A New Scheme for Two-Dimensional NMR Shift Correlation

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We demonstrate a new method for the automated analysis of two-dimensional NMR chemical shift correlation spectra.[1] It relieves the spectroscopist of the task of making subjective judgements about the presence or absence of cross peaks, and it functions effectively in overcrowded spectra. Applied to the 400 MHz correlation (COSY) spectrum[1] of chrysanthelline A, this procedure identifies spin−spin interactions between 22 protons in a region spanning only 0.8 ppm and displays this information as a two-dimensional matrix, showing the assignments and the respective confidence levels.

Two-dimensional NMR based on the spin−spin interaction (COSY)[1] or on cross-relaxation (NOESY)[2] has enjoyed remarkable success but still suffers from certain shortcomings. Decisions about the existence of a cross peak at a particular location are seriously compromised in regions of severe overlap, and the assignment can be ambiguous if chemical shifts are very close or if there are spurious responses from impurities, the solvent, or instrumental artifacts. Ideally, we would prefer to have a simplified correlation chart without the complication of spin−spin coupling, or merely a table of chemical shifts showing the correlations. The confidence levels would be estimated from the relative intensities of the respective cross peaks; these are far from uniform, being determined by the interplay of line broadening effects and interference between adjacent multiplet components.

The proposed method is based on a line-shape transformation[3] of the two-dimensional proton J-spectrum[4−6] followed by a projection at 45° to give a one-dimensional spectrum containing chemical shift information but no fine structure. These values are used to construct a two-dimensional "shift grid". Every genuine cross peak of the correlation spectrum must be centered at an intersection on this shift grid, although not *all* such locations correspond to cross peaks. The presence or absence of a cross peak is determined by examining the local symmetry of a suitable test zone, in the present case, a 28 × 28 Hz square. For TOCSY[7] or NOESY spectra, where the peak intensities are all positive, the search program tests for mirror symmetry in the F_1 and F_2 frequency dimensions; for COSY spectra, the local antisymmetry of the cross peak is exploited. This is a far less extensive search than those used in pattern recognition methods,[8,9] where every point in the data matrix must be explored as a possible center.

The search program compares pairs of symmetry-related locations within the test zone and replaces both intensities by the lower value.[10] In this way, any overlapping responses that lack the required symmetry are suppressed, leaving the cross peak exactly symmetrical in both frequency dimensions. Artifacts in the projected J-spectrum (arising, for example, from strong coupling effects) generate spurious points on the shift grid but are unlikely to survive the local symmetry test. The integral of all intensities within the symmetrized test zone (ignoring signs) serves as a measure of our confidence that there is a true cross peak centered there.

The 400 MHz proton double-quantum-filtered correlation spectrum[11] of chrysanthelline A contains a crowded region arising from 25 chemically distinct sites (Figure 1). The chemical shift values were obtained from a previous investigation[3] of the two-dimensional J-spectrum and were used to construct a grid comprising 22 × 22 intersections, of which 22

