Conformational Study of Lipophilic Ligands in Phospholipid Model Membrane Systems by Solution NMR

Jianxin Guo,† Spiro Pavlopoulos,† Xiaoyu Tian,† Dai Lu,† Spyros P. Nikas,† De-Ping Yang,‡ and Alexandros Makriyannis*,†,§
Department of Pharmaceutical Sciences, Center for Drug Discovery, The University of Connecticut, Storrs, Connecticut 06269; Department of Physics, College of the Holy Cross, Worcester, Massachusetts 01610; and The Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received September 5, 2002

Phospholipid bicelles were employed as a membrane bilayer model in the conformational studies of two lipophilic cannabinoids, Δ⁸-THC and its O-methyl ether analogue, Me-Δ⁸-THC using conventional high-resolution NMR. A preparation of 8% (w/v) phospholipid concentration and a high DMPC/DHPC ratio (q = 2.0) was found to be optimal for not only effectively incorporating our ligands, but also providing a more bilayerlike environment suitable for conformational studies. While the conformational differences between the two cannabinoids could not be observed in chloroform and were barely detectable in SDS micelle solution, there is an increasing preference for the pentyl tail of Δ⁸-THC to bend toward the tricyclic ring system with increasing proportions of DMPC in the bicelle preparation. Our results highlight the advantages of exploring the conformational properties of cannabinoids using bicelle preparations as a medium that more closely resembles biological membrane bilayers and eliminates the need for isotopic labeling. This approach should also be of more general value for studying the interactions of other cannabinoids and biologically active, hydrophobic or amphipathic, small molecules with membranes.

Introduction

The ability of many drugs and some endogenous ligands to reach their sites of action is believed to be associated with molecular properties governing cell membrane penetration and lateral diffusion within the membrane leaflet. Thus the conformation, orientation, and location of the ligand in the membrane is critical in understanding its ability to reach its site of action in the proper orientation and conformation so that it can interact productively with that site.¹,² Traditionally, solid-state NMR spectroscopy and small-angle X-ray diffraction were utilized to investigate the ligand interactions with model membrane bilayers;³,⁴ however, these approaches require extensive efforts in sample preparation that involve specific isotopic labeling. Alternatively, a sodium dodecyl sulfate (SDS) micellar medium⁵ has been used in high-resolution NMR conformational studies to mimic the membrane environment, even though the highly curved nature of SDS micelles does not accurately represent the cellular membrane bilayers. As phospholipid bicelles have been reported to more closely resemble the bilayer structure of cellular membranes,⁶,⁷ we became interested in exploring their suitability as model membrane systems for ligand conformational studies using high-resolution NMR.

The ligands used in this study belong to the class of classical cannabinoids, which are the tricyclic terpene analogues of (−)-Δ⁹-tetrahydrocannabinol (Δ⁹-THC), the key psychoactive constituent in marijuana. Many of the pharmacological effects of cannabinoids, including psychotropic activity, analgesia, and reduced intraocular pressure, have been attributed to their interactions with two cannabinoid receptors, CB1 and CB2.⁸,⁹ As cannabinoids are generally very lipophilic, it has been postulated that they reach their receptor active sites by lateral diffusion within the cell membrane where they preferentially accumulate.¹⁰ We have shown that using solid-state deuterium NMR and small-angle X-ray diffraction, two representative classical cannabinoids, Δ⁸-THC and Me-Δ⁸-THC (Figure 1), interact with DPPC multilamellar membrane bilayers quite differently even though they are closely related structurally.¹¹⁻¹⁸ These two cannabinoids are essentially water insoluble and expected to be partitioned within the lipid bilayer. We were interested in elucidating the conformations of these two ligands in a membrane bilayer environment and establishing whether the differences in the membrane interactions would be reflected in their conformational properties.

Phospholipid bicelles are typically composed of DMPC lipids with DHPC detergent, where the DMPC-rich domain has been demonstrated to be similar to those
in multilamellar membrane bilayers. Bicelle conditions can be tuned to be either isotropic or anisotropic over wide ranges of lipid:detergent ratio (roughly 5:1-1:2) and water content (roughly 60-97%). Early conformational studies using this bicelle system were able to measure anisotropic parameters of molecules that were either associated with the surface, or partially imbedded into the bicelle; however, these approaches generally involved isotopic labeling. By decreasing the DMPC to DHPC ratio to 0.5, the system can be tuned to isotropic conditions with smaller sized bicelles that permit high-resolution NMR structural determination of membrane-associated peptides.

