

The NMR Spectra of Samples Dissolved in Liquid–Crystalline Phases: Automatic Analysis with the Aid of Multiple Quantum Spectra—The Case of Flexible Molecules

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Received February 25, 1999; revised August 26, 1999

Homonuclear $N^S = 0$ and heteronuclear $N^S \neq 0$ multiple quantum spectra, involving changes in the magnetic number m^I by $(N^I - 1)$, $(N^I - 2)$, and $(N^I - 3)$, with N^I and N^S the number of interacting nuclei of magnetogyric ratio γ^I and γ^S , are used for the automatic analysis of ^1H NMR spectra of flexible molecules dissolved in liquid–crystalline phases. The automatic procedure has been applied to study molecules of general formula $\text{Ph-CH}_2\text{-X}$ starting from a parameter set having all the spectral parameters set to zero. The results of such an analysis are then used as starting parameters for analysis of the single quantum spectrum. The method was first tested when $X = \text{Br}$ and $X = \text{H}$ in order to compare strategies differing for the types of parameters used and was then applied to the analysis of 3-phenylprop-1-yne. © 2000

Academic Press

INTRODUCTION

Multiple quantum spectroscopy involves transitions with a change in the magnetic quantum number $|\Delta m| = N, (N - 1), (N - 2), \dots, 0$ for N spin-1/2 interacting nuclei (I). This technique is a powerful aid in the spectral analysis procedures used for the analysis of the very complex SQ ($\Delta m = \pm 1$) NMR spectra generated by up to eight to nine spin system molecules dissolved in liquid crystals (2). Recently we have described a new approach to fully automatic analysis of single quantum spectra, which uses $(N - 1)$ MQ spectra. The method worked properly for rigid molecules of C_{2v} symmetry with a maximum of six interacting protons (3).

Anisotropic spectra are difficult to analyze since, even for the simplest spin system, it is not easy to guess reasonable values of the spectral parameters. This happens because dipolar couplings D_{ij} , whose values range typically from -3000 to 1500 Hz (4), dominate spectra. As a consequence, no first-order features can be observed and it is not possible to guess parameter values from frequency/intensity patterns as is usually done for isotropic spectra. On the other hand the available standard procedures for the analysis of SQ spectra need a starting parameter set as similar as possible to the final one, the

condition becoming more strict as the spectrum complexity increases.

High-order $(N-1)$ and $(N-2)$ MQ spectra have a limited number of lines, a maximum of $2N$ and $N(2N-1)$, respectively, whose frequencies can be easily calculated by a modified version (3) of standard computer programs which use the Castellano Bothner-By (5) (CB-B) algorithm to optimize the parameters by fitting the calculated versus the experimental frequencies. It is important to note that the algorithm does not make use of line intensities; on the other hand intensity calculation in MQ spectra is a time-consuming task even for the simplest spin system (6).

In principle it is possible, using a trial and error approach, (i) to find a set of parameters which approximately reproduces the $(N-1)$ and/or the $(N-2)$ MQ frequencies, (ii) to refine them by the iterative algorithm, and then (iii) to calculate a SQ spectrum to be matched against the experimental one. The parameter set obtained from steps (i) and (ii) by no means assures us that the experimental SQ spectrum will be properly reproduced. Since frequencies and intensities depend on linear combinations of parameters, many combinations of parameters reproducing the few experimental frequencies of the $(N-1)$ and $(N-2)$ spectra will easily be found, but only a few of them, if any, could properly reproduce the SQ spectrum. When this happens, only assigning the highest possible number of lines in the experimental SQ spectrum assures that individual parameters can be determined with high accuracy and low correlation.

For rigid molecules (3) this problem can be overcome if the Saupe order matrix elements (7) are used as independent variables and the dipolar couplings are calculated from assumed bond lengths and angles. Only a maximum of five variables are needed and their number is further reduced for symmetric molecules. Using only the $(N-1)$ multiple quantum refocused (MQR) spectra (no frequency dependence on chemical shifts and symmetric spectra), starting with the order parameters set to zero, and cycling over all the possible permutations of the experimental frequencies, the correct set of direct couplings will be calculated. Some bias is introduced in the procedure

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since the geometry must be properly guessed, but the individual parameters are correctly determined within the linear combinations since order parameters are not correlated. The chemical shifts can be determined by the correspondent MQ spectrum, using the D_{ij} just found. As the last step, the 1Q spectra can be analyzed with a DANSOM-like algorithm (8) iterating on the full set of spectral parameters.

Unfortunately such an approach cannot be extended *sic et simpliciter* to molecules with internal rotation degrees of freedom since (i) the couplings connecting different rigid subunits cannot be predicted from geometrical considerations alone, with a consequent large increase in the number of independent parameters, and (ii) the inclusion of experimental frequencies from lower MQ order spectra is not feasible since the number of assignments increases to impracticable values.

In this paper we present some implementations of the previously proposed approach in order to verify whether the automatic procedure can be extended to nonrigid molecules too. The methodology is based on the following steps:

(1) Acquisition of the SQ, ($N-1$), ($N-2$), and possibly ($N-3$) MQ and MQR spectra.

(2) Fully automatic analysis using the MQR frequencies to obtain approximate values of the D_{ij} . This step differs from the procedure described for rigid molecules in three ways. (i) The principal component regression method was applied in order to determine the linear combinations of parameters which have a large effect on the calculated frequencies from others which can be safely kept constant in the first iterations cycles when only ($N-1$) MQ frequencies are considered. (ii) The full *permutative approach* is limited to the ($N-1$) MQ spectrum but frequencies from lower order spectra can be automatically assigned, and (iii) mixed sets of parameters (order parameters to calculate the direct couplings between nuclei in the rigid subunits and the D_{ij} between nuclei belonging to different subunits) can be used. The chemical shifts are then calculated from the MQ spectra.

(3) Refining of the spectral parameters of step (2) on the SQ spectrum, either in an automatic way or following the traditional assignment method.

The procedure was tested on a series of flexible molecules of general formula $\text{ArCH}_2\text{-X}$ with $\text{X} = \text{Br}, \text{H}, \text{C} \equiv \text{CH},$ and CH_3 . The anisotropic spectra of the first two molecules have already been analyzed and the conformational equilibrium has been determined (9). For this reason they were used to test the procedure. No information is available in the literature about the preferred conformation of 3-phenylprop-1-yne, whereas the conformational equilibrium of ethylbenzene in a liquid phase is still the subject of debate (10). The selectively deuterated isotopomer [1-*d*]-3-phenylprop-1-yne was synthesized and its ^1H spin-echo MQ and SQ spectra were recorded.

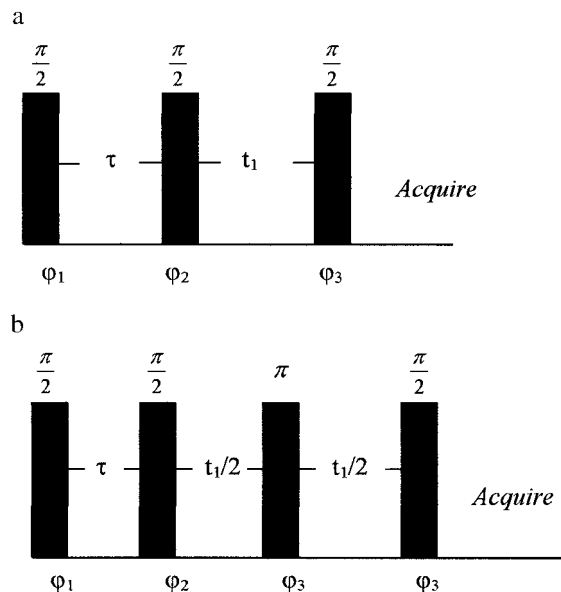


FIG. 1. Pulse sequence for obtaining multiple quantum spectra (a) which include (MQ spectra) and (b) which exclude chemical shifts (MQR spectra).

