^{13}C-^{15}N Connectivity Networks *via* Unsymmetrical Indirect Covariance Processing of ^{1}H-^{13}C HSQC and ^{1}H-^{15}N IMPEACH Spectra

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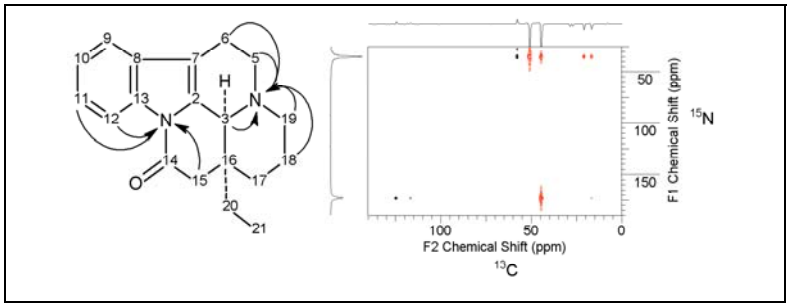

Long-range ^{1}H-^{15}N heteronuclear shift correlation methods at natural abundance to facilitate the elucidation of small molecule structures have assumed a role of growing importance over the past decade. Recently, there has also been a high level of interest in the exploration of indirect covariance NMR methods. From two coherence transfer experiments, A→B and A→C, it is possible to indirectly determine B↔C. We have shown that unsymmetrical indirect covariance methods can be employed to indirectly determine several types of hyphenated 2D NMR data from higher sensitivity experiments. Examples include the calculation of hyphenated 2D NMR spectra such as 2D GHSQC-COSY and GHSQC-NOESY from the discrete component 2D NMR experiments. We now wish to report the further extension of unsymmetrical indirect covariance NMR methods for the combination of ^{1}H-^{13}C GHSQC and ^{1}H-^{15}N long-range (GHMBC, IMPEACH-MBC, CIGAR-HMBC, *etc*.) heteronuclear chemical shift correlation spectra to establish ^{13}C-^{15}N correlation pathways.

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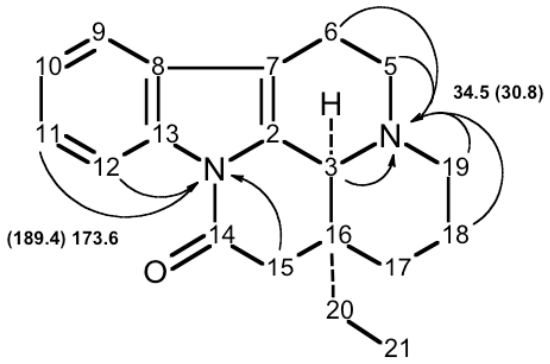
Long-range ^{1}H-^{15}N heteronuclear chemical shift correlation methods at natural abundance have been the subject of several recent reviews [1-5]. The growing importance of being able to access ^{1}H-^{15}N long-range heteronuclear shift correlation data for the characterization of natural products and pharmaceuticals has fostered two recent reports describing pulse sequences that allow the simultaneous acquisition of long-range ^{1}H-^{13}C and ^{1}H-^{15}N HMBC data [6,7]. Considerable recent attention has also been focused on the development of indirect covariance NMR methods, first reported by Brüschweiler and co-workers [8-13]. Insofar as potential for small molecule applications, to the authors, the most interesting report was that describing indirect covariance methods, which afford the capability of extracting carbon-carbon connectivity information from a GHSQC-TOCSY spectrum [11]. In their report, Brüschweiler and Zhang commented that proton resonance overlap could lead to artifacts in the calculated carbon-carbon correlation spectra although they did not explore or document that observation [11]. We subsequently described the analysis of two types of artifacts observed in IDR-(Inverted Direct Response)-GHSQC-TOCSY spectra with overlapped proton resonances, which, in turn, prompted us to explore the elimination of these artifacts *via* a method that we have named unsymmetrical indirect covariance [14]. Subsequent work has shown that it is also possible to mathematically combine various discretely acquired 2D NMR spectra. The calculation of hyphenated 2D NMR experiments from discretely acquired 2D spectra is based