It is highly desirable to obtain the conformational properties of small molecules in a bilayer environment without specific isotopic labeling. Unlike SDS micelles, which have been widely employed even though they are not ideal bilayers, the bicelle system has not been fully explored as a membrane model in ligand conformational studies. In this paper, we seek to obtain an isotropic bicelle preparation with the largest possible DMPC lipid content, and increased DMPC to DHPC ratio will result in increased proportions of bilayer-like domain. The lipid-to-detergent ratios were selected according to the temperature-composition phase diagrams. To maintain isotropic conditions at higher values of q, it is necessary to reduce the overall lipid concentration which allows faster tumbling of the bicelles. In addition, our preparations were free of KCl, which allowed us to use high values of q while maintaining isotropic conditions.

At q = 2.7, we estimated that the system conditions border the isotropic bicelle domain based on the temperature-phase diagrams. At a lipid concentration of 25% (w/v), lipid proton resonances are very broad, indicating a relatively high degree of orientation and a very slow tumbling rate of the bicelles. Similar broadening of the ligand proton resonances is consistent with the association of the ligands with the bicelles so that they assume a high degree of ordering. Reducing the lipid concentration does not adequately result in isotropic conditions.

Figure 1. Chemical structures of Δ^8-THC and Me-Δ^8-THC and their proposed orientations in model membrane bilayers. The dashed lines represent the direction of the lipid acyl chains.

Figure 2. ^1H spectra of Δ^8-THC at 38°C in bicelle solutions that have DMPC:DHPC ratios as indicated of q = 2.7 or q = 2.0 and with total lipid concentrations of 25% (w/v), 8% (w/v), and 3% (w/v).

Figure 3. ^1H spectra of Me-Δ^8-THC at 38°C in bicelle solutions that have DMPC:DHPC ratios as indicated of q = 2.7 or q = 2.0 and with total lipid concentrations of 25% (w/v), 8% (w/v), and 3% (w/v).

Results

Optimizing the Isotropic Bicelle Conditions. A series of isotropic bicelle solutions were prepared with the highest possible DMPC content for incorporating our ligands, as increased DMPC to DHPC ratio (q) will result in increased proportions of bilayer-like domain. The ^1H spectra of the ligands Δ^8-THC or Me-Δ^8-THC incorporated into different bicelle preparations are shown in Figures 2 and 3. The lipid-to-detergent ratios were selected according to the temperature-composition phase diagrams of bicelles with 20% (w/v) total lipid concentration. To maintain isotropic conditions at higher values of q, it is necessary to reduce the overall lipid concentration which allows faster tumbling of the bicelles. In addition, our preparations were free of KCl, which allowed us to use high values of q while maintaining isotropic conditions.

At q = 2.7, we estimated that the system conditions border the isotropic bicelle domain based on the temperature-phase diagrams. At a lipid concentration of 25% (w/v), lipid proton resonances are very broad, indicating a relatively high degree of orientation and a very slow tumbling rate of the bicelles. Similar broadening of the ligand proton resonances is consistent with the association of the ligands with the bicelles so that they assume a high degree of ordering. Reducing the lipid concentration does not adequately result in isotropic conditions. At the lower lipid concentrations of 8% and 3%, proton resonances are similarly broad, suggesting that the bicelles still retain a certain degree of orientation relative to the magnetic field. Two representative ^31P spectra that are of the 25% and 8% (w/v) preparations, with incorporation of Me-Δ^8-THC, are shown in Figure 4. The upfield phosphorus resonance indicates that at this lipid-to-detergent ratio, bicelles are partially oriented in the external magnetic field and are not in a fully isotropic state.
2.0 was chosen as an optimized bicelle preparation. Therefore, a total lipid concentration of 8% (w/v) and a precipitation was typically observed after several hours. However, the precipitated samples were very sensitive to temperature and lipid concentration and a slightly reduced value of q. The 3% (w/v) sample, which was observed from the 25% and 8% preparations over the course of several days. The 3% (w/v) sample was observed from the 25% and 8% preparations over the course of several days. The 3% (w/v) sample, which was observed from the 25% and 8% preparations over the course of several days. The 3% (w/v) sample, which was observed from the 25% and 8% preparations over the course of several days. The 3% (w/v) sample, which was observed from the 25% and 8% preparations over the course of several days. The solution with q = 2.0 are more within the isotropic bicelle condition as shown by the observed single resonance in the $^{31}$P spectrum (Figure 4). The phosphorus spectrum of the 25% solution, however, is quite broad, possibly as a result of long correlation times. This preparation does not yield sharp ligand proton resonances as shown in Figures 2 and 3. When the lipid concentration is reduced to 8% and 3%, there is a dramatic resolution increase in both $^1$H and $^{31}$P spectra. Well-resolved lines reflect the isotropic nature of these media, which results from the decrease of the total lipid concentration and a slightly reduced value of q. The isotropic state under these conditions may be due to a decrease in diameter of the bicelle disks combined with an increase in the spacing between them that results in faster motional averaging.

