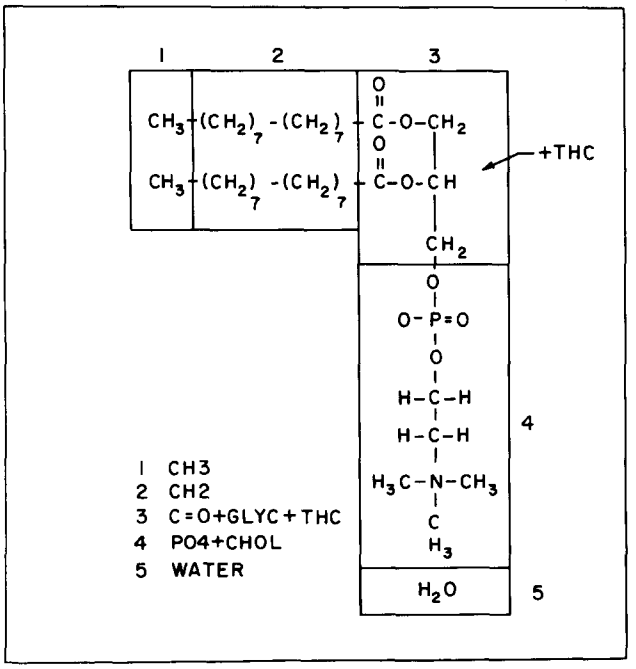

Fig. 7. Division or 'parsing' of DPPC into various regions in order to apply the quasimolecular model of Wiener and White [12]. The neutron scattering amplitude densities of each compartment are evaluated and Gaussian representations of each region are then employed in a fitting procedure to find the centroids and widths of these regions.

TABLE I

Gaussian parameters (in Å) for the quasimolecular model fit to the H10a pair and the pair with one sample having a deuterated methyl group

Regions as defined in Fig. 7.

Region	1	2	3	4	5	Sample pair
A_i	6 ±0.5	11±1	5±0.3	6±0.3	7±0.8	H10a label
A_i	5.5±0.6	10±1	5±0.5	6±0.5	6±0.7	Methyl label
X_i	0(fix)	6±1	16±0.3	21±1	26(fix)	H10a label
X_i	0(fix)	6±0.5	16±0.4	21±0.5	26(fix)	Methyl label

one region after another, evaluating the change in B_i, and obtaining a best fit to the measured structure factors with calculated structure factors $F_c(n)$. By this procedure the calculated F values can be related to an absolute scale. The Gaussian forms for $F_c(n)$ are given by:

$$F_c(n) = (2/s) \cdot \sum_{i=1}^{N} B_i \exp(1 - \pi A_i n/d)^2 \cos(2\pi X_i n/d) \qquad (4)$$

where S is the area per lipid and the other symbols have the meanings defined earlier. In our fitting procedure the number of water molecules per lipid was allowed to vary freely with the centroid of the water distribution fixed at $d/2$. At 53°C and a relative humidity of 96% the number of water molecules was determined to be 12 ± 4. The half-width A_i of the water layer was 7 ± 1 Å. In a free fit allowing the locations and half-widths of the cannabinoids to vary in the three $^1H_2O/^2H_2O$ mixtures, X_i and A_i were found to be 16.4 ± 0.8 and 4.4 ± 0.2 Å respectively for the sample pair utilizing the methyl label and 17.4 ± 0.9 and 4.3 ± 0.2 Å for those utilizing the H10a label. The latter result translates into a distance from the center of the water layer of 8.6 Å in excellent agreement with the value (9 ± 2 Å) derived from strip model analysis. The results of the fitting with Δ^8-THC added to the 'carbonyl plus glycerol' region are given in Table I.

Table I lists the results of a least squares fit to the five experimental structure factors for the deuterated and undeuterated cannabinoid samples with light water only between the bilayers. As with the free fit described above, the cannabinoid (whether deuterated or not) offered sufficient signal contrast to carry out a joint refinement. In itself this suggests that the Δ^8-THC molecule is distributed in a narrow band in the membrane with its long axis perpendicular to the normal to the bilayer surface. Note that either quasimolecular model places the cannabinoid in approximately the same region relative to the membrane center.

