A Comparison of the Hyphenated Experiments GHMQC-TOCSY and GHSQC-TOCSY

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Heteronuclear shift correlations generally may be established using either the multiple quantum-based GHMQC experiment or, alternately, the single quantum-based GHSQC experiment. A scant few reports contained in the literature have compared results obtained with both types of sequences. The F_1 resolution of the GHMQC and GHSQC experiments are compared using the polynuclear heteroaromatic naphtho-[2',1':5,6]naphtho[2',1':4,5]thieno[2,3-*c*]quinoline. Even when augmented by linear prediction in F_1, the single quantum-based GHSQC sequence gives better F_1 resolution than its multiple quantum counterpart. To date, no studies have compared the hyphenated analogs of these experiments, GHMQC- and GHSQC-TOCSY. Similar conclusions to GHMQC/GHSQC are drawn for the comparison of GHMQC- and GHSQC-TOCSY experiments with inverted direct responses with, and without, linear prediction. The latter is recommended whenever there is congestion in both the F_2 and F_1 frequency domains.

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Introduction.

Polynuclear aromatic and heteroaromatic molecules provide a significant nmr spectral assignment challenge. Relatively narrow spectral widths in both the F_2 and F_1 frequency domains generally make achieving moderately high digitization relatively easy. Despite the restricted spectral windows, the possibility still exists for severe congestion and spectral overlap, usually in F_2, thereby making successful utilization of some experiments much more difficult. Generally, while F_2 congestion or spectral overlap may be troublesome (thereby precluding the use of homonuclear TOCSY or similar experiments for the unequivocal elucidation of homonuclear spin systems), the investigator still has recourse to experiments such as GHMQC- or GHSQC-TOCSY that exploit the inherently much higher resolution usually found in the carbon or F_1 frequency domain to successfully sort homonuclear connectivity networks. In relatively fewer cases, molecules will have high levels of spectral congestion in both the F_2 and F_1 frequency domains. This group of molecules is the most difficult to successfully assign since extremely high levels of digital resolution are necessary in the F_1 frequency domain even when GHMQC- or GHSQC-TOCSY experiments are to be used. A comparison of the inherent resolution of the GHMQC- and GHSQC-TOCSY experiments is presented, coupled with a discussion of the advantages to be gained by using linear prediction in processing the data.

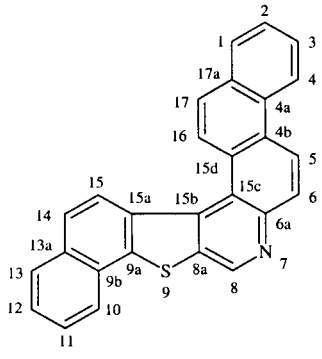
The polynuclear heteroaromatic, naphtho[2',1':5,6]naphtho[2',1':4,5]thieno[2,3-*c*]quinoline (**1**), is a molecule exhibiting a highly congested proton spectrum coupled with a high level of congestion in the carbon spectrum. Congestion in the former, F_2, generally precludes the successful usage of homonuclear TOCSY experiments for the establishment of proton-proton connectivity networks, mandating, instead, the use of hyphenated techniques such as GHMQC- or GHSQC-TOCSY. Congestion in F_1 makes the highest possible F_1 digital resolution a necessity if a hyphenated experiment such as GHXQC-TOCSY, (where X = M or S) is to be used successfully to unequivocally identify the component resonances of protonated heteronuclear spin systems.

Results and Discussion.

Comparison of GHMQC and GHSQC Spectra.

Whenever high levels of digital resolution are necessary for spectral assignments to be made unequivocally, it is appropriate to more carefully consider the experiments that will be performed. Assuming that insufficient samples are available for heteronuclear detection, as is generally the case with natural products, or impurities and degradants of pharmaceuticals, it becomes necessary to resort to proton, or inverse-detection. If proton-detected-methods are to be employed to establish the direct ^1H-^{13}C heteronuclear correlations, two fimdamental choices are available. One may elect to utilize the multiple quantum-based correlation sequences [1] or the single quantum-based sequences [2]. Unfortunately, the inherent resolution of the multiple quantum-based GHMQC pulse sequence [3,4] is limited by the fact that proton multiplet structure appears in both frequency domains. In contrast, the single quantum-based GHSQC pulse sequence [2] offers an F_1 resolution advantage in that the proton multiplet structure is limited solely to the F_2 frequency domain [4,5,6]. The inherent advantage of GHSQC based experiments can be further augmented by linear prediction. To date, for the aforementioned classes of molecules, there have been a scant few studies reported in the literature pertaining to small molecules that have addressed the inherent differences in digital resolution between multiple- and single-quantum based proton-detected heteronuclear shift correlation experiments. The first of which we are aware are a pair of comparative spectra of the

