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The orientation of (−)-Δ^9-tetrahydrocannabinol in DPPC bilayers as determined from solid-state ^{2}H-NMR

Alexandros Makriyannis [1,2], Alireza Banijamali [1], Harold C. Jarrell [3] and De-Ping Yang [1]

[1] *School of Pharmacy and Institute of Materials Science, University of Connecticut, Storrs, CT (U.S.A.),* [2] *Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA (U.S.A.) and* [3] *Division of Biological Sciences, National Research Council of Canada, Ottawa (Canada)*

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The orientation of the motional axis of (−)-Δ^9-tetrahydrocannabinol in dipalmitoylphosphatidylcholine model membrane was calculated from the ^{2}H quadrupolar splittings ($\Delta\nu_Q$) of individual deuterons strategically located on the cannabinoid tricyclic component. The molecule assumes an orientation in which its long axis is nearly perpendicular to the phospholipid chains and its most ordered axis is almost in the plane of the aromatic ring. This 'awkward' cannabinoid orientation in the membrane presumably occurs in order to allow the phenolic hydroxyl group to direct itself towards the polar bilayer interface.

Introduction

Many pharmacological actions of (−)-Δ^9-tetrahydrocannabinol (THC), the most active constituent of cannabis, can be related to its effects on cellular membranes. On the other hand, existing evidence indicates that these membrane effects, which include psychotropic properties, bronchodilation, increased heart rate intraocular pressure, and analgesia, at least in part, are due to interactions with those lipids that make up the environment of membrane-associated proteins [1]. There is also evidence that these cannabinoid : phospholipid interactions are governed by strict stereoelectronic requirements and that small changes in drug structure can result in dramatic changes in biological activity. The cannabinoid : lipid interactions presumably induce changes in the functions of a number of these proteins and thus produce a variety of physiological effects.

In our effort to understand the molecular features of this drug : membrane interaction we sought to determine the orientation of (−)-Δ^9-THC in DPPC bilayers using solid-state ^{2}H-NMR methods. Our choice of DPPC as the model membrane was motivated by the fact that phosphatidylcholines are a major membrane component whose phase properties have received a great deal of attention. Earlier studies in our laboratories [2] have showed that the interactions between amphipathic drug molecules and a number of phosphatidylcholines having different fatty acid chains are qualitatively similar. The present study necessitated the synthesis of (−)-Δ^9-THC specifically ^{2}H-labeled in the 2, 4, 8α, 8β, 10 and 10a positions of the tricyclic ring system (Fig. 1), which is reported elsewhere [3]. Solid-state ^{2}H-NMR spectra were generally obtained from membrane preparations containing (−)-Δ^9-THC with one or more ^{2}H labels for which the respective ^{2}H spectra could be unambiguously assigned.

During the past few years, solid-state ^{2}H-NMR has been applied as a method of choice for obtaining information on the conformational and dynamical properties of model and biological membranes [4]. The use of solid-state ^{2}H-NMR has also been extended successfully to study drug : membrane interactions [5,6]. This method finds application in systems undergoing anisotropic motions as is the case with phospholipid multilamellar bilayers (semi-solids). In such systems, the deuterium nuclei give rise to a 'powder spectrum' in which the doublet with the maximum intensity is the ^{2}H quadrupolar splitting ($\Delta\nu_Q$). The spectra due to the ^{2}H

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; THC, (−)-Δ^9-tetrahydrocannabinol.

Correspondence: A. Makriyannis, School of Pharmacy and Institute of Materials Science, University of Connecticut, Storrs, CT 06268, U.S.A.

labels on the drug molecule in the liquid crystalline phospholipid bilayer have quadrupolar splittings that depend on the angle between the individual C–D bond and the director axis. This axis is assumed to be parallel to the bilayer chains. The present communication describes how the average orientation of (−)-Δ^9-THC in the DPPC bilayer was calculated using the $\Delta\nu_Q$ values from ^{2}H spectra of the cannabinoid labeled in six different positions with deuterium atoms. How the orientation of (−)-Δ^9-THC in the membrane bilayer can be related to the molecular mechanism of cannabinoid activity is then briefly discussed.