NMR SPECTRA

All the spectra were recorded on a 7.04-T Bruker AC 300 spectrometer at room temperature using phase cycling as an order-selective detection methodology to obtain the desired p quantum order (2a, 11) with the pulse sequences shown in Fig. 1. The pulsed field gradient technique (12) was also tried, but the instrumental time needed to obtain spectra with good signal-to-noise (S/N) ratio was comparable to the time needed for the phase-cycling technique and so this latter method was preferred. For all these spectra the pathway $+p$ was selected by performing $4p$ experiments with the phases $\varphi_1 = \varphi_2$ advanced in steps of $2\pi/4p$ and the receiver cycling through 0° , 90° , 180° , and 270° .

The MQ spectra are obtained as the skyline projection (13) on the F_1 dimension of the 2D data set. For each system all spectra were recorded in a single long-lasting instrumental session; SQ spectra were recorded between MQ experiments to be sure that the experimental conditions did not change.

Homonuclear spin systems. The first step of the procedure consists of the acquisition of the complete series of NMR spectra: SQ, ($N-1$), ($N-2$), and ($N-3$), if needed, with (MQR) and without (MQ) a refocusing pulse in the middle of the t_1 evolution period.

Many spectra were recorded at different τ , since line intensity depends on τ , we were able to obtain spectra with better S/N ratio and to recognize and discard ghost lines due to pulse imperfections.

($N-1$) MQR spectrum. In general the ($N-1$) spectrum will contain $k \leq 2N$ lines for a total of $P^{(N-1)} = k!$ possible assignments of observed to calculated frequencies. Not all the

assignments are distinct since the refocusing pulse, removing the dependence on chemical shifts, makes the spectrum symmetric. In this way it is possible to halve the number of permutations, leaving it to the operator to decide the correct parameter sign. The number of permutations can be further reduced by noting that, as a consequence of the symmetry, all the independent permutations can be found by assigning just one-half of the spectrum but allowing the frequencies, f_i , to be either positive or negative. If $k = 8$, all independent permutations are to be found by performing the permutations within the following subsets:

$$\begin{aligned}
 &+ f_1, + f_2, + f_3, + f_4 & - f_1, + f_2, + f_3, + f_4 \\
 &+ f_1, - f_2, + f_3, + f_4 & + f_1, + f_2, - f_3, + f_4 \\
 &+ f_1, + f_2, + f_3, - f_4 & + f_1, - f_2, - f_3, + f_4 \\
 &+ f_1, - f_2, + f_3, - f_4 & + f_1, + f_2, - f_3, - f_4.
 \end{aligned}$$

The total number is then $P^{(N-1)} = 2^{k/2}/2 \cdot (k/2)!$. Thus $P^{(N-1)} = 192$ versus $P^{(N-1)} = \frac{8!}{2} = 20,160$ if all the permutations were calculated.

(N-2) and (N-3) MQR spectra. The lower order MQ spectra are characterized by a larger number of lines with respect to the $(N-1)$ MQR spectrum. The magnetization is spread over more transitions and some lines become too weak to be observed. This means that theoretical predictions of the number of lines always overestimate the number of transitions which can actually be observed and assigned in analyzing the spectrum. For the molecules benzylbromide and toluene, the $(N-2)$ MQR spectrum should have 40 lines and the $(N-3)$ 57; the number of transitions that can be safely recognized is less, depending on the liquid-crystal solvent used. However, not all the $(N-2)$ and $(N-3)$ frequencies are independent since transitions belonging to different quantum orders are related to each other. If the $k/2$ frequencies of the positive half of the $(N-1)$ MQR spectrum are assumed to be independent, only the $N(N-1)$ transitions $|r\rangle \rightarrow |s\rangle$ (from a total of $N(2N-1)$) between energy levels with $m_r = -N/2 \rightarrow m_s = (-N/2 + \Delta m)$ and $m_r = (N/2 - \Delta m) \rightarrow m_s = N/2$ are linearly independent and contain new information about the parameters; the remaining N^2 transitions, which involve energy levels with $m_r = -(N/2 - 1) \rightarrow m_s = (N/2 - 1)$, are a linear combination of the k frequencies calculated for the $(N-1)$ MQ spectrum.

This linear dependence can be easily demonstrated by considering the energy-level diagram for a simple four-spin system sketched in Fig. 2.

Each transition of order $(N-2)$ between energy levels $m_r = -1 \rightarrow m_s = 1$ is the sum of two $(N-1)$ MQR transitions, one with $m_r = -2 \rightarrow m_s = 1$ and the other with $m_r = -1 \rightarrow m_s = 2$; for example, $F_{2 \rightarrow 12} = E_2 - E_{12}$ can be expressed as

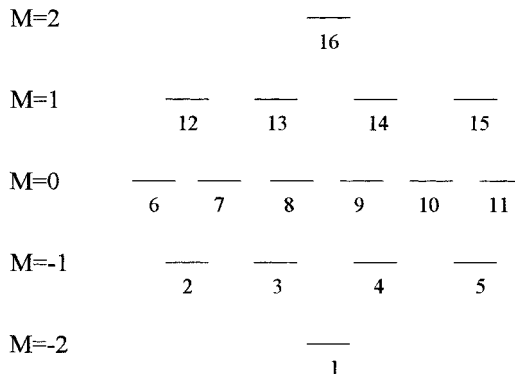


FIG. 2. Energy levels diagram for a four-spin system; M is the total magnetic quantum number.

$$F_{1 \rightarrow 12} + F_{2 \rightarrow 16} = E_1 - E_{12} + E_2 - E_{16} = E_2 - E_{12},$$

since $E_1 - E_{16}$ is a constant and can be set equal to zero, for a given set of spectral parameters.

This rule can also be usefully applied to the $(N-3)$ MQR spectrum, in which case $2N$ transitions are linearly independent and $N^2(N-1)$ frequencies are a linear combination of the $N(N-1)$ $(N-2)$ MQR and the $k/2$ frequencies of the $(N-1)$ MQR spectra. In fact, the transitions which involve energy levels with $m_r = (-N/2 + 1) \rightarrow m_s = [-N/2 + (N-2)]$ can be expressed as the sum of the $(N-2)$ MQR frequencies with $m_r = -N/2 \rightarrow m_s = (-N/2 + \Delta m)$ and the $(N-1)$ MQR transitions with $m_r = (-N/2 + 1) \rightarrow m_s = N/2$; in the same way the frequencies associated with $m_r = [N/2 - (N-2)] \rightarrow m_s = (N/2 - 1)$ are a linear combination of the $(N-1)$ MQR $m_r = -N/2 \rightarrow m_s = (N/2 - 1)$ and the $(N-2)$ MQR $m_r = (N/2 - \Delta m) \rightarrow m_s = N/2$ transitions.

In general, for each $(N-s)$ MQR spectrum with $1 < s < N$, a well-defined number of frequencies can be expressed as linear combinations of the $(N-1)$ MQR and $(N-(s-1))$ MQR frequencies, while just a few transitions are independent.

(N-1) and (N-2) MQ spectra. Spectra acquired without the refocusing π pulse depend upon the chemical shifts; as a consequence the number of independent frequencies is higher and it is not possible to reduce the total number of permutations as previously described. The same linear dependence of lower to upper quantum orders frequencies also applies to MQ spectra.