alkaloid cryptospirolepine presented in a review of the applications of inverse-detection methods in alkaloid chemistry [7]. More recently, Reynolds and co-workers [6] have conducted a rigorous comparison of the resolution attainable for HMQC and HSQC experiments when applied to the methylene groups of the steroid clionasterol. The former study suggested a modest improvement in resolution offered by the HSQC experiment for the alkaloid cryptospirolepine while a more dramatic enhancement in resolution for the HSQC experiment was noted in the latter study.



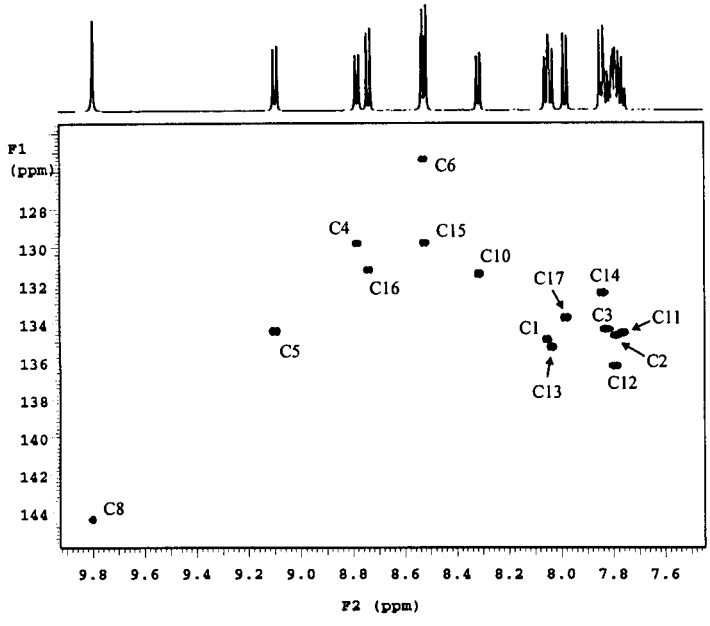


Figure 1. Full GHSQC spectrum of **1**, utilizing 128 files linear predicted to 512 points. The relative insolubility of the compound was counteracted with a drop of trifluroacetic acid, and, after the aquisition of the GHXQC data, the deuteriochloroform solvent evaporated. The sample was again redisolved using a drop of trifluroacetic acid, creating slightly different concentrations of trifluroacetic acid from the first to second sample, and hence minor shift changes, as can be noted from comparisons of the GHXQC and GHXQC-TOCSY plots. Direct comparisons of the experiments described in the test utilized the same sample. The contour plot above is the same sample as used in the GHXQC-TOCSY data.

In the present study, we were interested in evaluating more fully the differences in the inherent resolution of the GHMQC and GHSQC experiments, utilizing a polynuclear heteroaromatic compound with spectral congestion in both frequency domains. The direct correlations observed using the GHSQC experiment applied to **1** are shown in Figure 1. This annotated spectrum containing all resonances is presented for the purpose of a reference spectrum. The correlations for H8/C8 (9.79/144.4 ppm) and H6/C6 (8.53/125.4 ppm), which are further removed from the main grouping of resonances were intentionally omitted from all following plots to better illustrate the differences between the experiments. The aromatic region of the ^{1}H spectrum is presented along the top of the contour plot.

The direct correlations observed using GHMQC and GHSQC experiments applied to **1** are shown in Figures 2 and 3, respectively. The spectra shown are, counterclockwise from the top left, acquired with 32, 64, 128, and 256

