

Elimination of Spin–Spin Splittings from High-Resolution Proton NMR Spectra

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High-resolution proton spectroscopy of complex molecules would be far simpler if the resonance responses were determined only by chemical-shift effects with no spin–spin splitting, in analogy with broadband decoupled ^{13}C spectroscopy. A possible route to this goal was pioneered by Aue *et al.* (1) who showed that the appropriate 45° projection of a two-dimensional proton J spectrum would catch the spin multiplets in *enfilade*, leaving only chemical-shift information. Unfortunately there are considerable practical difficulties with this technique posed by the bizarre lineshapes recorded after Fourier transformation of phase-modulated signals. The phase-twist lineshape (2) has a vanishing 45° projection, while the alternative absolute-value mode has long tails and undesirable overlap properties. Heroic efforts have been made (3–10) to circumvent these problems, but no simple, universal and robust solution has yet emerged. Broadband decoupled proton spectra can be obtained but they all suffer from considerable distortion of relative intensities or unwieldy lineshapes.

Recently some success has been achieved with a method where the individual spin multiplets are separated by a pattern-recognition algorithm based on their symmetry properties (11). The two-dimensional J spectrum is recorded in a mode where there is no discrimination of the signs of the F_1 frequencies so that spin–spin multiplets that would normally lie on 45° diagonals are folded on themselves, superimposing reflected responses along 135° diagonals. This specific symmetry feature allows adjacent multiplets to be separated even in very crowded regions of the spectrum. The main drawback of this method is that the data-processing algorithm is rather complex and the calculation can be quite protracted.

We propose here a simpler and faster method which promises comparable results by attacking the lineshape problem directly. A two-dimensional proton J spectrum is recorded in the usual manner, the F_1 dimension being derived by Fourier transformation of the spin-echo modulation (1, 12, 13). In this case no folding is employed so the spin multiplets lie only along 45° diagonals. The idea is to convert the two-dimensional absolute-value lineshape with its exten-

sive tails running in the F_1 and F_2 dimensions into something more akin to a two-dimensional Gaussian function. In principle the lineshape transformation (deconvolution) may be implemented either as a multiplication in the time domain or as a convolution in the frequency domain. We chose frequency-domain processing because it is much faster since it avoids multiple forward and reverse Fourier transformations. Precautions were taken to work with experimental data that extended far enough in both dimensions so that there were no edge effects during the deconvolution.

The one-dimensional deconvolution function (14) was designed to convert a magnitude lineshape $m(f)$ into a Gaussian $g(f)$ with the linewidth at half-height reduced by $\sqrt{3}$ and with much less prominent tails. The deconvolution

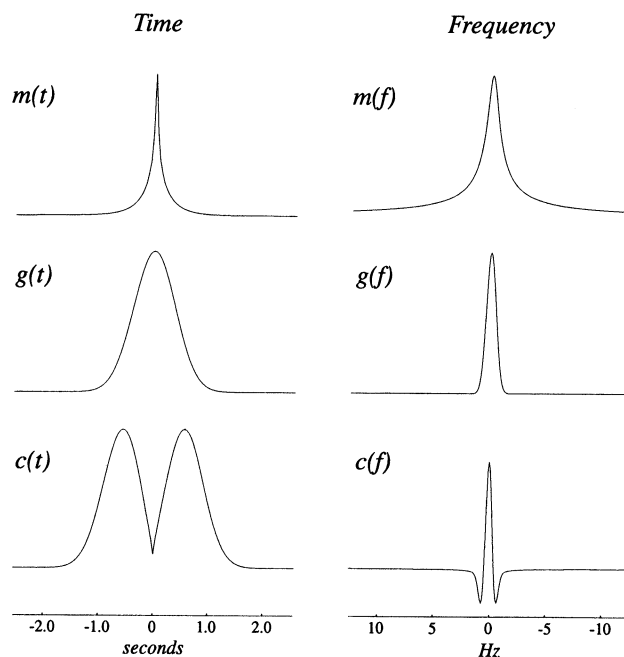


FIG. 1. Corresponding time-domain and frequency-domain functions used for the lineshape transformation. (Top) The experimental absolute-value mode. (Center) The target Gaussian with the width reduced by $\sqrt{3}$. (Bottom) The deconvolution function $c(f)$, the Fourier transform of $c(t) = g(t)/m(t)$.

