

Graphical Interactive Strategy for the Analysis of NMR Spectra in Liquid Crystalline Phases

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Spectral analysis of ^1H NMR spectra of molecules dissolved in liquid crystalline phases to obtain spectral parameters (chemical shifts, J_{ij} indirect and D_{ij} direct couplings) is usually a difficult and time-consuming task due to the peculiar characteristic of this kind of spectra. A procedure that links together a simulation/iteration program with graphic routines has been developed to be run on a Vax cluster. The procedure has proved to be very useful for the analysis of spectra due to molecules containing up to 11 interacting nuclei, needing reasonably low CPU times for the simulation/iteration step and providing an interactive, friendly graphic environment for such tasks as spectrum display and line assignment, whose importance increases quickly with the number of interacting nuclei.

INTRODUCTION

Graphical packages that deal with raw NMR data (i.e., the FIDs) and display 1D or 2D spectra on spectrometer-dedicated computers or on separate workstations are now available commercially from many different firms. In recent years software companies have produced many sophisticated packages, mainly for 2D experiments, which include features such as the maximum likelihood (MLM) and the maximum entropy (ME) deconvolution methods as alternatives to the fast Fourier transformation (FFT). They also include programs for interactive connectivity analysis, the simulation of NOE spectra from candidate structures, and so on. But, with some exceptions, few of these packages include simulation/iteration programs. This is probably because of the strong interest NMR researchers have in conformational studies of proteins, oligonucleotides, and similar high molecular weight systems containing many, but relatively simple, spin sub systems; the relevant problem is then to be able to establish the correct connectivity patterns among multiplet resonances of nuclei, which have similar chemical shifts but which are not directly coupled. Very often a sufficient dispersion of the signals can be achieved only at the highest fields available, which also yields first-order subspectra as a byproduct. The relevant spectral parameters (chemical shifts ν_i and indirect J_{ij} couplings) can then be determined simply by inspection of the spectra.

In our laboratory we are interested in studying the conformations of molecules from their NMR spectra as solutes in liquid crystalline solvents (LCNMR). The spectra we have to deal with are invariably of the tightly coupled type, and they cannot be converted to first-order spectra even if the highest available fields are used since the direct through-space spin spin couplings (D_{ij}) (which are nonzero because of the anisotropic potential the molecule experiences in the liquid crystalline phase) can have values as high as a few thousand hertz. Dipolar coupling is seen between all magnetically active nuclei in the molecule (usually ^1H), with couplings bigger than the line width so that the spectra due to even the simplest molecules are quite complex and very difficult to analyze. On the contrary in isotropic NMR the $^nJ_{ij}$ are expected to be nearly zero when n , the number of bonds between two coupled protons, is greater than four; each line is then composed of many transitions whose frequencies differ by less than experimental line width. Just to quote a relatively simple

case: which has been analyzed recently in our laboratory,¹ benzyl iodide, a 7-proton spin system with 10 independent direct couplings, gives when dissolved in a liquid crystalline phase the spectrum shown in Figure 1 (upper spectrum) composed of about 400 lines with higher than noise intensity. The spectrum was analyzed by assigning about 200 distinct transitions in the final iteration cycles (Figure 1, bottom).

A few strategies to extract the D_{ij} couplings have been developed by different researchers, some mainly instrumental,² others computer based;³ the one followed in our laboratory allows us to obtain sets of D_{ij} couplings from the analysis of the proton-deuterium decoupled spectra of selectively deuteriated molecules. The different sets, referring to different spin sub systems of the same molecule, can then be combined to give a set used as a starting point for the analysis of the spectrum of the fully protonated molecule. The iteration step, based on the algorithm developed by Castellano and Bothner-By,⁴ is the one used in many of the available programs.⁵ This is a very efficient algorithm for finding the correct set of spectral parameters, but it has a severe drawback: it requires the operator to make interactively the choices in assigning calculated to experimental lines. This is done by preparing an input file which contains, among other things, the previously calculated line identification numbers and their experimental frequencies. For small spin systems the CPU time needed for each iterative cycle is very short and the number of calculated frequencies is small: judgments can be made quickly and rapidly tested during short, intense sessions, but as the number of magnetically active nuclei increases, the CPU time increases quickly too and, much worse, the size of the output file which contains the calculated frequencies and intensities becomes so big as to make it unmanageable. All the wisdom and wizardry of the operator is wasted in trying to spot recognizable patterns of calculated lines in the overcrowded experimental spectrum.

According to the spectral analysis strategy we use, many spectra of increasing complexity must be analyzed before the analysis of the fully protonated molecule spectrum can be attempted; the time needed for each analysis and the manageability of the output files become crucial for the success of the adopted strategy.