A method that explicitly utilizes labelling involves direct use of the differences between structure factors $\Delta F_i(n)$ obtained with and without the ith label present

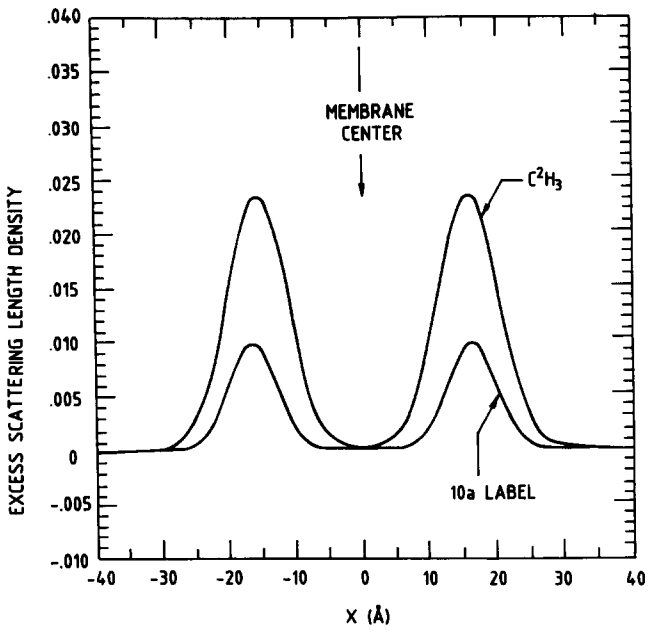

Fig. 8. Fits to the difference structure factors $\Delta F_{ij} = F_i - F_j$, where F_i and F_j are the structure factors of the labelled and unlabelled samples, respectively. It has been assumed that the labelled component has a Gaussian distribution. See text and Ref. 13 for details. Note the wide ranges over which the labels may be found in this and the previous figure. Because the lipid and cannabinoid are more fluid at high temperatures and humidities these distributions are physically relevant (See Ref. 12 and references therein). As a consequence it may be assumed that the methyl group at the tail end of the cannabinoid can penetrate well into the CH_2 region, as well as make occasional forays into the aqueous layer. Part of this wide distribution could arise from different conformers of Δ^8-THC.

[13]. With this method a fit is made to the calculated difference structure factors ΔF_{ci} given by:

$$\Delta F_{ci}(n) = 2m_i b_i \exp(-(\pi A_i n/d)^2) \cos(2\pi X_i n/d) \quad (5)$$

where m_i is the number of deuterium labels per lipid and b_i is the excess scattering length resulting from the deuteron substitution, the other symbols having the same meanings defined earlier. In this case no explicit account of the quasimolecular stucture is taken. Employing a scale factor K_i, the least-squares fit is actually made of all $K_i \cdot \Delta F_{ci}(n)$ to all the $\Delta F_i(n)$. The scale factor is given by:

$$K_i = \frac{\sum_{n=1}^{N} |\Delta F_i(n)|}{\sum_{n=1}^{N} |\Delta F_{ci}(n)|} \quad (6)$$

Fig. 8 illustrates the fitted curves that were obtained by this method. The centers of the Gaussian distributions shown here agree again with our other calculations. For the H10a samples A_i and X_i are 5.1 ± 0.8 and 16.4 ± 0.3 Å, respectively, and the corresponding values for the methyl label are 6.4 ± 1.6 and 15.8 ± 0.8. The overlap of the CH_3 distributions into the hydrocarbon and water regions suggests that the membrane is very fluid at 53°C and this probably permits various conformers of the tails of the THC molecule to be expressed.