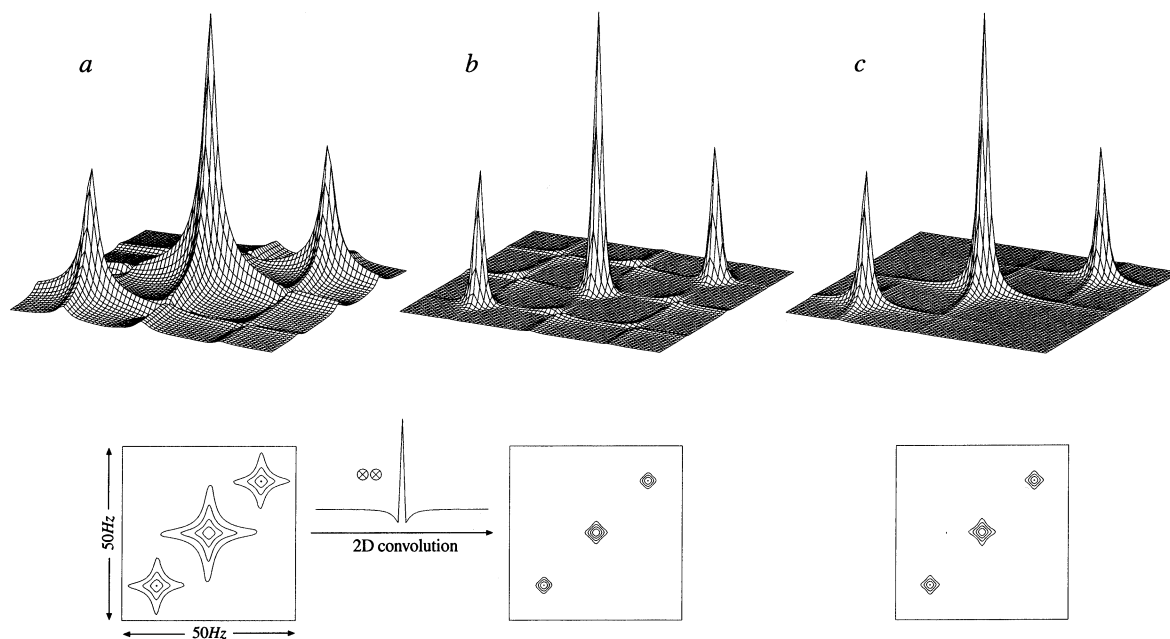


FIG. 2. Simulated two-dimensional lineshapes for a 1:2:1 triplet (a) in the absolute-value mode and (b) in the absolute-value mode after the lineshape transformation described in the text. Note the interference effects in regions where two tails overlap. The one-dimensional deconvolution function was applied trace-by-trace, first in the F_1 dimension and then in F_2 . (c) The pure absorption mode (Lorentzian).

function $c(f)$ was derived by Fourier transformation of the function $c(t) = g(t)/m(t)$, where $g(t)$ and $m(t)$ are the transforms of $g(f)$ and $m(f)$, respectively (Fig. 1). The only variable parameter required is the experimental linewidth of an absolute-value-mode singlet. This deconvolution function was applied successively in the F_1 and F_2 frequency dimensions.

This lineshape transformation is illustrated acting on a simulated absolute-value-mode triplet in Fig. 2 where the result is compared with the corresponding pure-absorption-mode spectrum. Note the interference effect where the tails of the absolute-value responses overlap. It arises because the dispersion-mode contributions have opposite phases in these regions. Owing to the discrete digitization steps the final