To address this problem, we have tried to fill the existing gap between the available graphical display routines, which include no, or relatively simple, simulation/iteration software and more sophisticated programs with, unfortunately, no graphical facilities. Therefore, we have developed a procedure

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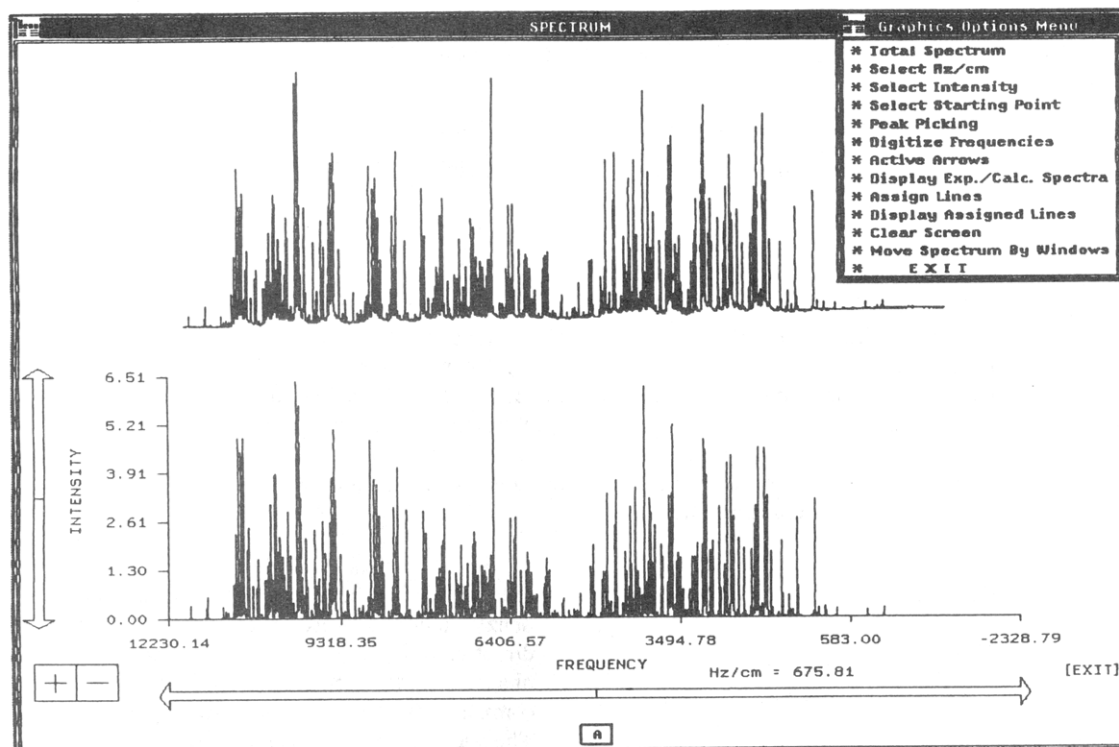


Figure 1. Experimental (upper) spectrum of benzyl iodide dissolved in the liquid crystal phase 135 registered at 300 MHz, displayed with the calculated one (bottom). In the upper right corner the "Graphics Option Menu" (see text for explanations) is displayed. In a real run the menu is displayed in an empty portion of the screen, but it has been moved over the "Spectrum" window to be shown with the spectra.

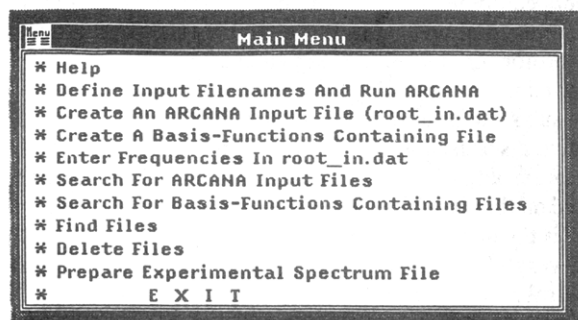


Figure 2. Main menu.

that (i) calculates the spectrum from a given set of spectral parameters, displays it on a monitor, and iterates on spectral parameters, (ii) allows for a mouse-driven graphically interactive line assignment step aimed to make the assignments fast and error free, and (iii) helps the operator in various tasks such as input file preparation and file managing. The simulation/iteration program available from QCPE has been modified to handle the complex spin systems in which we are interested, while the graphical routines have been designed to take into account the particular characteristic of anisotropic spectra.

PROCEDURE

The procedure has been designed to be run on a generalized system configuration ranging from a single graphic workstation up to a computer cluster composed of a powerful, high-speed computer (the cluster bootnode) where the time-demanding tasks are performed and many workstations. Through a *setup* file, prepared according to the available hardware configuration, the procedure recognizes the general parameters of the system (logical names of peripherals and queues, user names, and so on) and links them to its internal parameters.

