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NMR Experimental Evidence of the Differentiation of Enantiotopic Directions in C_s and C_{2v} Molecules Using Partially Oriented, Chiral Media

Christie Aroulanda, Denis Merlet, Jacques Courtieu, and Philippe Lesot*

Contribution from the Laboratoire de Chimie Structurale Organique, CNRS ESA 8074, ICMO, Bat. 410, Université de Paris-Sud, 91405 Orsay, France

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Abstract: We report new and explicit experimental evidence of the differentiation of ^{1}H−^{1}H, ^{13}C−^{1}H, and ^{13}C−^{2}H enantiotopic directions in prochiral molecules with C_s and C_{2v} symmetry dissolved in a chiral liquid-crystalline phase using ^{13}C and ^{2}H-{^{1}H} NMR spectroscopy at the natural abundance level. The case of *endo*-bicyclo[2.2.2]oct-5-ene-2,3-dicarboxylic anhydride, bicyclo[2.2.1]hepta-2,5-diene, and ethyl alcohol oriented in organic solutions of poly-γ-benzyl-L-glutamate (PBLG) or poly-ϵ-carbobenzyloxy-L-lysine (PCBLL) is investigated and discussed. Next, we describe the first NMR differentiation of enantiotopic directions in a C_{2v} molecule with no prostereogenic carbon using malononitrile as a model. The various results presented in this work experimentally validate our recent theoretical arguments which predict that NMR spectra of nonplanar C_s and C_{2v} compounds embedded in a chiral oriented solvent should differ from those recorded in nonchiral oriented media, because their effective molecular symmetry point group (interacting molecule) is different from their molecular point group (isolated molecule). In addition, the differentiation of enantiotopic directions in C_{2v} molecules exhibiting no prostereogenic tetrahedral center illustrates for the first time an old stereochemical hypothesis which speculates that "for molecules of the type CXXYY... the two X groups as well as the Y groups are equivalent and cannot be distinguished in chiral or achiral circumstances. However, the relationships between X and Y groups are not all equivalent. The four X−Y relationships may be ordered into two enantiotopic sets of two equivalent relationships" (Mislow, K.; Raban, M. *Top. Stereochem.* **1967**, *1*, 1) and validate the stereogenicity concepts proposed more recently by Fujita (Fujita, S. *J. Am. Chem. Soc.* **1990**, *112*, 3390).

Introduction

From point group theoretical arguments, it has been recently demonstrated that the effective molecular symmetry of some solutes embedded in any chiral liquid crystal (CLC) may differ from their molecular point groups defined for an isolated molecule.[1]Actually, the reduction in symmetry changes the location of the principal axes of the orientational order matrices for four molecular point groups, namely, C_s, C_{2v}, S_4, and D_{2d} (compared with a nonchiral liquid crystal).[1,2] From a stereochemical point of view, these symmetry groups correspond to organic compounds which have enantiotopic nuclei (or groups), directions, or faces. In other words, for these symmetries, all enantiotopic elements, which are exchangeable by a mirror plan but not by a C_2 axis, may be in principle distinguished using NMR spectroscopy in CLC.

The change in effective molecular symmetry for C_s, C_{2v}, S_4, and D_{2d} solutes in CLC has important consequences in the analysis of their NMR spectra. Until now, these effects were detected through carbon or deuterium NMR spectroscopy in a polypeptide liquid-crystalline solution made of poly-γ-benzyl-L-glutamate (PBLG) dissolved in various organic cosolvents, such as CH_2Cl_2, $CHCl_3$, DMF, and THF. Among the first illustrative results, reported elsewhere, the spectral changes expected in a CLC were easily and unequivocally evidenced in the case of ethanol-d_6 (**1**) or acenaphthene-d_{10} (**2**) oriented in the PBLG/$CHCl_3$ phase.[1,2] Thus in these nonplanar C_s and C_{2v} molecules, we have shown that the enantiotopic deuterons (in fact the C−D enantiotopic directions) of the methylene group were nonequivalent in terms of deuterium quadrupolar splittings, because two distinct quadrupolar doublets were detected on their ^{2}H spectrum. The same conclusion was drawn from inspection of the deuterium-coupled carbon-13 spectrum of **1** as well as the proton-coupled carbon-13 spectrum of protonated ethanol in PBLG. Indeed, in both cases, it was shown that the spin system associated with the methylene group was not of the AX_2 type but AXX′, due to the nonequivalence of ^{13}C−^{2}H (^{13}C−^{1}H) dipolar couplings.[2]

The spatial relationship between two enantiotopic directions that have a common prostereogenic center[4] (here a carbon atom) is easily seen (see Table 1). Obviously, this simplest case is not the generality, and various other occurrences exist and merit investigation. Herein, we will present and discuss three molecules involving different representative examples of enantiotopic directions. For this purpose, we will use both ^{2}H-{^{1}H} and ^{13}C NMR spectroscopies at the natural abundance level and two different polypeptide liquid-crystalline systems. First, we will show the possibility to differentiate between C−D enantiotopic directions which are not related by a common prostereogenic carbon atom in C_s and C_{2v} bridged ring systems. The cases of the *endo*-bicyclo[2.2.2]oct-5-ene-2,3-dicarboxylic anhydride (**3**) and the bicyclo[2.2.1]hepta-2,5-diene, more com-

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* To whom correspondence should be addressed. Fax: +33(0)1 69 15 47 70. E-mail: philesot@icmo.u-psud.fr.

[1] Merlet, D.; Emsley, J. W.; Lesot, P.; Courtieu, J. *J. Chem. Phys.* **1999**, *111*, 6890.

[2] Merlet, D.; Loewenstein, A.; Smadja, W.; Courtieu, J.; Lesot, P. *J. Am. Chem. Soc.* **1998**, *120*, 963.

[3] Mislow, K.; Raban, M. *Top. Stereochem.* **1967**, *1*, 1.

[4] Eliel, A. L.; Wilen, A. H. In *Stereochemistry of Organic Compounds*; John Wiley and Sons: New York, 1994; p 508.

