CHIRAL DISCRIMINATION OF L- AND D-N-ACYL-1-PHENYL-D_5-2-AMINOPROPANES IN A CESIUM N-DODECANOYL-L-THREONINATE CHOLESTERIC NEMATIC LYOMESOPHASE

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ABSTRACT

Molecular recognition based on chirality has fundamental importance in many biological processes. Deuterium quadrupole splittings from the aromatic ring and mesophase components of two series of optical isomers, L- and D-N-acyl-1-phenyl-d_5-2-aminopropanes, dissolved in anionic nematic cholesteric lyotropic liquid crystals of cesium N-dodecanoyl-L-threoninate, were measured using ^{2}H-NMR. The length of the acyl chain was 1, 2, 3, 4, 5, 7 and 10 carbon atoms. The two order parameters that fully characterize the average alignment of the aromatic ring were calculated. Both the L- and D- isomers are strongly attached to the aggregate. L-C_1, D-C_1, L-C_2 and D-C_2 derivatives have the same order parameters which suggests that are located in the same region of the interface, possibly H-bonded to the interstitial water molecules with the NH and/or CO groups. Increasing the hydrophobic chain length by one carbon atom decreases the overall alignment of the ring and differences between L-C_3 and D-C_3 were observed. Molecules with longer acyl chain progressively increase their quadrupole splittings, suggesting an increase in alignment. Increasing differences in the order parameter of the symmetry axis of the aromatic rings in both isomers were observed from C_3 to C_5, and almost no differentiation is detected between L-C_7 and D-C_7. However, differentiation appears again for C_{10}, and is attributable to interactions with a second chiral center of the head group.

INTRODUCTION

Most biologically interesting molecules and molecular assemblies found in living systems contain asymmetric carbons with a specific chirality. For instance, natural amino acids are L- and most sugars are D-. Specific interactions between chiral centers are of current interest, and may be crucial in many important biological processes.[1-4] Interactions between chiral atoms may influence the distribution as well as diffusion of biologically interesting chiral molecules across natural membranes. However these effects are hard to demonstrate *in vivo* because they are probably obscured by the possibility of differences in the enantioselectivity of blood plasma protein binding sites, among a number of other possible effects. The importance that enantioselective membrane permeation might have in fundamental processes such as drug absorption, transport and excretion, is not well understood.

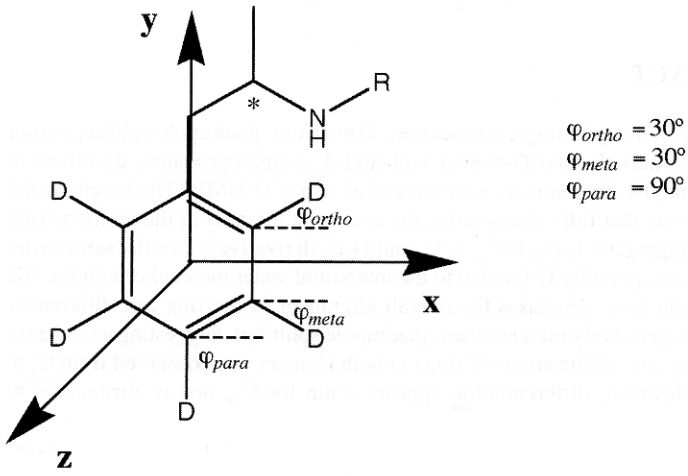
Only during the past few decades the pharmacological importance of chirality has been widely recognized. For instance, the D-isomer of amphetamine is a potent central nervous system stimulant while the L-isomer is significantly less active. The existence of stereospecific binding sites of D-amphetamine in membrane preparations from rat brain has been demonstrated. [5] There are many other examples of similar behavior and for this reason the pharmaceutical industry shows an increasing interest in developing asymmetric synthesis and chiral resolution technology.[6]

For many years it has been observed that different enantiomers of a molecule display different NMR spectra when dissolved in a chiral anisotropic media, such as cholesterics. NMR is one of the most direct techniques for the evaluation of enantiomeric enrichment and specific recognition interactions.[7-14] Experimental and theoretical efforts have also been devoted to understand the role of chiral interactions and recognition in chromatographic applications, also oriented to the resolution of racemic mixtures.[15]

Discotic lyotropic nematic liquid crystals prepared with a chiral head group, Ch_D, provide a magnetic field oriented bilayer with a chiral interface. These type of liquid crystals, containing different chiral amino acids as head group of the main component amphiphile, have been prepared before and a number of different studies were published.[4, 16-22]