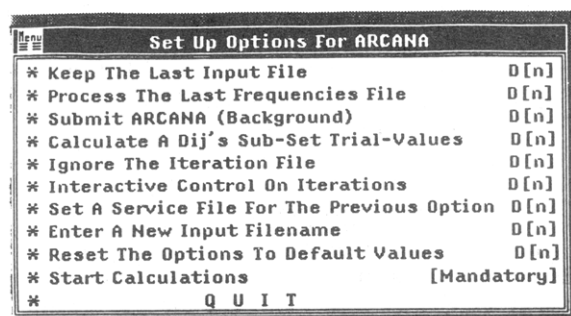


Figure 3. Submenu called by the "Define Input Filenames and Run Arcana" option of the "Main Menu". On the right the default value is reported. Clicking on the option reverses its value. Clicking on "Start Calculations" runs the ARCANA module. See text for its use.

The options available in the main menu (see Figure 2) fall into two different groups: (a) spectrum calculation and display options and (b) file managing.

Spectrum Calculations and Display Options. Two options are available for preparing input files to be used when the "Define Input Filenames And Run ARCANA" options is selected. In this last option the greatest part of the calculations is performed by three executable modules; the way the calculations are executed is fixed by the "Set Up Options For ARCANA" submenu (Figure 3), which is displayed after indications on the input file (its root name and the directory where it is kept) and on the basis functions containing file are entered via a keyboard. In Figure 4 the flow diagram of the relations among executable modules (thick bordered rectangles), input, output, and service files (thin bordered rectangles) is shown with some of the possible pathways that can be chosen through the options of Figure 3.

Module ARCANA. Defining as \mathbf{v} a proper basis set in an \mathcal{N} -dimensional space ($\mathcal{N} = 2^N$ if the *spin system* is composed of N 1/2 spin nuclei) and calling ϑ_i the generic element of the

