

Proton 1-D Double Quantum Filtering³ (DQF)

This simple experiment can be extremely useful even though the very mention of the word 'quantum' tends to turn away all but avid physical chemists. Basically a double quantum filter discriminates between coupled and non-coupled protons and allows coupled proton signals through while eliminating or suppressing signals from uncoupled protons. This means that solvent peaks such as uncoupled H₂O, HOD, dioxane, CHCl₃, DMSO or acetone singlets may be effectively removed by DQF experiments. The discrimination against singlets is especially valuable, for example, in the highly crowded methyl region of a steroid or triterpenoid, where methyl singlets and doublets overlap.

Although the utility is not well illustrated with sulcatol, the general effects of the experiment are clear from Figure 1B. The methyl singlets 7 and 8 are quite well suppressed while the methyl doublet, H1, remains intense. Residual signals from 7 and 8 arise primarily because these 'singlets' are in fact long-range coupled to H5.

In the DQF experiment, the coupling constants which can optimally pass through the filter are selected³ ($J \sim 7$ Hz for the above methyl singlet/methyl doublet discrimination).

Proton-Proton 2-D Shift Correlation (COSY)

A 2-D COSY experiment gives, in a single experiment, all the information obtainable from a complete series of decoupling experiments, i.e. all pairs of coupled protons (that is protons which share a coupling J) are revealed. It is a remarkably robust 2-D experiment⁵ and works reliably even if pulses are not set exactly. The number of possible artifacts in the spectrum is very small provided the system is at equilibrium before each sequence (hence the use of two or four dummy scans).

A simple COSY-45 spectrum of sulcatol is shown in Figure 2. A mixing pulse of 45° rather than the 90° pulse of the standard COSY experiment gives reduced diagonal peaks and is the favoured variant. For the reader not familiar with these 2-D spectra, start with H1 which is unambiguously assigned as the only methyl doublet. It is clearly correlated with H2 as evidenced by the correlation peak labelled (a). Similarly H2 correlates with H3 (b), H3 with H4 (c), H4 with H5 (e), and H5 with H7 and H8 (d) (due to longer-range allylic coupling). When the proton spectrum is well resolved, as is the case with sulcatol, it is sufficient to use even lower resolution — all the correlations are perfectly clear when 512 points are used in the F2 domain⁶ and 128 zero-filled to 256 in F1.

Although the number of variations of this experiment are enormous, the most popular are:

1. Simple homonuclear shift-correlated 2-D NMR experiment: COSY, as in Figure 2, results in a symmetric matrix with shifts and couplings in both dimensions (F1 and F2)⁶; off-diagonal peaks correlate spins which share a coupling J . If sufficient resolution is used in the COSY-45 variant, relative signs of coupling constants can be deduced by direct observation of the COSY-45 spectra².
2. COSY with F1 decoupling: This results in a matrix in which the F2 projection is the normal proton NMR spectrum while F1 is a proton-decoupled proton spectrum; i.e. each proton or group of equivalent protons appears as a single peak, without coupling. Since it is physically impossible to actually perform a 1-D experiment where you acquire the proton spectrum while decoupling all the protons, these 2-D methods which yield decoupled proton spectra as projections are often valuable. However, they work well only for weakly coupled systems and, since extra pulses are involved, the experiment is more prone to artifacts.
3. COSY with DQF: COSY with double (or multiple) quantum filtering eliminates solvent peaks and/or discriminates between coupled and non-coupled protons.
4. Long-Range COSY. In the long-range COSY experiment, delay periods in the pulse sequence are chosen so that long-range couplings (ones greater than the normal 2- and 3-bond coupling interactions) are emphasised; i.e. protons coupled by long-range coupling also give rise to strong off-diagonal correlation peaks. In this experiment with sulcatol, the spectrum is similar to that of Figure 2, the only