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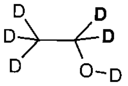
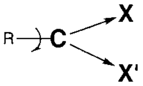
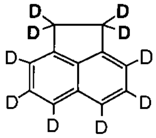
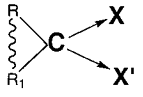
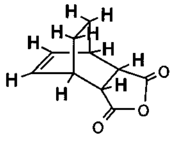
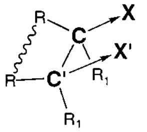
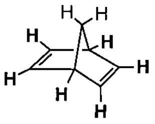
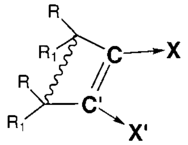
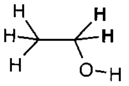
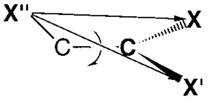
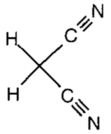
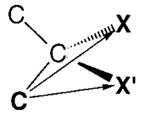
Table 1. Various Possible Cases of Enantiotopic Directions Differentiable in a CLC

Entry	Compound[a]	Symmetry group	Enantiotopic directions differentiated
1		C_s	
2		C_{2v}	
3		C_s	
4		C_{2v}	
5		C_s	
6		C_{2v}	

[a] The enantiotopic protons or deuterons in the prochiral molecules are displayed in bold.

monly known as the norbornadiene (**4**), using natural abundance deuterium (NAD) 2D NMR spectroscopy in PBLG will be shown as examples. Second, we will demonstrate the possibility to indirectly visualize the differentiation between two proton−proton enantiotopic directions in a molecule of C_s symmetry using proton-coupled carbon-13 NMR. For this purpose, we will examine the case of ethyl alcohol (**5**) dissolved in solutions containing poly-ϵ-carbobenzyloxy-L-lysine (PCBLL). Last, but not least, we will report the first NMR evidence of differentiation of C−H enantiotopic directions in a C_{2v} molecule exhibiting no prostereogenic tetrahedral center through proton-coupled carbon-13 NMR and a gradient-enhanced 1D heteronuclear single-quantum coherence (HSQC) experiment in PBLG using malononitrile (**6**) as a model molecule. Although rather academic, this result is the first successful attempt at illustrating the stereochemical hypothesis made by Mislow and Raban, who speculated that "for molecules of the type CXXYY... the two X groups as well as the Y groups are equivalent and cannot be distinguished in chiral or achiral circumstances. However, the relationships between X and Y groups are not all equivalent. The four possible X−Y relationships may be ordered into to two enantiotopic sets of two equivalent relationships".[3,4] For clarity, the different types of internuclear enantiotopic directions associated with compounds **1**−**6** are listed in the Table 1.

Experimental Section

Sample Preparation. The various liquid-crystalline NMR samples investigated in this work were prepared using a standard procedure described elsewhere. Details on the method and sample preparation can be found in the literature.[5] Note that all polypeptides used are commercially available from Sigma and all 5 mm o.d. NMR tubes were sealed to avoid solvent evaporation and centrifuged back and forth until an optically homogeneous birefringent phase was obtained. The exact composition of each oriented NMR sample (compounds **3**−**6**) investigated is shown in Table 2.

NMR Spectroscopy. Deuterium and carbon-13 NMR spectroscopies were performed at 9.4 T on a Bruker DRX 400 high-resolution spectrometer equipped with (unless otherwise specified) an inverse multinuclear probe (BBI) operating at 100.6 MHz for carbon and at 61.4 MHz for deuterium and with a standard variable temperature unit (BVT 3000). For the carbon-13 spectra, proton irradiation was applied during the relaxation delay period (2 s) so as to benefit from the nuclear Overhauser effect. For $^{13}C\text{-}\{^1H\}$ and $^2H\text{-}\{^1H\}$, the protons were decoupled using the broad-band composite pulse sequence WALTZ-16. Other experimental NMR parameters or details are given in the legends of the figures.

Results and Discussion

Differentiation between Enantiotopic C−D Directions in C_s and C_{2v} Molecules. Deuterium NMR spectroscopy in polypeptide chiral liquid-crystalline solutions is a very powerful tool to differentiate between two enantiotopic C−D directions.[1,2] The quadrupolar interaction is the largest order-dependent interaction. Thus, even when the difference between S_{C-D}^{pro-S} and S_{C-D}^{pro-R} in a C_s symmetry molecule is small, the magnitude of the quadrupolar coupling constant (170 ± 15 kHz for sp^3 carbon nuclei) for a C−D bond can make the difference in their residual quadrupolar splittings, $|\Delta\nu_{Q_{C-D}}^{pro-S} - \Delta\nu_{Q_{C-D}}^{pro-R}|$, measurable in the ^{2}H spectrum. The analysis of ^{2}H spectra is straightforward because when differentiation between the two enantiotopic C−D directions occurs, we observe simply two quadrupolar doublets, disregarding any $^2H-^2H$ scalar as well as dipolar couplings. Samulsky and co-workers reported this differentiation of the enantiotopic deuterons in the CD_2 of perdeuterated benzyl alcohol using the PBLG phase as an oriented solvent,[6] and we have also observed this effect for C_s and C_{2v} compounds (**1** and **2**).[1,2]

As emphasized in the Introduction, the spatial relationship between enantiotopic directions having a common prostereogenic center is quite evident but is, of course, a particular occurrence of the problem. To be convinced, we have explored the case of C−D enantiotopic directions which are not related by a common prostereogenic carbon atom. This stereochemical situation is typically encountered in rigid, bridged ring systems with C_s or C_{2v} symmetries. Among the numerous possible compounds, we have investigated the case of *endo*-bicyclo[2.2.2]-oct-5-ene-2,3-dicarboxylic anhydride (**3**) and of bicyclo[2.2.1]-hepta-2,5-diene (**4**), which are of C_s and C_{2v} symmetry, respectively. The molecular structure of both compounds and their numbering system are given in Figure 1. Due to the plane of symmetry, it should be remarked that the relationship between the various $^{13}C-^1H$ directions, and subsequently all $^{13}C-D$ directions, in the *Re* and *Si* faces of **3** is enantiomeric. In other words, we may, a priori, expect to differentiate between five pairs of enantiotopic directions in a PBLG phase and, hence, observe up to 10 distinct quadrupolar doublets through deuterium NMR spectroscopy, if all ^{2}H resonances are resolved. In the case of **4**, we can predict discrimination of only two $^{13}C-^1H$ ($^{13}C-^2H$) enantiotopic directions. They correspond to the ethylenic $^{13}C-^1H$ ($^{13}C-^2H$) directions that cannot be exchanged by the overall C_2 axis of the molecule, and subsequently two

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(5) Sarfati, M.; Lesot, P.; Merlet, D.; Courtieu, J. *Chem. Commun.* **2000**, 2069, and references therein.