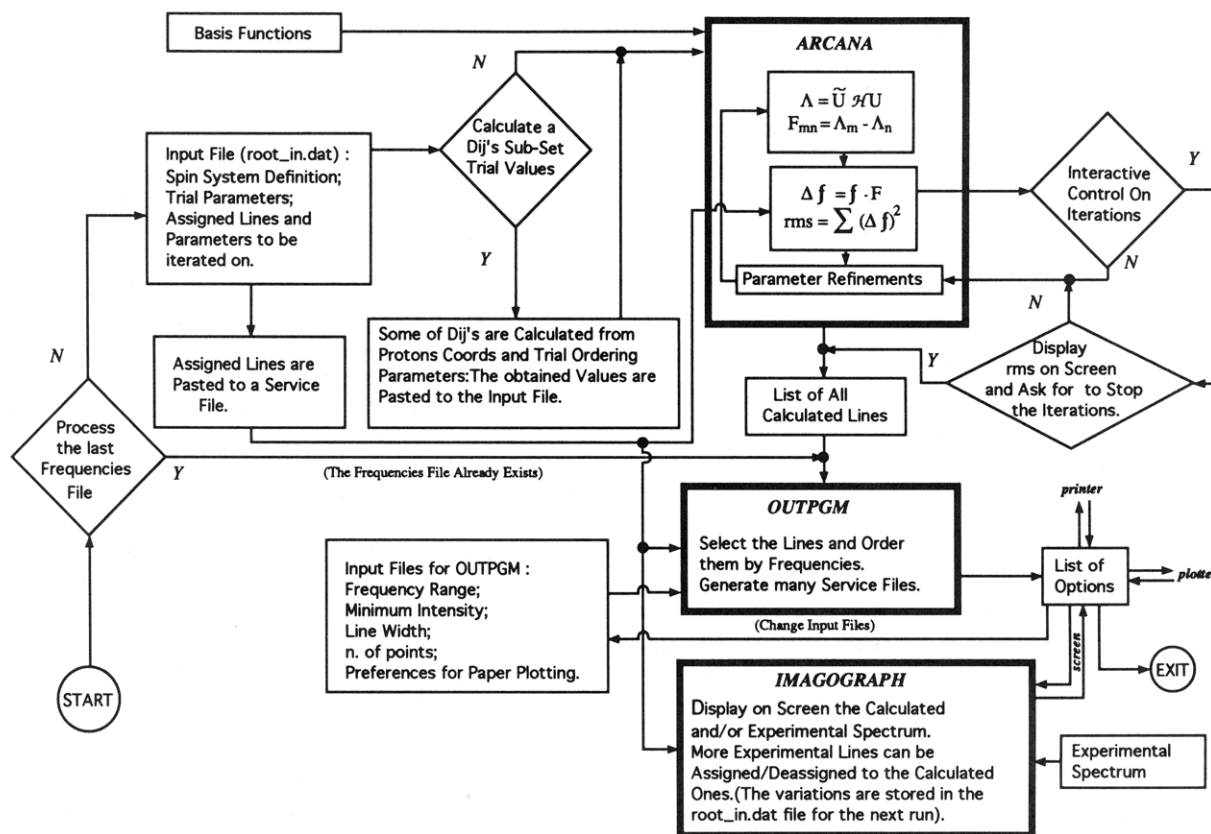


Figure 4. Flow diagram showing the relations among the ARCANA, OUTPGM, and IMAGOGRAF modules and related files.

basis, eigenvalues and eigenvectors can be calculated by diagonalization of the Hamiltonian matrix whose generic element $H_{ij} = H^*_{ji}$ is calculated according to $\langle \vartheta_i | H | \vartheta_j \rangle$. The diagonalization process can be formally written as

$$\tilde{U} H U = \Lambda$$

with Λ a diagonal matrix. The λ_{ii} elements of Λ will then be the eigenvalues, while the eigenvectors are the element of w given by

$$w = Uv$$

When the basis is chosen, a compromise has to be reached between the simplest algorithm which calculates the H_{ij} elements in that basis set and the maximum allowed factorization of H to reduce the computational time needed to diagonalize the matrix, since diagonalization is a time-consuming task. If, as in our case, the iterative procedure proposed by Castellano and Bothner-By⁴ is used, the choice of the basis is very important also to have a simple algorithm for the iterative step. If ϑ_i is defined as

$$\vartheta_i = \prod_{k=1}^N |J_k; m_k^{(i)}\rangle$$

[the so-called simple product spin functions (SPSF)], where J_k is the angular momentum operator eigenvalue for the nucleus k -th and m the eigenvalue of the angular momentum projection operator with $-J \leq m \leq +J$, then

$$H_{ii} = -[\sum_k \nu_k m_k^{(i)} + \sum_{k < k'} \sum m_k^{(i)} m_{k'}^{(i)} (J_{kk'} + 2D_{kk'})] \quad (1)$$

$$H_{ij} = -1/2 (J_{kk'} - D_{kk'}) \{ [J_k (J_k + 1) - m_k^{(i)} (m_k^{(i)} \pm 1)] [J_{k'} (J_{k'} + 1) - m_{k'}^{(j)} (m_{k'}^{(j)} \mp 1)] \}^{1/2}$$

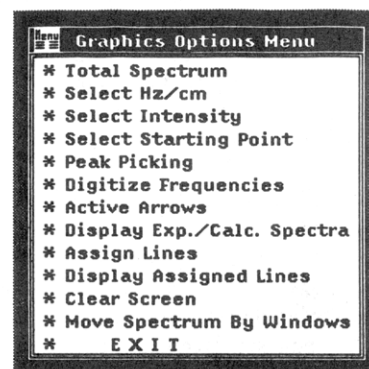


Figure 5. Menu displayed by the IMAGOGRAF module when called from "List of Options" following OUTPGM (see Figure 4; expanded from Figure 1).

The off-diagonal elements vanish unless

$$m_k^{(i)} = m_k^{(j)} \pm 1 \quad \text{and} \quad m_{k'}^{(j)} = m_{k'}^{(i)} \mp 1$$

$$m_s^{(i)} = m_s^{(j)} \quad \text{if } s \neq k, k'$$

$\nu_k, J_{kk'}$, and $D_{kk'}$ are the spectral parameters to be fed as input (see Figure 4). Off-diagonal elements cannot exist between SPSP with different values of $M^{(i)} = \sum_k m_k^{(i)}$, the eigenvalue of the total angular momentum projection operator; if the SPSP are ordered according to their M value, then the $(N \times N)$ H matrix is split into $N + 1$ submatrices of $\binom{N}{m}$ order, $m = 0, 1, \dots, N$. If nuclei with different magnetogyric ratios are present, each submatrix can be further split, according to the so-called X -factorization approximation, because the total angular momentum projection operator of nuclei with the same magnetogyric ratio value M_A, M_X , etc., nearly commutes with the Hamiltonian.

When magnetically equivalent nuclei are present in the *spin system*, then the composite particle formalism⁵ allows for a further splitting of **H**. According to this formalism, the magnetically equivalent nuclei can be formally considered as one particle *G*, whose angular momentum \vec{J}_G is given as the vectorial sum of the angular momenta of each group.