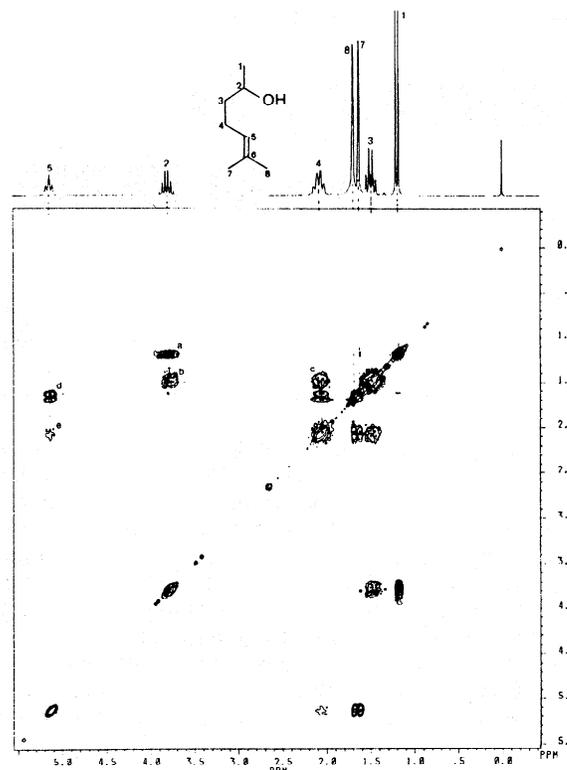


Figure 2: COSY-45 spectrum of sulcatol. (1024 by 256 zero-filled to 512). Time: 3.3 hr.

difference being the greater intensity of the correlation peaks (labelled d, Figure 2) between H5 and H7/H8 (4-bond coupling).

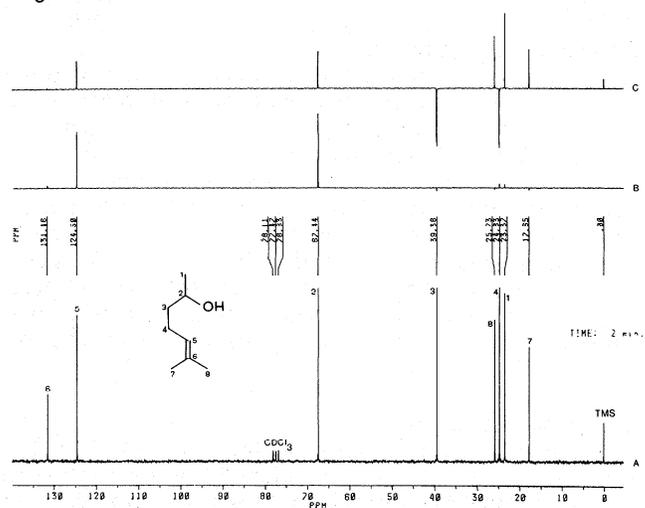
5. Others: Other sequences include simultaneous solvent suppression using presaturation, and a series of sequences which result in phase-sensitive spectra with⁷ or without⁸ DQF. Such sequences are particularly useful in analysing complex spectra since they provide good axial-peak suppression (which allows identification of cross-peaks very close to the diagonal) and improved spectral resolution. They are however more demanding on disk space. It is also possible to combine the COSY experiment with the NOESY experiment in a so-called CONOSY experiment and concurrently obtain information on protons which are near each other in space.

¹³C and ¹³C/¹H NMR Experiments

Standard and Edited Spectra

The standard ¹³C spectrum of sulcatol **1** along with two DEPT spectra are shown, with assignments in Figures 3A to

Figure 3: ¹³C spectrum, 32 scans, WALTZ-decoupled, 32K. A. Standard ¹³C spectrum. Time: 2 min. B. DEPT-90 experiment — CH's only. C. DEPT-135 experiment — CH, CH₃ positive, CH₂ negative.