Heteronuclear spin systems. For the deuterated compounds ^1H spin-echo HSQ spectra and ^1H spin-echo HMQ spectra were recorded. If the spin system is composed of N^I nuclei of spin I and N^S nuclei of spin S , $M_i^I = \sum_{k=1}^{N^I} m_{ki}^I$ and $M_i^S = \sum_{k=1}^{N^S} m_{ki}^S$ are good quantum numbers; i refers to the state $|i\rangle$ and m_{ki} is the magnetic quantum number of the k th nucleus in the i th state. If we define the quantities $n^I = M_i^I - M_j^I$ and $n^S = M_i^S - M_j^S$ as the differences in the Zeeman quantum numbers between the

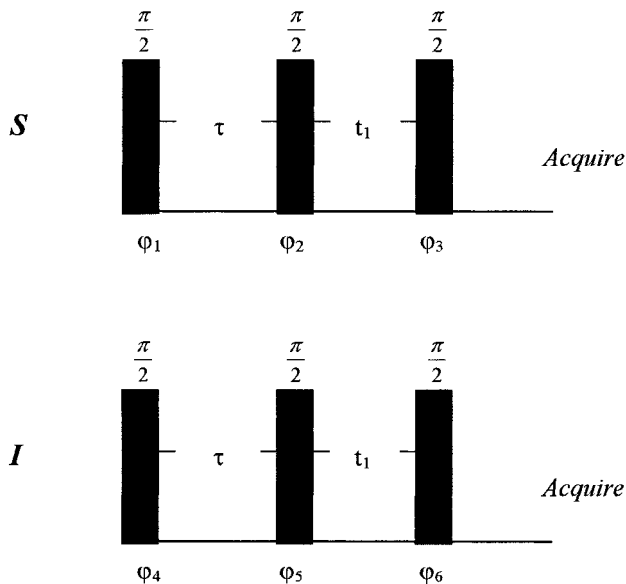


FIG. 3. Pulse sequence for generating heteronuclear multiple quantum spectra.

states $|i\rangle$ and $|j\rangle$ (14), then the density matrix operator describing the system can be written

$$\rho(\tau) = \sum_{n^I} \sum_{n^S} \rho_{n^I n^S}(\tau).$$

As a consequence of the commutation rules $[I_z, \rho_{n^I n^S}] = n^I \rho_{n^I n^S}$ and $[S_z, \rho_{n^I n^S}] = n^S \rho_{n^I n^S}$, each set of indices (n^I, n^S) represents a separate spectrum which can be isolated through its characteristic modulation properties under phase shifts of the irradiation frequencies in the pulse sequence of Fig. 3.

The HMQ sequence is closely analogous to the homonuclear sequence, the main difference being that the RF pulses are given at the resonance frequencies of both I and S nuclei and phase shifts are incremented independently for each channel. In this way it is possible to obtain MQ spectra of different quantum orders for nuclei I and S. ^1H spin-echo HMQ spectra characterized by $n^S = 0$ and $n^I = (N^I-1), (N^I-2), \dots$ will have the fewest transitions compatible with full determination of dipolar couplings and, if the heteronuclear couplings are alike and small (15), the π pulse can remove the heteronuclear interactions. Thereafter the $n^I = (N^I-1), n^S = 0$ spectrum will depend only on the D_{II} couplings. Since $n^S = 0$ the pulse sequence of Fig. 2 is equivalent to that of Fig. 1b. The following HMQ spectra were acquired: ^1H spin-echo 6HMQ, 5HMQ, and 4HMQ ($n^S = 0, n^I = (N^I-1), (N^I-2), (N^I-3)$, with $N^I = 7$). This method is an alternative to deuterium decoupling by on-resonance irradiation (16).

THE PRINCIPAL COMPONENT REGRESSION METHOD

Principal component analysis (17) can be used when only a few linear combinations of independent parameters influence

the data in a significant way; in this way it is possible to reduce the effective number of independent parameters, keeping constant those combinations whose influence is small. At a later stage, if more data are available, the restrictions on less effective linear combinations can be relaxed and each parameter can be determined with only a small correlation with the other parameters. For this reason the method seems promising, since it is well known that frequencies and intensities in NMR spectra often depend on linear combinations of parameters and that each parameter can be determined accurately only when nearly all the frequencies of the SQ spectrum can be assigned (4).

Let $p \in R^m$ be the parameter array, where m is the number of unknowns to be determined and \mathbf{dp} is the variation on the parameters which causes a \mathbf{ds} change in the calculated frequencies. The latter quantities are related through

$$\mathbf{C} \mathbf{dp} = \mathbf{ds},$$

where \mathbf{C} is an $(l \times m)$ matrix (l the number of assigned frequencies) whose elements are defined according to

$$C_{ij} = \frac{\partial s_i}{\partial p_j}.$$

If \mathbf{C} is known, according to the least-squares method, we have $\mathbf{dp} = \mathbf{H}^{-1} \mathbf{C}^T \mathbf{ds}$, where \mathbf{C}^T is the transposed matrix and $\mathbf{H} = (\mathbf{C}^T \mathbf{C})$ corresponds to the variance-covariance matrix (Hessian matrix).

The fundamental principle of the PCR method is that \mathbf{p} can be replaced by a new set of parameters, \mathbf{q} , whose generic element q_i is given by $q_i = \sum_j r_{ij} p_j$, such that in the new base the Hessian matrix is diagonal, $H'_{ij} = h_i \delta_{ij}$. If the value of the generic diagonal elements, h_i , is high, a given variation of q_i has a strong influence on \mathbf{ds} ; otherwise the i th linear combinations can be safely kept fixed. \mathbf{H}' is singular or nearly singular if $h_i \cong 0$.

If a threshold value ξ is fixed in such a way as to have m' q_i parameters with h_i greater than ξ , keeping fixed the remaining $m-m'$ parameters, the matrix \mathbf{H}' is not singular. The system can be solved and only those linear combinations which have the strongest influence on the calculated frequencies are varied in the iterations.

It is worthwhile to distinguish two cases: (i) the SQ spectra are made up of a number of transitions l much greater than m , so the value of ξ is calculated as a small percentage (0.1 or 1%) of the trace of \mathbf{H} , while (ii) for high quantum order spectra, the value of ξ is calculated in such a way to have, at each iteration, $m' \leq l$. Since in the latter case m' is imposed by the number of independent frequencies, some of the q_i with h_i significantly greater than zero will be kept fixed even if their influence on the calculated frequencies is not negligible and their number is likely to be higher when $m \gg l$. A strong bias is introduced in

the procedure and the probability that the true minimum will be found among the possible solutions decreases when the m to l ratio increases. This is a very important point since, as will be shown later, the way lower order frequencies act in the automatic procedure will only allow us to discharge false minima or to refine the parameters if the solution is within the convergence radius of the true minimum.

Thereafter a reasonable compromise between two contrasting needs must be found. (i) No bias has to be introduced since it could force the procedure toward false minima (the fewer the assumption the better), and (ii) no principal component analysis of the data will produce the right solution if the system is too undetermined (the smaller the parameter set the better).

SPECTRAL ANALYSIS STRATEGY

For flexible molecules the direct coupling constants are related to the molecular geometry by (18):

$$D_{ijz} = K_{ij} \sum_n p^n \sum_{\alpha, \beta} G_{ij\alpha\beta}^n S_{\alpha\beta}^n.$$

K_{ij} depends on the magnetogyric ratio of the i th and j th nuclei, p^n is the population of conformer n , $S_{\alpha\beta}^n$ are elements of \mathbf{S}^n , the Saupe orientational matrix for conformer n , and $G_{ij\alpha\beta}^n$ are related to the internuclear distance r_{ij} and to its orientation with respect to a Cartesian system x, y, z fixed on the molecule and chosen in such a way as to reduce the number of independent $S_{\alpha\beta}^n$. Dipolar couplings can be calculated from standard angles and bond lengths, assuming that the different subunits from which the molecule can be built up rotate rigidly, but now the number of independent variables is much higher. Even on the hypothesis that only the conformers corresponding to the minima of the internal rotation potential function have $p^n \neq 0$, the number of independent parameters $\sum (S_{\alpha\beta}^n + p^n) - 1$ is greater than $k/2$. The \mathbf{H} matrix could be made not singular only if the independent frequencies of the $(N-2)$ MQR spectrum were included in the permutative approach, but this hypothesis must be discarded because the total number of permutations $P^{(N-1)} P^{(N-2)}$ requires an unrealistic CPU time and computer resources; the only possible way is to apply the permutative approach to the $(N-1)$ MQ frequencies and then to reduce m by PCR at the very beginning of the analysis, but extending the parameter set as soon as assignments of the independent frequencies of the lower MQ order spectra are made possible.

Two possible strategies, (a) and (b), can be followed.