(6) Czarniecka, K.; Samulski, E. T. *Mol. Cryst. Liq. Cryst.* **1981**, *63*, 205.

Table 2. Compositions of Liquid-Crystalline NMR Samples Investigated

sample	solute	polymer	DP[a] (L-isomer/D-isomer)	cosolvent	solute/mg[b]	polymer/mg[b]	cosolvent/mg[b]	% of polymer in wt
1	**3**	PBLG	562	$CHCl_3$	100	100	350	18.2
2	**4**	PBLG	562	$CHCl_3$	100	100	350	18.2
3	**5**	PCBLL	992	$CHCl_3$	100	108	410	16.2
4	**6**	PBLG	562	CH_2Cl_2	20	100	350	21.3
5	**6**	PBG	562/914	CH_2Cl_2	20	50/50	350	21.3

[a] DP = degree of polymerization. [b] The accuracy on the weighting is ±0.5 mg.

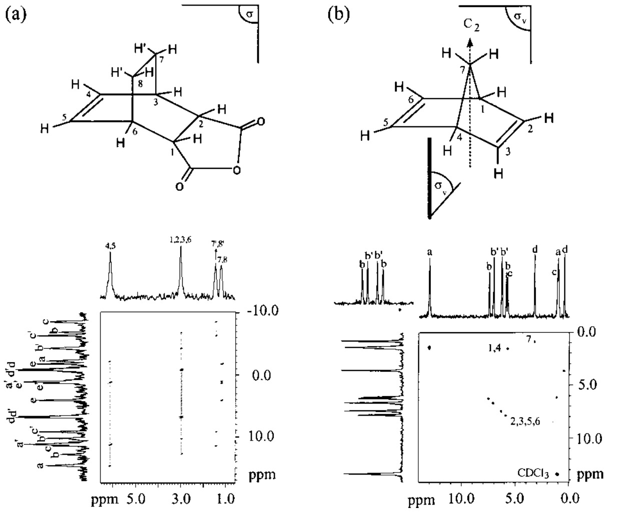

Figure 1. (a) 61.4 MHz natural abundance deuterium 2D Q-COSY tilted spectrum of *endo*-bicyclo[2.2.2]oct-5-ene-2,3-dicarboxylic anhydride at 300 K in the PBLG/$CHCl_3$ phase. (b) 61.4 MHz natural abundance deuterium 2D Q-COSY spectrum of 2,5-norbonadiene at 305 K in the PBLG/$CHCl_3$ phase. Both 2D spectra were recorded using 300 (t_1) × 1500 (t_2) data points, and the number of free induction decays added for each t_1 increment is 256 and 300, respectively. The contour plot is presented after symmetrizing without any filtering in (a) and with Lorentzian filtering in (b). In the left corner of panel b is presented a zoom of the NAD signal of ethylenic deuterons extracted from the tilted 2D spectrum.

different quadrupolar doublets should be detected in the deuterium spectrum. Actually, due to the C_2 axis, the deuterons on carbons 1 and 4, 2 and 5, and 3 and 6, and the pair of deuterons on single carbon 7 are stereochemically equivalent (homotopic).

To overcome isotopic labeling of solutes to be studied through ^{2}H NMR in polypeptide liquid-crystalline systems, we have recently shown that natural abundance deuterium (NAD) 1D and 2D NMR spectroscopies were possible using reasonable experimental time and provided analyzable ^{2}H spectra.[7-10] In addition, we have developed various 2D NMR sequences referred to as QUOSY and are dedicated to simplifying the analysis of overcrowded 1D spectra. Specific description of these 2D NMR sequences and related experiments may be found in refs 9 and 10. It could be argued here that, in NAD NMR, only monodeuterated isotopomers are detected, and so we observe molecules that are chiral by virtue of the isotopic substitution.[7] This argument is, however, not justified because it appears that the elements of the molecular ordering matrices are essentially identical for two isotopic enantiomers. Actually, in this chiral liquid crystal the order parameters are rather small (order parameter in the 10^{-4} to 10^{-3} range), and consequently, no isotopic effect on the ordering of solutes has been detected to date. Consequently, the origin of their chiral discrimination is only related to the fact that enantiotopic nuclei become nonequivalent in the presence of an asymmetric perturbation.[11]

In Figure 1 the 2D NAD Q-COSY spectra of **3** and **4** in the PBLG/$CHCl_3$ phase are displayed.[9] The analysis of these autocorrelation 2D contour plots is very simple and allows the two components of each quadrupolar doublet to be unambiguously identified. The assignment of each doublet is given on the respective 2D spectra. In the case of **3**, 10 quadrupolar doublets centered on the right chemical shifts (compared with ^{1}H spectrum) are observed as expected. This we interpret as evidence of the differentiation of all deuterium−carbon enantiotopic directions in **3**. The same conclusion can be drawn for the case of **4**. Indeed, as expected, a single quadrupolar doublet is observed for pairs of deuterons 1,4, and 7,7′, while two quadrupolar doublets centered on a chemical shift of the ethylenic deuterons are detected. Through these two examples, we show, therefore, that differentiation of deuterium−carbon enantiotopic directions in PBLG is possible even when they are not related by a common prostereogenic atom in the prochiral molecule.

The examination of the magnitude of various enantiotopic differentiations measured for **3** is of interest. Indeed the differences, $|\Delta\nu_{Q_{C-D}}^{pro-S} - \Delta\nu_{Q_{C-D}}^{pro-R}|$, vary from 20 to 418 Hz for **3**. These values are relatively large, compared with values measured in other prochiral solutes, and this situation seems to indicate that the PBLG helices interact very differently with the two prostereogenic faces of **3**. This result could, therefore, suggest a strong topological recognition of this type of rigid prochiral molecules in PBLG.