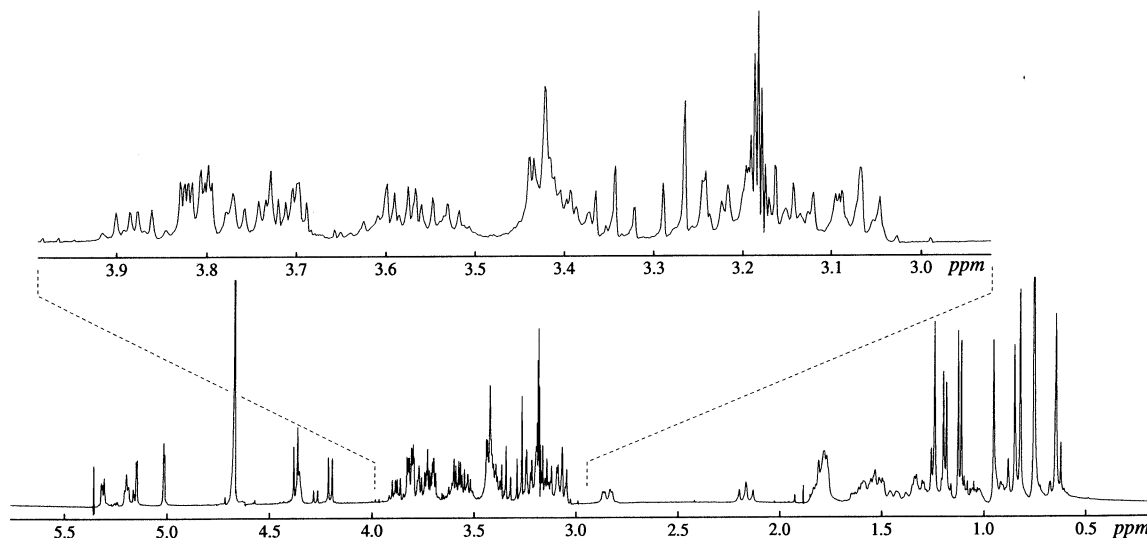


FIG. 3. Conventional 400 MHz proton spectrum of chrysaline with an expansion of the crowded region containing 25 chemically distinct sites, emphasizing the high degree of overlap.

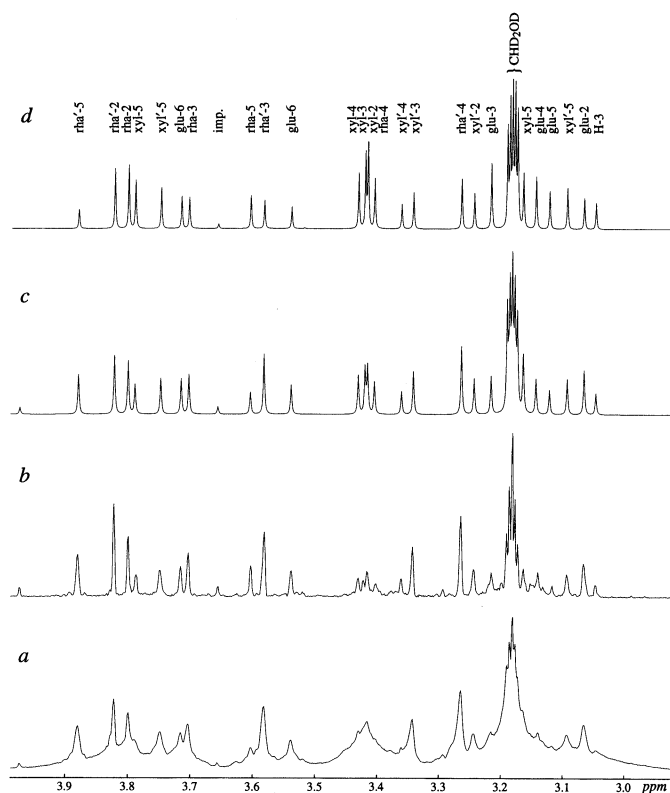


FIG. 4. Successive stages in the processing of the crowded region of the chrysantelline spectrum. (a) A 45° projection of the absolute-value-mode two-dimensional J spectrum. (b) The "search spectrum" obtained by lineshape transformation and 45° projection. (c) The "broadband-decoupled" spectrum, with chemical shifts obtained from (b) and intensities derived from (a). (d) The broadband-decoupled spectrum obtained by the alternative pattern-recognition algorithm acting on a "reflected" J spectrum.

lineshape retains vestiges of the four-pointed star shape but it is clearly much more suitable for projection than the absolute-value mode.