A DMPC:DHPC ratio of 2.0 is near the upper limit that may practically be employed as an isotropic model membrane preparation. A certain degree of broadening in the $^1$H spectrum still exists, which prevents accurate measurements of J-couplings and residual dipolar couplings between the multiple proton pairs. However, the resolution is adequate for conventional 2D-NMR experiments such as DQF–COSY and NOESY from which ligand conformational properties can be ascertained. In addition, no precipitation of either the ligand or lipid was observed from the 25% and 8% preparations over the course of several days. The 3% (w/v) sample, however, was very sensitive to temperature and lipid precipitation was typically observed after several hours. Therefore, a total lipid concentration of 8% (w/v) and q = 2.0 was chosen as an optimized bicelle preparation for the following ligand conformational study using two-dimensional NMR experiments.

**Assignments of the Ligand Resonances.** The DQF–COSY spectra, in conjunction with the NOESY spectra, were used for the assignment of Me-$\Delta^8$-THC resonances and partial assignment of $\Delta^8$-THC resonances in 8% (w/v), q = 2.0 bicelle preparations that are shown in Figure 5 (the lipid resonances were referenced according to Lee and Griffin 34). In general, the DQF–COSY spectra of $\Delta^8$-THC were not as well resolved as those for Me-$\Delta^8$-THC due to the slightly larger line widths of $\Delta^8$-THC resonances (Figure 6). Cross-peaks are observed that allow assignment of the pentyl-tail protons unambiguously; however, there are very few cross-peaks that reveal the positions of ring protons due to the increased line widths. There is a larger number of cross-peaks observed for Me-$\Delta^8$-THC, which allows a complete assignment of all the resonances.

Assignment of the aromatic protons of each ligand was confirmed by observed NOEs (discussed below) and carbon–proton heteronuclear multiple quantum coherence (HMQC) experiments, of which a representative spectrum is shown in Figure 7. The carbon chemical shift of C2 is upfield of C4 in CDCl3 solution (data not shown) due to the proximity to the phenolic hydroxyl or the arylmethoxy group. For Me-$\Delta^8$-THC there are no significant chemical shift changes in going from a chloroform solution to the bicelle preparation. For $\Delta^8$-THC, however, the aromatic H2 proton is shifted downfield of the H4 resonance, while there is a corresponding downfield shift in the C2 carbon.

Significant $^{13}$C chemical shift changes of $\Delta^8$-THC are also observed for resonances from the pentyl tail region of the HMQC spectra. The C1' resonance shifts upfield by ~1.7 ppm and the C2' resonance also shifts upfield by ~0.6 ppm. These chemical shift changes can be explained by a gauche C3–C1'–C2'–C3' dihedral angle.35 In the case of Me-$\Delta^8$-THC, however, the $^{13}$C chemical shifts are not significantly affected when compared to spectra acquired in chloroform, where the pentyl tail generally assumes a trans conformation.

