Unsymmetrical Covariance Processing of COSY or TOCSY and HSQC NMR Data to Obtain the Equivalent of HSQC-COSY or HSQC-TOCSY Spectra

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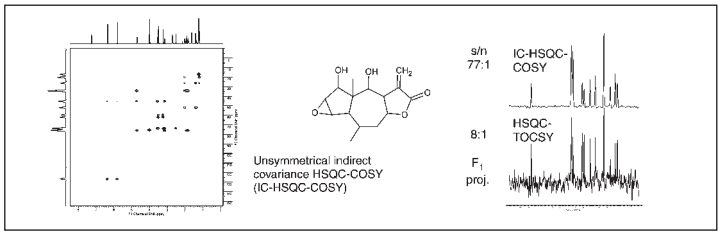

Artifacts observed in the indirect covariance NMR spectrum of HSQC-TOCSY data have recently been analyzed and a method for their elimination proposed. More recently, unsymmetrical covariance processing has been applied HSQC and HMBC spectral data to afford long-range carbon-carbon correlation information equivalent to that obtained from *n*,1-, 1,*n*- and *m,n*-ADEQUATE spectra. We now wish to describe the results obtained through the application of unsymmetrical covariance processing of HSQC and COSY or TOCSY data, which affords the equivalent of HSQC-COSY and HSQC-TOCSY data in a fraction of the time required to record these spectra directly and with considerably higher sensitivity.

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In a series of reports, Brüschweiler and co-workers have described various aspects of covariance NMR spectroscopy [1-6]. In the report that specifically dealt with indirect covariance NMR, Zhang and Brüschweiler [4] noted that artifacts can arise in the indirect covariance spectrum due to the overlap of proton resonances. In a subsequent report, we reported the analysis of two different types of artifact responses, as well as the means of eliminating these artifacts through the application of unsymmetrical covariance processing followed by symmetrization of the type used with simple COSY spectra [7]. More recently, we have shown that unsymmetrical covariance processing of HSQC and HMBC data [8] affords long-range carbon-carbon connectivity information that are equivalent to what can be obtained from *n*,1-, 1,*n*- and *m,n*-ADEQUATE spectra[9-12]. We now wish to communicate the extension of this approach to the unsymmetrical covariance processing of HSQC and either COSY or TOCSY data to yield the equivalent of HSQC-COSY and HSQC-TOCSY spectra. While the former represent high sensitivity data that are easy to acquire, the hyphenated latter group of experiments, in contrast, are lower in sensitivity than HMBC experiments and can take considerable time to acquire. The relative insensitivity of the HSQC-TOCSY experiment may unfortunately be a factor in limiting the application of this otherwise very useful experiment in structure elucidation efforts.

In the covariance procedure described by Zhang and Brüschweiler [4] the calculation of the covariance matrix, **C**,

$$\mathbf{C = R * R^T} \quad [1]$$

is accomplished by multiplying the real processed data matrix, **R**, by the transposed data matrix, $\mathbf{R^T}$. The result spectrum, **F**, is then calculated by taking the square root of the covariance matrix, **C**.

$$\mathbf{F = C^{1/2}} \quad [2]$$

In the unsymmetrical procedure that we described previously [8] the covariance matrix, **C**, is calculated by

$$\mathbf{C = R_1 * R_2^T} \quad [3]$$

Where $\mathbf{R_1}$ and $\mathbf{R_2^T}$ correspond to the real and transposed real data from two separate 2D NMR experiments, *e.g.* HSQC and HMBC in our previous report or COSY/TOCSY and HSQC in the present case. The data for $\mathbf{R_1}$ and $\mathbf{R_2}$ should be acquired so that there are equal numbers of columns in the data set, *i.e.* $\mathbf{R_1}$ is N by M_1 in size and $\mathbf{R_2}$ is N by M_2 is size, to allow multiplication of the data matrices. In the present case, the square root is not calculated because the result matrix, **C** of size M_1 by M_2 is not diagonally symmetrical in the general case

