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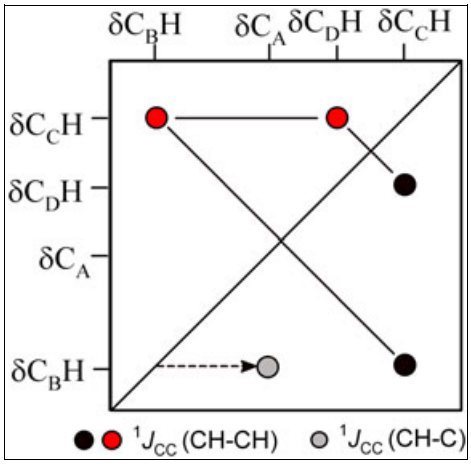

Posaconazole is a structurally complex triazole antifungal agent that, by virtue of its structural complexity, provides a good test molecule for the evaluation of NMR structure elucidation methodologies. Although GHMBC and related long-range ^{1}H–^{13}C heteronuclear shift correlation techniques are extremely powerful, at the same time, when dealing with unknowns, they can be problematic in that there is no way to readily differentiate adjacent ($^2J_{CH}$) correlations from longer range correlations, *e.g.*, $^3J_{CH}$ and $^nJ_{CH}$, $n > 3$. The 1,1-ADEQUATE experiment, in contrast, provides unequivocal experimental access to adjacent carbon–carbon correlation information, albeit with a sensitivity penalty, as the experiment involves an adjacent ^{13}C–^{13}C out-and-back magnetization transfer. In part, the sensitivity penalty can be overcome by using unsymmetrical indirect covariance or general indirect covariance processing methods. The application of these methods through the coprocessing of multiplicity-edited GHSQC and 1,1-ADEQUATE data to generate an HSQC-ADEQUATE correlation plot is demonstrated for posaconazole.

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INTRODUCTION

Two-dimensional (2D) NMR methods are irrefutably the cornerstone of modern structure elucidation methods and now allow the elucidation of complex molecular structures with far greater facility than even a few years ago. As molecules become more complex and their NMR spectra correspondingly more congested, the potential for ambiguity in structure elucidation protocols that rely on the simplest 2D NMR experiments increases. When dealing with complex molecules, investigators often resort to more sophisticated 2D NMR methods that make greater demands on instruments and probe technology for sensitivity. As an example, the 1980 reports of the INADEQUATE experiment by Freeman and coworkers [1] described an extremely powerful 2D NMR experiment that can establish the carbon skeleton of molecules *via* adjacent ^{13}C–^{13}C double quantum coherence in most cases. The down side of the 2D INADEQUATE experiment is the extreme insensitivity and prodigious sample requirement. Higher sensitivity alternatives such as the 1,1-ADEQUATE experiment have been developed [2–5] but still are relatively insensitive when compared to high sensitivity experiments such as multiplicity-edited GHSQC [6].

Recent work in several laboratories has shown that it is possible to utilize variants of indirect covariance processing methods to concatenate multiple 2D NMR experiment in useful fashion as long as they share a common frequency domain [7–12]. Coprocessing multiplicity-edited GHSQC and 1,1-ADEQUATE spectra using either unsymmetrical indirect covariance (UIDC) [7–10] or general indirect covariance (GIC) [12] processing methods has recently been shown to provide the means of elucidating adjacent carbon–carbon connectivity networks defined by the 1,1-ADEQUATE experiment with the multiplicity-editing and much high S/N ratio of the multiplicity-edited GHSQC spectrum with which the 1,1-ADEQUATE data are co-processed [13]. The resulting HSQC-ADEQUATE correlation spectra provide carbon–carbon connectivity information with the much higher S/N ratio inherent to the GHSQC spectrum.