3C. Each spectrum is a result of 32 scans, using WALTZ rather than broadband proton-decoupling. Figure 5B is from the DEPT experiment using the variable multiplicity section ^1H pulse of 90° . This gives rise to an edited spectrum in which only carbons bearing a single proton (CH 's) have significant intensity while other (C , CH_2 and CH_3) carbons are suppressed. It is instantly apparent that sulcatol contains two CH 's. Figure 3C is from the DEPT experiment with a 135° editing pulse. This results in positive signals for CH 's and CH_3 's, CH_2 's with negative intensities, and, since this pulse sequence transfers polarisation from the proton to the attached carbon, suppression of quaternary signals (carbons with no attached protons, e.g. C_6). It is again instantly clear that sulcatol contains two CH_2 's. There are also five CH 's plus CH_3 's (neglecting TMS at 0 ppm), three of which must be CH_3 's (since Figure 3B shows two CH 's).

The DEPT pulse sequence⁹ is probably the most widely used for this type of spectral editing. It is less sensitive than the earlier INEPT sequence to differences in C-H coupling constants and to incorrectly set pulse angles and is consequently a very "forgiving" experiment.

There are many variations of DEPT spectral editing procedures. Linear combinations of DEPT 45° , 90° and 135° spectra can give essentially pure CH , pure CH_2 and pure CH_3 spectra. However, the information is adequately contained in three spectra; normal carbon, DEPT-90, and DEPT-135, as shown in Figure 3. The differentiation of CH_3 's from CH 's is often trivial and in practice it is often sufficient to run only the DEPT-135 variant (Figure 3C) to identify the CH_2 's. Recently Pegg and Bendall¹⁰ have published a nice variation which actually acquires CH , CH_2 or CH_3 spectra with little breakthrough from the other components. This sequence was found to work very well in our laboratories and is particularly valuable for mixtures.

Spectra containing only resonances from quaternary carbons can be obtained by a variety of techniques, the most popular being the method of Bendall and Pegg.¹¹

Carbon-Proton J-Resolved

While the above DEPT sequences are excellent for determining carbon multiplicity (i.e. the number of protons attached to each carbon) and have completely replaced older methods based on single-frequency off-resonance decoupling, the ^{13}C - ^1H coupling constants are not extractable from these spectra. It is possible, in favourable instances to obtain coupling constants from fully coupled spectra (i.e. spectra obtained without proton decoupling) but spectral crowding, long-range coupling, and second-order effects often make the determination difficult. Directly bonded (i.e. one-bond) C-H coupling constants are ideally measured by the 2-D carbon-proton J-resolved experiment.

A 2-D heteronuclear J-resolved spectrum is given in Figure 4A. The multiplicities (or number of attached protons) are quickly apparent (6 is C, 5 is CH, 2 is CH, 3 is CH_2 , 8 is CH_3 , 4 is CH_2 , 1 is CH_3 , and 7 is CH_3). Note that the F1 scale⁶ represents $J/2$, not the full coupling J . The coupling constants can be measured directly off the 2-D contour plot but it is normal to make projections parallel to F1 through each carbon from which interpolation and measurement is easier.

$^{13}\text{C}/^1\text{H}$ Correlation

One of the most useful of all 2-D experiments, $^{13}\text{C}/^1\text{H}$ correlation is a brilliant experiment which allows ^{13}C chemical shift data along one axis (F2)⁶ to be correlated with ^1H data along the other (F1) via an INEPT-like polarisation transfer from ^1H to ^{13}C . An example is shown in Figure 5A. The correlation peaks (contours) in the figure show that C_6 has no attached protons, H_5 is attached to C_5 , H_2 to C_2 etc. It is from this experiment that the assignment of H_8 and H_7 can be made in the proton NMR. Although these two methyl singlets have very similar proton chemical shifts (and one would not confidently assign them from their shifts) the ^{13}C NMR chemical shifts are substantially different; C_7 , being *trans* to a proton is predictably at higher field than C_8 , *trans* to a carbon side chain¹². Once C_7

