The Application of Two-dimensional N.M.R. Cross Relaxation Spectroscopy to Natural Product Structure Determination: Talaromycin B

William C. Hutton,* Nancy J. Phillips,† David W. Graden,† and David G. Lynn†

Department of Chemistry, University of Virginia, Charlottesville, VA 22901, U.S.A.

Two-dimensional, cross relaxation, correlated n.m.r. spectroscopy is a powerful method for elucidating intramolecular, dipolar interactions of molecules in the extreme narrowing domain.

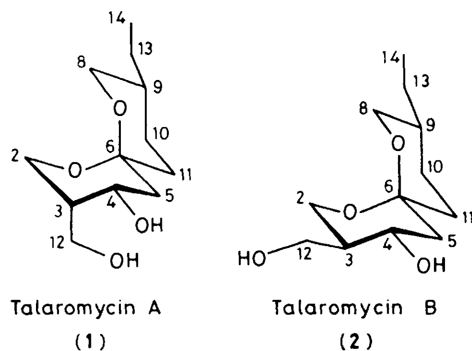
Ernst and co-workers have demonstrated a homonuclear, two-dimensional n.m.r. experiment that simultaneously maps out cross relaxation connectivities.[1] This technique has proved to be a powerful method for correlating proton cross relaxation in macromolecules such as proteins.[2] For large molecules, in medium to high magnetic fields, cross relaxation is often dominated by simple spin diffusion ($\omega\tau_c >> 1$). In this case the

† Present address: Department of Chemistry, 5735 S. Ellis Avenue, University of Chicago, Chicago, Illinois 60637, U.S.A.

two-dimensional spin exchange spectra contain intense cross-peaks.

The elucidation of intramolecular, proton cross relaxation is also an established n.m.r. method for determining the solution structure of small to medium sized molecules[3] where $\omega\tau_c << 1$. Cross relaxation is typically probed by evaluating nuclear Overhauser effects. In these experiments the selective irradiation of a resonance can cause a transfer of magnetization to other protons close in space. Recently nuclear Overhauser enhancement (n.O.e.) difference spectra have been used to provide sensitive, accurate measurements of cross relaxation.[4,5] Careful selection of irradiation position and intensity, prolonged signal averaging, and considerable data manipulation are required to obtain quality difference spectra.[5] However, n.O.e. difference experiments fail altogether when selective irradiation is not possible due to resonance overlap. Consider the two toxic metabolites talaromycin A (**1**) and B (**2**)‡. Nuclear Overhauser difference spectra established the relative configuration of the acetal in talaromycin A, where a 3.7% enhancement for H-2a was measured upon irradiation of H-8a.[6] Unfortunately the H-8a and H-2a protons of talaromycin B are separated by only 39 Hz at 8.5 T. N.O.e. difference experiments were not able directly to determine the acetal configuration in this case because attempts to irradiate H-8a and H-2a selectively failed.

The two-dimensional cross relaxation correlation spectra for talaromycin B are shown in Figure 1. This technique uses non-selective pulses and circumvents the selectivity requirement of the n.O.e. difference experiment. In Figure 1, the one-dimensional spectrum is reproduced on the diagonal. Cross-peaks are symmetrically located off the diagonal and correlate protons that exchange magnetization through cross relaxation pathways. Figure 1(a) reveals unambiguously that H-8a and H-2a are close enough in space to exchange magnetization *via* dipolar cross relaxation. The relative acetal conformation of talaromycin B is the same as in talaromycin A. Cross peaks are found in Figure 1(b) indicating dipolar interactions between H-5a and H-11e giving further support to the acetal conformation shown for (**2**). The cross relaxation connectivities for talaromycin B are summarized in Table 1.