Various values have been obtained for the locations of the labels. For example, for the 10a label (53°C, Rh = 96%) values ranging from 8.6 to 10.4 Å inwards from the center of the water layer have been given. This variation is due to differences in calculational technique, the older strip-function method utilizing difference Fourier spectroscopy being the least reliable. This technique yielded a value of 9 ± 2 Å. With 5 reflection orders explicit Gaussian analysis (the last one reported in this section) yields a value of 9.6 ± 0.3 Å. The latter technique is thought to yield the best value for the label location. The method [13] is a modification of the one originally outlined by Buldt et al. [17] who showed that with only 4 orders of reflection (hence data with 15 to 20 Å resolution) it is possible with this technique to obtain the location of a deuterium label with an 'accuracy better than 1.5 Å'. These numbers refer to mean values [12].

Discussion and Conclusions

The intralamellar conformation of Δ^8-THC in DPPC has been determined by neutron diffraction from deuterium-labelled samples. In most of these measurements the Bragg repeat indicated that the DPPC was in a phase such that shortening of the hydrocarbon region occurred in a manner typical of the liquid crystal phase. The results of all methods of analysis are in agreement regarding the location and orientation of the intercalated cannabinoid.

Agreement with previous neutron scattering results [9] is less satisfactory and there are several reasons for this. Firstly, the previous measurements were carried out on dispersions in excess water at 30°C, well below the liquid crystal phase region in DPPC. In the previous work the location of the acyl tail of THC was assumed to be parallel to the hydrocarbon core of the lipid and a fit to only two peak intensities was then carried out via a strip-function model which assumed values for many geometrical parameters. As a result the location of the tricyclic ring was not well determined. These early results did indicate identical scattering from inclusions of either Δ^8 or Δ^9-THC but again this is a low-resolution result. One interesting feature from this experiment still stands firmly; in excess water the aqueous layer increases in thickness when either THC is present.

The present measurements suggest a difference from the cannabinoid side-chain conformation determined by previous X-ray measurements on a related system

[3]. However the latter measurements on DMPC involved shorter hydrocarbon chains which are more mobile than those of DPPC at any given temperature. The headgroups are chemically identical. More recent X-ray measurements [14] with bovine synaptosomal membranes indicated that the marker atom (iodine) in (−)-5′-iodo-Δ^8-tetrahydrocannabinol is located at the same depth as the tricyclic component in agreement with our present results.

An examination of the *d*-spacing variation as a function of temperature in the DMPC experiment [3] indicates that at 35°C the samples were not entirely in the liquid crystal phase because a minimum value for the *d*-spacing had not been attained. Because of mesomorphic effects [3] this may account in part for the different tail conformation seen in DMPC. From a biological point of view attainment of the liquid crystal phase is desirable because artificial membranes only approximate the biophysical properties of natural membranes when the former are in this phase.

In spite of the greater motion associated with DMPC there seems to be little difference in the ease with which bilayers of this lipid and those of DPPC, accommodate THC molecules. This is borne out by comparing wide angle X-ray measurements on DMPC [3] and wide angle neutron diffraction from DPPC [9]. In the case of DMPC there was no significant expansion in the intralamellar lattice with a 15% abundance of Δ^8-THC molecules, and the same was found with a 19% abundance of Δ^8-THC or Δ^9-THC in DPPC.

In a lateral diffusion model first advanced by Rhodes et al. [15] and subsequently elaborated by Makriyannis et al. [16], lipid-soluble molecules like Δ^8-THC enter the lipid cell wall from outside and then diffuse laterally to a region in a membrane-bound receptor near the hydrophobic-hydrophilic boundary of the membrane. Recalling that the OH-group on the various cannabinoids is necessary for biological activity, lateral movement of the cannabinoid could involve short forays to the phosphate moity in the headgroup where it is tethered by means of its hydroxyl for short periods between diffusional jumps. This picture is in accord with the substantial widths of the Δ^8-THC distributions found by Gaussian function analysis. Elaboration of this theme on the basis of the present results requires caution, however, since there is evidence that headgroup type may influence the drug location in phospholipids. For instance, in synaptic plasma membranes [14] Δ^8-THC is located some 2–5 Å deeper into the hydrocarbon region than in a DMPC bilayer system.

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