Analysis of natural abundance deuterium NMR spectra of enantiomers in chiral liquid crystals *via* 2D auto-correlation experiments

Denis Merlet,[a] Bernard Ancian,[b] William Smadja,[a] Jacques Courtieu[a] and Philippe Lesot*[a]†

[a] *Laboratoire de Chimie Structurale Organique, ICMO, URA CNRS 1384, Université de Paris-Sud, 91405 Orsay, France*

[b] *Université Paris VII-Denis Diderot, Département de chimie, 2 Place Jussieu, 75251 Paris cedex 05, France*

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Natural abundance deuterium auto-correlation 2D NMR experiments of enantiomers orientated in a chiral polypeptide liquid crystalline solvent (PBLG) are used as a novel analytical tool for the study of chiral solutes.

Enantiomeric analysis *via* NMR spectroscopy is a real challenge for organic chemists.[1–3] In a recent study we have shown the feasibility of visualisation of chiral molecules orientated in the poly(γ-benzyl L-glutamate) (PBLG) liquid crystalline system using proton-decoupled natural abundance deuterium NMR spectroscopy.[4] The proton-decoupled deuterium (^{2}H-{^{1}H}) NMR spectrum of an oriented molecule containing a single deuterium nucleus ($I = 1$) consists of a quadrupolar doublet separated by $\Delta\nu_Q = 3/2(e^2qQ/h)S_{C-D}$, where (e^2qQ/h) is the quadrupole coupling constant and S_{C-D} is the order parameter along the C–D bond.[2,4,5] In a chiral medium the enantiomeric discrimination is observed *via* the measurement of the quadrupolar splitting differences ($\Delta\nu_Q^R - \Delta\nu_Q^S$) and we have shown that the sensitivity of natural abundance deuterium NMR is sufficient to measure the 'Differential Ordering Effects' (DOE) without isotopic enrichment.[4]

In natural abundance deuterium NMR spectroscopy the spectral analysis in an anisotropic medium is simplified due to the absence of residual dipolar couplings between two rare atoms. However, since all deuterons of the chiral solute are simultaneously probed, the spectrum may appear extremely complex for large molecules, due to the excessive overlapping of peaks. In order to illustrate this, the natural abundance ^{2}H-{^{1}H} NMR spectrum of (±)-but-3-en-2-ol dissolved in the PBLG–$CHCl_3$ phase at 301 K is shown in Fig. 1(*a*).‡ The spectrum was recorded at 38.4 MHz using a Bruker AM 250 spectrometer equipped with a 5 mm diameter inverse broadband probe in the unlocked mode.§

In the racemic mixture of but-3-en-2-ol, twelve different chiral isotopomers exist. Consequently, a maximum of 24 peaks (12 doublets), disregarding the solvent doublet, are expected in the natural abundance ^{2}H spectrum, assuming that all deuteron sites are discriminated in a chiral medium. In the experimental 1D spectrum 14 lines of various intensities are clearly observed [Fig. 1(*a*)]. A first analysis of the spectrum based on the chemical shifts allowed us to attribute some of the signals, such as the two quadrupolar doublets for the methyl group (δ 1.19) [Fig. 1(*a*),(*b*)] and the doublet of solvent, which is used as internal reference (δ 7.30) [Fig. 1(*a*),(*g*)]. But we were enable to assign easily the other signals visible in the spectrum. When the assignment problem becomes acute, the use of 2D auto-correlation deuterium NMR experiments is needed in order to attribute the two components of each quadrupolar doublet. A method for the assignment of quadrupolar doublets in perdeuterated liquid crystal molecules was developed many years ago by Emsley and Turner using the pulse sequence $\pi/2 - t_1/2 - \pi/2 - t_1/2 -$ acq.(t_2).[6] However, the analysis of the 2D spectrum is only possible when the deuterons are dipolar coupled, which is not the case in natural abundance ^{2}H-{^{1}H} NMR. In order to resolve specifically the analysis of overcrowded natural abundance deuterium spectra, we propose applying a new 2D pulse sequence developed using the product-operator formalism for spin $I = 1$ nuclei. The basic idea of this sequence is to maximise the intensity of cross peaks which auto-correlate the components of each quadrupolar doublet in the 2D contour plot. This 2D experiment is then complemented by a composite pulse decoupling using the WALTZ-16 sequence to remove the proton–deuterium scalar and dipolar couplings.