‡ Talaromycin B, 360 MHz ^{1}H-n.m.r. in $CDCl_3$: H-2a, δ 3.28 (t, *J* 11 Hz); H-2e, δ 3.56 (dd, *J* 11, 4.6 Hz); H-3a, δ 1.82 (m); H-4a, δ 4.04 (dt, *J* 11, 5.1 Hz); H-5a, δ 1.42 (dd, *J* 12, 11 Hz); H-5e, δ 1.97 (dd, *J* 12, 5.1 Hz); H-8a, δ 3.17 (t, *J* 11 Hz); H-8e, δ 3.49 (dm); H-9a, δ 1.43 (m); H-10a, δ 1.38 (dq, *J* 13, 3.6 Hz); H-10e, δ 1.60 (dm); H-11a, δ 1.52 (dt, *J* 13, 4.0 Hz); H-11e, δ 1.68 (dm); H-12, δ 3.68 (2H, d, *J* 6.0 Hz); H-13, δ 1.13 (2H, m); H-14, δ 0.85 (3H, t, *J* 7.5 Hz).

The two-dimensional spectra shown here were obtained using the pulse sequence:[7] $(\pi/2\text{–}t_1\text{–}\pi/2\text{–}\tau_m/2\text{–}t_1/4\text{–}\pi$ comp–$\tau_m/2\text{–}\pi/2\text{–}t_2)n$ where t_1 is the evolution period, τ_m is the mixing time, and t_2 is the detection period. The $\pi/2$ pulses are phase-shifted to allow elimination of axial peaks and quadrature detection in both dimensions.[8] The incremented, composite π pulse, combined with data set symmetrization, serves to suppress *J* cross-peaks. This pulse sequence produces two-dimensional data free from ambiguities due to instrumental artifacts and *J* cross-peaks.

Qualitative interpretation of the two-dimensional spin exchange spectra is straightforward. Quantitative interpretation can be complicated as cross-peak intensities may not always be directly related to the magnitude of the dipolar interactions. Some general features of two-dimensional spectra in the extreme narrowing domain are shown in Figure 1 and in Table 1. It is not uncommon to observe significant variations in the transition intensities of a given multiplet. The variations could arise from at least two sources: filtering of selected transitions by data collection and processing parameters and strong spin–spin coupling where unique relaxation cannot be defined.

Examples are observed for H-10a and H-10e and H-11a and H-11e. The centre of cross-peak intensity in such instances may not be equal to the actual proton chemical shift. Accurate analysis will be aided by comparing data sets obtained with at least two mixing times. In general, cross relaxation interactions which give at least 3% intensity changes with one-dimensional n.O.e. difference spectra will be easily detected in the two-dimensional spin exchange spectra. As in one-dimensional n.O.e. experiments, the lack of cross-peaks in a two-dimensional exchange spectrum does not necessarily mean that nuclei are not close in space.

Two-dimensional cross relaxation spectroscopy simultaneously maps out the cross relaxation interactions in small to medium sized molecules. This experiment requires about the same amount of instrument time as does a series of one-dimensional, n.O.e. difference spectra where multiple, selective irradiations are employed. When quantitative interpretation of cross relaxation interactions is indicated, the two-dimensional technique can point to useful n.O.e. difference experiments. Combining n.O.e. difference data with two-dimensional exchange spectra in rigid molecules can provide

Table 1. Cross relaxation connectivities for talaromycin B in $CDCl_3$.

Cross peak co-ordinates below the diagonal δ(*x*)–δ(*y*)	Protons correlated[d]
3.17–3.49[a,b,c]	8a–8e
3.17–3.28[a,b,c]	8a–2a
3.28–3.56[a,b,c]	2a–2e
3.28–3.68[c]	2a–12
3.56–3.68[c]	2e–12
1.13–1.43[b]	13–9a
1.82–3.68[b]	3a–12
1.42–1.97[a,b,c]	5a–5e
1.42–1.68[a,b,c]	5a–11e
1.53–1.68[a,b,c]	11a–11e
1.38–1.60[b,c]	10a–10e
0.87–1.43[b,c]	14–9a
0.87–1.13[b,c]	14–13
1.97–4.04[b,c]	5e–4a

[a] Mixing time = 2.7 s. [b] Mixing time = 0.5 s. [c] Mixing time = 1.0 s. [d] Assignments (from Ref. 6).