The use of order parameters derived from the ^{2}H-NMR spectra of specifically labeled molecules, is one of the simplest methods to estimate the average alignment of ions and molecules in anisotropic media, such as oriented bilayers. In the past, we have studied the incorporation of a number of ions and molecules in cationic and anionic nematic lyotropic liquid crystals, using deuterium quadrupole splittings of deuteriated aromatic rings.[3,4,23-25] In this work, we have measured ^{2}H-NMR quadrupole splittings from the fully deuteriated aromatic ring of two series of seven optical isomers each, L- and D-N-acyl-1-phenyl-2-aminopropane 40% d_5. Both series of isomers, derivatives of L- and D-amphetamine, contain linear acyl substituents with 1, 2, 3, 4, 5, 7 and 10 carbon atoms in the chain, and were dissolved in a nematic discotic cholesteric lyomesophase, prepared using cesium N-dodecanoyl-L-threoninate (CsD-L-Thr) as the main component, providing the chirality. The identification of the different guest molecules is related to the

number of carbons in the linear acyl substituent: the formyl derivative is named C_1; C_2 is N-acetyl; C_3 is N-propanoyl, and so on. Figure 1 corresponds to the basic structure of the molecules including the designation of the Cartesian axis and structural parameters of the aromatic ring. From the quadrupole splittings of the deuterium nuclei at the *ortho*, *meta* and *para* positions, we calculated the two order parameters that completely describe the average alignment of the aromatic ring. Using this information we postulate a possible solubilization process of the molecules within the mesophase. The influence of the incorporation of the guest molecule on the integrity of the aggregate was examined by adding 20% 1,1-dideuterodecanol to the decanol necessary to form the mesophase. The interaction between water molecules and the bilayer interface was observed by adding 0.05% of D_2O to the water used in the preparation of the mesophase.



R = Formyl, acetyl, propanoyl,butanoyl, pentanoyl, heptanoyl, decanoyl

Fig. 1. Basic structure of the studied molecules and designation of the symmetry axis of the aromatic ring. The principal axis of the electric field gradient is assumed to be along the direction of the C-D bonds.

Experimental Section

Synthesis and Enantiomeric Resolution of L- and D-N-acyl-1-phenyl-d_5-2-aminopropanes. The details of the synthesis and enantiomeric resolution were published in a previous paper.[4]

Sample preparation. Cesium-N-dodecanoyl-L-threoninate was prepared using a method described before, except that CsOH was used in the neutralization of the acid.[17-19] The mesophase was prepared dissolving 284 mg of CsD-L-Threo, 25 mg KCl and 60 µl of decanol (15% 1,1-dideuterodecanol) in 0.48 ml of H_2O (0.05% D_2O). Decanol-α-d_2 was prepared by reduction of decanoyl ethyl ester with $LiAlD_4$. Between 3 to 5 mg of amide, depending on molecular weight, was dissolved in 0.6 ml of mesophase and transferred to the NMR tube. A batch of approximately 15 ml of liquid crystal was prepared and used in all the experiments, to avoid differences in mesophase composition for each sample. This particular threoninate mesophase, with potassium instead of cesium as counter ion, has been prepared before and in this work we have used the previously reported composition.[19] The optical birefringence of the solution and the ^{2}H- NMR spectra obtained in all samples corroborate the anisotropic nature of the aggregate. No further characterization of the mesophase was performed.

NMR Spectra. NMR spectra were recorded at 300 K on a Bruker AMX-300 spectrometer in the Facultad de Ciencias, Universidad de Chile. Proton spectra were obtained from the ^{1}H channel of a broadband probe and ^{2}H spectra were obtained by using the X channel. A 30 kHz spectral window, 32 kB file size and 10 µs pulse length (30° flip angle), at a rate of 30 pulses/second, were employed when measuring the deuterium spectra. The experimental errors in the measured quadrupole splittings were estimated as: ±2 Hz for HDO; ±10 Hz for the *ortho* and *meta* deuterium nuclei; ±30 Hz for decanol-α-d_2 and ±90 Hz for the *para* deuterium nucleus.