RESULTS AND DISCUSSION

Multiplicity-edited GHSQC experiments require little description or discussion, as the experiment is routinely used for structure elucidation. In contrast, the 1,1-ADEQUATE experiment is less familiar to many investigators and perhaps warrants brief discussion [3,4]. The pulse sequence begins with the establishment of coherence between a proton and its directly attached carbon. Magnetization is next transferred from the directly bound ^{13}C to adjacent neighbor ^{13}C resonances *via* $^{1}J_{CC}$. That magnetization, now labeled with the ^{13}C frequency of the adjacent carbon(s), is transferred back to the starting ^{13}C resonance and finally detected *via* the directly attached proton. Consequently, the 1,1-ADEQUATE experiment has much higher sensitivity than the related INADEQUATE experiment. Indeed, work by Klein and Cheatham [14] and more recent work from this laboratory [15,21] has shown that it is possible to acquire 1,1-ADEQUATE data for submilligram samples when using a 600 MHz spectrometer equipped with a 1.7-mm Micro CryoProbe™.

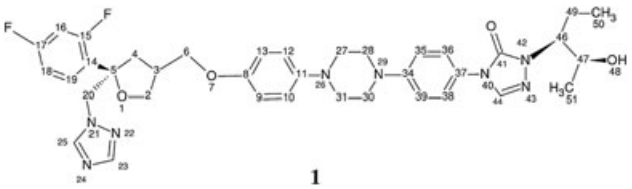
During the development of unsymmetrical indirect covariance processing methods, it has been shown that various individual 2D NMR experiments can be mathematically combined. Examples include the coprocessing of HSQC and COSY spectra to afford HSQC-COSY spectra [8,9]. Snyder and Brüschweiler have recently shown an alternative route to HSQC-TOCSY spectra by double indirect covariance processing that yields similar results [12]. HSQC and NOESY spectra can be combined to afford low-sensitivity HSQC-NOESY spectra in a fraction of the time required to actually perform the GHSQC-NOESY experiment [10]. Similarly, ^{1}H-^{13}C GHSQC and ^{1}H-^{15}N GHMBC spectra can be coprocessed to afford experimentally inaccessible ^{13}C-^{15}N correlation spectra [16]. Kupcče and Freeman have described an alternative concatenation method to obtain this correlation information [19].

The interpretation of 1,1-ADEQUATE data is typically undertaken side-by-side with GHSQC data or with the two spectra overlaid to facilitate interpretation. The interpretation process requires the investigator to have assignments made for the GHSQC data, which correspondingly facilitates the identification of the adjacent neighbor carbon(s). In most instances, the data will be unambiguous unless resonances are overlapped. In those cases, as with simpler experiments, the interpretation becomes problematic and structural ambiguity may be introduced.

Coprocessing multiplicity-edited GHSQC and 1,1-ADEQUATE spectra are logical step to facilitate the utilization of 1,1-ADEQUATE spectra [13]. Mathematical combination of these data retains the multiplicity-editing information of the GHSQC experiment, leading to diagonally symmetric vicinal carbon–carbon correlations that are observed in a familiar "COSY" format with the intrinsic sensitivity of the GHSQC spectrum rather than the much lower sensitivity of the 1,1-ADEQUATE experiment.

Adjacent nonprotonated carbons give rise to diagonally asymmetric responses that are detected at the F_2 frequency of the nonprotonated carbon and the F_1 frequency of the protonated ^{13}C resonance to which the quaternary carbon is vicinally coupled.