It should be emphasized that the enantiotopic ethylenic carbons of **4** were also discriminated on the basis of chemical shift anisotropy (CSA) differences using proton-decoupled carbon-13 NMR spectroscopy.[12,13] The signal of ethylenic carbons (not reported here) exhibits two distinct resonances separated by a chemical shift difference of 0.027 ppm at 100.6 MHz (2.7 Hz). Although this difference is rather small (the chemical shift anisotropy is the less sensitive order-dependent NMR interaction), this result points to the possibility of directly distinguishing between enantiotopic carbons of prochiral molecules through ^{13}C-{^{1}H} NMR spectroscopy.

Differentiation between Enantiotopic H···H Directions in *C_s* Molecules. The first successful example of differentiation between enantiotopic directions using proton-coupled carbon-13 NMR was reported in the case of ethyl alcohol dissolved in the PBLG/$CHCl_3$ phase.[2] In this flexible molecule, we have

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[9] Merlet, D.; Ancian, B.; Courtieu, J.; Lesot, P. *J. Am. Chem. Soc.* **1999**, *121*, 5249.

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[11] Meddour, A.; Canet, I.; Loewenstein, A.; Péchiné, J. M.; Courtieu, J. *J. Am. Chem. Soc.* **1995**, *116*, 9652.

[12] Lesot, P.; Merlet, D.; Meddour, A.; Loewenstein, A.; Courtieu, J. *Faraday Trans.* **1995**, *91*, 1371.

[13] Meddour, A.; Berdagué, P.; Hedli, A.; Courtieu, J.; Lesot, P. *J. Am. Chem. Soc.* **1997**, *119*, 4502.

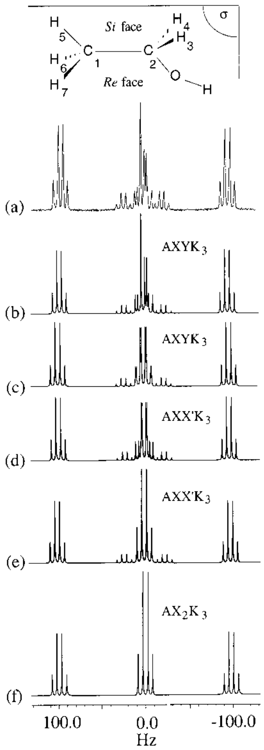

Figure 2. Top: Definition of the numbering system of atoms for the ethyl alcohol. Bottom: (a and b) Experimental and simulated 100.6 MHz proton-coupled carbon-13 NMR signals of the C2 carbon of **5** dissolved in the PCBLL/$CHCl_3$ system recorded at 300 K. A total of 2000 scans of 3K data points are added for each spectrum, and a recycle delay of 1.5 s was used. A Gaussian filtering and zero filling to 16K data points were employed to improve the spectral appearance and the digital resolution. (b−f) Simulations of carbon-13 spectral patterns associated to the proton-coupled carbon-13 signal of the methylene carbon in a fragment RCH_2CH_3 portrayed as a function of the equivalence or the inequivalence of $^1H-^1H$ dipolar couplings between the CH_2 and CH_3 groups and the ^1H chemical shift of enantiotopic protons. As expected for spectrum f, the $^{13}C-^1H$ dipolar couplings between the enantiotopic protons and the prostereogenic carbon are inequivalent. The spin systems associated with the various patterns, from b to f, are $AXYK_3$ with $D_{AX} \neq D_{AY}$ and $D_{XK} \neq D_{YX}$, $AXYK_3$ with $D_{AX} \neq D_{AY}$ but $D_{XK} = D_{YK}$, $AXX'K_3$ with $D_{AX} \neq D_{AX'}$ and $D_{XK} \neq D_{X'K}$, $AXX'K_3$ with $D_{AX} \neq D_{AX'}$ but $D_{XK} = D_{X'K}$, and AX_2K_3, respectively. These simulations were performed using the $^1H-^1H$ and $^1H-^{13}C$ scalar and dipolar couplings reported in Table 3. When the equivalence between $^1H-^1H$ dipolar couplings and/or ^1H chemical shift is required, the average of experimental values was considered.

shown that the C−H enantiotopic directions in the methylene group are nonequivalent in terms of $^{13}C-^1H$ dipolar couplings, and hence the spin system associated with the $^{13}CH_2$ group cannot be classified as an AX_2 spin system but as an AXX' spin system (X and X′ are the two protons). No clear experimental evidence, however, was established to suggest the differentiation of two other possible pairs of enantiotopic directions in this molecule, namely, between the enantiotopic protons (3,4) and the carbon (1) or the protons (5,6,7) of the methyl group (see the numbering in Figure 2). Consequently, no measurable difference in the long distance dipolar couplings D_{1-3} and D_{1-4} or $D_{3-(5,6,7)}$ and $D_{4-(5,6,7)}$ was found. These results were rather frustrating because we also expected to differentiate these enantiotopic directions.