RESULTS AND DISCUSSION

Figure 2 shows the ^{2}H-NMR spectrum of D-C_2 dissolved in the CsD-L-Threo mesophase. Quadrupole splittings from decanol-α-d_2, HDO, and the *ortho-meta* and *para* positions of the aromatic rings were observed. All quadrupole splittings were measured directly from the spectrum. The experimental results, including the splittings of the mesophase with no guest molecule dissolved, are listed in Tables 1 and 2. These tables show that the quadrupole splitting from HDO for the L-C_1 and D-C_1 samples decreased by about 20 Hz as compared with the value observed in the pure mesophase. The same effect is observed for the splitting of decanol. For the longer chain derivatives these splittings increase progressively to a maximum value of about 250 Hz for HDO and about 14500 for decanol in sample C_{10}. Both signals arise from deuterium nuclei in the same region of the aggregate, most likely the interface. There are two possible explanations for this observation: i) the guest molecule affects the internal dynamics of the interface of the aggregate or ii) it modifies its size. Since the amount of added guest molecule is less than 3% w/w it is more likely that C_1 is located at the interface, modifying the local organization of this region. More hydrophobic derivatives should incorporate deeper into the aggregate and the amide fragment may act as non-charged spacer between carboxylate head groups, in a similar way to the role of decanol, introducing order into the interface.

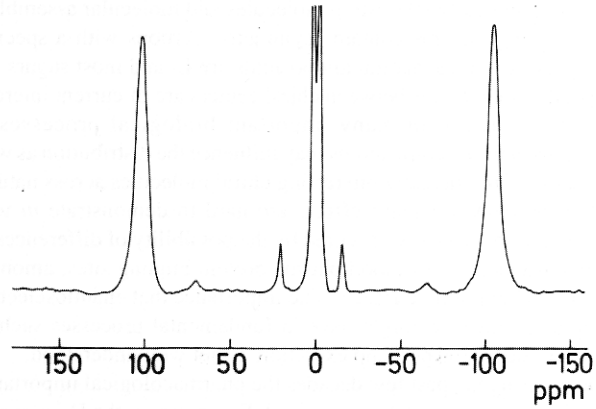


Fig. 2. ^{2}H-NMR spectrum of D-N-acetyl-1-phenyl-2-aminopropane dissolved in CsD-L-Threo mesophase. The central doublet arises from HDO, the next external doublet comes from the ortho and meta positions of the aromatic ring, the smallest pair of signals arises from the para position and the external splitting corresponds to decanol-α-d_2.

Tables 1 and 2 show that the splittings from the *ortho* and *meta* positions were never resolved, suggesting the existence of a fast averaging rotational motion around the ring torsion angle of the guest

molecules. The splittings from the *para* positions were always several times larger than those from positions *ortho- meta*, suggesting that the motion of the y-axis of the ring is probably much more restricted than the torsional motion. The *para* splitting from C_{10} were not observed for either series. It was assumed that the peak from decanol-α-d_2 is overlapping it and the value from decanol-α-d_2 was used in the order parameter calculations.

Table 1. L-Amide Splittings

N° Carbons	$\Delta\nu_{HDO}$	$\Delta\nu_{DeOH}$	$\Delta\nu_{ortho}$	$\Delta\nu_{meta}$	$\Delta\nu_{para}$
1	171.7	10730	1824	1824	-7078
2	188.8	11522	1962	1962	-7721
3	195.5	12032	2068	2068	-6800
4	216.0	12888	2635	2635	-7249
5	224.0	13095	3204	3204	-7335
7	208.1	12740	3297	3297	-9786
10	246.0	14375	3216	3216	-14375
Pure Phase	190.7	11983			

Table 1. Deuterium quadrupole splittings (Hz) from decanol-α-d_2, from HDO, and from the ortho, meta and para positions of the aromatic ring of the L-N-acyl-1-phenyl-2-aminopropanes series. The experimental errors in the measured quadrupole splittings are: ±2 Hz for HDO; ±10 Hz for the ortho and meta deuterium nuclei; ±30 Hz for decanol-α-d_2 and ±90 Hz for the para deuterium nucleus.

Table 2. D-Amide Splittings

N° Carbons	$\Delta\nu_{HDO}$	$\Delta\nu_{DeOH}$	$\Delta\nu_{ortho}$	$\Delta\nu_{meta}$	$\Delta\nu_{para}$
1	174.5	10950	1910	1910	-6937
2	184.9	11549	2020	2020	-7475
3	190.7	11857	2177	2177	-6617
4	201.2	12261	2689	2689	-7027
5	182.1	11914	2848	2848	-7524
7	217.4	13159	3570	3570	-9728
10	255.5	14757	3776	3776	-14757
Pure Phase	190.7	11983			

Table 2. Deuterium quadrupole splittings (Hz) from decanol-α-d_2, from HDO, and from the ortho, meta and para positions of the aromatic ring of the D-N-acyl-1-phenyl-2-aminopropanes series. The experimental errors in the measured quadrupole splittings are: ±2 Hz for HDO; ±10 Hz for the ortho and meta deuterium nuclei; ±30 Hz for decanol-α-d_2 and ±90 Hz for the para deuterium nucleus.