Several recent studies utilizing posaconazole (**1**) as a model compound have been reported [20,21]. The overlaid multiplicity-edited GHSQC and 60-Hz optimized 1,1-ADEQUATE spectra of posaconazole, **1**, are shown in Figure 1(A). The multiplicity-edited GHSQC data are plotted in black for positive CH and CH_3 resonances and red for negative CH_2 resonances. The correlations for the 60-Hz optimized 1,1-ADEQUATE spectrum are overlaid and plotted in green. The connectivity network for the side chain (C_{46}–C_{51}) is traced out in Figure 1(A). As will be noted, even using this approach, there are overlaps for the C_{46} and C_{47} resonances (due to the very similar chemical shifts of H_{46} and H_{47}) that obscure the adjacent carbon connectivity information from the 1,1-ADEQUATE experiment as shown by the expansion in Figure 1(B). In contrast, when the GHSQC and 1,1-ADEQUATE data were subjected to generalized indirect covariance processing (power = 0.5) [12], the diagonally symmetric HSQC-ADEQUATE spectrum shown in Figure 1(C) was obtained. As is readily observed, the overlap ambiguity associated with the C_{46}/C_{47} resonances is clearly resolved in the HSQC-ADEQUATE spectrum shown in Figure 1(C). Returning to Figure 1(A), no correlation was observed for the closely spaced C_3 and C_4 resonances in the tetrahydrofuran moiety within the structure of posaconazole. However, as shown in the inset of Figure 1(C), there is a weak correlation between these resonances that is observed despite being below the threshold of the contour plot shown in Figure 1(C).



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When the S/N ratios are compared for F_1 projections through the various spectra, the observation of the C_3–C_4 correlation in the HSQC-ADEQUATE spectrum but not in the overlaid GHSQC and 1,1-ADEQUATE spectra can be better understood. Using the C_6 methylene resonance as a reference point, a noise region from 90 to 100 ppm was defined. The multiplicity-edited GHSQC spectrum had a S/N ratio for the C_6 methylene of 95:1. In contrast, the C_6