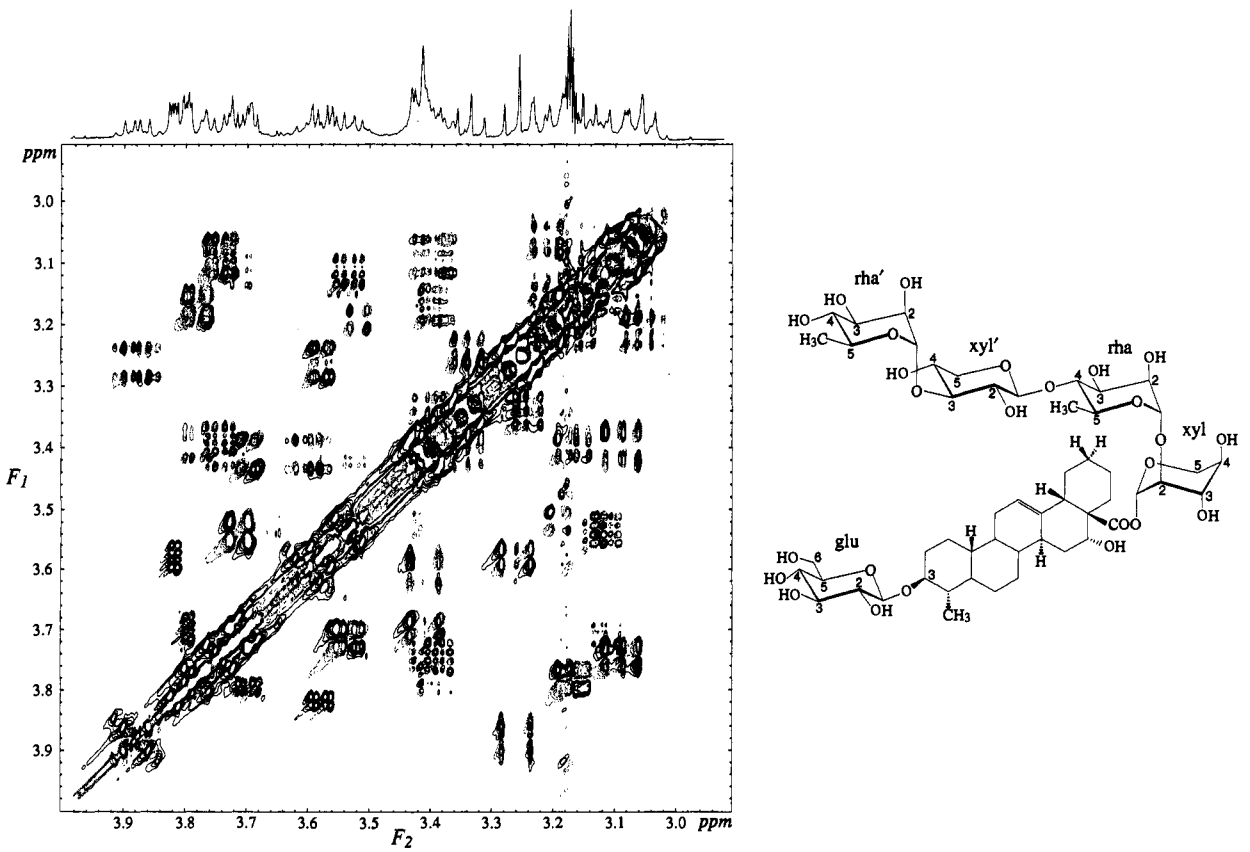

Figure 1. Crowded region of the conventional 400 MHz proton double-quantum-filtered correlation spectrum of chrysanthelline A, with the one-dimensional spectrum (top).

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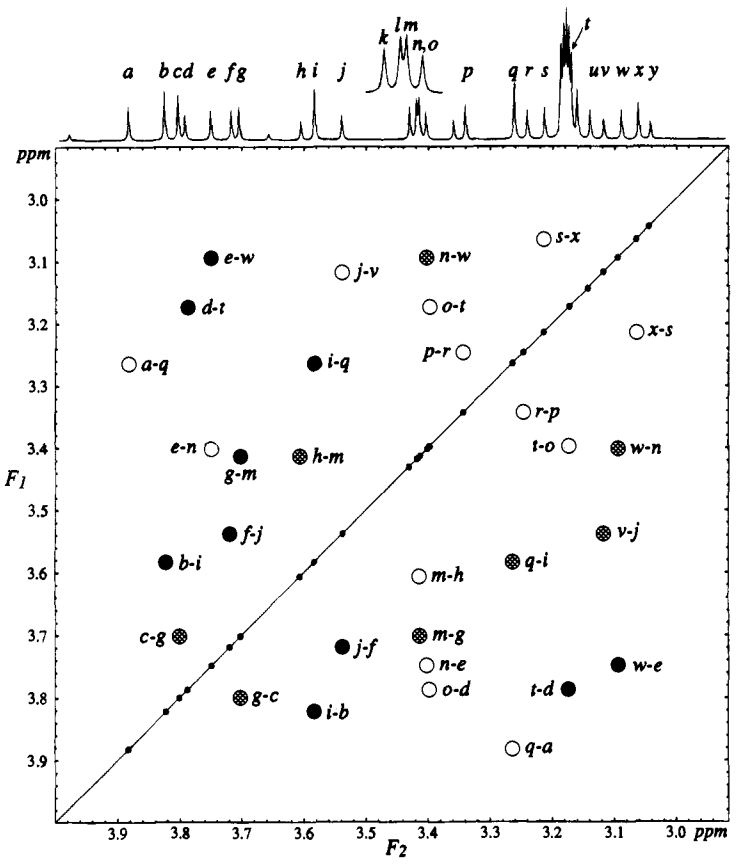