Assignment of the ring proton resonances for Me-$\Delta^8$-THC was further confirmed based on observed NOEs. For example, H10x can be distinguished from H10a due to a weak NOE between the 9CH3 protons (1.55 ppm) and H10x (3.14 ppm) and the expected close proximity of 9CH3 to H10x. All NOEs are unambiguous except for

![Figure 4](image-url) **Figure 4.** $^{31}$P spectra of Me-$\Delta^8$-THC in bicelle preparations with 25% and 8% (w/v) lipids and q = 2.7 and q = 2.0.

![Figure 5](image-url) **Figure 5.** A comparison of the $^1$H NMR spectra of $\Delta^8$-THC and Me-$\Delta^8$-THC in an 8% (w/v) and q = 2.0 bicelle preparation (left panel) and in CDCl3, (right panel). Resonances from the phospholipid and water are labeled with asterisk.
two cross-peaks to the overlapped ligand OCH₃ and lipid choline 2-CH₂ resonances to 3.73 ppm. One of these is an extremely weak NOE to the overlapped H₃′ and H₄′ resonances of the ligand at 1.21 ppm. The second of these NOEs is to the aromatic resonance at 6.00 ppm. Taking into account the ortho position of the H₂ relative to the arylmethoxy group and lack of NOEs to other protons of the lipid headgroup, this is most likely an intramolecular cross-peak between H₂ (6.00 ppm) and the OCH₃ protons (3.73 ppm) of the ligand. No NOEs are observed between the overlapped ligand OCH₃ and lipid headgroup 2-CH₂ resonance at 3.73 ppm with the other aromatic proton (H₄) at 6.13 ppm, which is congruent with the assignments of the ligand H₂ and H₄ aromatic proton resonances and the idea that the aromatic ring is not just associated with the surface but is within the bilayer.

To differentiate the overlapped resonances between the Me-Δ⁸-THC methoxy protons and lipid choline 2-CH₂ protons, an HMQC experiment was performed on Me-Δ⁸-THC with a 13C-enriched methoxy group (Figure 8). This resolved the overlapping by splitting the methoxy proton resonances of 143 Hz. No intermolecular NOEs were observed from any of the lipid headgroup protons to the ligand, which supports that the ligand is partitioned within the bilayer rather than associated with the surface.

**Figure 6.** Selected regions from DQF-COSY and 100 ms NOESY spectra of the ligands in a preparation with a total deuterated lipid concentration of 8% (w/v) and a DMPC/DHPC ratio of 2.0:1. Panel A is an expansion of the DQF-COSY spectrum of Δ⁸-THC focusing on the upfield aliphatic region. Panel B is an expansion of the NOESY spectrum, correlated to panel A, showing NOEs between the aromatic protons and protons of the pentyl-tail. Likewise, Panel C and D are correlated expansions of the DQF-COSY and NOESY spectra of Me-Δ⁸-THC.

**Figure 7.** Expansions of the HMQC spectra of Δ⁸-THC (top panels) and Me-Δ⁸-THC (bottom panels) highlighting the assignment of the aromatic resonances (left panels) as well as the resonances from the pentyl tail (right panels).

NOE and Dihedral Angle Restraints. Long-range NOEs observed in the 100 ms NOESY spectra were classified as medium, weak, or very weak using the H₈-H₇ NOE as a standard for a strong NOE, which provides internuclear distance restraints in the subsequent molecular modeling. Three long-range NOEs were observed in the case of Δ⁸-THC, that could be assigned unambiguously (Table 1) and for Me-Δ⁸-THC, seven NOEs were identified between proton pairs not adjacent to each other (Table 2).

Of greatest interest are the NOEs between the aromatic ring protons and protons on the pentyl-tail (Figure 6B and 6D). For Me-Δ⁸-THC, the only NOEs observed are those between the H₁′ protons and the H₂/H₄ protons of the aromatic ring. For Δ⁸-THC, however, additional NOEs are observed between the aromatic H₄...
H10a was observed from Me-8-THC while there was another very weak NOE from the H2 proton and H3 proton to the H3 proton. In Table 2.

