

An Improved Method for Heteronuclear Chemical Shift Correlation by Two-Dimensional NMR

Since the use of two-dimensional NMR for correlating the spectra of coupled heteronuclei was first proposed in 1977 (1), a considerable number of papers have appeared describing different techniques for heteronuclear correlation (2-8). The most widely used method (5, 9-12) produces a two-dimensional spectrum in which one signal appears for each directly bonded carbon-hydrogen pair in a molecule. The f_1 domain of the spectrum is governed by the proton resonance frequencies and the f_2 by the carbon-13, so that the correlation signal for a given CH_n group appears at frequency coordinates which are just the proton chemical shift in f_1 , and the carbon-13 chemical shift in f_2 . Heteronuclear couplings are absent from both frequency domains, although proton-proton scalar coupling structure remains in f_1 . Such experiments are relatively simple to perform, and show good sensitivity; data for a typical shift correlation two-dimensional spectrum may be obtained in about 10 times the time required to produce a good proton-decoupled carbon-13 spectrum.

A number of chemical applications of such shift correlation experiments have been published (9-11), all using the basic pulse sequence of Ref. (5). This sequence generates proton-decoupled carbon-13 free-induction decays which are modulated as a function of t_1 by the frequency difference between the proton chemical shift and the proton transmitter frequency. Since the signals are amplitude modulated with respect to t_1 , it is not possible to distinguish between positive and negative f_1 frequencies (13), so that to avoid ambiguity it is necessary to position the proton transmitter either to low field or to high field of the proton spectrum. This is unfortunate in that much of the proton transmitter power is thus wasted, which can lead to severe heating problems with ionic samples if the same proton frequency is used for pulses and for decoupling, as is usually the case. A second problem is that twice as many t_1 samples are needed to digitize the f_1 domain of the spectrum if the proton transmitter lies to one side of the proton spectrum. Both from the point of view of optimum decoupling and from that of minimizing data storage needs and complexity of data processing, it would be desirable to be able to place the proton transmitter in the middle of the proton spectrum.

This communication suggests the use of phase cycling of the proton and carbon-13 transmitter pulses, similar to that recently introduced in homonuclear correlation experiments (14-16). This converts amplitude modulation as a function of t_1 into phase modulation, allowing positive and negative f_1 frequencies to be distinguished. Thus in the usual experiment (5), examination of f_2 spectra for successive t_1 values would show the amplitudes of individual signals oscillating while their phases remained fixed. The proposed modification would lead to f_2 spectra with signals oscillating between absorption and dispersion mode while

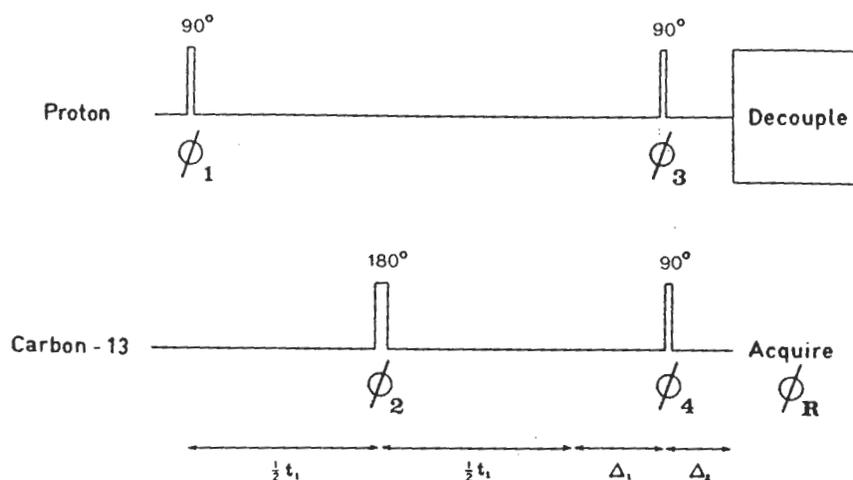


FIG. 1. Pulse sequence used for measuring two-dimensional chemical shift correlation spectra; the phases ϕ_{1-4} of the various pulses and of the detection ϕ_R are cycled on successive transients during signal averaging, according to the scheme summarized in Table 1.