Site	Proton	F_2 (ppm)	Correlations (% confidence)	
a	rha′-5	3.88	*q* (18)	
b	rha′-2	3.82	*i* (51)	
c	rha-2	3.80	*g* (33)	
d	xyl-5	3.79	*o* (11)	*t* (83)
e	xyl′-5	3.75	*n* (21)	*w* (98)
f	glu-6	3.72	*j* (91)	
g	rha-3	3.70	*c* (35)	*m* (53)
h	rha-5	3.61	*m* (29)	
i	rha′-3	3.58	*b* (57)	*q* (68)
j	glu-6	3.54	*f* (100)	*v* (23)
k	xyl-2	3.43		
l	xyl-3	3.41		
m	rha-4	3.41	*g* (44)	*h* (19)
n	xyl′-4	3.40	*e* (22)	*w* (34)
o	xyl-4	3.40	*d* (13)	*t* (15)
p	xyl′-3	3.34	*r* (20)	
q	rha′-4	3.26	*a* (25)	*i* (45)
r	xyl′-2	3.25	*p* (23)	
s	glu-3	3.21	*x* (23)	
t	xyl-5	3.17	*d* (91)	*o* (18)
u	glu-4	3.14		
v	glu-5	3.12	*j* (29)	
w	xyl′-5	3.09	*e* (95)	*n* (38)
x	glu-2	3.06	*s* (21)	
y	H-3	3.04		

Figure 2. Correlation map and table corresponding to the spectrum shown in Figure 1. The proton-decoupled spectrum is shown at the top. Cross peak integrals have been normalized to the geometric mean of the four strongest peaks and are represented by open circles (12.5−25%), shaded circles (25−50%), and solid circles (50−100%). The strong quintet is from solvent CHD_2OD. Protons k and l have only cross peaks very close to the diagonal, and proton y has no couplings in this region.

fall on the principal diagonal and the remaining 462 indicate the centers of potential test zones. When these zones were examined for C_2 symmetry in the F_1 and F_2 dimensions, 16 pairs of cross peaks were found (Figure 2).

Of particular interest in Figure 2 are three well-separated correlations, m−g, n−e, and o−d, that are not clearly resolved in Figure 1. We would have expected three pairs of cross peaks close to the principal diagonal, but the search routine fails to find them, presumably because of overlap from intense diagonal peaks. It also misses a weak pair, f−v and v−f, close to the very intense cross peaks e−w and w−e. The chemical shift between protons n and o (0.004 ppm) is so small that the program cannot distinguish them and reports four sets of cross peaks instead of two. Apart from these minor ambiguities, the program gives a comprehensive picture of the correlations in this very congested region.

This is an example of the use of results from one type of two-dimensional NMR technique (J-spectroscopy)[3-6] to analyze a complex spectrum obtained by a different method (correlation spectroscopy).[1,2] It replaces subjective decisions with an unbiased data reduction algorithm.

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