To provide an alternative to the PBLG system when this polymer gives poor or no successful results, we recently investigated the enantioselectivity of other liquid-crystalline homopolypeptides.[14] Thus we have shown that organic solutions of poly-ϵ-carbobenzyloxy-L-lysine (PCBLL) or poly-γ-ethyl-L-glutamate (PELG) may also provide noteworthy chiral liquid-crystalline NMR solvents. In this context, it was of interest to check if such chiral oriented systems could differentiate between the enantiotopic direction 1−3 and 1−4, as well as 3−(5,6,7) and 4−(5,6,7) in ethyl alcohol, which were not discriminated in the PBLG phase. For this purpose, we prepared a liquid-crystalline NMR sample (sample 3) using 16.2% of PCBLL in $CHCl_3$ (see Table 1 for details). The analysis of this new spectrum shows that the differentiation between enantiotopic directions 1−4 and 1−3, in terms of dipolar couplings, was not experimentally evident, meaning that these couplings are either fortuitously identical or that their difference is too small to produce a significant effect. This situation is, however, very different for the enantiotopic directions 3−(5,6,7) and 4−(5,6,7) as shown by the proton-coupled carbon-13 signal of the C2 carbon of ethanol embedded in the PCBLL/$CHCl_3$ system in Figure 2a. This spectrum is rather different from those obtained in PBLG. In both cases, the spectral pattern associated with the CH_2 group is a second-order spectrum mostly due to the inequivalence between the dipolar couplings, D_{2-4} and D_{3-4}. The main difference with the results obtained in PBLG (see ref 2 or the simulated spectrum in Figure 2e) lies, however, in the fact that resonances in the internal structure of signal are not equally centered on the chemical shift of C2 carbon. This we interpret as a consequence of the inequivalence between the $^1H-^1H$ enantiotopic directions, 3−(5,6,7) and 4−(5,6,7), in terms of dipolar couplings as well as in terms of proton chemical shift anisotropy for H_3 and H_4. Indeed, the simulation of the experimental spectrum performed using the program PANIC (supplied by Bruker) and displayed in Figure 2b has shown that all signals of the ^{13}C spectrum recorded in PCBLL are only reproduced when $D_{3-(5,6,7)}$ and $D_{4-(5,6,7)}$ and δ_3 and δ_4 were both different. For this simulation, the magnitude and the sign of isotropic scalar couplings, J_{ij}, used here were taken from our previous work on ethyl alcohol in the PBLG phase.[2] These values were kept fixed in the iterative analyses of the proton and carbon-13 spectra in the anisotropic medium. Also note that the $^1H-^1H$ dipolar couplings were kept constant during the fitting process for the analysis of the carbon-13 spectrum. Using these assumptions, the spectral iterative analysis of the proton-coupled carbon-13 spectrum for C2 gives a maximum deviation in line positions between the simulated and experimental spectra which is smaller than 0.15 Hz, and the root-mean-square (rms) error of the fit was equal to 0.14 Hz. The set of spin−spin couplings of the spin system that corresponds to the best fit between the experimental and calculated values (for the C2 carbon) is listed in Table 3. Note that the calculated difference between δ_3 and δ_4 is 0.0036 ppm at 400.1 MHz. To complete this study, the various other possible spectral patterns associated with the proton-coupled carbon-13 signal of the methylene carbon in a fragment RCH_2CH_3 have been considered and reported in Figure 2c−f. These simulations show clearly that a symmetrical spectral pattern is only obtained when there is equivalence in terms of dipolar couplings ($D_{2-(3,4)}$ and $D_{3,4-(5,6,7)}$) or the chemical shift anisotropy but not when these two spectral parameters are simultaneously different. Also, it should be remarked that when the couplings D_{2-3} and D_{2-4} are equivalent, the effects due to the inequivalence between $D_{3-(5,6,7)}$ and $D_{4-(5,6,7)}$ are never revealed in the proton-coupled carbon-13 spectrum.

Although no visible effects were detected using proton NMR, the indirect spectral consequences of the inequivalence between

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Table 3. Scalar and Dipolar Couplings Used To Fit the ^{13}C NMR Spectrum of the Ethylenic Group of **5** in the PCBLL/$CDCl_3$ Phase at 300 K

type of atoms	interacting nuclei[a]	no. of bonds	J_{ij}^{iso}/Hz[b]	D_{ij}^{exp}/Hz[b]
C⋯H	2−3 (or 2−4)	1	141.2 ± 0.2	−17.9 ± 0.2
C⋯H	2−4 (or 2−3)	1	141.2 ± 0.2	−26.2 ± 0.2
C⋯H	2−(5,6,7)	2	−4.6 ± 0.1	−0.4 ± 0.1
H⋯H	3−4	2	−9.4 ± 0.1[c]	−30.1 ± 0.2
H⋯H	3−(5,6,7)	3	7.1 ± 0.1	−2.6 ± 0.1
H⋯H	4−(5,6,7)	3	7.1 ± 0.1	−0.9 ± 0.1
H⋯H	(5,6,7)	2		+2.0 ± 0.1

[a] See Figure 2 for the atom numbering. [b] Experimental fitted values with the program PANIC. [c] The magnitude and the sign of J_{ij} are taken from ref 2.

$D_{3-(5,6,7)}$ and $D_{4-(5,6,7)}$, observed on the proton-coupled carbon-13 signal of the C2 atom, are evidence unequivocally of the first differentiation of $^1H-^1H$ enantiotopic directions in C_s symmetry molecules as well as the discrimination of enantiotopic protons on the basis of a proton chemical shift anisotropy difference. Until now such differentiation has never been directly observed in proton spectra, mainly due to the line width of ^1H resonances. In addition, we show that the enantiotopic discrimination in a CLC can also be observed in flexible molecules, namely, between nuclei which can be considered as fixed in the molecular structure and nuclei which are in fast rotation on the NMR time scale (here, the rotation of protons in the methyl group about the C1−C2 bond). To easily understand this result, we can consider that the methyl group has a 3-fold symmetry axis coincident with C1−C2.[15] The barrier to rotation of methyl groups with a 3-fold symmetry axis is generally of the order of 12 kJ·mol^{-1}, and so at 300 K the protons in this group can be confidently assumed to spend all of their time in the three equivalent minimum energy positions generated by rotation through 120°. The motion of the methyl group is therefore equivalent to jumps between these three sites, and we may assume that the location of the methyl protons is such that the C1−H5 bond lies in the symmetry plane, σ (see Figure 2).[2] In this model, the value for $D_{3-(5,6,7)}$ and $D_{4-(5,6,7)}$ experimentally measured can be seen as the weighted average of dipolar couplings D_{3-5}, D_{3-6}, D_{3-7} and D_{4-5}, D_{4-6}, D_{4-7}, respectively. The results obtained with ethyl alcohol oriented in a PCBLL imply that the mean values corresponding to $D_{3-(5,6,7)}$ and $D_{4-(5,6,7)}$ couplings are different.