keeping nearly the same absolute intensity, the sense of the phase rotation being determined by the sign of the offset of the appropriate proton resonance from the proton transmitter frequency. The pulse sequence of Fig. 1 is retained (5), but the receiver and the proton and carbon-13 transmitter phases are changed in cyclic fashion on successive transients during time averaging, as summarized in Table 1. The resultant technique is thus significantly less complex than the data manipulation of the "hypercomplex Fourier transformation" method proposed by Müller and Ernst (7).

The mechanism of chemical shift correlation experiments has been extensively discussed in the literature (1-3, 6, 7, 9, 11) and will not be described here. A single transient obtained using the pulse sequence of Fig. 1 unadorned with phase shifts will (1-3, 6, 11) give rise to the following signal for a simple carbon-13-proton AX spin system,

$$S_0(t_1, t_2) = M_0 \sin(2\pi\delta_H t_1) \sin(\pi J \Delta_1) \sin(\pi J \Delta_2) \exp(-2\pi i \delta_C t_2), \quad [1]$$

TABLE I
RADIOFREQUENCY PHASES USED IN THE
PULSE SEQUENCE OF FIG 1 ON
SUCCESSIVE TRANSIENTS

Transient number	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_R
1	270	0	0	0	0
2	180	0	0	0	90
3	90	0	0	0	180
4	0	0	0	0	270
etc.

FIG. 2. Two-dimensional chemical shift correlation spectrum in deuteriochloroform, obtained in a spectrometer operating at 50.3 MHz.

where J is the carbon-proton and proton chemical shifts, and the second proton 90° pulse are not

$$S_{90}(t_1, t_2) = iM_0 \cos(\dots)$$

so that summing the two transients

$$S_{0+90}(t_1, t_2) = 2M_0 \exp(\dots)$$

allowing discrimination between

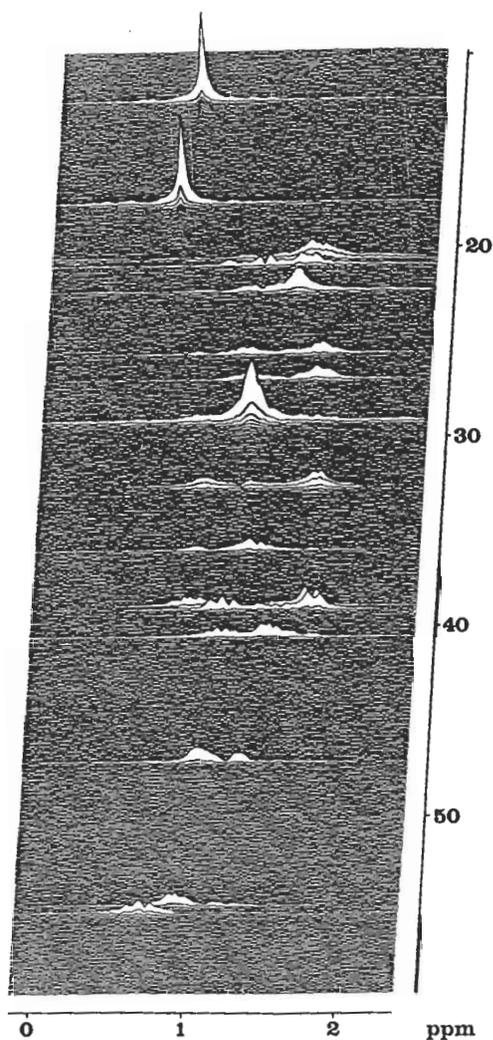


FIG. 2. Two-dimensional chemical shift correlation spectrum of a 1.5 *M* solution of 5- α -androstane in deuteriochloroform, obtained in approximately 30 min of time averaging using a Varian XL-200 spectrometer operating at 50.3 MHz for carbon-13.