(a) *Order parameters and direct couplings as a mixed variable set.* Dipolar couplings are divided into two subsets. The D_{ij} between nuclei belonging to the rigid subunits (the aromatic ring in the cases treated) are calculated from an assumed geometry using the order parameters of the subunit as variables. The D_{ij} between nuclei belonging to different subunits

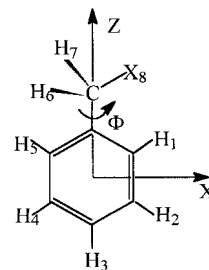


FIG. 4. The structure of a molecule Ph-CH₂-X.

or to the other rigid subunits make up the remaining variables. Then for ArCH₂-Br the parameter set will be S_{zz} ; $S_{xx} - S_{yy}$ (to calculate the six interaromatic proton direct couplings); D_{16} ; D_{26} ; D_{36} ; D_{67} ($m = 6$). See Fig. 4 for axis definition and numbering.

Since the geometry of the aromatic ring can be safely assumed to be a regular hexagon, this approach is biased very little but unfortunately m will be typically greater than $k/2$. The PCR approach must be used to reduce the number of independent parameters. At this point, it is possible (a) to use the parameter sets as input of a standard procedure for the analysis of the SQ spectrum or (a') to discard or refine them using lower order MQR spectra.

(b) *Order parameters as independent variables.* No conformational equilibrium is considered; the molecule is frozen in a single conformation, leaving the system with a maximum of five order parameters to be determined. In this way the approach proposed for rigid molecules can be followed and the dipolar couplings obtained fitting the $(N-1)$ MQR frequencies used as the starting point for the analysis of the SQ spectrum (strategy (b)). This approach keeps m very low ($\leq k/2$) but it is strongly biased since the chance of success relies on conformational assumptions whose reliability cannot be assessed. However the solution (or solutions), if found, can be discarded or refined using lower order MQR frequencies. In this case, l increases considerably and so the mixed variable set strategy (a') can be followed. We will refer to this mixed approach as strategy (b'). Strategies (a') and (b') share the same set of mixed variables to be iterated on but they differ in a significant way with respect to the parameter set which fit the $(N-1)$ MQ frequencies. The PCR approach used in strategy (a) gives many different solutions with similar (and low) RMS while strategy (b) usually gives either one solution with a relatively low RMS or none.

The computer program developed for rigid molecules (3) has been modified to include the assignment procedure of lower order MQ spectra and mixed input parameters.

Algorithm for $(N-1)$, $(N-2)$, and $(N-3)$ MQ spectra analysis (see Fig. 5). With the dipolar couplings determined according to strategy (a) or (b), the $(N-1)$ MQ frequencies are calculated and the permutations performed by the routine PERM. For each permutation, iteration cycles according the

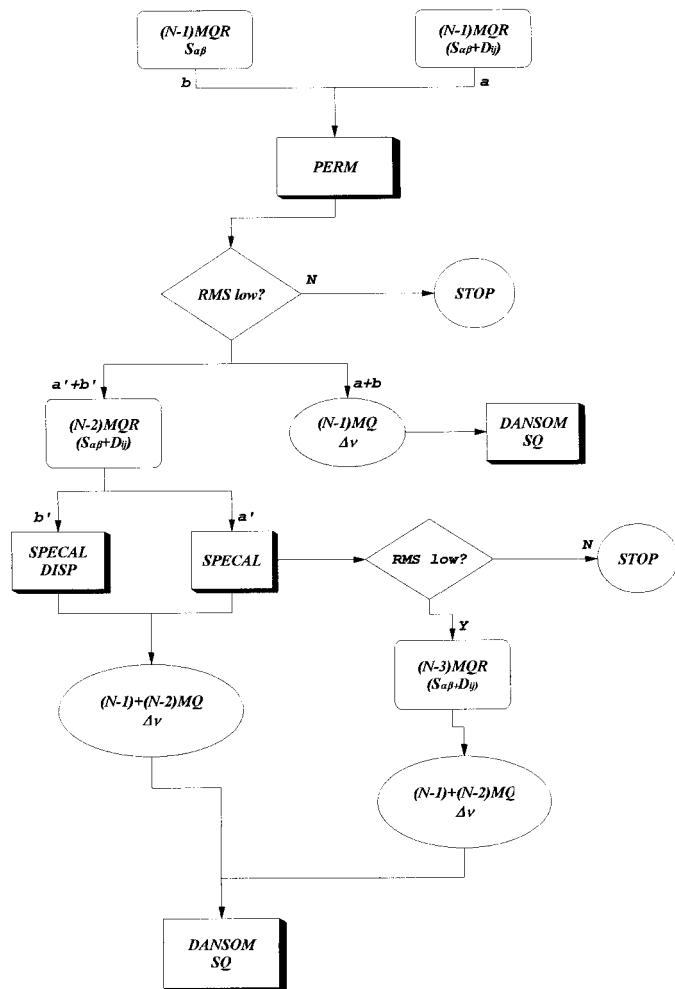


FIG. 5. The flow diagram summarizes the four strategies of spectral analysis. The shaded boxes contain the codes used. The rectangles stand for using MQR spectra and the iteration variables, while the ellipses stand for MQ spectra iterating only on the chemical shifts.

CB-B algorithm (with or without the PCR approach) are performed in order to find the best fit (RMS) between calculated and experimental frequencies; if strategy (a') or (b') is followed, for MQ spectra of a given order (defined in the input file), the subroutine SPECAL will compute the linear combinations of higher order frequencies and assign them to the corresponding experimental values. The frequencies left will be assigned following different strategies: (i) to the nearest experimental value if, within a fixed interval (approximately 100 Hz), no other calculated or experimental frequency is found or (ii) by the DISP routine which computes all the permutations P_s of the calculated versus the experimental values. To perform the assignments, the routine will compute $P_s = S!$ permutations if $S = R$ (S and R are the numbers of experimental and calculated frequencies within the interval); if $S < R$ the $P_{R,S} = R \cdot (R - 1) \cdot (R - 2) \cdot \dots \cdot (R - S + 1)$ permutations will be calculated. With the new assignment, new

iteration cycles will be repeated in which the threshold is gradually lowered. At this stage, solutions with high RMS and/or poor assignment can be rejected. Eventually, the solution which shows a good agreement between experimental and calculated frequencies and which allows all the $(N-2)$ to be assigned is refined with lower order MQ frequencies (if available).

The choice of the interval is very important; if the true minimum is not found in the first critical iteration cycles, only a very large value would still let the algorithm find the correct solution, but this stresses the computer performance to unacceptable limits. Short intervals are useful to refine the parameter set and will not increase the CPU time. It was found that a value of 80 Hz represents a good compromise.

The procedure is controlled by a *script* file written in Open VMS 1.5, while the programs are written in Fortran 77. All the calculations are performed on a Digital Server 2000 ALPHA-AXP computer.

Chemical shifts will be obtained using the MQ frequencies; the D_{ij} calculated from the MQR spectra and the isotropic ν_i values will be used as starting parameters and iterations will be performed only on the chemical shifts by the modified CB-B algorithm.

Single quantum spectrum analysis. The SQ spectrum will be then analyzed using DANSOM. DANSOM is run on a SG Indy workstation within a UNIX script. The output files are then displayed by an original graphical shell (19) together with the experimental spectrum; the operator intervention is limited at this stage.

It is worthwhile, at this point, to outline briefly a few features of the DANSOM procedure (for more details see the original papers by Stephenson and Binsch (8)). In order to find the global minimum, the procedure makes use of smoothing functions, typically a lorentzian or a gaussian, whose action is controlled by the operator. The smoothing parameters α and β fix the initial flatness of the error hypersurface and they are stepwise increased by a correlation multiplier (CM) factor. These parameters are varied in a so-called *iteration grand cycle*, starting from very low values (10^{-8} or lower) and increased up to 1 in a number of iteration cycles. Lorentzian and gaussian functions can be used alternatively or consecutively. The lower the smoothing parameter value used as a starting parameter, the greater the number of iterations in an iterative grand cycle and the farther from the correct values are the starting spectral parameters. The whole strategy is depicted in Fig. 5.