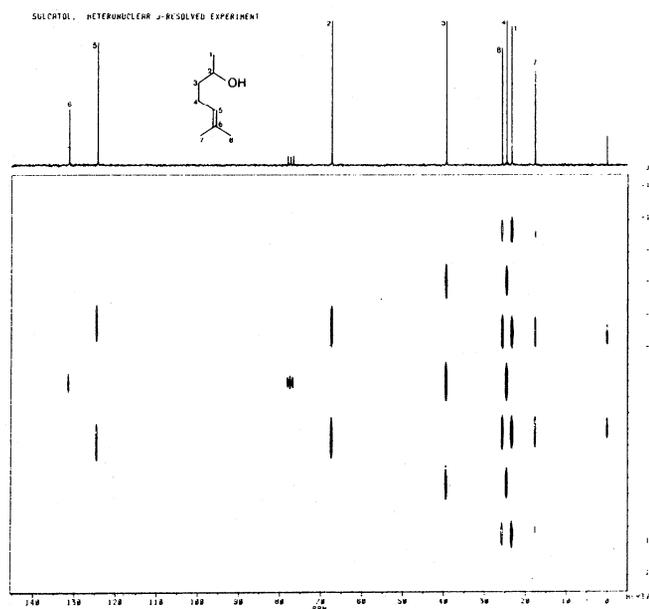


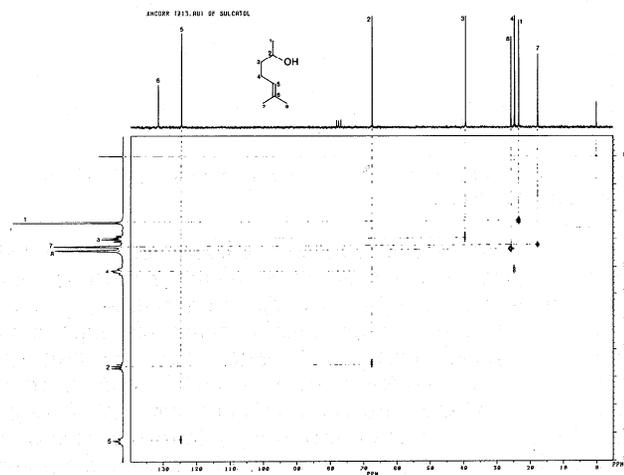
Figure 4A: 2-D Heteronuclear J-resolved spectrum of sulcatol (2K by 64 zero-filled to 128). Time: 2 hr.

and C_8 are assigned in the ^{13}C NMR, the correlations allow unambiguous assignments of H_7 and H_8 protons in the proton NMR.

The $^{13}\text{C}/^1\text{H}$ correlation experiment has a number of valuable features in addition to aiding assignment. Because ^{13}C resonances are sharp and dispersed over a large chemical-shift range, peaks seldom overlap in ^{13}C spectra of normal-sized molecules. Proton resonances however often overlap. Hence, the $^{13}\text{C}/^1\text{H}$ correlation experiment can be used to thoroughly dissect the proton spectrum. Projections through the carbon peaks (i.e. parallel to F1) give proton spectra of only the proton(s) attached to that carbon. For sulcatol, there are no overlapping resonances so the projections (Figure 5B) are no more revealing than the proton spectrum itself (and are of course of lower resolution). With more complicated molecules where the proton NMR spectrum is far from fully resolved but the ^{13}C spectrum is well resolved (for example with steroids and terpenoids), the projections give a series of fully resolved proton spectra. They effectively separate out all the overlapping proton resonances, a feat which may not be achieved in a normal proton spectrum even at 800 MHz or more! Only protons which are tightly coupled and have close chemical shifts will not be fully interpretable.

As with the COSY experiment, there are numerous variations, some of which are exceedingly useful. Firstly, equivalent DEPT-like versions exist, and there is therefore the possibility,

Figure 5A: $^{13}\text{C}/^1\text{H}$ 2-D correlation. (1K by 512 zero-filled to 1K). Time: 5.3 hr.