on the premise that using coherence transfer experiments of the type A→B and A→C, one can indirectly determine B↔C. The first effort in this direction demonstrated the combination of ^{1}H-^{13}C GHSQC and GHMBC spectra to afford the equivalent of an *m,n*-ADEQUATE spectrum [15]. Subsequent studies have demonstrated the calculation of GHSQC-COSY [16,17] and GHSQC-NOESY [18] spectra from discretely acquired COSY, NOESY, and ^{1}H-^{13}C GHSQC 2D NMR spectra. Recently, Kupče and Freeman [7] have demonstrated the use of projection reconstruction techniques to establish ^{15}N-^{13}C correlations at natural abundance, using vitamin B-12 as a model compound for their study, also noting in parallel that indirect covariance methods can be used to obtain homonuclear correlation spectra indirectly. Thus, we would now like to demonstrate specifically, that unsymmetrical indirect covariance NMR methods can also be used to derive ^{15}N-^{13}C connectivity information from discretely acquired ^{1}H-^{13}C GHSQC and ^{1}H-^{15}N HMBC spectra.

For the present study, multiplicity-edited ^{1}H-^{13}C GHSQC and ^{1}H-^{15}N IMPEACH-MBC (IMPEACH hereafter) spectra were acquired using an ~5 mg sample of (-) eburnamonine (**1**) dissolved in ~180 μL $CDCl_3$ in a 3 mm NMR tube. The spectra were recorded at 600 MHz using a Varian three channel spectrometer equipped with a 3 mm gradient inverse detection probe at 26° C. The ^{1}H-^{13}C GHSQC spectrum was recorded in 23 m as 1024 x 96 data points; the ^{1}H-^{15}N IMPEACH spectrum was recorded in 16.2 h as 1024 x 66 data points. The spectra were acquired with identical proton spectral widths and the data were processed to yield data matrices that were identically digitized with F_1,F_2 dimensions of 512 x 2048 points.

The processed ^{1}H-^{13}C GHSQC and ^{1}H-^{15}N IMPEACH spectra were subjected to unsymmetrical indirect covariance processing to yield the ^{13}C-^{15}N HSQC-IMPEACH long-range correlation spectrum shown in Figure 1. The ^{1}H-^{15}N IMPEACH spectrum is shown in the top left panel and corresponds to the A→B coherence transfer spectrum; the multiplicity-edited ^{1}H-^{13}C GHSQC spectrum is shown in the bottom right panel and can be considered the A→C coherence transfer experiment. The GHSQC spectrum has been transposed to reflect the eventual orientation of the ^{13}C spectrum as the F_2 axis in the indirectly determined ^{13}C-^{15}N correlation spectrum shown in the top right panel, which corresponds to the B↔C coherence transfer spectrum. In the ^{13}C-^{15}N correlation spectrum, ^{15}N chemical shift information is displayed in the F_1 frequency domain while ^{13}C chemical shift information is presented in the F_2 frequency domain. ^{13}C-^{15}N correlation responses in the spectrum shown in Figure 1 arise from transfer between ^{13}C and ^{15}N *via* the $^{n}J_{NH}$ correlation response in the ^{1}H-^{15}N IMPEACH spectrum, the ^{13}C chemical shift information derives from the chemical shift of the carbon directly bound to the proton in question in the ^{1}H-^{13}C GHSQC spectrum. $^{2}J_{NH}$ long-range correlations lead to ^{13}C-^{15}N direct correlation responses in the spectrum shown in Figure 1. $^{3}J_{NH}$ and $^{4}J_{NH}$ long-range correlations in the ^{1}H-^{15}N GHMBC give rise to ^{13}C-^{15}N responses corresponding to correlations *via* two- and three-bonds, respectively.



^{13}C-^{15}N Heteronuclear correlation responses observed in Figure 1 are shown on the structure above. The ^{15}N neural network shift calculations are shown parenthetically (ACD/Labs, NNMR Predictor v10.02), accompanied by the observed ^{15}N shifts. Correlations plotted with red contours in the ^{13}C-^{15}N correlation spectrum shown in Figure 1 arise from correlations between methylene carbons and the nitrogen; correlations plotted in black arise from correlations from methine carbons to nitrogen (or methyl carbons, although there are no methyl groups correlated to nitrogen in the^{1}H-^{15}N IMPEACH spectrum of (-) eburnamonine). The phase of the ^{13}C resonance from the multiplicity-editing of responses in the GHSQC spectrum is carried forward into the ^{13}C-^{15}N HSQC-IMPEACH unsymmetrical indirect covariance processed spectrum shown in the top right panel in Figure 1. Presumably, methine and methylene carbons with the same ^{13}C chemical shift that correlate to the same nitrogen could partially or completely cancel, hence it may be useful to consider the acquisition of both conventional and multiplicity-edited ^{1}H-^{13}C GHSQC spectra when the data are acquired since these data can be accumulated in a very reasonable period of time. For weaker samples, the sensitivity loss associated with multiplicity-editing (~20%) may obviate the acquisition of a multiplicity-edited GHSQC spectrum in any case. It is also interesting to note that in the ^{13}C-^{15}N HSQC-IMPEACH correlation spectrum shown in Figure 1, the C10 and C19 resonances, which have identical ^{13}C chemical shifts (~44.7 ppm), correlate to N1 and N4, respectively, but do not produce artifacts as seen in the case of IDR-GHSQC-TOCSY spectra with overlapping proton resonances as in our previous work [14]. An interesting corollary arises in the case of overlapped