**Table 1. Intramolecular NOEs Observed from Δ8-THC in q = 2.0 Bicelle**

<table>
<thead>
<tr>
<th>proton A</th>
<th>proton B</th>
<th>intensity</th>
<th>distance Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3', 4'</td>
<td>medium</td>
<td>2.5-4.0</td>
</tr>
<tr>
<td>4</td>
<td>2'</td>
<td>weak</td>
<td>3.5-5.0</td>
</tr>
<tr>
<td>2</td>
<td>3', 4'</td>
<td>very weak</td>
<td>4.0-6.5</td>
</tr>
<tr>
<td>8</td>
<td>7α</td>
<td>strong</td>
<td>1.5-2.5</td>
</tr>
</tbody>
</table>

**Table 2. Intramolecular NOEs Observed from Me-Δ8-THC in q = 2.0 Bicelle**

<table>
<thead>
<tr>
<th>proton A</th>
<th>proton B</th>
<th>intensity</th>
<th>distance Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1'</td>
<td>medium</td>
<td>2.5-4.0</td>
</tr>
<tr>
<td>2</td>
<td>-OCH3</td>
<td>medium</td>
<td>2.5-4.0</td>
</tr>
<tr>
<td>4</td>
<td>1'</td>
<td>medium</td>
<td>2.5-4.0</td>
</tr>
<tr>
<td>8</td>
<td>7α</td>
<td>strong</td>
<td>1.5-2.5</td>
</tr>
<tr>
<td>8</td>
<td>9Me</td>
<td>medium</td>
<td>2.5-4.0</td>
</tr>
<tr>
<td>10α</td>
<td>9Me</td>
<td>weak</td>
<td>3.5-5.0</td>
</tr>
<tr>
<td>10a</td>
<td>6α</td>
<td>medium</td>
<td>2.5-4.0</td>
</tr>
<tr>
<td>OCH3</td>
<td>3', 4'</td>
<td>very weak</td>
<td>4.0-6.5</td>
</tr>
</tbody>
</table>

For the tricyclic ring proton resonances, an NOE between the axial C6 methyl protons (0.89 ppm) and H10a was observed from Me-Δ8-THC while there was no NOE between the equatorial C6 methyl protons (1.18 ppm) and H10a. This suggests that the tricyclic ring system is quite rigid for Me-Δ8-THC with the axial C6 methyl group is on the same side of the molecule (α-side) as H10a. However, there were no well-resolved NOEs for Δ8-THC due to the increased line widths.

The methoxy group orientation with respect to the aromatic ring of Me-Δ8-THC was elucidated from its 13C chemical shift (55.0 ppm) in the HMQC experiment. It has been demonstrated that the 13C chemical shift of the methoxy group can be utilized to predict whether the O-CH3 bond is coplanar with the phenyl ring. The 13C chemical shift of the arylmethoxy group would be very similar to that of unsubstituted anisole (55.1 ppm) for a coplanar conformation whereas a chemical shift of 5-7 ppm downfield from that of anisole is expected for an out-of-plane conformation. The OCH3 chemical shift of Me-Δ8-THC in our bicelle preparation provided clear evidence that the O-CH3 bond is coplanar with the phenyl ring. The dihedral angle C2-C1-O-CH3 of Me-Δ8-THC was then set to between -10.0° and +10.0° and used as a restraint in the subsequent molecular modeling studies.

**Ligand Conformations.** Two families of Δ8-THC conformations were found from the NMR constrained molecular modeling calculations. In each of these families, the pentyl tail is generally perpendicular to the long axis of the tricyclic ring system (Figure 9A). The difference between these two families arises from the orientation of the pentyl-tail relative to the plane of the tricyclic ring system. In one set of the conformers, the ligand pentyl-tail is oriented toward the α-face of the tricyclic ring at a maximum of ~30° out of the plane. The second conformer is oriented toward the β-face of the ring, also to a maximum of ~30° out of the plane. In the case of Me-Δ8-THC (Figure 9B), the most favorable conformations are those where the pentyl-tail extends away from the tricyclic ring system. As for the O-CH3 conformation, the dihedral angle C2-C1-O-CH3 is found to be within ~5.7° and 3.8° out of a total of 100 conformers recorded from the simulation, illustrating that the energetically favorable conformations place the O-CH3 bond coplanar with the aromatic ring.