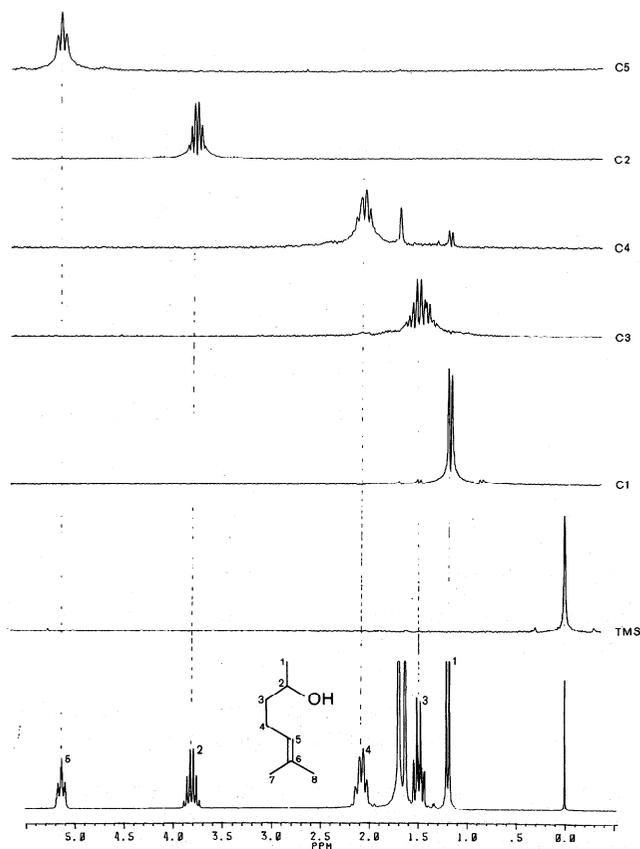


Figure 5B: Projections parallel to F1 through the indicated carbons in the 2-D $^{13}\text{C}/^1\text{H}$ correlation experiment.

by choosing the variable multiplicity selection pulse of a) 45° , b) 90° or c) 135° , of obtaining 2-D spectra with a) CH, CH_2 and CH_3 's all positive (as for Figure 5A), b) CH's only or c) CH and CH_3 's positive and CH_2 's negative as for the 1-D DEPT experiments in Figure 3. A decoupled version gives a correlation map with the carbon spectrum (as normal) in the F2 dimension and a proton-decoupled proton projection in the F1 dimension. Note that only couplings between spins not attached to the C3-H3 correlation peak. This procedure gives not only another method for obtaining the proton-decoupled proton spectrum but also enhanced sensitivity over the normal correlation experiment due to the fact that correlation signals are no longer smeared over proton multiplets.

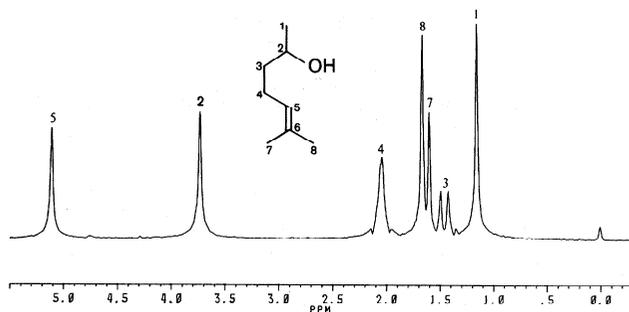


Figure 5C: F1 projection from proton-decoupled 2-D $^{13}\text{C}/^1\text{H}$ correlation experiment.

An exciting alternative to the above sequences for $^{13}\text{C}/^1\text{H}$ correlation appeared in the literature¹³ as this manuscript was being completed. Reynolds *et al* claim enormously greater sensitivity and reduced accumulation times (as little as 10 minutes) to achieve similar results (with lower proton resolution) to the proton decoupled version of the $^{13}\text{C}/^1\text{H}$ correlation experiment. This new sequence should enable $^{13}\text{C}/^1\text{H}$ correlations to be run as routinely as normal spectra and is likely to become a welcome addition to the library of valuable pulse sequences.