APPLICATIONS

Spectral analysis of $\text{ArCH}_2\text{-X}$ when $X = \text{Br}$ and H (Fig. 4) will be discussed first and then the procedure will be applied to the more challenging analysis of 3-phenylprop-1-yne and ethylbenzene spectra.

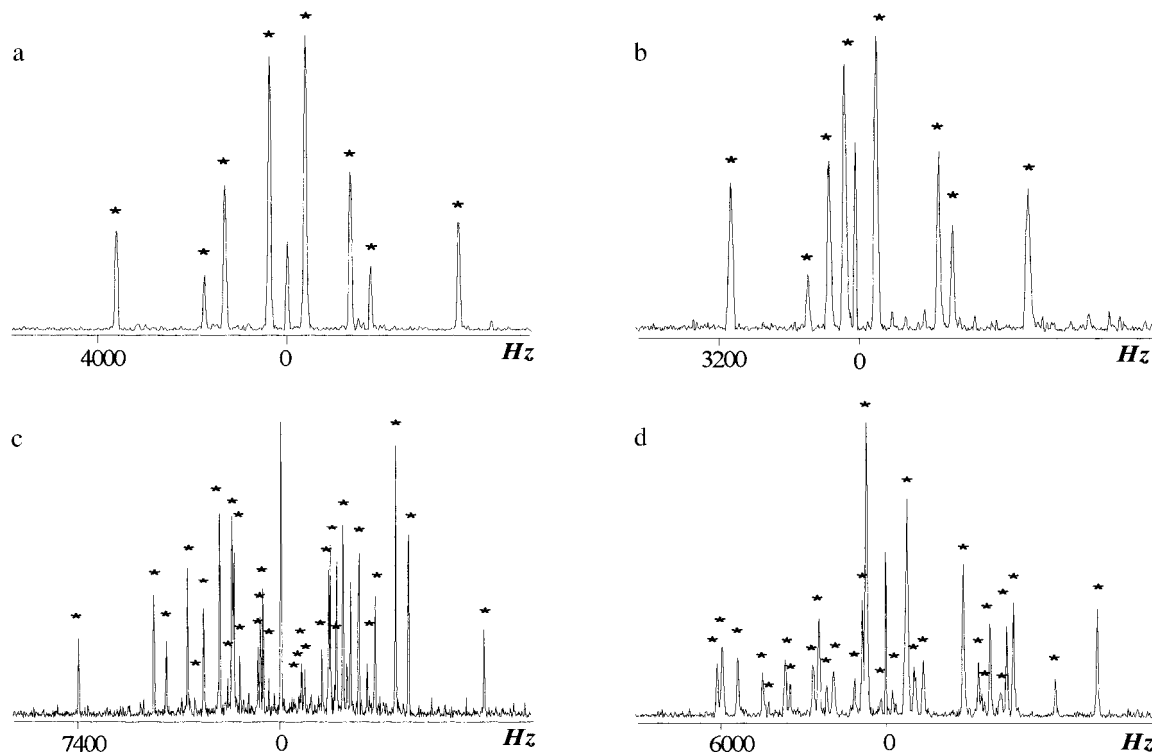


FIG. 6. The 300-MHz ^1H spectra at 300 K of a sample of benzylbromide dissolved in the nematic solvent ZLI-1132: (a) 6MQR, (b) 6MQ, (c) 5MQR, and (d) 5MQ spectra acquired with 512 increments, 10 KHz in F_1 . (a) $\tau = 10$ ms, 24 scans; (b) $\tau = 18$ ms, 24 scans; (c) $\tau = 10$ ms, 20 scans; (d) $\tau = 20$ ms, 24 scans. The real lines are marked with * in (a), (b), and (c) to distinguish them from extra lines arising from the use of imperfect pulses; in (d) only the lines assigned in the iteration process are marked with *.

Test Molecules

Due to the molecular symmetry and to the presence of magnetic equivalent groups, benzylbromide and toluene MQ spectra are similar and will be described together.

MQ analysis. There are eight symmetric lines (four independent frequencies) in the $(N-1)$ MQR spectra of the homonuclear spin systems $\text{AA}'\text{BB}'\text{CD}_2$ and $\text{AA}'\text{BB}'\text{CD}_3$. Just two of the lines at $\pm f_1$ depend on D_{67} (see Fig. 4). Their frequencies are given by a linear combination of the D_{67} , D_{16} , D_{26} , and D_{36} couplings. The three remaining pairs of lines depend on the aryl-aryl and aryl-alkyl couplings. It is then very important that all the allowed transitions can be observed. In the $(N-1)$ MQR spectra of both molecules the expected number of transitions is observed.

There are 40 calculated transitions in the $(N-2)$ MQR spectrum; 8 pairs, belonging to the totally symmetric irreducible representations, are linear combinations of the lines in the $(N-1)$ MQR spectrum, leaving 12 independent pairs that give additional information and can be used in the spectral analysis. Since a permutative approach performed on $P \cong 2 \times 10^{14}$ distinct permutations of the 20 lines in the $(N-2)$ MQR spectrum is out of the power of today's computers, the critical step is to find a suitable starting point using only the four independent lines of the $(N-1)$ MQR spectrum.

Benzylbromide

NMR spectra. The whole series of MQ (6QR, 5QR, 6Q, 5Q) and SQ spectra of a sample of benzylbromide (from Aldrich) dissolved in the liquid crystal ZLI-1132 (from Merck) (15 wt%) has been acquired in a single instrumental session lasting a week. The spectra are shown in Fig. 6.

MQ analysis. All strategies will be followed.

Strategy (a). There are four independent $(N-1)$ MQR frequencies to be reproduced by a total of six parameters. Performing the PCR approach, 16 possible solutions were found in 16 min of CPU time; the solutions have approximately the same RMS but the parameter values differ in a significant way. Only 1 solution, whose values are nearest to the ones found following the procedures described under Spectral Analysis Strategy, is reported in Table 1 (column a).

Strategy (a'). If the $(N-2)$ MQR frequencies are considered, 12 different parameter sets reproducing the experimental frequencies with similar RMS are obtained in about 40 min of CPU time. Some of these can be disregarded since the procedure could assign a limited number of $(N-2)$ frequencies. In Table 1 are shown the three parameter sets with the highest number of $(N-2)$ MQR frequencies assigned: column b with 15 transitions assigned, column c with 6, and column d with 5. For

TABLE 1

Dipolar Coupling D_{ij} and Chemical Shifts ν_i Obtained from the Analysis of MQ and SQ Spectra of a Sample of Benzylbromide Dissolved in the Nematic Solvent ZLI 1132, Following the Different Strategies (a) (column a), (a') (columns b, c, and d), (b) (column e), and (b') (columns f and g)

i,j	a	b	c	d	e	f	g	SQ	J_{ij}/Hz^a
	(D_{ij}/Hz)								
1,2	-1757.	-1739.	-1789.	-2165.	-1808.	-1757.	-1787.	-1697.13	7.8
1,3	-250.	-245.	-253.	-269.	-257.	-247.	-252.	-241.16	1.3
1,4	-48.	-44.	-46.	16.	-49.	-43.	-44.	-47.47	0.62
1,5	13.	21.	19.	171.	14.	25.	23.	11.8	1.2
1,6	-442.	-467.	-439.	-467.	-368.	-439.	-413.	-523.37	0.
2,3	-385.	-351.	373.	130.	-396.	-341.	-353.	-370.26	7.47
2,4	13.	21.	19.	171.	14.	25.	23.	10.91	1.39
2,6	-168.	-192.	-171.	-192.	-158.	-216.	-196.	-149.23	0.
3,6	-87.	-126.	-89.	-126.	-129.	-131.	-117.	-119.47	0.
6,7	1089.	1172.	518.	1172.	1012.	1173.	1109.	1175.0	10.
	ν_i/Hz								
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
2	-3.	-4.	2.	-3.	-8.	-3.	-3.	26.09	
3	107.	94.	209.	242.	160.	91.	100.	123.8	
6	505.	-522.	-457.	-565.	-485.	-522.	-527.	-485.68	
RMS	19.	27.	73.	79.	57.	29.	52.		