The size of the splittings listed in tables 1 and 2 suggest that all the studied molecules are significantly attached to the aggregate. The quadrupole splittings from the ring increase with the size of the molecule revealing either a modification in the average orientation or a change in the dynamics of it, due to the increase in molecular mass. A more detailed picture of the process can be obtained from the two order parameters that describe the average alignment of the aromatic ring in the magnetic field, Sxx and Syy. [26] The order parameter of the z axis, Szz, is obtained from the traceless condition of the order parameters matrix.[27] These parameters may have values from –0.5 for an axis perpendicular to the magnetic field, to +1 for an axis parallel to the field. The value zero corresponds either to a freely rotating axis or to an axis oriented at the magic angle. To obtain the necessary geometrical information for the order parameters calculation, AM1-MO full geometry optimization calculations were carried out for all guest molecules. The deviation of the optimized angles of the ring from 120° is small enough for the calculated order parameters to become insensitive to the difference. Therefore 120° was used in all the calculations. The value of the quadrupole coupling constant Q is 185 kHz and the electric field gradient, η, is 0.05. [28-30] As observed, the addition of the guest molecules may introduce a significant effect on the dynamics of the aggregate, and to make the order parameters from different samples comparable, we normalized the quadrupole splittings. Dividing the splittings from the *ortho-meta* and *para* positions of the ring by the splitting of decanol-α-d_2 of each sample, and multiplying the result by the average value of decanol splitting, provides a set of normalized splittings for the calculation of the order parameters of the ring. To distinguish a true reorientational process from a simple slowing down of motion by increase in molecular weight, the absolute value of the ratio Syy/Sxx was also calculated. A slow down in motion due to an increase in molecular mass should affect both order parameters in the same amount. All these results appear in tables 3 and 4 and figures 3 and 4 are plots of the normalized Sxx and Syy against the acyl chain length for both series. The values of the ratio Syy/Sxx suggest the existence of a reorientational process of the ring along both series. The calculated values of Sxx, Syy and Szz indicate that the torsional dynamics of the ring, mainly represented by Sxx and Szz, seems to be faster than the motion of the symmetry axis, Syy.

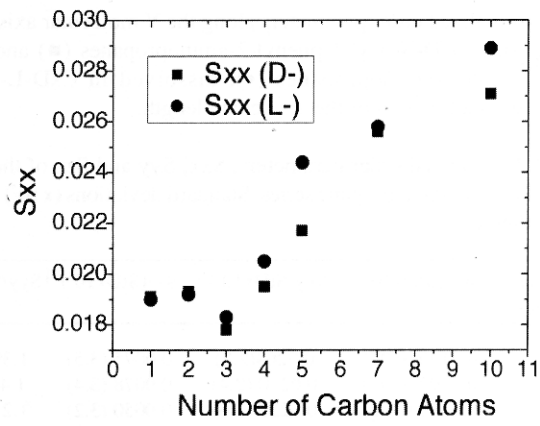


Fig. 3. Normalized order parameters along the X molecular axis of the aromatic ring of D-N-acyl-1-phenyl-2-aminopropanes (g) and L-N-acyl-1-phenyl-2-aminopropanes (•), dissolved in CsD-L-Threo mesophase as a function of the acyl chain length.

From tables 3 and 4 a small difference among the order parameters of L-C_1 D-C1, L-C2 and D-C_2 derivatives is observed. The values strongly suggest that these four derivatives are located in the same region of the mesophase, most likely at the interface. In this situation, the chiral centers of the guests should be away from the chiral centers of the aggregate. These derivatives seem to be stabilized by hydrogen bond between the amide fragment of the guest molecule and the water molecules at the interface. Adding a third carbon atom to the guest molecule augments its hydrophobicity. This effect should become comparable to the magnitude of the stabilization by H-bonding, inducing an increase in the overall molecular mobility. This is observed in both series of isomers. However, the splittings and order parameters from

L-C_3 and D-C_3 suggests that they are located in a region of the aggregate where chiral discrimination between both isomers is observed, particularly in the value of the Sxx parameter. Our results indicate that the torsional coordinate of the ring is clearly more sensitive to the chiral nature of the interface, suggesting either a difference in average orientation or a difference in the reorientational dynamics of the x-axis of the ring. This motion appears more restricted in the D- series. The same differentiation process is not equally observed for Syy, where the values are similar for both isomers along the series. The parameter Syy mainly represents the behavior of the quadrupole splitting from the *para* position of the ring, the one with the largest experimental error.