If, for example, three $1/2$ -spin magnetically equivalent nuclei were present, they could be described as a single particle having $J_G = 3/2$ with degeneracy 1 and $J_G = 1/2$ with degeneracy 2. The H_{ij} elements will be given as in eq 1, while a third term

$$\sum_{k=1}^N D_{kk} (3/2 (m_k^{(i)})^2 - 1/2 J_k^{(i)} (J_k^{(i)} + 1))$$

for which $D_{kk} \neq 0$ only for composite particles, has to be added to the formula which gives H_{ii} in the same equation. Now also the *J* value depends on the basis function index.

Again, because $H_{ij} = 0$ if $J_k^{(i)} \neq J_k^{(j)}$, ordering the SPSF according to the *J* value, the Hamiltonian will be split further. All of these factorizations are implemented in LEQUOR.^{5,6}

The symmetry of the *spin system* can play a very important role in factorizing the Hamiltonian if a basis set whose elements are eigenvectors of the symmetry operators can be prepared as a linear combination of the SPSF (LCSPSF). No simple general form as in eq 1 can be written in this case to calculate the M_{ij} elements, and the resulting algorithm will be more complex.

For a C_2 symmetry a simulation/iterative program is available (LACX)⁵ but for higher symmetries only simulation programs exist.^{3a} Many of the molecules in which we were interested in the past presented both magnetic and C_2 symmetry equivalence. To factorize as much as possible the **H** matrix, and still keep the iterative part, an algorithm was implemented which included the four different kinds of factorizations just described. Each pair of symmetrically equivalent nuclei or group of magnetically equivalent nuclei was considered as a group *G* and identified by the following: its J_G , its m_G , and two flags, the first indicating the symmetric/antisymmetric nature of the group *G* and the second identifying the group as a single nucleus with $J_G > 1/2$ or as a composite particle. The basis functions obtained as a combination of these parameters were ordered (a) according to each set of J_G values, (b) according to their symmetry character within each set, (c) according to their $M_i = \sum_G m_G^{(i)}$ value, and (d) finally according to their M_A , M_X , etc. values. Basis function sets, corresponding to different *spin systems*, can be prepared by the proper option and stored in files (see Figure 2). The file is then fed as input in ARCANA as shown in Figure 4.

Each *subsubsubmatrix* was diagonalized, and the relative eigenvalues and eigenvectors were stored in a file. The intensity of each transition of frequency $F_{lm} = \lambda_l - \lambda_m$ was calculated according to

$$\Gamma'_- = \tilde{U} \Gamma_- U$$

where Γ_- is the representation of the single quantum transition operator in our basis and Γ'_- is the representation in the basis which diagonalizes **H**. No simple general form can be given for the generic element of Γ_- when the C_2 symmetrized eigenfunctions are used, as happens for the composite particle formalism,⁵ but the various different situations were taken into account in the algorithm. Note that transitions can occur only between eigenvectors with the same symmetry, the same set of J_G values, and consecutive values of M_i . The algorithm is schematically represented in Figure 4 in the upper box of the ARCANA module.

The module will output a file containing line identification number, frequency, and intensity. This procedure, first used to analyze a four-spin, six-deuteron AA'A''A'''X₃X'₃,⁷ has been further implemented allowing us to deal with *spin systems* such as the 13-spin $1/2$ nuclei AA'BB'CC'DD'E2X3, although with a reasonable throughput only when a vectorial computer such as the CONVEX C240 and the Convex implemented IMSL⁸ diagonalization routines are used (see Experimental Section).

The details of the interactive algorithm are discussed elsewhere;^{4,5} the ARCANA module follows the same design but has some needed modifications. For clarity's sake it will be only noted (i) that because the derivatives $(\partial H / \partial p_j)$, p_j being the generic spectral parameter, are calculated in an analytical way, the choice of the allowed basis set is limited to the few which do not make the relative algorithm too difficult to handle and (ii) that the iterative procedure will produce the correct final spectral parameter set only if the experimental frequencies (or at least a relevant percentage of them) are correctly assigned to the calculated ones through their identification number. The assignment step then becomes the critical point in spectral analysis. No systematic approach is possible because there are *Z!* assignments to be considered if *Z* is the number of transitions, and no sophisticated frequency/intensity pattern recognizing algorithm that could do the assignment in a semiautomatic way is known to us. The task has then to be done by the operator in two steps: (a) when the input file is prepared and (b) during the iterations by suppressing from the list of the assigned lines the ones that contribute in a significative way to the rms as sketched in Figure 4 on the right side of the ARCANA module.

Remembering that the transition number, rising quickly with the number of interacting spins, can be in the order of a few thousand, there is no doubt that every action which simplifies the assignment step will help to speed spectral analysis.