^a Taken from the analysis of the isotropic spectrum and kept fixed.

each set the chemical shifts were calculated from the 6 and 5MQ spectra.

Strategy (b). Using standard bond lengths and angles (9a), assuming the C–X bond perpendicular to the aromatic ring, the dipolar couplings can be calculated from a set of three nonzero order parameters. Since our goal is to test the ability of the procedure to produce a good starting set of spectral parameters for the analysis of the SQ spectra, we did not worry whether the conformational hypotheses were acceptable or wrong but rather we tried to keep the system as overdetermined as possible. For this reason we opted to iterate only on two S values (S_{zz} and $S_{xx} - S_{yy}$). Starting with a null value for the parameters, the $P^{(N-1)} = 192$ permutations were performed in approximately 14 min. Only 1 permutation gave an acceptable RMS value; the D_{ij} and the ν_i obtained from the 5Q spectrum are shown in Table 1 (column e).

Strategy (b'). Since only one minimum was found, the ($N-2$) MQR spectrum can be used only to refine the direct couplings calculated according to strategy (b); 15 pairs of the expected 40 transitions are recognizable in the experimental spectrum. The automatic code assigned experimental lines to the nearest calculated frequency for 12 pairs of lines. Regarding the remaining three pairs of experimental lines, the code performed the disposition of 1 experimental line over 3 calculated and a permutation of the remaining 2. The needed CPU time is about 40 min. An inspection of the solutions reveals that a group of 4 and a group of 2 have parameters which differ by just a few hertz. Only one set of parameters for each group (column f for

the group of 4 and column g for the group of 2) are reported in Table 1. The chemical shifts calculated from the 6MQ spectrum are also reported.

SQ analysis. All solutions obtained were used as input for DANSOM. Starting with the parameters obtained with strategy (b) (column e) and the two of strategy (a') with the lowest number of assigned frequencies (columns c and d), no solution was found even when several tests stressing the convergence capability of DANSOM to its maximum were performed.

DANSOM reached the global minimum (i) with the parameter set of columns f (strategy (b')) and b (strategy (a')) with only 56 iterations, $\alpha = 10^{-4}$ (a relatively high value) and $\text{CM} = 10$, and (ii) with the solutions of columns a and g after a few attempts and with some difficulties (126 iterations, $\alpha = 10^{-8}$, $\text{CM} = 10$).

Toluene

NMR spectra. The whole series of MQ (7QR, 6QR, 7Q, 6Q) and SQ spectra of a sample of toluene (from Aldrich) dissolved in liquid crystal ZLI-1132 (15 wt%) were acquired in a single instrumental session lasting a week.

MQ analysis. All strategies will be followed.

Strategies (a) and (a'). There are four independent ($N-1$) MQR frequencies to be reproduced by a total of six parameters. Performing the PCR approach, eight possible solutions were found in 35 min of CPU time, all with approximately the same

TABLE 2

Dipolar Coupling D_{ij} and Chemical Shifts ν_i Obtained from the Analysis of MQ and SQ Spectra of a Sample of Toluene Dissolved in the Nematic Solvent ZLI-1132, Following the Different Strategies (a), (columns a, b) (b) (column c), and (b') (column d)

i, j	a	b	c	d	SQ	J_{ij}/Hz^a
	(D_{ij}/Hz)					
1,2	-1401.	-1509.	-1312.	-1316.	-1346.96	7.8
1,3	-210.	-174.	-200.	-198.	-202.07	1.27
1,4	-58.	37.	-61.	-58.	-57.97	0.62
1,5	-30.	173.	-42.	-35.	-41.87	1.79
1,6	-408.	-436.	-441.	-473.	-451.53	0.
2,3	-467.	300.	-491.	-464.	-239.54	7.47
2,4	-30.	173.	-42.	-35.	-42.19	1.39
2,6	-128.	-573.	-125.	-120.	-126.63	0.
3,6	-95.	-590.	-95.	-108.	-96.59	0.
6,7	1735.	2197.	1790.	1786.	1776.16	10.
	ν_i/Hz					
1	0.0	0.0	0.0	0.0	0.0	
2	6.	55.	42.	11.	31.55	
3	78.	769.	58.	78.	67.67	
6	-1346.	-1062.	-1329.	-1347.	-1329.31	
RMS	42.	76.	53.	30.		

Note. Parameters obtained from the SQ step are reported.

^a Taken from the analysis of the isotropic spectrum and kept fixed.

RMS but differing widely for the values of each parameter. To speed up the calculations, we processed all the solutions with the ($N-2$) MQR frequencies: it was possible to assign (i) the whole spectrum and (ii) seven frequencies (columns a and b). The other solutions allowed for a smaller number of assignments and are not reported. The chemical shifts were calculated from the 7 and 6MQ spectrum as previously described.

Strategy (b). Using standard bond lengths and angles (9b), assuming a C–H bond perpendicular to the aromatic ring, the dipolar couplings have been calculated from a set of two nonzero order parameters. Only one solution is obtained with RMS lower than the threshold value in 16 min of CPU time. The chemical shifts were calculated from the 7MQ spectrum as previously described. The parameter values are reported in Table 2, column c.

Strategy (b'). This set was refined using the 6MQR frequencies. In order to assign the lines in the more crowded central part of the spectrum two permutations of two calculated versus experimental frequencies were needed. The procedure gave four acceptable solutions and took about 35 min of computational time. For each set the chemical shifts were calculated. The parameter values differ by just a few hertz and so only one solution is reported in Table 2, column d. The chemical shifts were calculated from the 7 and 6MQ spectrum as previously described.

SQ analysis. DANSOM reached a global minimum starting with the parameters of columns a, c, and d of Table 2 after 122 iterations starting with $\alpha = 10^{-8}$ and $\text{CM} = 13$. With the data of columns b and the other five solutions of strategy (a') no convergence was achieved.

These initial tests proved that the permutative approach can be extended to some simple flexible molecules and that both strategies can be used. Their behavior is quite different: strategy (b') gives only one solution with reasonably low RMS and the ($N-2$) MQ frequencies simply refine the parameter set. When the PCR approach is used, a higher number of solutions are found with similar RMS values but only a few of them allow for a full assignment of the ($N-2$)-order MQ frequencies. Moreover the global minimum is reached by DANSOM only for those solutions which allow the assignment of all the ($N-2$) frequencies.

3-Phenylprop-1-yne

The anisotropic spectrum of this molecule has not been analyzed before nor its conformational equilibrium investigated. The number of parameters is greater (18) than for the test molecules (13), and its analysis proved helpful to point out some limits of the PCR approach.

NMR spectra. The whole series of MQ (7QR, 6QR, 5QR, 4QR, 7Q, 6Q) and SQ spectra of a sample of 3-phenylprop-1-yne (from Aldrich) dissolved in liquid crystal ZLI-1132 (15 wt%) has been acquired in a single instrumental session lasting more than a week. Some of the MQR spectra are shown in Fig. 7.

MQ analysis. There are eight interacting protons (spin system AA'BB'CD₂E) and 14 direct couplings, 8 of them depending on the conformational equilibrium. The ($N-1$) MQR spectrum contains five pairs of lines for a total of $P^{(N-1)} = 1920$ possible assignments. Two pairs of frequencies depend on D_{67} and D_{68} (see Fig. 4 for numeration) as shown:

$$\pm f_1 = \pm (c_1 D_{18} + c_2 D_{28} + c_3 D_{38} + c_4 D_{68})$$

$$\pm f_2 = \pm (c_5 D_{68} + c_6 D_{16} + c_7 D_{26} + c_8 D_{36} + c_9 D_{67}).$$

The ($N-2$) MQR spectrum shows 36 of the 57 expected transitions, of which only 24 are independent.

Strategy (a'). There are 10 independent parameters (S_{zz} and $S_{xx} - S_{yy}$ and eight dipolar couplings) to be determined but only five frequencies. All the $P^{(N-1)}$ assignments have been tested, but the code was not able to assign a reasonable number of the lower order MQR frequencies.