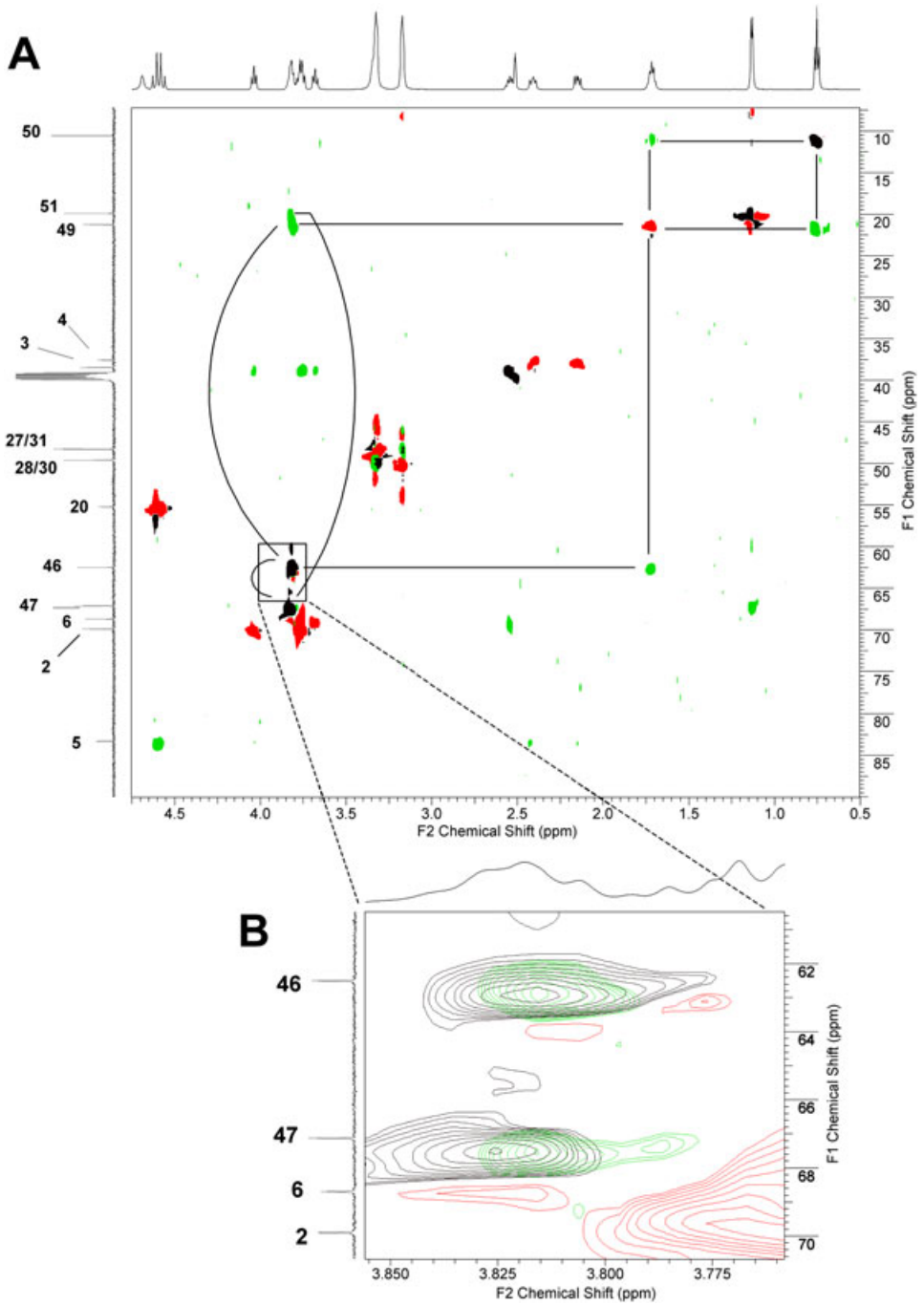

Figure 1. (A) Overlaid multiplicity-edited GHSQC and 60-Hz optimized 1,1-ADEQUATE spectra. Correlations in the GHSQC spectrum are plotted in black for methine and methyl resonances and in red for methylene resonances. Correlations from the overlaid 1,1-ADEQUATE spectrum are plotted in green. The connectivity network for the side chain (C_{46}–C_{51}) is traced out in the spectrum. The overlap of the correlations for the C_{46} and C_7 methine resonances creates a point of ambiguity that could be problematic if dealing with an unknown structure. It should also be noted that there is no correlation observed linking the C_3 methine and C_4 methylene resonances in the tetrahydrofuran moiety in the structure. (B) Expansion showing the overlap of the C_{46} and C_{47} methine resonances. (C) HSQC-ADEQUATE spectrum calculated using general indirect covariance (GIC) processing (power 0.5) [12]. Correlations between adjacent protonated carbons are diagonally symmetric [13] analogous to the distribution of connectivity information in a COSY spectrum. Correlations between nonprotonated and adjacent protonated carbons are observed at the F_2 frequency of the nonprotonated carbon and the F_1 frequency of the protonated member of the pair. Correlations to the C_5 nonprotonated carbon from the C_4 and C_{20} methylene resonances are denoted by dashed horizontal lines. Correlation networks for all of the aliphatic resonances of the molecule are traced out. The ambiguity of the C_{46} and C_{47} methine resonances is clearly resolved in the HSQC-ADEQUATE spectrum (compare boxed region of (C) with the expansion of the overlaid GHSQC and 1,1-ADEQUATE spectra shown in Figure 1(B)). As shown in the inset in panel C, there is a diagonally symmetric correlation establishing the connectivity between the C_3 methine and C_4 methylene resonances, although it is below the threshold used to prepare the contour plot. This correlation was not visible at all in the overlaid spectra and is visualized by the enhanced S/N ratio of the HSQC-ADEQUATE spectrum that results from covariance processing.