Comparison of Ligands in Chloroform, Micelles, and Bicelles. NOESY spectra were also acquired from ligands dissolved in chloroform, SDS micelles, and a bicelle preparation with 8-THC in a q = 2.0, 8% w/v bicelle preparation.
In the q = 0.5 bicelle solution, the H4′-H2 and H4-H3′/H4′ NOEs are similar in intensity to the H4-H1′, H2-H1′ NOEs, while in the q = 2.0 solutions, the former set of NOEs are significantly stronger than the latter. The above observation shows that with increasing proportions of DMPC in the bicelle, there is an increasing preference for ω-THC where the pentyl tail is oriented toward the H4 side of the tricyclic ring system.

For Me-ω-THC, however, the only NOEs observed in membrane mimetic media are those between the H1′ protons and the H2/H4 protons of the aromatic ring, which are similar to those observed in chloroform (data not shown) except that the NOEs are positive from the chloroform solution due to a much shorter correlation time. The NOEs observed in chloroform may reflect rapid transitions between all possible conformations and that NOEs with protons in the tail that are further away from the aromatic ring are not observed. Alternatively, the observation of a similar pattern of negative NOEs in membrane mimetic media may reflect a predominantly extended conformation of the tail with respect to the tricyclic ring system.

It is also worthy to note that in both the SDS micelle and the q = 0.5 bicelle preparations, ω-THC was observed to precipitate over 3–6 h whereas it was still well solubilized in the q = 2.0 bicelle solution for days. These results suggest that the choice of lipid–detergent composition can greatly influence the degree of ligand solubilization. Our earlier investigation has shown that DPPC multilamellar model membrane bilayers can effectively incorporate ω-THC at a ratio as high as

Figure 9. (A) Two representative structures from each of the two families of Δ9-THC conformers. The structures have been superimposed and the pentyl tail carbons of the conformer in which the tail is toward the ω-face of the tricyclic ring system are colored pink; while the pentyl tail carbons of the conformer in which the tail is toward the β-face are colored blue. (B) A typical structure from the derived set of conformations of Me-Δ9-THC.

Figure 10. Expansions of NOESY spectra showing NOEs between the aromatic ring and the pentyl-tail of Δ9-THC in (A) CDCl3, (B) SDS micelles, (C) q = 0.5 bicelle, and (D) q = 2.0 bicelle.
The fact that our ligands can be well incorporated into the q = 2.0 bicelle at 10% suggests that our bicelle preparation provides a more bilayerlike environment compared to the SDS micelle and the q = 0.5 bicelle systems.

**Discussion**

Comparing the isotropic solutions in Figures 2 and 3 (i.e., 8% w/v and 3% w/v at q = 2.0), it is evident that there are significant differences between the interactions of Δ⁸-THC and Me-Δ⁸-THC with the bicelles. The ¹H signals observed for Me-Δ⁸-THC at 8% and 3% w/v are much narrower than those observed for Δ⁸-THC, which can be easily seen from the aromatic resonances near 6.0 ppm highlighted in Figure 5. Given that there is no corresponding change in the line widths of the lipid resonances, we cannot attribute the decrease in ligand line widths to differences in the overall dynamic properties of the bicelle disks; however, the explanation may lie in the orientations adopted by these molecules in membrane bilayers. The orientation of Me-Δ⁸-THC where the long axis of the molecule is generally parallel to the DMPC acyl chains in bilayers (Figure 1) may result in a greater degree of motional averaging within the bilayer relative to Δ⁸-THC. The “awkward” orientation of Δ⁸-THC where the long axis of the molecule is perpendicular to DMPC acyl chains is not as easily accommodated among the acyl chains and so is more restricted.

The downfield shift of the aromatic H2 proton of Δ⁸-THC in the q = 2.0 bicelles compared to the chloroform solution may be attributed to a polar environment. It has been reported that protons situated ortho to hydroxyl groups in phenolic systems experience stronger deshielding effects in polar solvents such as pyridine. The downfield shift of the ortho H2 proton reflects that the aromatic hydroxyl group resides in the lipid/water interface region. The interaction between the Δ⁸-THC phenolic OH and lipid polar headgroup results in an anchoring effect that may further restrict the molecule. Such a strong anchoring effect may orient the psychoactive Δ⁸-THC with the long axis of its tricyclic ring system almost parallel to the bilayer surface, which is consistent with our previous orientational study using DPPC multilamellar model membrane.