Materials and Methods

Sample preparation and NMR experiments

2,4-d_2-(−)-Δ^9-THC, 10a-d-(−)-Δ^9-THC, 8α,8β-d_2-(−)-Δ^9-THC and 8α,8β,10-d_3-(−)-Δ^9-THC were synthesized in our laboratory [3]. L-α-Dipalmitoylphosphatidylcholine (99 + %) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Samples of model membranes for the solid-state NMR experiments were prepared by dissolving the phospholipid (150 mg) with and without (−)-Δ^9-THC in 2 ml of dichloromethane. The solvent was then evaporated by passing a stream of nitrogen over the solution at 50 °C and the residue was placed under vacuum (0.1 mmHg) for 12 h. The phospholipid or the drug : phospholipid (molar ratio 3 : 7) mixture was subsequently introduced in 7-mm glass tubes appropriately constricted, and ^{2}H-depleted water (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) was added to produce a 50% lipid : water (w/w) preparation. The samples were sealed under high vacuum (0.005 mmHg) and equilibrated before recording the spectra by heating them 10 C° above the phase transition temperature for 15 min.

^{2}H-NMR spectra were obtained on a home-built solid-state pulse spectrometer operating at 6.9 T (45.1 MHz) using the quadrupole echo pulse sequence, which consists of a pair of phase-shifted 90° pulses separated by a time (typically 30–40 μs) longer than the spectrometer recovery time (90°_x–τ–90°_y) [7].

Computational methods

Solid-state ^{2}H-NMR spectra are dominated by the nuclear electric quadrupole interaction, which is generally anisotropic, i.e., orientation dependent. When a rigid structure with deuterium labels is incorporated in the uniaxial liquid crystalline model membrane, each ^{2}H-label results in a different residual quadrupolar splitting which can be described by [8]

$$\Delta\nu_Q = \tfrac{1}{4}A_Q S_{C-D} \qquad (1)$$

where A_Q is the deuterium quadrupolar coupling constant (e^2qQ/h) and S_{C-D} is the order parameter of the C–D bond vector. S_{C-D} varies from one C–D bond to another because different C–D bonds have different orientations with respect to a director axis, about which the rigid structure undergoes a certain fluctuational motion. The effect of this motion is described by a traceless, symmetric second-rank tensor, S, which has a maximum of five independent components.

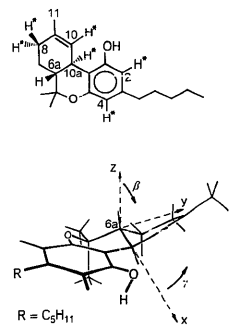

Fig. 1. Above: structure of (−)-Δ^9-THC. Asterisks indicate the ^{2}H-labeled positions. Below: the molecular-fixed axis system is shown, with the definition of the polar angles (β and γ) for the orientation of the motional axis.

When an axis system (x, y, z) is attached to the rigid molecule (Fig. 1), it follows from the transformation properties of second-rank tensors that each S_{C-D} is related to the components of S by [9,10]

$$S_{C-D} = \sum_{ij} S_{ij} \cos\alpha_i \cos\alpha_j \qquad i = x, y, z; \quad j = x, y, z \qquad (2)$$

where cos α_x, cos α_y and cos α_z are the direction cosines of the C–D bond vector in the molecular coordinate system. Since S_{C-D} values can be obtained from the spectra using (1) and the direction cosines can be calculated from the geometry of the molecule, the values of S_{ij} can be easily solved from a linear system of five equations like (2) and one equation: $S_{xx} + S_{yy} + S_{zz} = 0$. Therefore, five different S_{C-D} values should be sufficient for determining the order parameter tensor. Diagonalization of the order parameter matrix gives the principal components and the orientation of the principal axis (X, Y, Z) with respect to the molecular fixed axis system (x, y, z). The principal Z-axis is chosen such that it has the largest component S_{ZZ} and thus the most ordered axis. The orientation of the Z-axis is represented by a unit vector having direction cosines

(cos γ sin β, sin γ sin β, cos β) relative to the molecular frame. This most ordered axis is the director of the fluctuational motion of the rigid structure and coincides with the normal of the bilayer, therefore the orientation of the molecule in the bilayer is determined by positioning the molecule in the bilayer such that the principal Z-axis is parallel to the lipid chains.