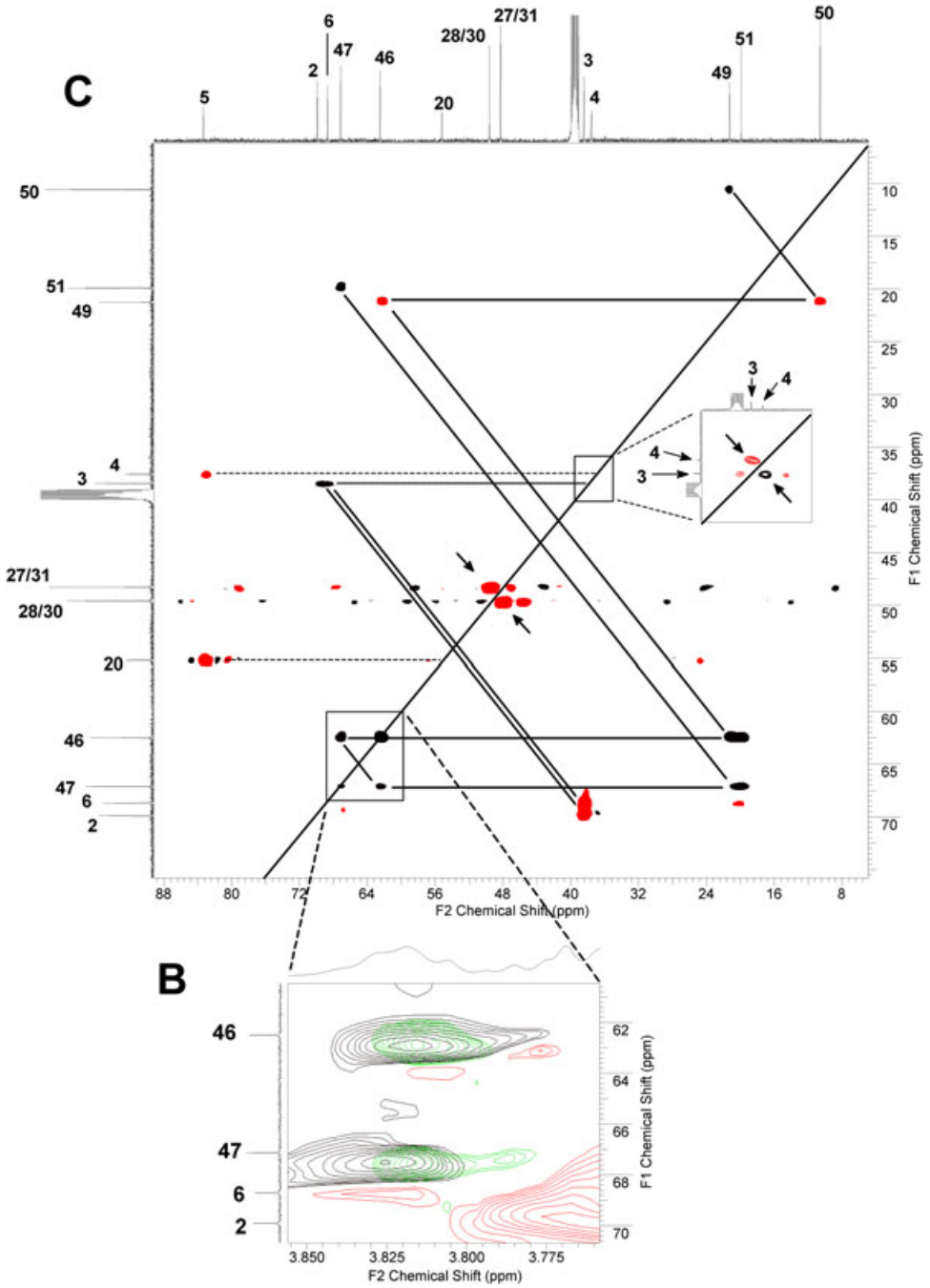

Figure 1. *(Continued)*

methylene resonance in the projection of the 1,1-ADEQUATE data had a much more meager S/N ratio of 15:1. Finally, in contrast, the S/N ratio for the C_6 methylene resonance of the GIC calculated HSQC-ADEQUATE spectrum was 195:1. UIDC or GIC coprocessing of HSQC and 1,1-ADEQUATE data not only resolved ambiguities due to resonance overlaps as shown above but also provided access to correlations that were too weak to be readily observed in the 1,1-ADEQUATE data [15]. To date, a rigorous direct comparison of UIC [7] and GIC [12] processed spectra has not been reported although comparable S/N ratios have been reported for data processed using both methods [13].