Differentiation between Enantiotopic C−H Directions in C_{2v} Molecules Exhibiting No Prostereogenic Tetrahedral Center. Organic molecules of C_{2v} symmetry such as malononitrile (**6**) or difluoromethane (**7**) may be superimposed upon themselves by an overall π radian rotation and thus produce a structure that is indistinguishable from the original. Consequently, the protons and carbon nuclei of the nitrile group in **6** as well as the protons and fluorine nuclei in **7** are isochronous whatever the solvent used. In addition, the one-bond $^{13}C-^1H$ and $^{13}C-^{19}F$ directions in these molecules are homotopic and so cannot be differentiated even using a chiral liquid-crystalline medium. This stereochemical situation is no longer true when we consider the $^{13}C\cdots{}^1H$ and $^1H\cdots{}^{19}F$ directions in **6** and **7**, respectively, because the spatial relationship between them is enantiomeric (Table 1).[3] Under these conditions in chiral liquid crystals it should be possible to distinguish between enantiotopic directions in C_{2v} molecules exhibiting no prostereogenic tetrahedral center.[16] Therefore, we may expect to observe an AXX′ spin system for **6** (A is the carbon of the nitrile group and X and X′ are the protons) and an AA′XX′ spin system for **7** (A and A′ are the fluorine atoms and X and X′ are the protons). In fact, this point was enlightened long ago by Mislow and Raban, who speculated that in the case of "...molecules such as CH_2F_2, there should, in principle, be two H−F coupling constants in chiral solvent" because "the relationship of H_a with F_a and F_b in CH_2F_2 is enantiomeric".[3] The results (not shown here) obtained using proton or fluorine-19 NMR in the case of **7** oriented in the PBLG phase were very deceptive because whatever the sample temperature, the spin system associated with the proton and fluorine of the molecule cannot be classified as an AA′XX′ spin system but as an A_2X_2 spin system. This, of course, indicates that no inequivalence in the couplings D_{H-F} can be experimentally observed. It means also that these dipolar couplings are either fortuitously identical or that their difference is too small to provide a significant effect in the ^1H and ^{19}F spectra.

The results were more exciting when investigating malononitrile oriented in the PBLG/CH_2Cl_2 phase (sample 4) through proton-coupled carbon-13 NMR. As can be seen in Figure 3a, the pattern associated with the C2,3 carbons of **6** is a second-order spectrum mostly manifested as a triplet whose relative intensities are not 1:2:1 and flanked by two small external peaks, centered on $\delta_{C2,3}$. This spectral pattern seems to indicate that the spin system cannot be considered as an AX_2 but as an AXX′. In this situation, the couplings D_{2-4} and D_{2-5} (D_{3-5} and D_{3-4}, respectively) (see the numbering of **6** in Figure 3) are imperatively different, meaning that the corresponding $^{13}C-^1H$ enantiotopic directions are effectively differentiated in the PBLG system. The calculation of dipolar couplings derived from the iterative analysis of the proton-coupled carbon-13 spectrum for C2,3 carbons gives a difference of 5.3 Hz between D_{2-4} and D_{2-5}, their respective signed value being 0.0 ± 0.1 Hz and 5.3 ± 0.1 Hz. The root-mean-square deviation of the calculated D_{ij}'s, compared to the experimental values, is 0.06 Hz. The complete set of spectral parameters for **6** oriented in PBLG is summarized in Table 4. For comparison, Figure 3b shows the simulated spectrum using the NMRSIM program.[18]

To validate our hypothesis on the origin of the additional small external peaks, centered on $\delta_{C2/C3}$, two different approaches were explored. In a first step we recorded the carbon-13-coupled proton spectrum of **6** using the gradient-enhanced 1D HSQC sequence. The purpose of this sequence is to purge the signal of protons attached to carbon-12 atoms by applying pulse field gradients. Such a multiple-pulse 1D experiment directly derives from the 2D HSQC experiments, which were previously introduced by Bax and co-workers to determine the residual X−H dipolar couplings in proteins dissolved in weakly oriented liquid crystals.[19] For our purpose, we have used the basic pulse scheme of the gradient-enhanced HSQC experiment but kept constant (at 3 μs) the incremental delay of the 2D experiment. In fact, our aim was simply to extract the $^{13}C-^1H$ dipolar splittings from the carbon-13-coupled proton spectrum

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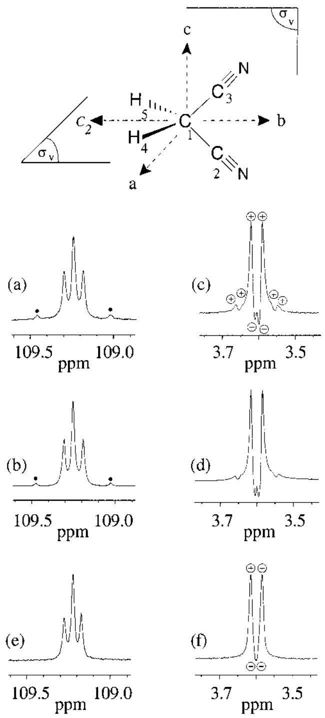

Figure 3. Top: Definition of axes labels (*a*, *b*, *c*) of the reference molecular coordinate frame and numbering system of atoms for the malononitrile. Bottom: (a and b) Experimental and simulated 100.6 MHz proton-coupled carbon-13 NMR signals of the C2,3 carbons of **6** embedded in the PBGL/CDCl$_3$ phase at 295.5 K. This spectrum was recorded using a 5 mm selective dual probe. A total of 23000 scans of 3K of data points are added, and a recycle delay of 1.5 s was used. Zero filling to 16K data points was employed to increase the digital resolution. No filtering window was used. Additional peaks due to the second-order effect are labeled using a black circle. (c and d) Experimental and simulated 400.1 MHz carbon-13-coupled proton NMR signals of the H5,6 protons of **6** using the gradient-enhanced 1D HSQC sequence. The proton gradient-enhanced HSQC experiment was achieved by adding 1024 scans of 4K data points and zero filling to 16K data points. The recycle delay was 2 s. Sinusoidally shaped *z*-gradient pulses in the ratio 80:30:20.1 were applied. A Gaussian filtering window was applied (GB = 25%, LB = −2 Hz). (e and f) Same 1D NMR experiments as in (a) and (c) but recorded in the PBG/CDCl$_3$ phase at 295 K. For both series of spectra the experimental parameters are identical. Note the disappearing of external small peaks in the proton-coupled carbon-13 NMR spectrum.