The sequence proposed is derived from a COSY experiment in which a π read pulse must be used to transfer coherences in the proper way. In this experiment the diagonal peaks cancel out and only the '*N*-type' cross peaks[7] are observed in the 2D spectrum with a single scan. Disregarding all relaxation terms and phase factors, the expression of the signal during the acquisition period (t_2) is given by eqn. (1).

$$S(t_1,t_2) = e^{[-i(\omega + \pi\Delta\nu_Q)t_1]}.e^{[i(\omega - \pi\Delta\nu_Q)t_2]} + e^{[-i(\omega - \pi\Delta\nu_Q)t_1]}.e^{[i(\omega + \pi\Delta\nu_Q)t_2]} \quad (1)$$

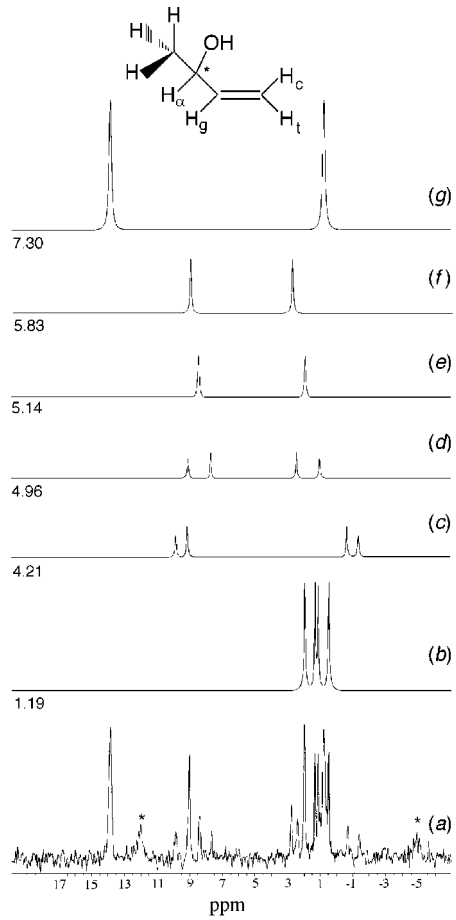

Fig. 1 (*a*) Natural abundance ^{2}H-{^{1}H} NMR spectrum of (±)-but-3-en-2-ol. (*b*)–(*g*) Fitted subspectra of all deuterated isotopomers in the mixture: (*b*) CDH_2, (*c*) D_α, (*d*) D_t, (*e*) D_c, (*f*) D_g and (*g*) $CDCl_3$. The signals of the hydroxy group (*) could not be clearly separated from noise and were not fitted.

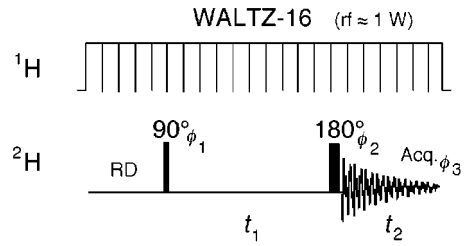

Fig. 2 Schematic diagram of the 2D Q-COSY pulse sequence.

Table 1 Phase cycling[a] of the Q-COSY sequence

ϕ_1	ϕ_2	ϕ_3
x	x	x
x	y	$-x$
x	$-x$	x
x	$-y$	$-x$

[a] The total phase cycling (16 steps) is obtained by applying the CYCLOPS procedure.

The pulse scheme and the phase cycling of the sequence denoted 'Quadrupole-COSY' (Q-COSY) are shown in Fig. 2 and Table 1, respectively. The phase cycling is identical to that used in a classical COSY experiment. As the pulse sequence basically produces a 'pseudo-quadrature' in the F_1 dimension, the 2D spectrum should be displayed in the magnitude mode to avoid the so-called 'phase-twist' lineshape.[7] The analysis of the Q-COSY sequence using the product operator-formalism shows that during the t_2 period only single quantum coherences are different from zero. Thus, this sequence is more sensitive, by a factor of 2, than the sequence of Emsley and Turner.[6] In other words it means that the Q-COSY sequence drives to maximum sensitivity, which is a major advantage for the observation of molecules *via* natural abundance deuterium NMR spectroscopy.

In order to explore the potentialities of the sequence, the natural abundance ^{2}H Q-COSY 2D spectrum of (±)-but-3-en-2-ol dissolved in the PBLG–$CHCl_3$ phase at 301 K was recorded (Fig. 3). The 2D spectrum was obtained using a spectral digitisation of 256 (t_1) × 1024 (t_2) data points, 848 transients per FID were added, leading to a total number of 217088 scans and a total experiment time of around 53 h.¶

As expected, the observation of the cross peaks in the 2D surface enable us to separate all sets of quadrupolar doublets. The comparison with the 1D spectrum recorded under the same conditions then allows us to attribute unambiguously each doublet. Thus, from the 2D contour plot, we can clearly deduce that the peak located at δ9.05 on the 1D spectrum is fortuitously made of three different lines corresponding to three different isotopomers. The same situation arises for the NMR line at δ1.95. These two examples illustrate the usefulness of 2D auto-correlation experiments in enantiomeric analysis *via* natural abundance ^{2}H-{^{1}H} NMR.

From the spectral analysis of the 2D spectrum all the clearly observable quadrupolar doublets in the 1D spectrum could be independently fitted for each isotopomer of mixture as presented in Fig. 1(b)–(g). The analysis shows that only three out of the six possible couples of chiral isotopomers are discriminated, *i.e.* the methyl group, the chiral centre (D_α) and *trans*-position (D_t) in the vinyl group. In addition, because the C–D bonds for the *cis*- and *gem*-deuterons (D_c and D_g) in the vinyl group are more or less parallel, they show a very similar orientation relative to the magnetic field and they must then exhibit the same behaviour relative to the chiral discrimination. In our case the quadrupolar splitting difference between the *cis*- and *gem*-positions is < 3%, and both are not discriminated, thus confirming our spectral assignment.