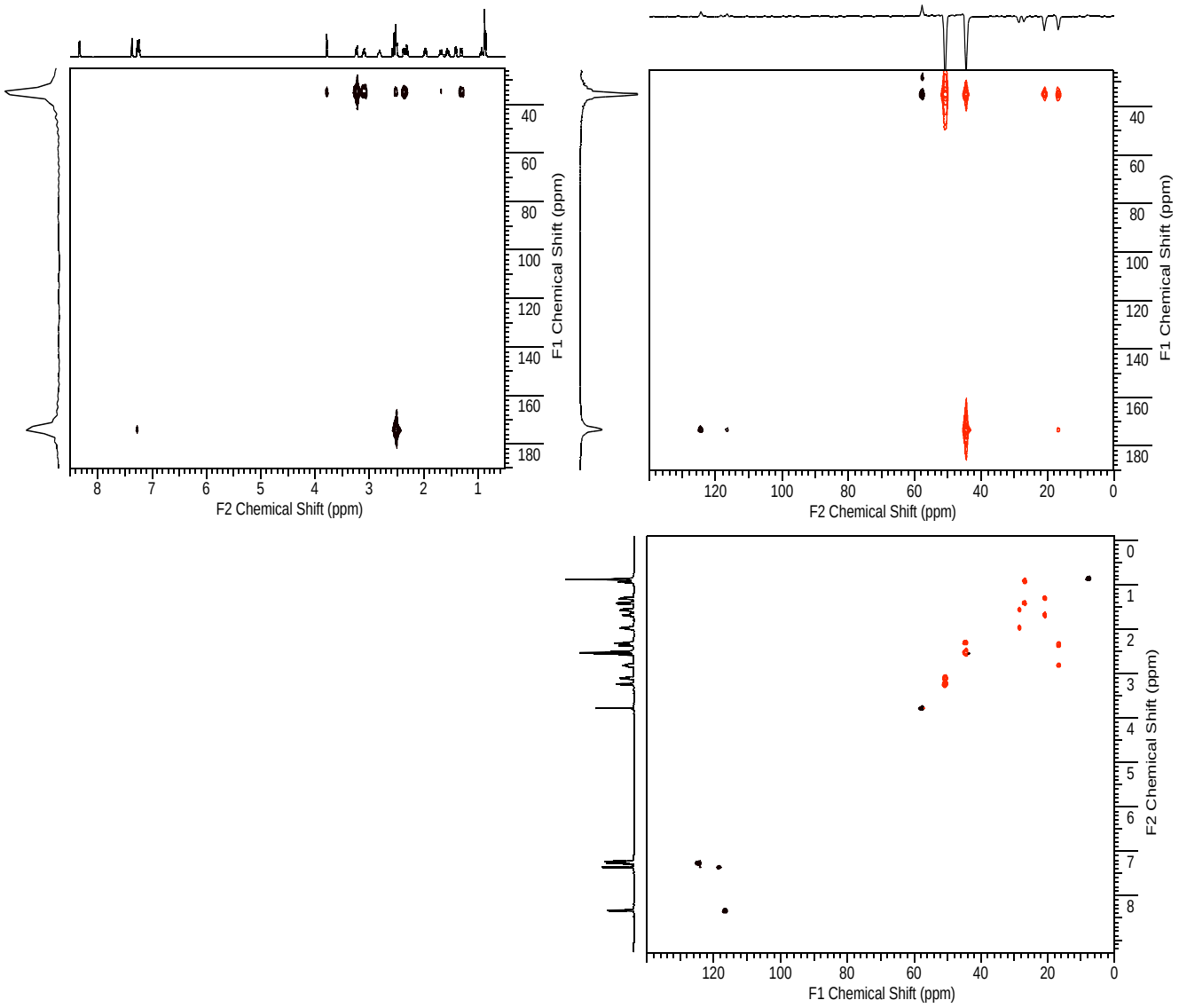


Figure 1. ^{13}C-^{15}N HSQC-IMPEACH (F_2,F_1) heteronuclear chemical shift correlation spectrum (upper right panel) derived by the unsymmetrical indirect covariance processing of discretely acquired ^{1}H-^{13}C GHSQCAD (bottom right panel, transposed to reflect the orientation of the ^{13}C chemical shift axis in the final ^{13}C-^{15}N correlation spectrum) and the ^{1}H-^{15}N IMPEACH spectrum (top left panel). The spectral traces flanking the ^{13}C-^{15}N HSQC-IMPEACH spectrum (upper right panel) are projections through both frequency domains; proton reference spectra are plotted along the F_2 axis of the ^{1}H-^{13}C GHSQC and ^{1}H-^{15}N IMPEACH spectra (bottom right and top left panels, respectively). The threshold level of the ^{1}H-^{15}N IMPEACH spectrum (top left panel) was above the weaker of the two responses in the ^{13}C-^{15}N HSQC-IMPEACH spectrum was below the threshold. The data were recorded at 600 MHz using an ~5 mg sample of (-) eburnamonine (**1**) in 180 µL $CDCl_3$ at 26° C. The individual 2D NMR spectra were processed to yield a pair of spectra comprised of 512 x 2048 points that were then subjected to unsymmetrical indirect covariance processing. Processing time was approximately 4 sec. The N1 and N4 resonances of (-) eburnamonine (**1**) are observed at 173.6 and 34.5 ppm, respectively. Correlations are observed, as expected, between C15 and the N1 amide nitrogen resonance in addition to much weaker correlations to the C11 and C12 aromatic carbons. Correlations are observed from the C3, C5, C6, C18, and C19 resonances to the N4 aliphatic nitrogen resonance. The C15 and C19 resonances are overlapped at ~44.7 ppm and correlate to the N1 and N4, resonances, respectively, with no observed artifacts of any type. The phase of the responses in the ^{13}C-^{15}N correlation spectrum arises from the multiplicity-editing of the GHSQC spectrum; methine and methyl carbons give rise to correlation responses with positive intensity; methylene resonances are negatively phased.