where J is the carbon-proton scalar coupling, and δ_C and δ_H are the carbon-13 and proton chemical shifts, respectively. If the phases of the receiver and of the second proton 90° pulse are now advanced by 90° in a second experiment, the result is

$$S_{90}(t_1, t_2) = iM_0 \cos(2\pi\delta_H t_1) \sin(\pi J\Delta_1) \sin(\pi J\Delta_2) \exp(-2\pi i\delta_C t_2), \quad [2]$$

so that summing the two transients restores phase modulation as a function of t_1 ,

$$S_{0+90}(t_1, t_2) = 2M_0 \exp(2\pi i\delta_H t_1) \sin(\pi J\Delta_1) \sin(\pi J\Delta_2) \exp(-2\pi i\delta_C t_2), \quad [3]$$

allowing discrimination between positive and negative f_1 frequencies.

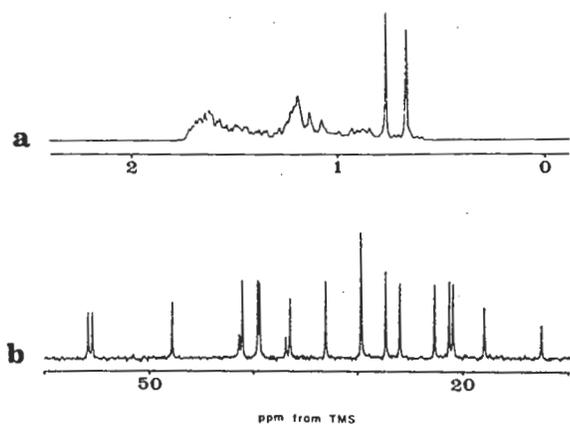


FIG. 3. Conventional proton (a) and carbon-13 (b) spectra for 5- α -androstane.

The remainder of the phase cycling scheme of Table 1 serves to suppress residual carbon-13 signals not due to polarization transfer from protons, and to remove the f_2 zero frequency artifact introduced by imperfect receiver phase balance. Depending on the relative sign of the phase shift introduced between the two proton 90° pulses, the two exponential terms of Eq. [3] may have the same or, as is the case in [3], opposite senses. The physical significance of choosing opposite senses for the t_1 and t_2 precession terms is that this leads to a partial cancellation of static field inhomogeneity effects, giving rise to a coherence transfer echo (17) and thus improving the sensitivity of the experiment slightly. In homonuclear correlation experiments it can be advantageous to select the opposite phase relationship (16), as this can help to discriminate in favor of resonances with natural linewidths.

Figure 2 shows the result of applying the technique described to a solution of the steroid 5- α -androstane in deuteriochloroform; for comparison purposes the conventional proton decoupled carbon-13 and proton spectra for this molecule are shown in Fig. 3. Clearly an analysis of the normal proton spectrum would not be feasible even at the highest magnetic fields currently available, whereas the spectrum of Fig. 2 allows all the proton shifts of androstane to be determined using the known carbon-13 assignments (18). Although the method proposed differs only very slightly from that currently in routine use (5), the advantages of reducing decoupler power requirements by a factor of 2 to 4, reducing the size of data matrix by nearly one-half, and halving the number of t_1 increments required, enhances significantly the utility of one of the most effective of the many two-dimensional NMR techniques now available. Analogous phase cycling schemes are possible in many other two-dimensional NMR experiments, including the important new techniques for mapping chemical exchange and cross-relaxation pathways (19).