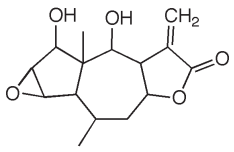
By definition and with respect to the transposition of the $\mathbf{R_2}$ matrix to afford $\mathbf{R_2^T}$, the following formula is used to calculate each element, $\mathbf{C_{ij}}$ of matrix **C:**

$$\mathbf{C^{ij} = R_1^{i1} * R_2^{j1} + R_1^{i2} * R_2^{j2} + \ldots + R_1^{ik} * R_2^{jk} + \ldots + R_1^{iN} * R_2^{jN}} \quad [4]$$

where each element of matrix **C** is the sum of products of values $\mathbf{R_1^{ik}}$ and $\mathbf{R_2^{ik}}$. A necessary condition for the sum of a matrix element to be non-zero is to have non-zero elements in equal positions in the rows of $\mathbf{R_1^i}$ and $\mathbf{R_2^j}$. For two "ideal" 2D NMR spectra, assuming zero noise in the data matrix, the sum of a matrix element will be non-zero when rows $\mathbf{R_1^i}$ and $\mathbf{R_2^j}$ have crosspeaks in the same position.

In the case of combining COSY or TOCSY with HSQC spectra, each element of the covariance matrix $\mathbf{C^{ij}}$ corresponding to the *i* and *j* chemical shifts (F_1 proton shift in COSY and F_1 carbon shift in HSQC) will be non-zero when these spectra have crosspeaks in the *i* and *j* F_1 chemical shifts and equal F_2 proton positions. This means that element $\mathbf{C^{ij}}$ of the covariance matrix is non-zero when a proton with chemical shift *i* and a carbon with chemical shift *j* are connected in the chemical structure, *i.e.* the result matrix contains long-range connectivities between carbons and protons obtained from COSY crosspeaks and $^1J_{CH}$ connectivities between carbons and protons obtained from COSY diagonal peaks. Thus, the result matrix will be equal to a HSQC-COSY or -TOCSY spectrum, depending on which was used as the source of the proton-proton connectivity information.

Using the sesquiterpene lactone autumnolide (**1**), as a model compound, a series of two-dimensional NMR spectra were acquired that included HSQC, COSY, and HSQC-TOCSY. The HSQC data were acquired without multiplicity editing and the HSQC-TOCSY data were acquired without direct response inversion [13,14]. The 18 msec HSQC-TOCSY spectrum of **1** is shown in the left panel of Figure 1. These data required just over 16 h to acquire. In contrast, the right panel shows an HSQC-COSY spectrum calculated by unsymmetrical indirect covariance processing of an HSQC and COSY spectrum using the method described above. The HSQC and COSY spectra were acquired, in series, in 60 and 10 minutes, respectively. Unsymmetrical coprocessing using ACD/SpecManager v9.0 required approximately 4 sec on an office PC. Visual comparison shows that the two spectra are comparable in terms of the responses contained in the spectra but that there are some additional legitimate responses observed in the calculated HSQC-COSY spectrum shown in the right panel that are not observed in the HSQC-TOCSY spectrum shown in the left panel. The acquisition time savings reflected in the calculated spectrum shown in the right panel of Figure 1 is nearly a factor of 16. However, the advantage inherent to the unsymmetrical indirect covariance method is even more striking when projections through the F_1 frequency domain are compared as shown in Figure 2. The F_1 projection through the HSQC-TOCSY spectrum is shown in Figure 2A and had a s/n ratio of 8:1. In contrast, the F_1 projection through the unsymmetrical indirect covariance calculated HSQC-COSY spectrum shown in the right panel of Figure 1 gave a s/n ratio of 77:1, which is nearly an additional 10-fold advantage over the conventionally acquired HSQC-TOCSY spectrum. As with other indirect covariance NMR spectra overlap in the proton spectrum can lead to artifact responses [4], which we have previously analyzed [7]. In addition, either of the data matrices shown in Figure 1 can be further subjected to indirect covariance processing to afford the equivalent of a carbon-carbon COSY presentation as previously described by Zhang and Brüschweiler [4].