Module OUTPGM. The main functions of this module are (a) to tailor the ARCANA output file according to the user-given parameters such as frequency range, minimum intensity, line width, and hertz/point ratio, (b) to order lines by frequency, and (c) to prepare output files for printing and/or displaying and plotting. Lines are ordered by a "Shell-Metzner-like" order algorithm.⁹ This algorithm is much more efficient than the "Bubble Sort" routine usually found in similar programs. The gain in CPU time is quite impressive mainly when, according to the number of magnetically active nuclei, the number of transitions is high. For a AA'BB'CC'XX'Y3 ($I_A, I_B, I_C = 1/2$; $I_X, I_Y = 1$) spin system we ran on a VAX 11/780, the CPU time needed to order about 50 000 transitions shortened from 102 min to about 2 min.

Module IMAGOGRAPH. This module produces the graphical display of the spectrum on a graphic device, opening a new window called "Spectrum" (top of Figure 1). Because the module has been written using the VWS Digital Graphics Libraries,¹⁰ the calls to this module can be done only when the procedure is run from a VaxStation. The module has been designed to perform three main tasks: (a) to display the calculated and/or experimental spectrum, (b) to assign interactively experimental to calculated frequencies, and (c) to manipulate the experimental spectrum which has been previously transferred from a personal computer where it has been transformed and phased by means of Bruker WIN-NMR software¹¹ (see Figure 5 for a list of options a and b).

The module can be called by the "Prepare Experimental Spectrum File" option in "Main Menu" (Figure 2) or by the

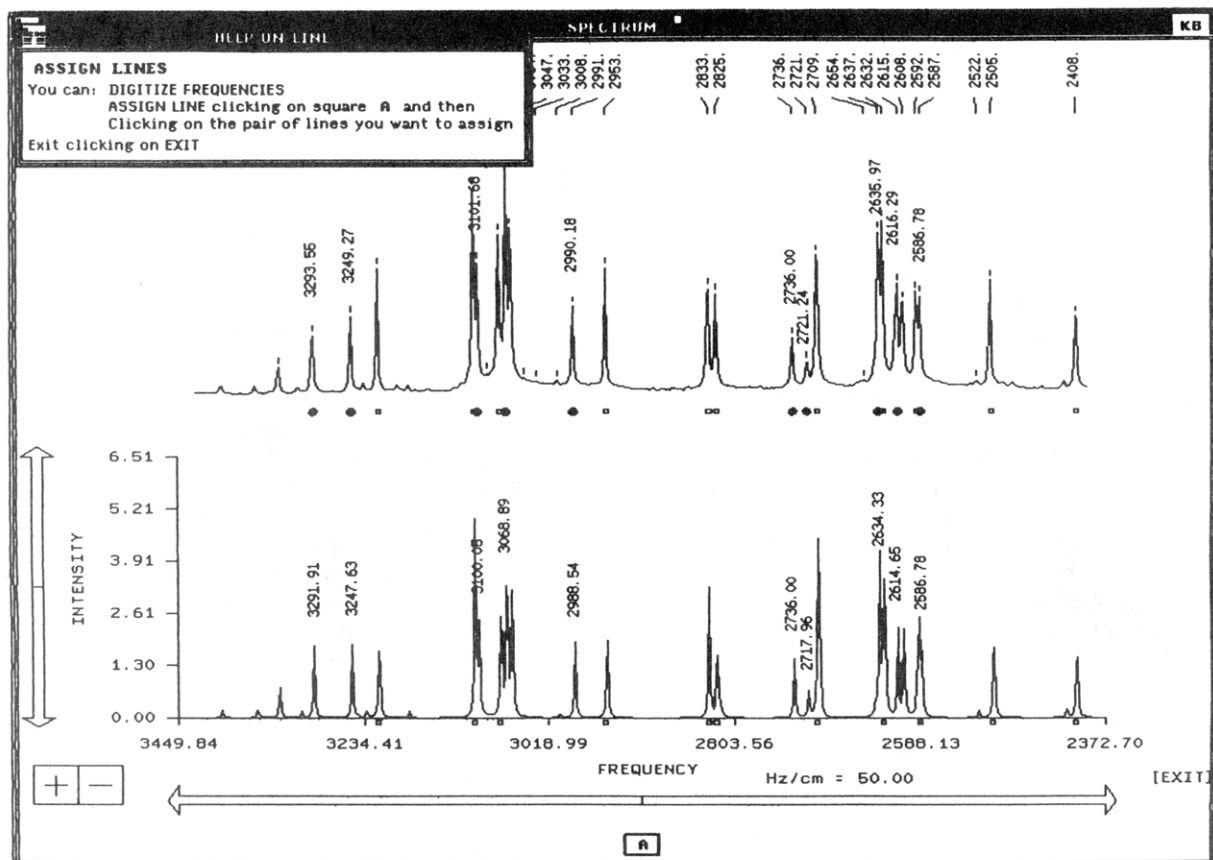


Figure 6. Typical assignment session. Part of the benzyl iodide spectrum of Figure 1 is displayed at 50 Hz/cm. The procedure displays the "Help on Line" window in an empty portion of the screen, but here it is moved over the "Spectrum" window to be shown with the spectra. See text for explanations.