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the advice, encouragement, and stimulation of Ray Freeman, who very generously made available the facilities used in the work described. G.A.M. gratefully acknowl-

edges discussions with Geoffr
The XL-200 spectrometer was
Council, while A.B. thanks th

1. A. A. MAUDSLEY AND R.
2. A. A. MAUDSLEY, L. M.
3. G. BODENHAUSEN AND F.
4. G. BODENHAUSEN AND F.
5. R. FREEMAN AND G. A.
6. P. H. BOLTON AND G. B.
7. L. MÜLLER AND R. R. E.
8. G. BODENHAUSEN, *J. M.*
9. R. FREEMAN AND G. A.
10. L. D. HALL, G. A. MORI
11. G. A. MORRIS AND L. D.
12. G. A. MORRIS AND L. D.
13. G. BODENHAUSEN, R. F.
- 133 (1977).
14. K. NAGAYAMA, K. WÜ
- 305 (1979).
15. K. NAGAYAMA, A. KUM
16. A. BAX, R. FREEMAN, A.
17. A. A. MAUDSLEY, A. W.
18. H. EGGERT AND C. J. D.
19. S. MACURA AND R. R. E.

*Physical Chemistry Lab
University of Oxford
South Parks Road
Oxford OX1 3QZ, Engl*

Received November 20

* Present address: Departm
† Author to whom corresp

edges discussions with Geoffrey Bodenhausen, who has independently proposed similar experiments. The XL-200 spectrometer was purchased with the aid of an equipment grant from the Science Research Council, while A.B. thanks the Delft University Fund for a stipend.

REFERENCES

1. A. A. MAUDSLEY AND R. R. ERNST, *Chem. Phys. Lett.* **50**, 368 (1977).
2. A. A. MAUDSLEY, L. MÜLLER, AND R. R. ERNST, *J. Magn. Reson.* **28**, 463 (1977).
3. G. BODENHAUSEN AND R. FREEMAN, *J. Magn. Reson.* **28**, 471 (1977).
4. G. BODENHAUSEN AND R. FREEMAN, *J. Am. Chem. Soc.* **100**, 320 (1978).
5. R. FREEMAN AND G. A. MORRIS, *J. Chem. Soc. Chem. Commun.*, 684 (1978).
6. P. H. BOLTON AND G. BODENHAUSEN, *J. Am. Chem. Soc.* **101**, 1080 (1979).
7. L. MÜLLER AND R. R. ERNST, *Mol. Phys.* **38**, 909 (1979).
8. G. BODENHAUSEN, *J. Magn. Reson.* **39**, 175 (1980).
9. R. FREEMAN AND G. A. MORRIS, *Bull. Magn. Reson.* **1**, 5 (1979).
10. L. D. HALL, G. A. MORRIS, AND S. SUKUMAR, *J. Am. Chem. Soc.* **102**, 1745 (1980).
11. G. A. MORRIS AND L. D. HALL, *J. Am. Chem. Soc.*, in press.
12. G. A. MORRIS AND L. D. HALL, *Can. J. Chem.*, in press.
13. G. BODENHAUSEN, R. FREEMAN, R. NEIDERMEYER, AND D. L. TURNER, *J. Magn. Reson.* **26**, 133 (1977).
14. K. NAGAYAMA, K. WÜTHRICH, AND R. R. ERNST, *Biochem. Biophys. Res. Commun.* **90**, 305 (1979).
15. K. NAGAYAMA, A. KUMAR, K. WÜTHRICH, AND R. R. ERNST, *J. Magn. Reson.* **40**, 321 (1980).
16. A. BAX, R. FREEMAN, AND G. A. MORRIS, *J. Magn. Reson.* **42**, 164 (1981).
17. A. A. MAUDSLEY, A. WOKAUN, AND R. R. ERNST, *Chem. Phys. Lett.* **55**, 9 (1978).
18. H. EGGERT AND C. J. DJERASSI, *J. Org. Chem.* **38**, 3788 (1973).
19. S. MACURA AND R. R. ERNST, *Mol. Phys.* **41**, 95 (1980).

AD BAX*
GARETH A. MORRIS†

*Physical Chemistry Laboratory
University of Oxford
South Parks Road
Oxford OX1 3QZ, England*

Received November 20, 1980

* Present address: Department of Applied Physics, University of Technology, Delft, The Netherlands.

† Author to whom correspondence should be addressed.