Strategy (b'). The angle ϕ (see Fig. 4) was varied from 0° (planar molecule) to 90° in steps of 10° and, for each ϕ , iterations have been performed on three or five order parameters, depending on the symmetry. No acceptable solution was found. The results obtained with the test molecules persuaded us not to try the SQ automatic step.

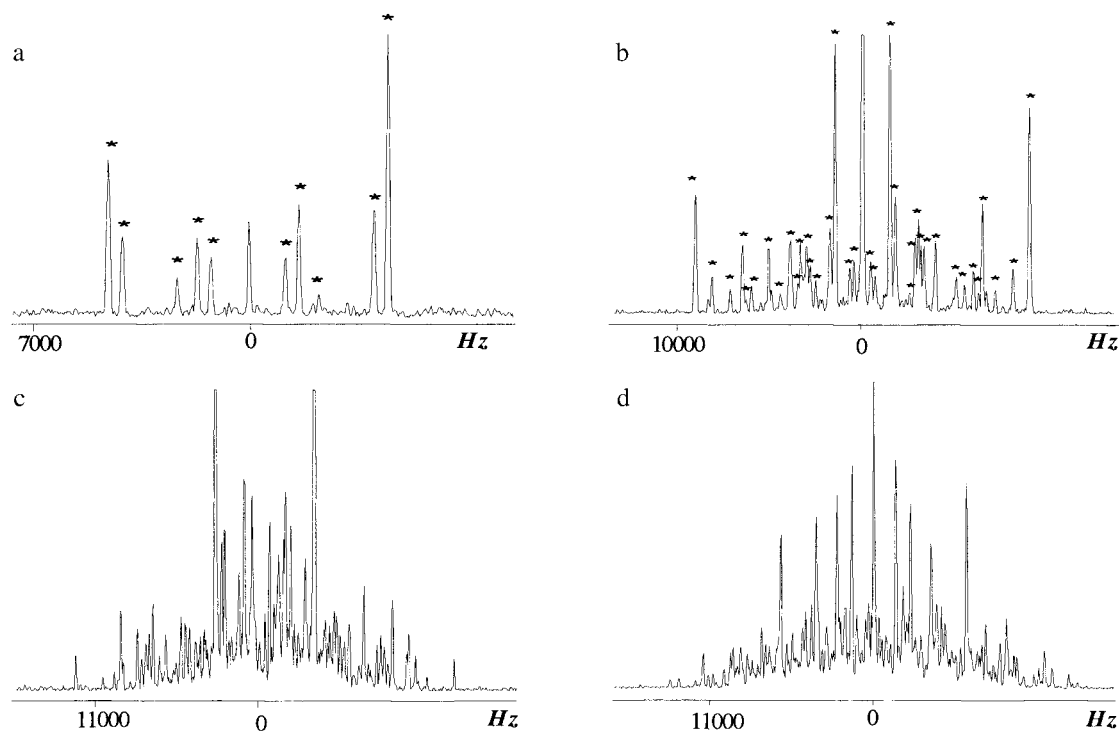


FIG. 7. The 300 MHz ^1H spectra at 300 K of a sample of 3-phenylprop-1-yne dissolved in the nematic solvent ZLI-1132: (a) 7MQR, (b) 6MQR, (c) 5MQR, and (d) 4MQR spectra acquired with 512 increments, 22730 Hz in F_1 . (a) $\tau = 2.212$ ms, 28 scans; (b) $\tau = 0.755$ ms, 24 scans; (c) $\tau = 0.3812$ ms, 20 scans; (d) $\tau = 0.3812$ ms, 16 scans. The real lines are marked with * in (a) and (b) to distinguish them from extra lines arising from the use of imperfect pulses.

As a way of reducing the number of direct couplings, we deuterated selectively the molecule in position 1 according to the standard acid–base exchange reaction (20) and ran heteronuclear ^1H MQ spin–echo spectra on the deuterated sample. Since the D_{HD} couplings are expected to be of the same order and small compared with the D_{HH} we hope that the decoupling will be effective, reducing in this way the number of direct couplings.

[1-*d*]-3-Phenylprop-1-yne

NMR spectra. Using the pulse sequence of Fig. 1b on a sample of [1-*d*]-3-phenylprop-1-yne dissolved in the liquid crystal ZLI-1132 the following spectra were recorded: ^1H spin-echo 6HMQ, 5HMQ, and 4HMQ characterized by $n^s = 0$, $n^1 = (N^1 - 1)$, $(N^1 - 2)$, $(N^1 - 3)$, where $N^1 = 7$ and I refers to ^1H . The spectra obtained with the optimum τ are shown in Fig. 8.

MQ analysis. The (N^1-1) MQ spectrum contains just eight lines symmetrically positioned as predicted for an AA'BB'CD₂ spin system showing that the decoupling is effective. The same procedure followed for the two test molecules can be applied to this case.

Strategy (a') was followed, in this way avoiding a conformational hypothesis. There are now six independent parameters versus four frequencies. Also using the (N^1-3) MQ to discard wrong solutions the parameter set reported in Table 3,

column a, was obtained. The 7, 6, and 5MQR spectra of the fully protonated isotopomer were analyzed in a single run using as starting set (i) the D_{ij} found for the deuterated samples scaled according to the $\gamma_{\text{H}}/\gamma_{\text{D}}$ ratio and (ii) the $D_{i8} = 0$ Hz for $i = 1, 7$. The chemical shifts were then determined from the 7 and 6MQ spectra. The full set of parameters is shown in Table 3, column b.

SQ analysis. Using these spectral parameters as input, DANSOM reached the global minimum in 88 iterations and with an initial value of $\alpha = 10^{-5}$ and CM = 10 to give the final parameters reported in Table 3, column c. The experimental and simulated spectra are shown in Fig. 9.

Ethylbenzene

The SQ anisotropic spectrum of ethylbenzene (Etb) with its 15 independent dipolar couplings and 5 chemical shifts has defied analysis for many years. As proved for 3-phenylprop-1-yne, the use of ^1H spin-echo HMQ spectra of some selectively perdeuterated Etb, combined with the $(N-1)$, $(N-2)$, and $(N-3)$ MQ and MQR spectra of the fully protonated isotopomer, let us hope that such a complex spectrum could be analyzed following the procedure described here, using the standard CB-B algorithm for the analysis of the SQ spectrum instead of the DANSOM algorithm.

Unfortunately only the 9QR spectrum of Etb was successfully acquired (the spreading of coherences over density ma-

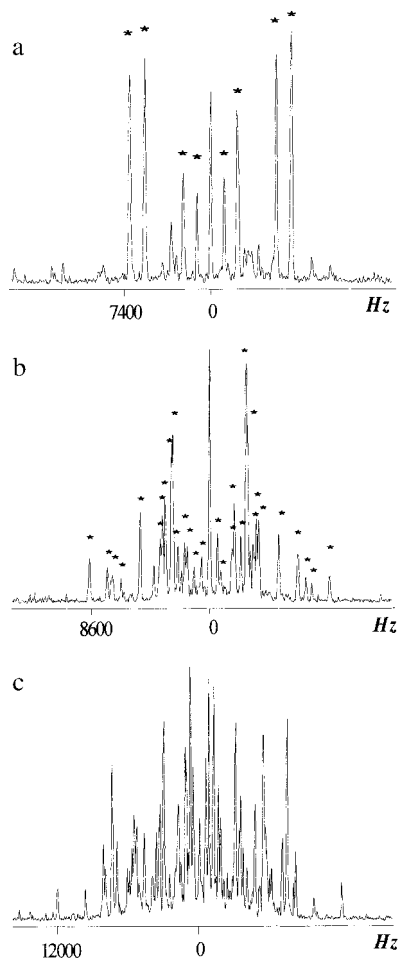


FIG. 8. The 300 MHz ^1H spectra at 300 K of a sample of [1- d]-3-phenylprop-1-yne dissolved in the nematic solvent ZLI-1132: (a) 6HMQ, (b) 5HMQ, (c) 4HMQ spin-echo spectra with 512 increments, 16 KHz in F_1 . $\tau = 0.8897$ ms for all experiments, (a) 24 scans; (b) 20 scans; (c) 16 scans. The real lines are marked with * in (a) and (b) to distinguish them from extra lines arising from the use of imperfect pulses.

trices of increasingly higher order— 2^{10} in our case—poses serious limits to MQ techniques since sensitivity decreases very quickly); thereafter the procedure described for 3-phenylprop-1-yne could not be applied. The more complex and cumbersome procedure described in Ref. (21) and based on the analysis (i) of ^1H spin-echo HMQ spectra and (ii) of the SQ spectra of different isotopomers allowed at the end the analysis of the SQ spectrum.