protons, one of which is long-range correlated to a nitrogen resonance. By calculating the ^{13}C-^{15}N correlation spectrum, as shown in Figure 1, the specific proton (*via* the $^{1}H/^{13}C$ response in the GHSQC spectrum) correlating to the ^{15}N resonance could be determined *via* the ^{13}C-^{15}N correlation response.

CONCLUSION

In conclusion, ^{13}C-^{15}N HSQC-IMPEACH heteronuclear chemical shift correlation spectra can be derived through the unsymmetrical indirect covariance processing of ^{1}H-^{13}C GHSQC and ^{1}H-^{15}N long-range heteronuclear chemical shift correlation spectra (^{1}H-^{15}N IMPEACH in the present example, but we have obtained the comparable results by co-processing ^{1}H-^{13}C GHSQC with ^{1}H-^{15}N IMPEACH [19,20] and ^{1}H-^{15}N CIGAR-HMBC [21] spectra). We propose the "labeling" convention ^{13}C-^{15}N HSQC-HMBC, ^{13}C-^{15}N HSQC-IMPEACH, *etc*. to denote the component 2D experiments used in the unsymmetrical indirect covariance processing of the data. The utilization of ^{13}C-^{15}N heteronuclear chemical shift connectivity information may prove useful in the structural characterization of pharmaceuticals (>80% contain nitrogen in their structures), alkaloids, as well as in the characterization of other nitrogen-containing heterocyclic compounds. The availability of ^{13}C-^{15}N heteronuclear connectivity information may also prove useful in computer-assisted structure elucidation studies, as have ^{1}H-^{15}N long-range heteronuclear chemical shift correlation data [5,22,23]. These data may also allow investigators to differentiate between overlapped protons, one of which is long-range coupled to ^{15}N, provided that the carbons directly bound to the overlapped protons are resolved, which is almost always the case. It will be quite interesting to see what other applications for ^{13}C-^{15}N heteronuclear chemical shift correlation data may arise. We are currently engaged in the exploration of further unsymmetrical indirect covariance processing possibilities; the results of that work forms the subject of forthcoming reports.

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