Table 4. Scalar and Dipolar Couplings Used To Fit the ^{1}H and ^{13}C NMR Spectra of **6** in the PBLG/CDCl$_3$ Phase at 295 K

type of atoms	interacting nuclei[a]	no. of bonds	J_{ij}^{iso}/Hz[b]	D_{ij}^{exptl}/Hz[c]	D_{ij}^{calcd}/Hz[d]
C···H	1−4 (or 1−5)	1	140.0 ± 0.1	47.5 ± 0.2	47.4 ± 0.3
C···H	2−4 (or 3−5)	2	−11.1 ± 0.2	0.0 ± 0.3	0.7 ± 0.3
C···H	2−5 (or 3−4)	2	−11.1 ± 0.2	5.3 ± 0.3	6.0 ± 0.3
H···H	4−5	2	−20.3 ± 0.2[c]	1.1 ± 0.3	1.2 ± 0.3

[a] See Figure 4 for the atom numbering. [b] The value and the sign of J_{3-4} are taken from literature (ref 23). [c] Experimental fitted values with the program PANIC. [d] Dipolar couplings calculated from the program SHAPE.

in a relatively short experimental time. In Bruker's pulse program library, the program is referred to as *invigpnd1d*. Note that as the sample does not contain deuterated solvent, the lock blanking commands employed for avoiding the field drift when the gradients are opened were removed. To optimally observe the carbon-13-coupled proton signal (H5,6) of isotopomers containing a carbon-13 atom in the nitrile group, the interpulse delay for the evolution of the two-bond ^{1}H−^{13}C total spin−spin couplings must be carefully adjusted. In our case, the optimal delay was set to 30.1 ms.

Figure 3c shows the carbon-13-coupled proton spectrum of **6** in the PBLG phase. The analysis of this proton spectrum is not straightforward. To facilitate its interpretation, we have simulated the proton 1D HSQC spectrum of **6** using NMRSIM and the spectral data listed in Table 4. Simulated and experimental parameters for the pulse sequence are identical. The theoretical spectrum is displayed in Figure 3d. Due to the inequivalence between the two carbon−proton dipolar couplings, D_{2-4} and D_{2-5}, we obtain a second-order eight-line multiplet (the XX′ part of an AXX′ spin system), featured by six in-phase (⊕) and two antiphase (⊖) components. The in-phase and antiphase components are a consequence of the signal modulation under the proton−proton dipolar coupling effect in the HSQC sequence, but this effect does not affect the analysis of the spectrum. The comparison between the calculated and experimental 1D HSQC spectra exhibits no spectral difference and so confirms the inequivalence between the dipolar couplings, D_{2-4} and D_{2-5}.

To definitely establish that D_{2-4} and D_{2-5} are different, we have, in a second approach, recorded the proton-coupled carbon-13 spectrum in a nonchiral nematic solvent made of a racemic mixture of PBLG and its enantiomer, the PBDG, in CH$_2$Cl$_2$ (sample 5). In such a racemic mixture, which is denoted as PBG hereafter, the *S* and *R* isomers of a chiral molecule are in the fast exchange limit by diffusing very rapidly, on the NMR time scale, from the vicinity of PBLG and PBDG. Consequently, we observe only an average of these situations, thus eliminating all chiral discriminations.[20] Obviously because of the loss of chirality of the solvent in the PBG system, all enantiotopic discriminations, which were visible in the PBLG phase, are also eliminated. In this situation, the magnetic inequivalence between the enantiotopic direction is lost, and hence a simple triplet with a 1:2:1 relative intensity is expected to be observed for the C-13 signal of carbons 2 and 3. In Figure 3e, it can be effectively seen that the spin system can no longer be considered as an AXX′ but as an AX$_2$ spin system. The dipolar couplings D_{2-4} and D_{2-5} are now identical, and both are equal to +2.9 ± 0.1 Hz. Here again, the analysis of the experimental gradient-enhanced 1D HSQC spectrum presented in Figure 3f confirms that the dipolar couplings D_{2-4} and D_{2-5} are equivalent, since the small in-phase external resonances which were visible in spectrum c have now disappeared. Finally, note that, disregarding a small discrepancy due to solvent effects, the dipolar couplings, D_{2-4} and D_{2-5}, measured in the PBG phase correspond to the algebraic average of D_{2-4} and D_{2-5} measured in the PBLG phase, accordingly with what can be expected to be found.[19]

The analysis of the proton−carbon spectrum in PBLG and in PBG and their confirmations using HSQC experiments proves undoubtedly that the differentiation of the enantiotopic directions in C_{2v} molecules exhibiting no prostereogenic tetrahedral center is possible using NMR spectroscopy in CLC. In other words, we have demonstrated that it is possible to turn magnetically equivalent nuclei of such compounds in isotropic media into magnetically inequivalent ones when dissolved in chiral oriented phases. Although rather academic, this result is the first successful attempt[3,4] illustrating theoretical speculations made by Mislow and Raban, who predicted, in the case of CH$_2$F$_2$, that an enantiotopic proton (or fluorine nuclei) would become anisogamous in a chiral solvent.[3]

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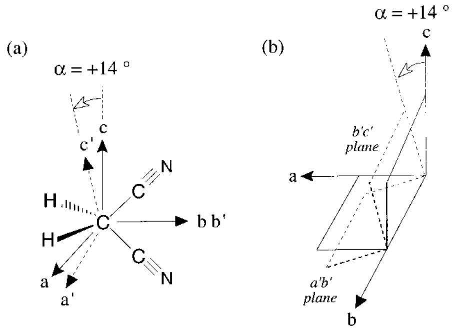

Figure 4. (a) Space representation of the principal axes of malononitrile in the PBLG system relative to the initial axes. The eigenvectors associated to the eigenvalues of the diagonal matrix are orthonormal. (b) Representation of the *b*′*c*′ and *a*′*b*′ planes relative to the initial *bc* and *ab* planes seen from the *b* axis. The tilting angle α between the initial and final planes is +14°.

Table 5. Principal Components, $S_{\alpha'\alpha'}$, of the Diagonalized Matrix and Biaxiality Term

parameters[a]	values[b]
$S_{a'a'}$	−0.0005
$S_{b'b'}$	−0.0064
$S_{c'c'}$	+0.0069
$(S_{aa'} - S_{b'b'})/S_{c'c'}$	+0.855

[a] The $S_{\alpha'\alpha'}$ values are reported with respect to the principal molecular axis system pictured in Figure 6. [b] The error on the $S_{\alpha'\alpha'}$ values is ±0.0002.