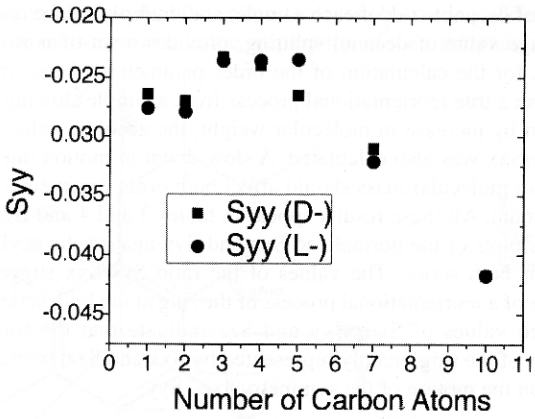


Fig. 4. Normalized order parameters along the Y molecular axis of the aromatic ring of D-N-acyl-1-phenyl-2-aminopropanes (■) and L-N-acyl-1-phenyl-2-aminopropanes (•), dissolved in CsD-L-Threo mesophase as a function of the acyl chain length.

Table 3. Normalized order parameters, Sxx, Syy and Szz of the L-N-acyl-1-phenyl-2-aminopropane series. Standard deviations (x10^5) appear in parenthesis.

Number of Carbons	Sxx (StDx10^5)	Syy(StDx10^5)	Szz(StDx10^5)	|Syy/Sxx|
1	0.0190 (0.95)	-0.0264(2.2)	0.0075 (3.5)	1.395
2	0.0192 (0.91)	-0.0270 (2.4)	0.0078 (3.4)	1.408
3	0.0183 (0.98)	-0.0233 (2.6)	0.0050 (3.2)	1.274
4	0.0205 (0.94)	-0.0240 (2.3)	0.0035 (3.6)	1.173
5	0.0244 (0.89)	-0.0265 (2.6)	0.0040 (3.8)	1.180
7	0 0258 (0.98)	-0.0310 (2.8)	0.0052 (3.9)	1.201
10	0.0289 (1.10)	-0.0417 (3.2)	0.0128 (4.6)	1.445

Table 4. Normalized order parameters, Sxx, Syy and Szz, of the D-N-acyl-1-phenyl-2-aminopropane series. Standard deviations (x10^5) appear in parenthesis.

Number of Carbons	Sxx (StDx10^5)	Syy (StDx10^5)Szz (StDx10^5)	|Syy/Sxx|
1	0.0191 (0.96)	-0.0275 (2.1)	0.0084 (3.4)	1.440
2	0.0193 (0.94)	-0.0279 (2.4)	0.0087 (3.3)	1.450
3	0.0178 (0.89)	-0.0236 (2.3)	0.0058 (3.6)	1.327
4	0.0195 (0.97)	-0.0236 (2.6)	0.0040 (3.5)	1.207
5	0.0217 (0.99)	-0.0235 (2.2)	0.0019 (3.8)	1.086
7	0 0256 (1.11)	-0.0321 (2.9)	0.0066 (4.1)	1.258
10	0.0271 (0.12)	-0.0417 (3.5)	0.0145 (4.3)	1.536

An increment in the acyl chain length of the guest molecule should incorporate it more deeply into the aggregate and/or increases the alignment, because of the increase in the hydrophobicity. This should increase the possibility of chiral discrimination by close encounter of the chiral centers in the guest molecule and the aggregate. From C_3 to C_5 chiral discrimination between both series of isomers is observed, manifested mainly in the torsional motion of the ring. The discrimination almost disappears for L-C_7 and D-C_7, where a small difference is observed. However, for L-C_{10} and D-C_{10} a significant difference in Sxx is observed again. These results may be interpreted in terms of the nature of the chiral interface. Because threonine has two chiral centers, probably there is more than one region of the mesophase that contains asymmetric carbons and, on the average, they should be located at different distances from the surface.

The obtained result can also be interpreted in terms of a difference in the average orientation of the X symmetry axis of the ring, relative to the direction of the magnetic field. Particularly, for intermediate chain derivatives, the average angle between the X-axis of the ring in the L-series and the magnetic field direction is closer to the magic angle than the D-series. Finally, an inspection to table 2 reveals that the Syy/Sxx ratio keeps changing for the long chain derivatives. This strongly suggests that the average alignment of the ring should still being modified by the increase in the length of the acyl chain.

ACKNOWLEDGEMENTS

The authors are pleased to acknowledge financial assistance from FONDECYT, Grant No. 1010211. R.M. and H.A. acknowledge Doctoral Fellowships from CONICYT.

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