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If the universality of unsymmetrical indirect covariance can be demonstrated, the time advantages inherent to this approach over the direct acquisition of hyphenated two-dimensional NMR data may lead to the more widespread utilization of these data in structure elucidation studies. It

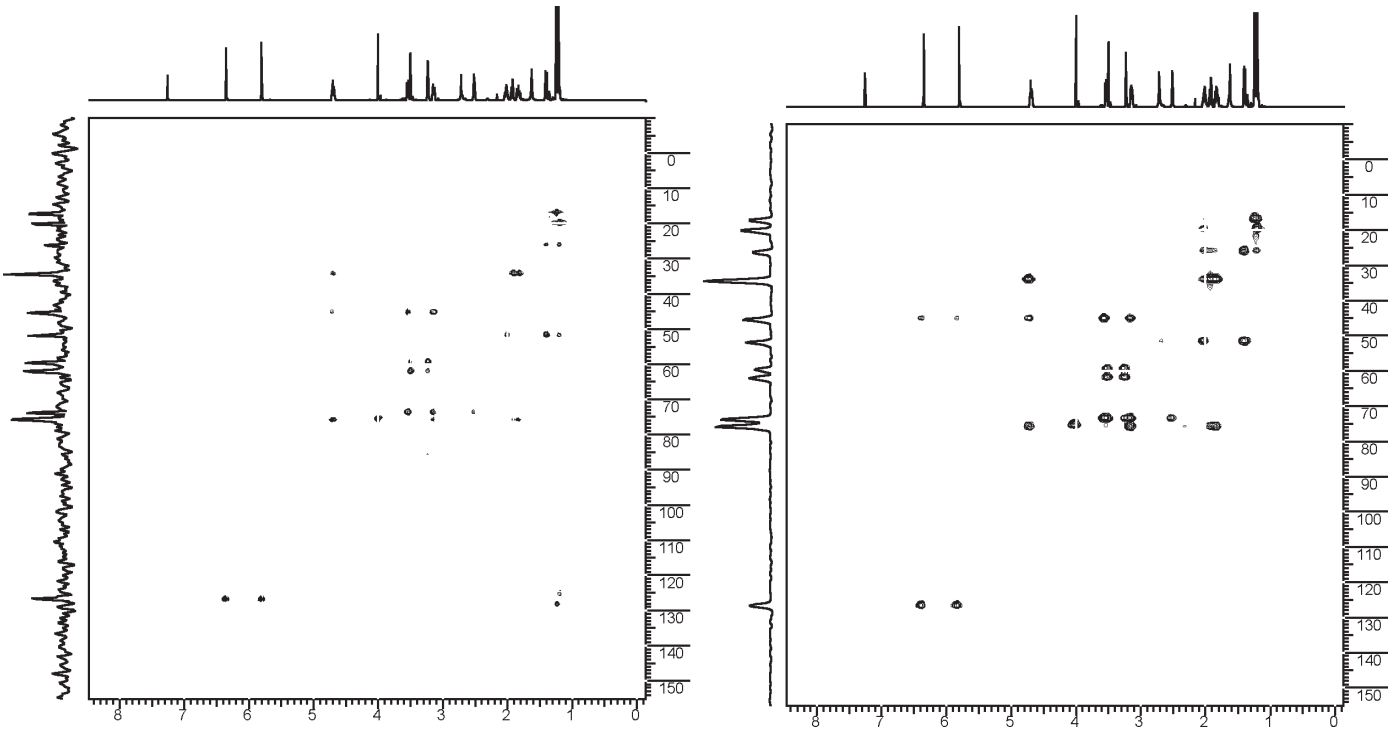

Figure 1. The left panel shows the HSQC-TOCSY spectrum of autumnolide (**1**) acquired in just over 4 h at a ^{1}H observation frequency of 600 MHz. The right panel shows the HSQC-COSY spectrum of autumnolide (**1**) prepared by the unsymmetrical covariance processing of a COSY and HSQC spectrum which required <20 minutes for the acquisition of both. The spectra are plotted at approximately the same threshold. Small differences in the responses contained in the spectra arise through the difference between the homonuclear connectivities observed in the COSY spectrum used to prepare the data shown in the right panel *vs*. the TOCSY transfer in the HSQC-TOCSY spectrum shown in the left panel. All data were acquired using a Varian Inova 600 MHz spectrometer operating at a proton observation frequency of 599.707 MHz and equipped with a Nalorac Z•SPEC™ MIDTG-600-3 gradient micro inverse detection probe. The sample used contained ~2 mg of autumnolide (**1**) dissolved in ~150µL of deuterochloroform. All data were digitized with 2048 points in the observed frequency domain. Pulse sequences used were from the vendor-supplied pulse sequence library and were used without modification. The indirect covariance NMR spectrum shown in Figure 1 was computed using the 2D NMR processing module of a modified beta version of ACD/SpecManager developed initially to operate on an IDR-HSQC-TOCSY data set as described in our previous report [7]. The processing was performed using a PC with a 2.8 GHz Pentium IV processor with 1 Gbyte of RAM. Processing time for the unsymmetrical indirect covariance spectrum shown in Figure 1 was approximately 4 sec.