option that redirects the output of OUTPGM to a graphical device in "List of Options" (Figure 4). According where it is called from two different subsets of all the available options become active: options a and c in the former and options a and b in the latter case.

The experimental free induction decays are processed by the spectrometer software, in our case Bruker, and the spectrum is transferred to the VaxStation. However, the commercially available baseline flattening procedures are unsatisfactory for our needs because anisotropic spectra have very complex baselines. Also, the cubic spline interpolation routine provided by Bruker is unsuitable because the maximum number of reference points allowed is too small for anisotropic spectra. We have written our own baseline procedure (a cubic spline interpolation routine with up to 1000 reference points with a counter to show how many are left). The experimental spectrum shown in Figure 1 had its baseline flattened using about 500 reference points.

Three other options intended (i) to cut out unwanted parts of the experimental spectrum, (ii) to choose an arbitrary frequency reference point, and (iii) to calculate the noise height were added so that these useful spectral features can be changed after transfer of the experimental spectrum to the cluster.

IMAGOGRAPH is written with the particular characteristics of anisotropic spectra in mind. These spectra are composed of many thousands of closely spaced, well-resolved, single transitions dispersed over a range of a few kilohertz with few eye-catching features. Displaying the full spectrum requires a large hertz/centimeter ratio which makes it useful only for quick, superficial comparisons between experimental and calculated spectra, as is clearly shown in Figure 1. The spectrum is for seven spins, and the situation is much worse

for the more complex spin systems the procedure can handle. Only a relatively narrow frequency range compared with the full spectral width can be displayed with a hertz/centimeter ratio suitable for options such as comparing the experimental and calculated spectra, peak picking, and assigning transitions. A quick and reliable way to move around the spectrum is then very important, and we have introduced many options to fulfill this task.

The same particular characteristics of anisotropic spectra make error prone the line assignment task, the crucial point in spectral analysis, because the lines, clustering together in many unpredictable ways, can produce quite complex signals: since the iterative step is very sensitive to incorrect assignments, a good algorithm should ensure that unwanted assignments are very unlikely. Of course, when single isolated lines are present, the requisite is easily satisfied, but for more complex situations (in Figure 6 there are at least two such cases) a more sophisticated procedure is needed.

Given the nature of the problem, the way the procedure works can be described only by following a real case: the analysis of the benzyl iodide spectrum dissolved in a nematic mesophase. An initial parameter set was derived from spectral parameters of previously analyzed similar molecules (benzyl fluoride,¹² chloride,¹³ and bromide¹ in our case) by varying a few relevant couplings and the methylenic proton chemical shifts. The calculated spectra were quickly compared with the experimental one until a spectrum was found with a reasonable number of the more intense lines unmistakably recognizable (nearly 20 in our case). These lines were assigned and the iterative cycles started. First it was iterated on just two parameters at times, but quickly, as more lines were recognized and assigned, it was possible to increase the number

of parameters we were iterating on. Since the chosen starting set was quite a good one, a set of spectral parameters which gave the calculated spectrum partially reproduced in Figure 6 (bottom trace) was quickly obtained. At first glance, by comparison of the upper (experimental) and bottom traces, it can be seen that spectral analysis is nearly accomplished (in fact, the calculated and experimental frequencies differ on the average by less than 3 Hz), but some features in the two more crowded regions are still not fully resolved. Many lines are already assigned, but before the last iteration cycles can be started, more have to be assigned. The spectral region we are interested in is displayed with some of the options previously described (in this case fixing first a hertz/centimeter ratio of 200, moving through the spectrum with the "Move Spectrum By Window" option, and then fixing via the mouse the upper frequency value and entering a more suitable hertz/centimeter value of 50). The line frequencies are written on the screen with the "Peak Peaking" option (it was decided to display only the experimental ones), while the pairs of calculated and experimental transitions already assigned are marked with a white rectangle by clicking on the "Display Assigned Lines" option. This has to be done to avoid assigning the same pair twice, which would produce a fatal error in the following run of ARCANA. For this reason the procedure checks all of the new assigned lines against the old ones for duplicate line number as soon as it exits from the IMAGOGRAPH module. The "Assign Transition" routine is now entered: as shown in the "Help on Line" window (Figure 6, top), the line frequencies can be read by moving the cursor on the top of the lines through a calling to the "Digitize Frequencies" option (such a case is not shown) or, after clicking on the "A" marked square, a pair of lines is selected and a dialogue with the user started to make sure the correct lines were selected. In fact, it has been found that even if a very restricted frequency range is fixed, it is possible to select more than one experimental and/or calculated line; when this happens, the identification number, frequency, and intensity of all the selected lines are displayed and the user is asked to make a choice. In the example illustrated in Figure 6 the very intense experimental line at 3608 Hz is actually a superposition of three less intense lines falling in a 2-Hz interval; at a lower hertz/centimeter ratio more than one experimental line can be picked up by the "Peak Picking" option near the line at 2637 Hz, and these lines correspond to many calculated ones. In both cases the correct assignments were made possible only by using the interactive dialogue. Many spectra can be even more complex and some typing and deleting of assigned lines have to be done directly on the input file by calling it later from the procedure or (better) opening a new window on the screen and modifying it with the preferred editor.