From the spectral data, we have calculated the three nonzero independent elements of the molecular ordering matrix $S_{\alpha\beta}$ to describe correctly the molecular ordering of **6** in a CLC,[1] with respect to the molecule fixed Cartesian coordinate system reported in Figure 4. These values, derived from the measurements of the ^{1}H−^{1}H and ^{13}C−^{1}H dipolar couplings, were obtained by a least-squares fitting method using the SHAPE program initially developed by Diehl et al.,[21] assuming a model geometry for **6**. The values of S_{bb}, S_{cc}, and S_{ac} are equal to -6.4×10^{-3}, $+6.5 \times 10^{-3}$, and $+1.7 \times 10^{-3}$, respectively. The error of these various values is evaluated to be $\pm 2 \times 10^{-4}$. The ^{13}C−^{1}H and ^{1}H−^{1}H dipolar couplings calculated from these order parameters are included in Table 4. The calculated values of D_{ij} agree rather well with all experimental values, and the root-mean-square error of the calculated D_{ij}'s, compared to the experimental values, is 0.49 Hz. The maximum deviation between the calculated and experimental dipolar couplings is below 0.7 Hz. Note that no significant variations in the calculation of $S_{\alpha\beta}$ were found when using the geometry given in ref 22 or calculated geometries derived from classical, semiempirical, or ab initio models. Actually, this result could probably be enhanced if the dipolar couplings were corrected for the effects of molecular harmonic vibrational motions. The ordering tensor of **6** can be diagonalized to give both their eigenvalues, $S_{\alpha'\alpha'}$, and their associated eigenvectors (a',b',c').[24] The three nonzero diagonalized order parameters are listed in Table 5 together with the biaxiality term ($S_{a'a'} - S_{b'b'}/S_{c'c'}$). Mathematically, the transformation of the initial axis system (a,b,c) into the principal axis system (a',b',c') can be viewed as a simple rotation around the *b* axis. In this example, the α tilt angle is equal to +14 ± 2°. This rotation implies that the principal axes *a*′ and *c*′ are not included in the symmetry plane of the molecule. In other words, the *a*′*b*′ and *b*′*c*′ planes formed by the eigenvectors *a*′,*b*′,*c*′ of the ordering principal axis system no longer coincide with the *ab* and *bc* molecular planes of symmetry, respectively, as shown in Figure 4. Actually, the value of the α angle provides a measure for the magnitude of the discrimination of enantiotopic directions in C_{2v} molecules. Also, note that the sign of the S_{ac} order parameter and, subsequently, the sign of the α tilt angle depend on the assignment of D_{2-4} and D_{2-5}, which is totally arbitrary here. By simply reversing their respective values (given in the Table 4), we obtain $S_{ac} = -1.7 \times 10^{-3}$ and $\alpha = -14 \pm 2°$. This situation shows that, from the order parameter calculations only, we are not able to define a priori the absolute configuration of the 2−4 (3−5) and 2−5 (3−4) enantiotopic directions.

Conclusions

In this paper, we have presented and discussed NMR experimental evidence of differentiation of enantiotopic directions in C_s molecules as well as in C_{2v} molecules with or without a prostereogenic tetrahedral center, using ^{13}C and ^{2}H-{^{1}H} NMR in the natural abundance level. The various results described in this work experimentally validate the theoretical arguments which predict that the NMR spectra of nonplanar C_s and C_{2v} compounds embedded in chiral oriented media should differ from those recorded in nonchiral oriented media, because the molecular point group of these molecules and their effective molecular point group in a CLC are not the same.[1] The final step of this demonstration will consist of examining the NMR spectra of S_4 and D_{2d} molecules that are also expected to be affected by the symmetry reducing effect when they are dissolved in a chiral oriented phase. This study is currently underway.

Although rather academic the experimental differentiation of enantiotopic directions in malononitrile is of interest in stereochemistry because this result raises a fundamental question about the definition of prochirality. The results reported here suggest that malononitrile can be regarded as prochiral when interacting with the chiral liquid crystal phase. Consequently, it is legitimate to define whether this type of molecule can be considered as a prochiral molecule in isolation even though it does not possess a prostereogenic tetrahedral center. Actually, this occurrence has already been conceptually discussed by S. Fujita, who considers that "the compounds (CX_2Y_2) can be regarded as prochirals, since the four edges (X−Y) construct an enantiospheric C_{2v}(C_1) orbit".[16] The concept of enantiospheric orbits is not simple, and to date this theoretical approach of the problem is not commonly in use among organic (bio)chemists who generally define prochirality in terms of the pratical possibility that an achiral molecule becomes chiral in the course of a single-step chemioselective chiral process. In other words, the main (and earliest) rules used by the majority of chemists avoid treating such classes of C_{2v} compounds as prochiral.[17] This is mainly because, to date, the symmetry or substitution criteria employed as a test for the definition of prochirality involve only groups (atoms)[3] but never intramolecular directions. Since all CX_2Y_2 molecules contain enantiotopic directions between nonbonded nuclei which can be discriminated in a CLC in the same way as all CX_2YZ molecules which contain enantiotopic directions involving bonded or nonbonded atoms, consequently they can be called prochiral. Our own conclusion is therefore in total agreement with Fujita's terminology. In conclusion, this last experimental result illustrates for the first

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time the purpose of Raban and Mislow as well as it experimentally validates the extended concept of prochirality proposed by Fujita. In light of his theoretical approach and our experimental work, a better recognition of this new stereogenicity definition could rapidly emerge in the community of organic chemists.

Finally, this investigation shows the practical interest in examining the various possible order-sensitive NMR interactions (averaged to zero in isotropic liquid) in oriented media for observing enantiotopic elements in molecules. It originates from the remarkable analytical potential of NMR in CLC compared with classical isotropic NMR methods which are based only on ^{1}H shift anisochronies and generally required the preparation of specific chiral solvating agents for a class of prochiral molecules.

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