Since the NMR spectra do not provide the signs of the quadrupolar splittings, each $\Delta\nu_Q$ can be either positive or negative, leading to a total of 32 different sign combinations for five $\Delta\nu_Q$ values. To determine the correct sign combination, we have strategically placed six deuterium labels on the rigid segment of the molecule and used the following self-consistent method [11,12]. First, we choose five splittings, assign the sign combinations one at a time and calculate the components S_{ij}. For each case, we use Eqn. (2) to predict the value of the sixth splitting. Among the 32 possible combinations, only those for which prediction agrees with the observed $\Delta\nu_Q$ within certain tolerance are considered further. In addition, the prediction also provides the sign of the sixth $\Delta\nu_Q$. Next, we choose another set of five from the six available splittings and use the same method to eliminate the wrong combinations. This procedure can be done repeatedly six times. The right sign combination can be recognized because it always predicts the correct values and signs of the sixth $\Delta\nu_Q$ in all six rounds of calculations. In addition, the order parameter matrices calculated from different choices of five $\Delta\nu_Q$ values are consistent with each other only when the correct sign combination is used.

The matrix, containing five independent order parameter elements in the molecular frame obtained from the above analysis, was diagonalized to give the principal components S_{XX}, S_{YY} and S_{ZZ}, the asymmetry parameter $\eta = (S_{YY} - S_{XX})/S_{ZZ}$ and the orientation of the principal axes X, Y, Z with respect to the molecular frame (x, y, z) is expressed in terms of the Euler angles β, γ and α. All calculations were performed on an IBM 370 computer.

Results and Discussion

Solid-state NMR spectra from ^{2}H-labeled (−)-Δ^9-THC in the DPPC bilayer in the liquid crystalline phase appeared to be axially symmetrical or nearly so, and had different quadrupolar splittings depending on the position of the ^{2}H label in the molecule. Fig. 2 shows two representative spectra from 2,4-d_2-(−)-Δ^9-THC and 10a-d-(−)-Δ^9-THC in DPPC at 42°C. In 2,4-d_2-(−)-Δ^9-THC, we were able to distinguish between the 2- and 4-deuterons on the basis of selective deuteration experiments [3]. The observed values of $\Delta\nu_Q$ are listed in Table I. The quadrupolar coupling constants were taken to be 170 kHz, 175 kHz and 180 kHz for sp^3 [13], sp^2 [14] and the aromatic sp^2 [15] C–D residues, respectively. The absolute values of S_{C-D} were calculated using Eqn. (1), and are also listed in Table I. We have carried out parallel experiments with DPPC bilayer preparations containing lower THC concentrations, and showed that the calculated orientation for THC in fact did not change.

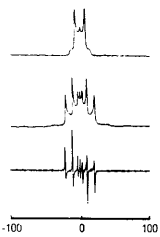

Fig. 2. Above and middle: representative solid-state ^{2}H-NMR spectra of 10a-d-(−)-Δ^9-THC and 2,4-d_2-(−)-Δ^9-THC (0.3 M) in hydrated DPPC bilayers at 42°C. The central doublet in the middle spectrum is due to the phenolic deuteron. Below: first derivative of the middle spectrum, from which ^{2}H quadrupolar splittings could be measured more accurately.