By appropriate choice of delay times it is also possible to obtain correlations for long-range $^{13}\text{C}-^1\text{H}$ couplings. Such an experiment additionally gives valuable correlations with quaternary carbons (e.g. C6). Another experiment, RELAY gives H to H to C connectivity information.

These correlations from more distant protons can be valuable in determining carbon-carbon connectivities and often are ideal alternatives to the insensitive INADEQUATE experiments. The RELAY example shown in Figure 5D was far from optimised but even at this level gives the C1 to C2 connectivity as well as C4 to C5 and C3; C8 and C7 weakly to C5 (long range), C3 to C4 etc.

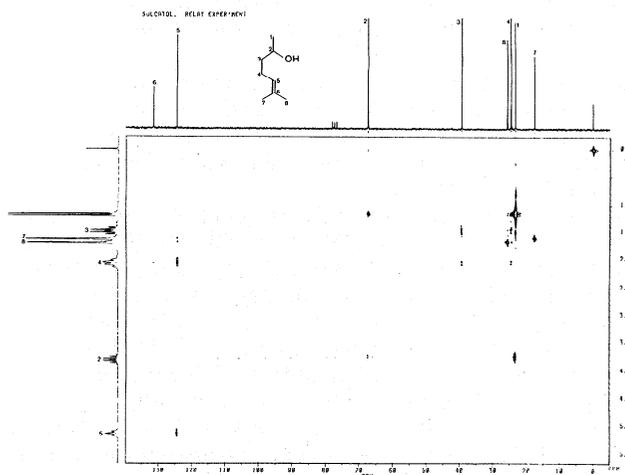
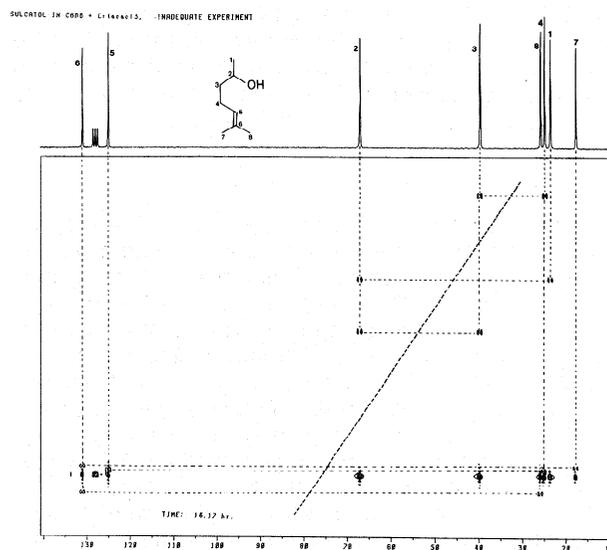


Figure 5D: $^{13}\text{C}/^1\text{H}$ Relay experiment. (1K by 512 zero-filled to 1K). Time: 5.3 hr.

Carbon-Carbon Coupling

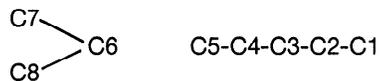
It is generally possible, by use of the preceding experiments, to obtain full connectivity information far more quickly than can be accomplished using the insensitive 1-D and 2-D INADEQUATE experiments. INADEQUATE experiments detect $^{13}\text{C}-^{13}\text{C}$ coupling and, since ^{13}C is only about 1% abundant, the sensitivity for this experiment is quite low. INADEQUATE experiments require either a high sample concentration or long data-accumulation times and are best for small molecules or those isotopically enriched with ^{13}C . However, they do yield unambiguous and straightforward connectivity information. The 2-D INADEQUATE experiment takes a little

Figure 6: 2-D INADEQUATE spectrum of sulcatol (1K by 256 zero-filled to 512). Note that the centers of each coupled pair all lie on a diagonal straight line (dotted). Time: 14.17 hr.



more time than the 1-D experiment but avoids the problems of overlapping lines and spectral congestion present in the 1-D spectrum of even a relatively simple molecule.