After deconvolution, the two-dimensional J spectrum is projected at 45° . We call this the "search spectrum" since it is used only to find the chemical-shift frequencies but not the intensities. A peak-finding routine locates the centers of all lines that exceed a predetermined threshold level. In the search spectrum the intensities are perturbed, but more reliable values can be obtained by suitably processing one resonance line at a time in the magnitude spectrum. At each chemical-shift frequency the magnitude spectrum is weighted with a Gaussian function that suppresses all but the chosen line; the integral of this line is taken as the intensity of that particular resonance. Finally, all the peaks are assigned an appropriate lineshape and then reassembled to form the "decoupled" proton spectrum.

Since there is no difficulty in determining chemical shifts in sparse high-resolution proton spectra, the technique is of most interest for very crowded regions where the individual spin multiplets interpenetrate and are not separable by in-

spection. We therefore chose to examine a dense cluster of resonances in the 400 MHz proton spectrum of chrysantelline (15, 16) in methanol- d_4 . This region contains overlapping spin multiplets from 25 chemically distinct protons together with a 1:2:3:2:1 quintet from the proton in the residual CHD_2 groups of the solvent (Fig. 3). It is clear that overlap effects make the assignment far from straightforward.

Figure 4 shows the successive steps in the data processing. The absolute-value-mode spectrum (Fig. 4a) exhibits the broad lineshape and the interference effects characteristic of overlapping resonances in this mode. After the lineshape transformation in both dimensions and projection at 45° , a workable search spectrum is obtained (Fig. 4b) which yields values for the 25 chemical shifts. A threshold level of 0.02 times the maximum signal height was used to avoid locating artifacts or noise spikes close to the baseline. These chemical-shift frequencies were used to extract more reliable values for the intensities from the absolute-value spectrum, allowing the construction of a "broadband-decoupled" spectrum (Fig. 4c).

As a check on the new processing method we reexamined the same sample by the C_4 pattern-recognition technique described earlier (11). The resulting spectrum (Fig. 4d) is of comparable quality. Both methods give 25 chemical-shift values with a weak impurity line near 3.66 ppm. Note that the lack of baseline artifacts and baseline noise is illusory; they have been eliminated from Fig. 4c by the imposition of a threshold level and from Fig. 4d by a search algorithm that starts with the most intense signal and works down, stopping before it reaches the baseline noise.

A stringent test of the deconvolution method is afforded by the close group of four protons from xylose-4, xylose-3, xylose-2, and rhamnose-4, between 3.40 and 3.43 ppm. (The ordering of the assignment of the three xylose resonances is not known.) The contour diagram of this region of the J spectrum in the absolute-value mode is shown in Fig. 5. It is clear that any attempt at separation into four components by inspection is doomed to failure. As a guide, the appropriate 45° diagonals (obtained with hindsight) are indicated by the dashed lines. After the lineshape transformation the peaks are much better resolved and it is possible to identify four chemically distinct sites.

We conclude that the more complex pattern-recognition program (11) and the present deconvolution approach offer comparable "decoupled" spectra but both suffer from a lack of uniformity in the intensities in very crowded regions. The deconvolution method is more direct and between one and two orders of magnitude faster. This could prove a powerful method for extracting chemical-shift information from recalcitrant proton spectra. There are many other possible applications. We mention just one. The automated reduction of two-dimensional correlation spectra by pattern-recognition techniques (17) would surely be simplified by first establishing a two-dimensional matrix of chemical-shift co-