The molecular fixed co-ordinate system shown in Fig. 1 has its origin at C(6a) and the x-axis along the bond C(6a)–C(10a). The z-axis is in the plane of atoms C(6a), C(10a) and H(6a). The definitions of the two Euler angles β and γ for the orientation of the principal Z-axis are indicated in Fig. 1. The direction cosines for the six C–D bond vectors in this molecular frame were derived from the X-ray crystallographic data of (−)-Δ^9-tetrahydrocannabinolic acid as a model for the atom positions in (−)-Δ^9-THC [16]. The choice of (−)-Δ^9-tetrahydrocannabinolic acid was made because (−)-Δ^9-THC molecules cannot be crystallized. An alternative approach involves the dihedral angles obtained from high-resolution ^{1}H-NMR analysis of (−)-Δ^9-THC in solution [17]. Analysis of dihedral angles revealed that

TABLE I

Observed values of $\Delta\nu_Q$ and the corresponding S_{C-D} for (−)-Δ^9-THC in DPPC at 42°C

Position	Observed $\Delta\nu_Q$ (kHz)	A_Q (kHz)	S_{C-D}
2	20.8	180	0.1541
4	43.0	180	0.3185
8α	5.1	170	0.0400
8β	12.2	170	0.0957
10	16.7	175	0.1272
10a	14.9	170	0.1169

TABLE II

Results of calculations using six different choices (I to VI) of five S_{C-D} values

Each column lists the self-consistent solution only, which comes from one of the 32 trials with different sign combinations.

		I	II	III	IV	V	VI
S_{C-D}	2		+0.1541	+0.1541	+0.1541	+0.1541	+0.1541
used	4	+0.3185		+0.3185	+0.3185	+0.3185	+0.3185
	8α	−0.0400	−0.0400		−0.0400	−0.0400	−0.0400
	8β	+0.0957	+0.0957	+0.0957		+0.0957	+0.0957
	10	+0.1272	+0.1272	+0.1272	+0.1272		+0.1272
	10a	−0.1169	−0.1169	−0.1169	−0.1169	−0.1169	
Predicted		2	4	8α	8β	10	10a
S_{C-D}		+0.1560	+0.3333	−0.0990	+0.0907	+0.1251	−0.1121
Calculated [a]	S_{xx}	0.2835	0.2832	0.2851	0.2781	0.2820	0.2795
results	S_{xy}	0.2510	0.2670	0.2536	0.2529	0.2501	0.2557
	S_{xz}	−0.0915	−0.0905	−0.0928	−0.0862	−0.0927	−0.0884
	S_{yy}	−0.0129	−0.0129	−0.0174	−0.0129	−0.0109	−0.0175
	S_{yz}	−0.0852	−0.0868	−0.0231	−0.0834	−0.0838	−0.0864
	S_{zz}	−0.2706	−0.2702	−0.2677	−0.2652	−0.2711	−0.2620
	S_{XX}	−0.2969	−0.2970	−0.2849	−0.2906	−0.2967	−0.2896
	S_{YY}	−0.1506	−0.1638	−0.1562	−0.1529	−0.1494	−0.1574
	S_{ZZ}	0.4475	0.4608	0.4412	0.4435	0.4461	0.4470
	η	0.3269	0.2891	0.2917	0.3104	0.3301	0.2957
	β	−80.36	−80.50	−82.59	−80.66	−80.33	−80.39
	γ	30.19	30.93	29.27	30.50	30.26	30.41
	α	11.27	12.58	11.63	12.22	10.37	13.57

[a] S_{ij} ($i, j = x, y, z$) are order parameters in the molecular frame as derived from the experimental order parameters while S_{IJ} ($I = X, Y, Z$) are order parameters in the coordinate system in which the order parameter tensor S is diagonal.

the conformation of the tricyclic ring system of (−)-Δ^9-THC in solution is consistent with that obtained from the X-ray crystallographic data.

Table II lists the results for the molecular orientation and ordering derived from the six different combinations of five S_{C-D} values. Choice I uses the S_{C-D} values for deuterium positions 4, 8α, 8β, 10 and 10a, and the calculation predicts the sign and values of S_{C-D} for position 2. Each of the choices II to VI uses another set of five S_{C-D} values and predicts the S_{C-D} of the sixth position. The self-consistent results of the order parameter tensor elements S_{xx}, S_{xy}, S_{xz}, S_{yy}, S_{yz} and S_{zz} in the molecular fixed x-y-z-frame and S_{XX}, S_{YY} and S_{ZZ} in the principal X-Y-Z-frame are listed in the column of each choice. The asymmetry parameter and the Euler angles that relate the x-y-z-frame to the X-Y-Z-frame are also tabulated.