In conclusion we have demonstrated that natural abundance deuterium 2D auto-correlation experiments with routine magnetic field strengths (5.87 T) and standard NMR instrumentation are feasible and provide crucial information for enantiomeric analysis in chiral liquid crystals. The total experimental time needed to obtain a reasonable signal-to-noise ratio for each deuterium signal on a 250 MHz spectrometer may appear as an important limitation of this technique. However the use of higher magnetic fields will greatly reduce the experimental time.[4] Thus, operating on a 400 MHz spectrometer, only *ca*. 15 h would be needed to reach the same signal-to-noise ratio for (±)-but-3-en-2-ol. Finally, we note that natural abundance deuterium 2D experiments have sensitivities comparable with those of the carbon-13 2D INADEQUATE experiments which are commonly used.[7] Additional studies of the scope of the Q-COSY sequence (sensitivity, resolution improvement and phase sensitive mode) are currently underway. The sequence reported in this study belongs to a new class of 2D auto-correlation experiments for the NMR analysis of spins $I = 1$ in oriented media that we denote 'QUadrupole Ordered SpectroscopY' (QUOSY). Other 2D auto-correlation experiments are currently under development to assess their analytical potential in natural abundance deuterium NMR spectroscopy.

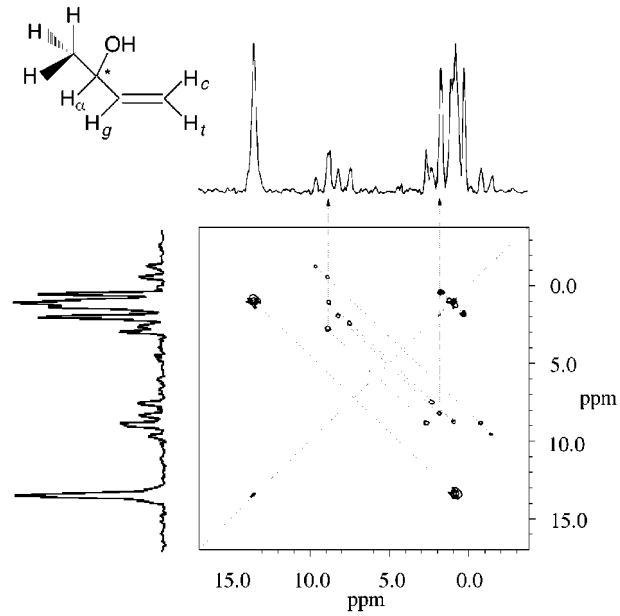

Fig. 3 2D Contour plot of proton-decoupled natural abundance deuterium Q-COSY experiment obtained for (±)-but-3-en-2-ol.

Notes and references

† Corresponding author: E-mail: philesot@icmo.u-psud.fr

‡ *Sample composition* : The NMR sample was made from racemic substrate (100 mg), PBLG (100 mg) with a DP = 854, MW ≈ 188000 (Sigma), and $CHCl_3$ (350 mg). The sample preparation is described in refs. 2–4.

§ *1D Experiment*: The sample was not spun in the field and the temperature was controlled by a BVT 1000 system. The spectrum was recorded using 60° pulses, a 0.5 s recycling delay and 150 000 scans with 1 K data points (around 20 h of experiment time) (see ref. 4). Zero filling to 4 K data points without apodisation was applied. The linewidths of the solute are 3–7 Hz.

¶ *2D experiment*: The recycling delay of the sequence is of 0.75 s. The spectral width in the F_1 and F_2 dimensions is 1500 Hz. A Gaussian filtering in both dimensions ($LB_1 = -4$ Hz, $GB_1 = 20\%$ and $LB_2 = -3$ Hz, $GB_2 = 15\%$) and a symmetrisation procedure were applied.

1 D. Parker, *Chem. Rev.*, 1991, **91**, 1441.
2 I. Canet, J. Courtieu, A. Loewenstein, A. Meddour and J. M. Péchiné, *J. Am. Chem. Soc.*, 1995, **117**, 6520 and references cited therein.
3 A. Meddour, P. Berdagué, A. Hedli, J. Courtieu and P. Lesot, *J. Am. Chem. Soc*., 1997, **119**, 4502.
4 P. Lesot, D. Merlet, A. Loewenstein and J. Courtieu, *Tetrahedron*: *Asymmetry*, 1998, **9**, 1871.
5 J. W. Emsley and J. C. Lindon, *NMR Spectroscopy Using Liquid Crystal Solvents*, Pergamon, Oxford, 1975.
6 J. W. Emsley and D. L. Turner, *Chem. Phys. Lett.*, 1981, **82**, 447.
7 R. R. Ernst, G. Bodenhausen and A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Clarendon, Oxford, 1987.

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