CONCLUSIONS

Concatenation of 2D NMR spectra can be used to advantage when a high sensitivity experiment is combined with a lower sensitivity experiment, reducing data acquisition

times for the latter and affording higher S/N ratios for the investigator. For complex molecules such as posaconazole, **1**, HSQC-ADEQUATE spectra calculated from multiplicity-edited GHSQC and 1,1-ADEQUATE data affords a presentation that can resolve ambiguities in select cases.

REFERENCES AND NOTES

[1] (a) Bax, A.; Freeman, R.; Kempsell, S. P. J Am Chem Soc 1980, 102, 4849; (b) Bax, A.; Freeman, R.; Kempsell, S. P. J Magn Reson 1980, 41, 349; (c) Bax, A.; Freeman, R. J Magn Reson 1980, 41, 507; (d) Bax, A.; Freeman, R.; Frenkiel, R. A.; Levitt, M. H. J Magn Reson 1981, 43, 478.

[2] Köck, M.; Reif, B.; Frenical, W.; Griesinger, C. Tetrahedron Lett 1996, 37, 363.

[3] Reif, B.; Köck, M.; Kerrsebaum, R.; Kang, H.; Fenical, W.; Griesinger, C. J Magn Reson 1996, 118A, 282.

[4] Köck, M.; Kerrsebaum, R.; Bermel, W. Magn Reson Chem 2003, 43, 65.

[5] Thiele, C. M.; Bermel, W. Magn Reson Chem 2007, 48, 889.

[6] Martin, G. E.; Hilton, B. D. J Nat Prod 2010, 73, 1465.

[7] Blinov, K. A.; Larin, N. I.; Williams, A. J.; Zell, M.; Martin, G. E. Magn Reson Chem 2006, 44, 107.

[8] Blinov, K. A.; Larin, N. I.; Williams, A. J.; Mills, K. A.; Martin, G. E. J Heterocycl Chem 2006, 43, 163.

[9] Martin, G. E.; Hilton, B. D.; Irish, P. A.; Blinov, K. A.; Williams, A. J. J Nat Prod 2007, 70, 1393.

[10] Blinov, K. A.; Williams, A. J.; Hilton, B. D.; Irish, P. A.; Martin, G. E. Magn Reson Chem 2007, 45, 544.

[11] Martin, G. E.; Irish, P. A.; Hilton, B. D.; Blinov, K. A.; Williams, A. J. Magn Reson Chem 2007, 45, 624.

[12] Snyder, D. A.; Brüschweiler, R. J Phys Chem A 2009, 113, 12898.

[13] Martin, G. E.; Hilton, B. D.; Blinov, K. A. Magn Reson Chem 2011, 49, 248.

[14] Cheatham, S. F.; Kline, M.; Sasaki, R. R.; Blinov, K. A.; Elyashberg, M. E.; Molodtsov, S. G. Magn Reson Chem 2010, 48, 571.

[15] Martin, G. E.; Hilton, B. D.; Willcott, M. R., III; Blinov, K. A. Magn Reson Chem 2011, 49, 350.

[16] Martin, G. E.; Hilton, B. D.; Irish, P. A.; Blinov, K. A.; Williams, A. J. J Hetrocycl Chem 2007, 44, 1219.

[17] Martin, G. E.; Hilton, B. D.; Irish, P. A.; Blinov, K. A.; Williams, A. J. Magn Reson Chem 2007, 45, 883.

[18] Martin, G. E.; Hilton, B. D.; Blinov, K. A.; Williams, A. J. J Nat Prod 2007, 70, 1966.

[19] Kupcče, E.; Freeman, R. Magn Reson Chem 2007, 45, 103.

[20] Hilton, B. D.; Feng, W.; Martin, G. E. J Heterocycl Chem 2011, 48, 948.

[21] Hilton, B. D.; Martin, G. E. J Heterocycl Chem 2012, 49.