CONCLUSIONS

The procedure here described is really automatic, since it requires neither a reasonable set of parameters nor an experienced operator. It also requires less CPU time than the automatic procedure applied directly to the SQ spectrum (19a) but is a little more demanding of instrumental time, since MQ spectra must be recorded.

The method, as currently formulated, works for the important class of flexible molecules of general formula $\text{Ph-CH}_2\text{-X}$ (X either containing or not containing protons). Spectra whose analysis requires a number of spectral parameters (order parameters and/or dipolar couplings) slightly exceeding the number of lines in the $(N-1)$ MQR spectrum can also be dealt with by the procedure. The use of deuterated compounds becomes important for the more complex spin systems (eight spin- $1/2$ nuclei in our case).

Strategy (a) gives better results but needs more instrumental time since lower order MQ spectra are needed to discriminate among the many solutions and give to DANSOM only the correct solution to be refined on the SQ spectrum, in this way avoiding multiple runs with different starting parameters. On the other hand, for simpler spin systems, strategy (b) can give excellent results with very short instrumental and CPU times.

For Etb, among the most difficult exempla known to us, it was not possible to test the procedure since the loss of sensitivity of the MQ technique associated with the high number of interacting spins did not allow us to acquire the 9, 8, and 7MQR spectra. High field spectrometers are becoming quite

TABLE 3
Dipolar Couplings D_{ij} and Chemical Shifts ν_i Obtained from (1) the Analysis of the MQ Spectra of the 1-Deutero 3-Phenylprop-1-yne (column a), (2) the Analysis of the MQ Spectra of the 3-Phenylprop-1-yne (column b), and (3) the Analysis of the SQ Spectrum

i, j	a	b	SQ	J_{ij}/Hz^a
	(D_{ij}/Hz)			
1,2	-1646.	-1653.	-1632.81	7.81
1,3	-261.	-254.	-249.88	1.27
1,4	-76.	-82.	-81.79	0.62
1,5	-55.	-62.	-63.41	1.79
1,7	-683.	-702.	-703.87	
1,8		-297.	-322.7	
2,3	-663.	-652.	-642.38	7.47
2,4	-55.	-62.	-62.85	1.39
2,7	-163.	-151.	-144.7	
2,8		-95.	-102.25	
3,7	-86.	-80.	-97.59	
3,8		-71.	-77.12	
6,7	4555.	4587.	4572.08	10.
6,8		-218.	-195.64	2.
		ν_i/Hz		
1		0.0	0.0	
2		10.	13.57	
3		60.	69.49	
7		-887.	-864.	
8		-1858.	-1813.47	

Note. Proton coordinates are calculated from standard bond lengths and angles.

^a Taken from the analysis of the spectrum in isotropic solution and kept fixed.

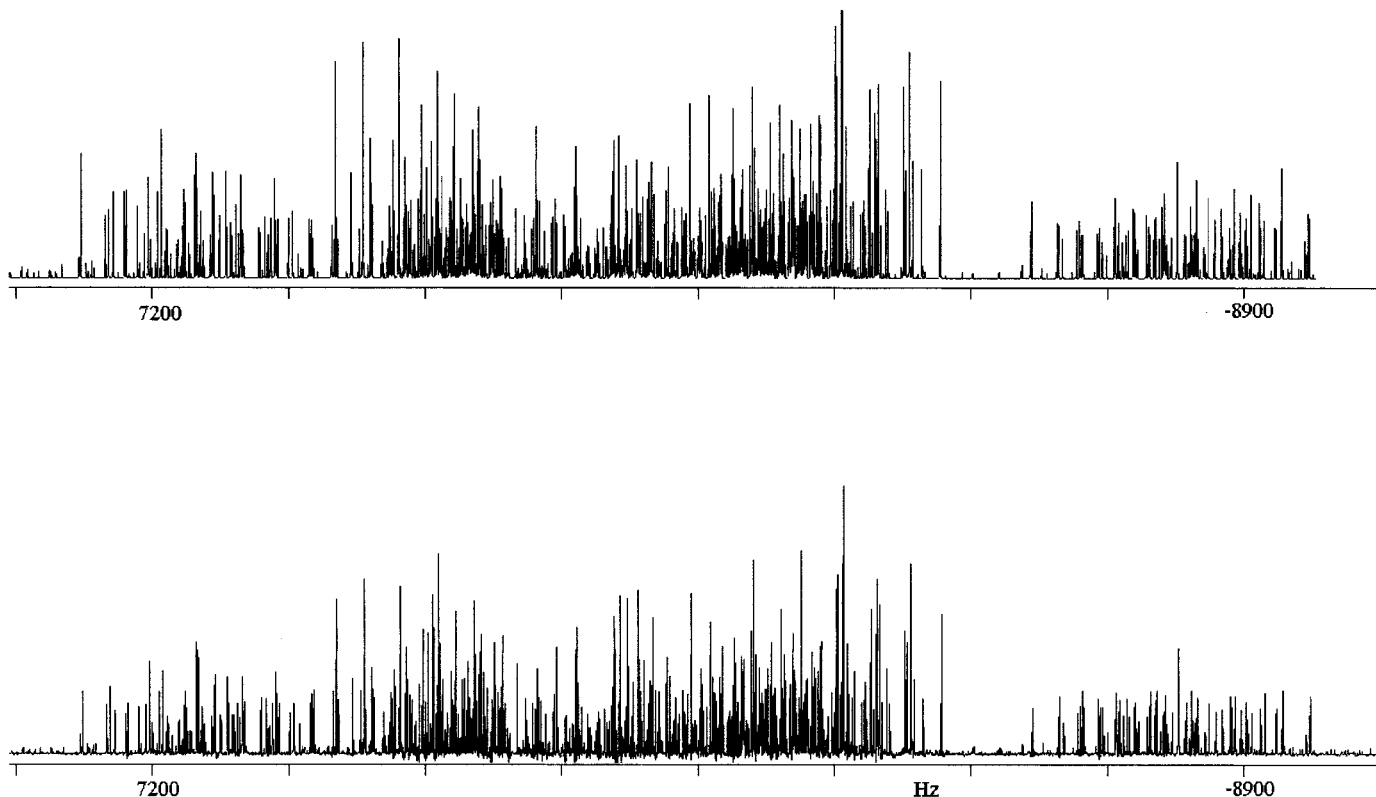


FIG. 9. The 300 MHz ^1H SQ spectrum at 300 K of a sample of 3-phenylprop-1-yne dissolved in the nematic solvent ZLI-1132: (bottom) experimental spectrum acquired with 16 K points, 16 scans in 22727 Hz; (top) simulated with the parameters obtained by iterative analysis.

common and so (i) the latter difficulty could be overcome and (ii) the higher line dispersion could make the fully automated analysis of the SQ spectrum possible (3). However, the possibility of obtaining well-resolved SQ spectra, whose analysis can be accomplished at least with the standard CB-B algorithm, would constitute the limiting factor even if a suitable series of deuterated isotopomers could be synthesized and the ^1H spin-echo HMQ spectra effectively decoupled.

The method has been applied to molecules characterized by the presence of just one rigid subunit (the aromatic ring); extension to other, more complex, situations (i.e., the presence of two rigid subunits) is under investigation.

ACKNOWLEDGMENTS

This work was supported by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (ex 40% "Cristalli Liquidi" Programme) and by the Consiglio Nazionale delle Ricerche (Italy). We thank Professor J. W. Emsley (University of Southampton) for his invaluable contribution to this work.

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