also remains to be determined how much proton resonance overlap, which can lead to artifact responses in indirect covariance processed spectra [7] will impact the use of these data. In part, as shown in this communication, considerable time savings are possible using the unsymmetrical indirect covariance approach. Alternatively, the unsymmetrical indirect covariance method may make hyphenated 2D NMR data more accessible in limited sample situations where the direct acquisition of these data would be out of the question because of the limited sensitivity of experiments such as HSQC-TOCSY.

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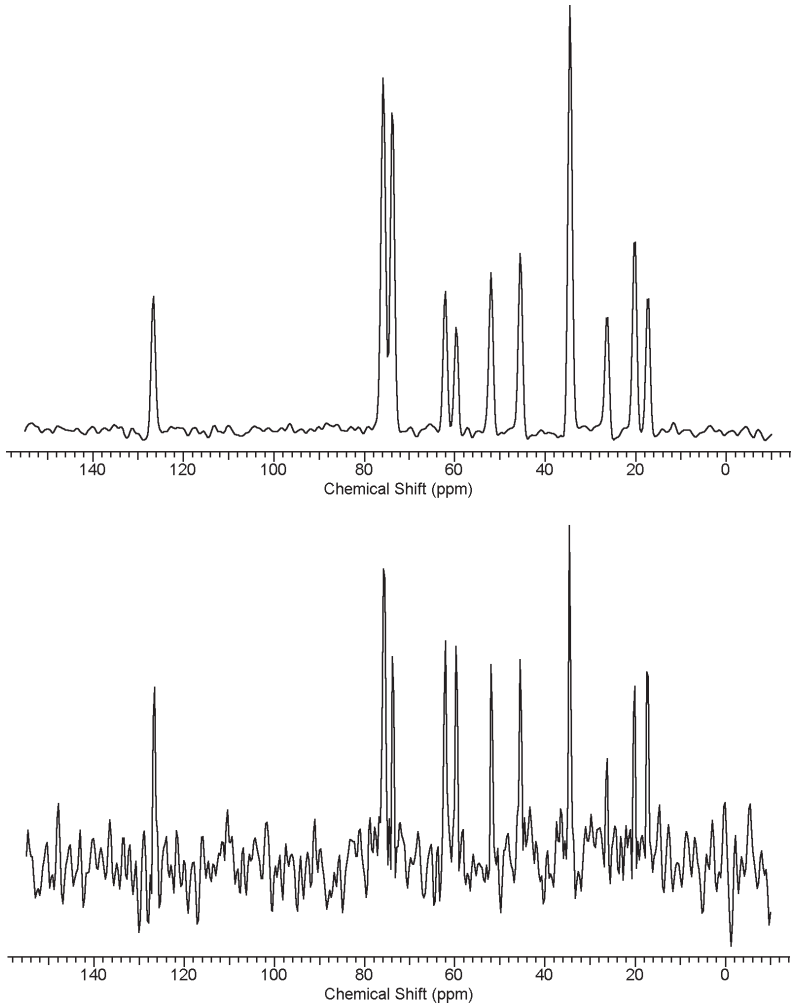

Figure 2. Projection through the F_1 frequency domain of the HSQC-TOCSY and unsymmetrical indirect covariance calculated HSQC-COSY spectra shown in Figure 1. The s/n ratio in the F_1 projection through the HSQC-TOCSY was 8:1 (bottom panel). In contrast, the projection through the F_1 frequency domain of the unsymmetrical indirect covariance calculated HSQC-COSY spectrum (top panel) was 77:1. The nearly 10-fold s/n advantage reflected by the projections above, when coupled with the time advantage inherent to acquiring an HSQC and COSY spectrum rather than the direct acquisition of an HSQC-TOCSY spectrum affords a very significant performance advantage.

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