The assigned pair is then marked with a shadowed circle, and the identification number and the experimental frequency are stored in a file. The same procedure can be repeated in different portions of the spectrum until all of the necessary assignments are done. The file is then properly pasted in the input file ready for a further run of module ARCANA.

CONCLUSIONS

The procedure described here has been thoroughly tested in recent years in our laboratory, making possible the analysis of anisotropic spectra from such molecules as biphenyl^{14a} (a 10-spin system with 12 independent direct couplings) or 4-methoxy-4'-cyanobiphenyl and 4-(α,α,α -trifluoromethoxy)-4'-cyanobiphenyl^{14b} (11-spin systems with 17 independent

direct couplings). The anisotropic spectra of these spin systems could not be analyzed by the most widespread, commonly used commercial program for spectral analysis, Bruker's PANIC¹⁵ (which allows for many of the graphical facilities we have discussed) simply because the number of interacting nuclei is higher than its maximum value and the program cannot be easily modified. For simpler spin systems such as the seven-spin system benzyl bromide (5-s CPU time without iterations on a 3100 VaxStation), the resulting running times are exceedingly long. On the other hand, programs such as LEQUOR,⁶ which could be easily modified to include spin systems even larger and which run on very fast computers, lack interactive graphic procedures.

Our main aim in writing the procedure was to link together an efficient and fast simulation/iteration program with a powerful graphical routine and to make it run in a computing environment completely independent from the NMR spectrometer dedicated computer. We chose the VWS Graphical Library as graphical language as it was the only one available in our laboratory when we started to develop the procedure, but work is in progress to rewrite the procedure and IMAGOGRAPH module using C language and the OSF/Motif¹⁶ graphical library to increase portability.

EXPERIMENTAL SECTION

The NMR spectra are registered on a AC 300-MHz Bruker spectrometer using the Bruker software package and transferred using Kermit¹⁷ to a Bull Z-386/20 Zenith personal computer, where they are Fourier transformed and phased by means of WIN-NMR.¹¹ The file is translated to ASCII code and then transferred with Kermit¹⁷ to the Vax cluster. The cluster (Operating System VMS 5.5) is composed of a Digital MicroVax 3600 (boot node, main memory 32 Mb, 2.5 mips) and two Digital VaxStations (a 2000 with main memory 6 Mb, 0.7 mips, and a 3100 with main memory 8 Mb, 2.5 mips). The cluster is connected through an optical link at 100 Mbits with a Convex C240 computer (>100 mips; O. S. UNIX Berkeley 3.2), where a procedure containing ARCANA has been installed. Selecting a proper option, the ARCANA input files are transferred from the Vax cluster to the C240, where the case is run. The output files are transferred back to the Vax cluster to be displayed.

We succeeded in analyzing spectra due to molecules with up to 11 interacting nuclei^{14b} on the 3100 MicroVax also if the CPU times are quite high [1.5 h needed for four iteration cycles, 15 min without iterations for the 4-(α,α,α -trifluoromethoxy)-4'-cyanobiphenyl where the X-factorization too can be used]; more complex spin systems can be handled with a reasonable turnover only using the C240 computer (elapsed times within minutes).

All of the figures have been obtained using the Digital "Print (portion of) screen" facility and printed on a Digital LA210 printer (Figures 1 and 6) or a Digital laser printer LNO3.

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- (15) PANIC is a package developed by Bruker Spectrospin.
- (16) OSF/Motif is an Open Software Foundation Inc. trademark.
- (17) Kermit is a communication protocol developed by Columbia University.