In this table, one can see that the calculations converge to a set of self-consistent solutions that have demonstrated without exception that the signs of $\Delta\nu_Q$ for positions 2, 4, 8α, 8β, 10 and 10a must be +, +, −, +, + and −, respectively. Furthermore, when the correct sign combination is used in any choice of the starting data of five S_{C-D} values, the calculation predicts the correct sign of the sixth S_{C-D}. The predicted values of the sixth S_{C-D} is generally within 0.02 compared with the experimentally observed values. The consistency between the results of calculations using different choices of five S_{C-D} values is self-evident when we examine the six sets of S_{ij} values in the x-y-z-frame and S_{XX}, S_{YY} and S_{ZZ} in the X-Y-Z-frame. These six sets are virtually identical with only minor numerical differences. The largest principal components S_{ZZ} has values between 0.44 and 0.46 while the asymmetry parameter η lies between 0.29 and 0.33. The results for the three Euler angles, β, γ and α are $-81° \pm 1°$, $30° \pm 1°$ and $11° \pm 2°$, respectively. The orientation of the motional axis of (−)-Δ^9-THC in DPPC bilayers is shown schematically in Fig. 3.

Our calculations show that the motional axis is nearly in the plane of the aromatic ring and makes an angle of about 27° with the C4-^{2}H bond. Therefore, the long axis of the molecule is nearly perpendicular to the

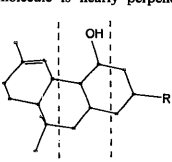

Fig. 3. The orientation of (−)-Δ^9-THC in hydrated DPPC bilayers. The dashed lines represent the direction of the lipid acyl chains.

phospholipid chains. The molecular order parameter S_{mol} or S_{ZZ} was found to be 0.45, which is less than that reported for the glycerol backbone region of phospholipids (0.65) [12] and similar to that of the plateau region of the lipid acyl chains (0.4).

We have also calculated the orientation of (−)-Δ^9-THC using a simplified method, the quadrupolar splitting ratio method [9,12], which assumes implicitly an axially symmetrical order parameter tensor. We found that the calculated angles β, γ and the order parameter S_{mol} are surprisingly close to the results of the formal calculation using the complete tensor analysis. This factor indicates that the simplification by assuming $\eta = 0$ in the calculation can lead to a reasonable first-order estimate of the orientation.

The calculated orientation of (−)-Δ^9-THC relative to its motional axis places the phenolic hydroxy at the water-lipid interface. This orientation allows the cannabinoid OH group to direct itself towards the polar side of the bilayer indicating that this group serves as the orienting anchor in the molecule. We refer to this cannabinoid : bilayer interaction as 'amphipathic interaction', in which its primary incentive is to direct the polar and hydrophobic components of the drug molecule towards the respective sites in the amphipathic bilayer. The amphipathic interaction has also been explored in detail in the case of weakly polar lipids [19]. Solid-state ^{2}H-NMR has been applied to study the orientations of amphipathic molecules such as cholesterol [9] and α-tocopherol (vitamin E) [19] in model membranes. The results of these studies showed that both molecules orient in such a way that the OH group of the respective molecule points towards the polar side of the bilayer. Our calculations show that, because of the particular OH position on cannabinoids, (−)-Δ^9-THC orients with its long axis almost orthogonal to, rather than parallel to, the bilayer chains, as is the case with cholesterol [9].

The anchoring effect of the THC phenolic OH on the membrane is intriguing and deserves more extensive investigation. Indeed, the requirement of a free OH activity in THC is well established. Also, differences in activities have been reported between cannabinoid isomers differing only in the presence and/or orientation of hydroxyl and keto groups in the C-ring [20]. Since it is reasonable to assume that these hydrogen bonding groups may also be involved in the orientation of the cannabinoid molecule, one may speculate that cannabinoid orientation in the membrane may have an important effect on its biological activity. The drug orientation in the amphipathic membrane may, thus, play an important (although not unique) role in determining the nature of the cannabinoid : membrane interaction and its ability to induce a 'productive membrane perturbation'. We are currently investigating the above hypothesis.

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