For the INADEQUATE experiment a 90% solution of sulcatol in benzene- d_6 , containing a few milligrams of chromium (III) acetylacetonate (relaxation reagent) to reduce the recycle delay, was used. The INADEQUATE plot, Figure 6, shows nearly complete connectivity.



Because the delay time was chosen to compromise between the optima for sp^3-sp^3 and sp^3-sp^2 $^{13}C-^{13}C$ coupling constants (40 Hz), the C5-C6 correlation peak is not present; sp^2-sp^2 couplings are typically about 70 Hz.

Experimental

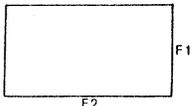
Materials: Sulcatol 1 was obtained from Aldrich Chemical Company and used without purification. For Carbon and 2-D experiments, a 25% solution in $CDCl_3$ was used.

Spectra: All spectra were run on a Bruker AC-200 FT NMR spectrometer equipped with a 5-mm $^{13}C/^1H$ dual probe using standard Bruker pulse programmes and software. The 90° pulse widths were: 1H observe, 7.8 μs ; 1H decouple, 13.8 μs ; ^{13}C observe, 8.3 μs . The INADEQUATE experiment was run on a 10-mm broad-band multinuclear probe. Some conditions are indicated in the figures.

Times shown on the figures are actual acquisition times but should not be considered minima or even representative — the signal to noise ratio is often far greater than is necessary. All Fourier transform times are less than 3 minutes with just an FT processor. An instrument equipped with an array processor would perform any of these 2-D transforms in well under a minute.

Further details, other experiments, and listings of acquisition parameters are available from the authors.

References and Notes

1. R. Benn and H. Gunther, *Angew. Chem. Int. Ed. Engl.*, **22**, 350-380 (1983).
2. A. Bax, *Two-Dimensional NMR in Liquids*, D. Reidel Publ. Co., USA, 1982.
3. U. Piantini, O. W. Sorensen and R. R. Ernst, *J. Amer. Chem. Soc.*, **104**, 6800-1 (1984).
4. Since J-modulation is present in the resulting spectrum, processing is favoured by software which can easily perform resolution enhancements (e.g. sine-bell, sine-bell squared, or Gaussian) and a magnitude calculation.
5. See the work of Alex Bain and his use of the program "SIMPLTN" for examining pulse-sequence artifacts. A. D. Bain, Bruker Spectrospin (Canada) Ltd. CANADA. The PASCAL program SIMPLTN is available from the ABACUS Bruker Users Group.
6. The convention for 2-D spectra is 
7. M. Rance, O. W. Sorensen, G. Bodenhausen, G. Wagner, R. R. Ernst and K. Wuthrich, *Biochem. Biophys. Research Communications*, **117** (2), 479-485 (1983).
8. D. Marion and K. Wuthrich, *Biochem. Biophys. Research Communications* **113** (3), 967-974 (1983).
9. D. M. Doddrell, D. T. Pegg and M. R. Bendall, *J. Magnetic Resonance*, **48**, 323-327 (1982).
10. D. T. Pegg and M. R. Bendall, *J. Magnetic Resonance*, **53**, 347-351 (1985).
11. D. T. Pegg and M. R. Bendall, *J. Magnetic Resonance*, **63**, 556-572 (1985).
12. D. T. Pegg and M. R. Bendall, *J. Magnetic Resonance*, **60**, 347-351 (1984).
13. c.f. Geraniol: *cis*, 17.40; *trans* 25.50 — In "C-13 NMR Spectroscopy". E. Breitmaier and W. Voelter, Verlag Chemie, NY, 1978, p. 222.
13. W. F. Reynolds, D. W. Hughes, M. Perpich-Dumont and R. G. Euriqez, *J. Magnetic Resonance*, **64**, 304-311 (1985).