The conformational differences observed between Δ⁸-THC and Me-Δ⁸-THC are clearly a reflection of the conditions imposed by a bilayerlike environment on each ligand. The NOEs observed between the aromatic protons of Δ⁸-THC and protons in the pentyl-tail confirms the postulation in our previous publications that the pentyl-tail of Δ⁸-THC tends to align generally parallel to the lipid acyl chains and is in this way accommodated among the lipid acyl chains. Therefore, the pentyl-tail tends to bend toward the tricyclic ring system and adopt a gauche C3′−C1′−C2′−C3′ dihedral angle, which is demonstrated by the upfield ¹³C chemical shift change of the pentyl tail carbons. For Me-Δ⁸-THC, however, the observed pattern of NOEs favors a conformation with the pentyl-tail extended away toward the center of the bilayer, which is also consistent with our earlier orientational studies within DPPC model membrane bilayers.

The conformational properties of lipophilic ligands may be greatly influenced by the choice of its solubilizing membrane mimetic media. As can be seen from the observations of Δ⁸-THC, NOEs indicative of the conformation favored in bilayers (H4−H2, H4−H3′/4′) are barely observable in SDS micelles, suggesting that the tail has a greater degree of conformational freedom in the micellar environment. In going from the q = 0.5 to the q = 2.0 bicelle preparation, NOEs indicative of a bent conformation become more intense, and this may be a reflection of an environment that more closely resembles a bilayer. A fluorescent study has shown that at conditions of q < 0.5, the size of the planar bilayer domain is directly proportional to the ratio of DMPC/DHPC. Despite the fact that the morphology of these fast-tumbling isotropic bicelle preparations may in fact be quite complex, it is very unlikely that the observed NOEs arise from ligands partitioned into the DHPC domain, as in this case, a pattern more similar to that observed from SDS micelles would be expected. This notion is consistent with a recent study that the structural difference between bicelles and spherical micelles influence the conformation of peptides that are solubilized by them.

Our results obtained from higher proportions of DMPC (q = 2.0) bicelle preparation are congruent with the previous studies using DPPC multilamellar membrane bilayers, which suggests that the q = 2.0 bicelle provides an ideal membrane model for ligand conformational analysis. These bicelle disks can be tumbling isotropically in such a dilute solution (8% w/v) as a result of faster motional averaging combined with a decrease in bicelle size. Nevertheless, at the experimental temperature (38 °C), the bilayer domain of each bicelle disk is still in the liquid crystalline (Lα) phase where the DMPC acyl chains undergo rapid trans-gauche isomerization. At such isotropic conditions, conventional high-resolution NMR methods can be employed to ascertain ligand conformation within a membrane bilayer environment.

**Conclusions**

The conformations of Δ⁸-THC and Me-Δ⁸-THC were determined, and while there are no observable conformational differences in CDCl₃ solution, they are found to differ within membrane mimetic media due to the more amphipathic-like properties imparted by the phenolic hydroxy group of Δ⁸-THC compared to Me-Δ⁸-THC. The solubilizing medium influences the variations in the conformation of the pentyl tail. While differences between the two ligands are barely detectable in SDS micelles, there is an increasing preference for Δ⁸-THC where the pentyl tail bends toward the tricyclic ring system with increasing proportions of DMPC in the bicelle preparation. This is most likely a reflection of the more bilayerlike morphology of the lipid bicelles compared to SDS micelles. The congruency between observations from the bicelle preparations and multilamellar DPPC model membrane systems indicates that the q = 2.0 preparations provide a bilayer environment capable of incorporating our lipophilic cannabinoids in a manner similar to DPPC model membrane bilayers.

Without the need for isotopic labeling of the ligands, our bicelle preparations provide an avenue for obtaining ligand conformation in a lipid bilayer environment using conventional solution NMR spectrometers. Thus, this
technique is complementary to solid-state NMR methods such as REDOR\(^3\) that requires specific isotopic labeling. To further define the interactions between our cannabinoid ligands and the lipid membrane, work is currently in progress to measure the anisotropic parameters, which allows us to determine the orientation of the molecules within the bilayer. This can be accomplished by using specifically deuterated ligands and by modifying the bicelle preparations to yield oriented bicelles.