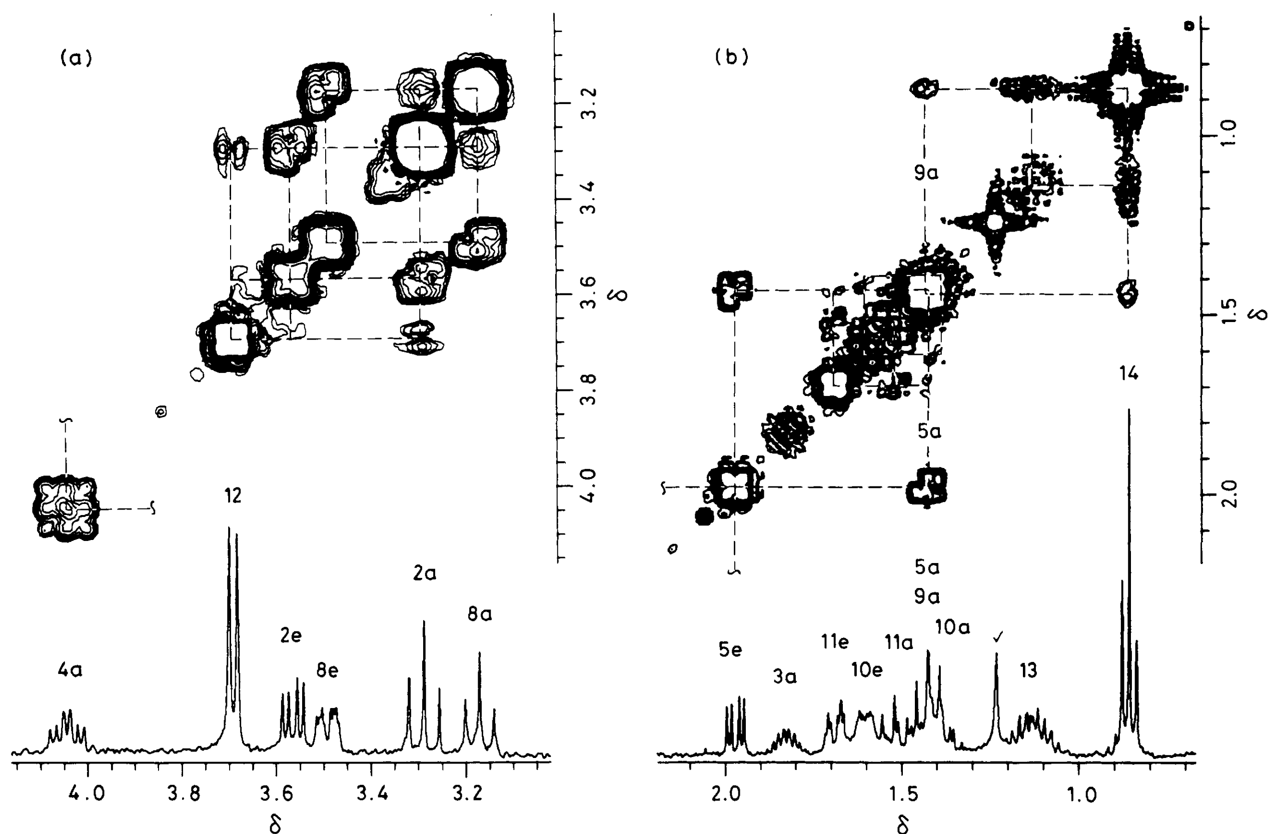


Figure 1. Expansions of the two-dimensional, spin exchange, correlation spectrum for 4.1 mg of talaromycin B at 360 MHz collected with a 1.0 s mixing time. The cross-peaks are connected with dashed lines on both sides of the diagonal. Proton assignments are indicated on the conventional spectrum. The complete, symmetrized data set contains 512 × 512 data points. Data collection and processing took 16.5 (32 scans per block) and 0.5 h, respectively. (a) Contour plot of the downfield region apodized with a cosine bell function in both dimensions. (b) Contour plot of the upfield region apodized with a sine bell function in both dimensions. An impurity is present at δ 1.28.

unambiguous structural and conformational analysis on a level that was previously obtained only by employing *X*-ray crystallography.

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