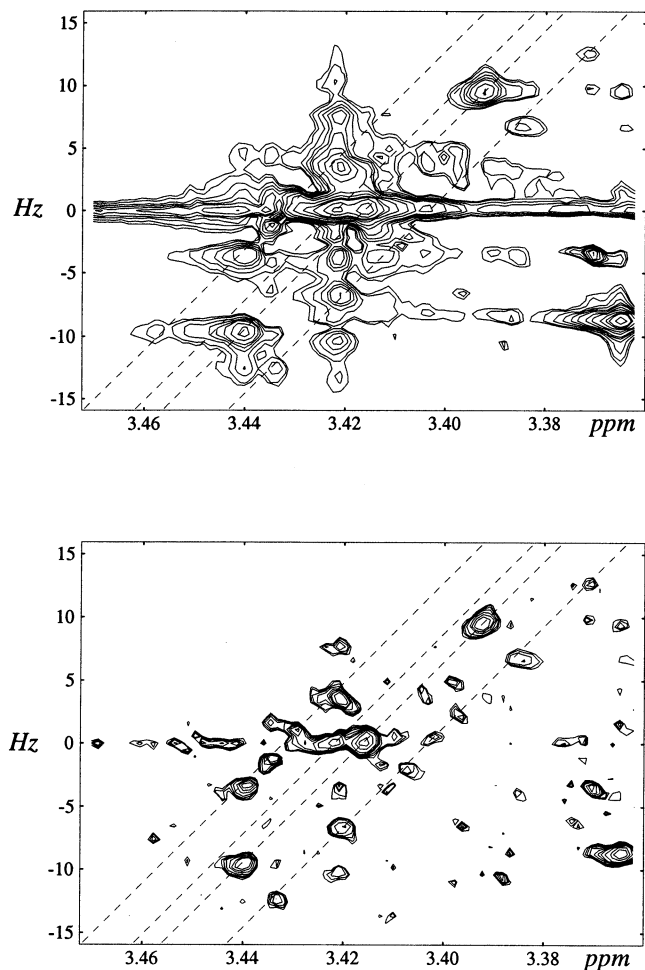


FIG. 5. Examination of a very crowded region of the two-dimensional J spectrum of chrysantelline containing four overlapping responses. (Top) Absolute-value mode. (Bottom) After the lineshape transformation. The 45° diagonals (dashed lines) indicate the positions of the four spin multiplets.

ordinates. The search program could then concentrate its attention on this very limited number of centers without having to explore the entire data matrix.

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REFERENCES

1. W. P. Aue, J. Karhan, and R. R. Ernst, *J. Chem. Phys.* **64**, 4226 (1976).
2. G. Bodenhausen, R. Freeman, R. Niedermeyer, and D. L. Turner, *J. Magn. Reson.* **26**, 133 (1977).
3. K. Nagayama, P. Bachmann, K. Wüthrich, and R. R. Ernst, *J. Magn. Reson.* **31**, 133 (1978).
4. A. Bax, A. F. Mehlkopf, and J. Smidt, *J. Magn. Reson.* **35**, 167 (1979).
5. A. Bax, R. Freeman, and G. A. Morris, *J. Magn. Reson.* **43**, 333 (1981).
6. A. Bax and R. Freeman, *J. Magn. Reson.* **44**, 542 (1981).
7. B. Blümich and D. Ziessow, *J. Magn. Reson.* **49**, 151 (1982).
8. A. J. Shaka, J. Keeler, and R. Freeman, *J. Magn. Reson.* **56**, 294 (1984).
9. P. Xu, X-L. Wu, and R. Freeman, *J. Am. Chem. Soc.* **113**, 3596 (1991).
10. P. Xu, X-L. Wu, and R. Freeman, *J. Magn. Reson.* **95**, 132 (1991).
11. M. L. Woodley and R. Freeman, *J. Magn. Reson. A* **109**, 103 (1994).
12. E. L. Hahn and D. E. Maxwell, *Phys. Rev.* **88**, 1070 (1952).
13. R. Freeman and H. D. W. Hill, *J. Chem. Phys.* **54**, 301 (1971).
14. R. R. Ernst, R. Freeman, B. Gestblom, and T. R. Lusebrink, *Mol. Phys.* **13**, 283 (1967).
15. M. Becchi, M. Bruneteau, M. Trouillard, H. Combier, J. Sartre, and G. Michel, *Eur. J. Biochem.* **108**, 271 (1979).
16. G. Massiot, C. Lavaud, and J. M. Nuzillard, *Bull. Soc. Chim. France* **127**, 100 (1991).
17. B. U. Meier, G. Bodenhausen, and R. R. Ernst, *J. Magn. Reson.* **60**, 161 (1984).