**Experimental Section**

The lipid molecules for the bicelle preparations were 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC), and the acyl-chain perdeuterated D\(\alpha\)-DMPC and D\(\beta\)-DHPC (Avanti Polar Lipids, Inc. Alabaster, AL). Deuterated sodium dodecyl sulfate (D\(\alpha\)-S DS) was purchased from Cambridge Isotope Laboratory (Andover, MA). (\(-\)\(\Delta\)\(\Delta\)-tetrahydrocannabinol (\(\Delta\)\(\Delta\)-THC), (\(-\)O-methyl-\(\Delta\)\(\Delta\)-tetrahydrocannabinol (Me-\(\Delta\)\(\Delta\)-THC), and \(\Delta\)\(\Delta\)-THC were synthesized in our laboratory. The ligand, \(\Delta\)\(\Delta\)-THC or Me-\(\Delta\)\(\Delta\)-THC, was first dissolved along with DMPC and DHPC in chloroform that was then evaporated using an \(N\)_\(2\) stream. The sample was vacuum-dried overnight followed by adding an appropriate amount of D\(\alpha\)O (Aldrich Chemical Co., Milwaukee, WI). To improve the stability of the bicelle and prevent phase separation in dilute solutions, a 0.5 mM deuterated SDS/D\(\alpha\)O solution, rather than pure D\(\alpha\)O, was added, which yielded an SDS:DMPC ratio of 1:60 for the 3\% (w/v) solution and 1:160 for the 8\% (w/v) bicelle system. The preparation then underwent a combination of mechanical blending, heating, and cooling until a clear and homogeneous system was obtained. Bicelle solutions were prepared in which the molar ratio of DMPC to DHPC was either 2:7:1, 2:0:1, or 0.5:1 and total lipid concentrations were 25\% (w/v), 8\% (w/v), or 3\% (w/v), where the ligand molar concentration relative to the long acyl-chain DMPC in each solution was 10\%. SDS micelle solutions containing 10\% ligand were prepared by first dissolving an appropriate amount of ligand in chloroform, evaporating by an \(N\)_\(2\) stream, and leaving a film coating at the bottom of a vial. A 12\% (w/v) SDS solution was then added, and the mixture was sonicated using a Branson probe sonicator for 15 min.

All NMR experiments were carried out on a Bruker DMX-500 high-resolution spectrometer. One- and two-dimensional NMR spectra from all the bicelle preparations were acquired at 38 °C, while the samples were allowed to equilibrate at least 30 min in the magnet.\(^{39}\) 1\(H\) spectra were recorded using a phase-cycled Hahn-echo pulse sequence with gated proton decoupling,\(^{39}\) and the chemical shifts were externally referenced to 1 M H\(\text{H}_{\text{3}}\)PO\(_4\). The NMR spectra for ligands in CDCl\(_3\) and SDS micelle solutions were recorded at 25 °C.

NMR constrained molecular modeling of the conformation of \(\Delta\)\(\Delta\)-THC and Me-\(\Delta\)\(\Delta\)-THC was achieved using the Biosym Insight/Discover molecular modeling package on an Al Inigo workstation. The Biosym integrated CVFF force field was employed in the calculation with a dielectric constant \(\epsilon = 2\), which has been generally accepted for the lipid hydrocarbon region within a membrane bilayer.\(^{40}\) A data file containing the NMR constraints was created by including the NOE-derived internuclear distances as well as dihedral angles derived from chemical shift observations. Initial energy minimization by molecular mechanics was performed to relieve any overly strained bonds. The resulting structures underwent NMR-constrained molecular dynamics, performed by heating to 1000 K and recording 100 atomic coordinate trajectories every 200 fs. The 100 frames recorded during the dynamics run were retrieved and minimized with a two-step energy minimization, using the steepest descent method for the first 100 iterations and then conjugate gradient method until the maximum derivative was less than 0.001 kcal/mol.

**Acknowledgment.** This research was supported by Grants DA3801, DA7251 from the National Institute on Drug Abuse. Helpful discussions with Dr. T. Mavromoustakos and Mr. R. Chari are highly appreciated.

**References**


J M 020385R