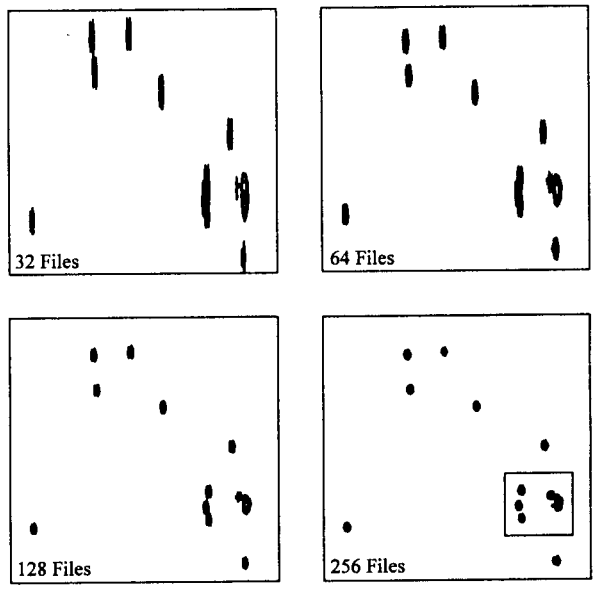


Figure 2. GHMQC spectra of **1**. Experiments were acquired identically with spectral windows on F_2 of 225 Hz and F_1 of 4525 Hz, 1024 complex points in F_2, 16 transients per F_1 file, a recycle delay of 1 sec, and an acquisition time of 0.230 sec. The temperature was held constant at 35°C, and 128 steady state scans were pulsed prior to acquisition. Data were acquired with 32, 64, 128, and 256 F_1 files, counterclockwise from top left, and zero-filled to 64, 128, 256, and 512, respectively. Gaussian multiplication was applied prior to both transformations.

increments in F_1, and are processed and plotted with identical parameters. As will be noted from even the most cursory inspection, the most heavily congested region is in the lower right hand corner (graphically designated by the boxed region in the 256 file spectra).

Under normal acquisition parameters, 32 files would typically be acquired for survey F_1 resolution (~1 file per ppm). As can be seen from this spectrum, the inherent congestion was far too great for this to be acceptable F_1 digital resolution.

Indeed, acceptable resolution is not reached until 256 files are acquired in the GHMQC experiment or, possibly, 128 files in the GHSQC experiment (Figure 3). It should be noted that even when 256 files are accumulated in the GHMQC experiment (Figure 2, lower right panel), all of the responses in the boxed region are still not resolved.

Comparing the 32, 64, and 128 increment spectra from both the GHMQC and GHSQC experiments will reveal only a modest enhancement in F_1 resolution of the GHSQC data, whereas the 256 increment GHSQC data exhibits complete F_1 resolution. This is most readily observed in the boxed regions, in which the GHSQC resonances are completely resolved while the corresponding resonances in the GHMQC spectrum are still partially overlapped.

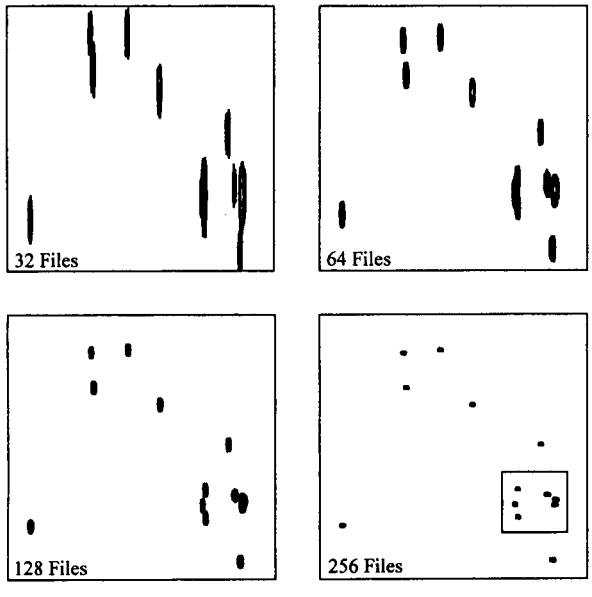


Figure 3. GHSQC spectra of **1**. Experiments were acquired identically with spectral windows on F_2 of 225 Hz and F_1 of 4525 Hz, 1024 complex points in F_2, 16 transients per F_1 file, a recycle delay of 1 sec, and an acquisition time of 0.230 sec. The temperature was held constant at 35°C, and 128 steady state scans were pulsed prior to acquisition. Data were acquired with 32, 64, 128, and 256 F_1 files, counterclockwise from top left, and zero-filled to 64, 128, 256, and 512, respectively. Gaussian multiplication was applied prior to both transformations.

Results obtained with both experiments when linear prediction is used are presented in Figures 4 and 5. The same spectral region is presented as shown in Figures 2 and 3, and the data are identical but processed with a linear prediction of three times the acquired number of files. Counterclockwise from the top left, the data is acquired with 32 files linear predicted to 128, 64 files linear predicted to 256, 128 predicted to 512, and 256 predicted to 1024 points in F_1. The boxed regions in Figure 5 exhibit a level of resolution that is unattainable through the multiple quantum experiment. The six resonances contained inside the box are completely resolved,

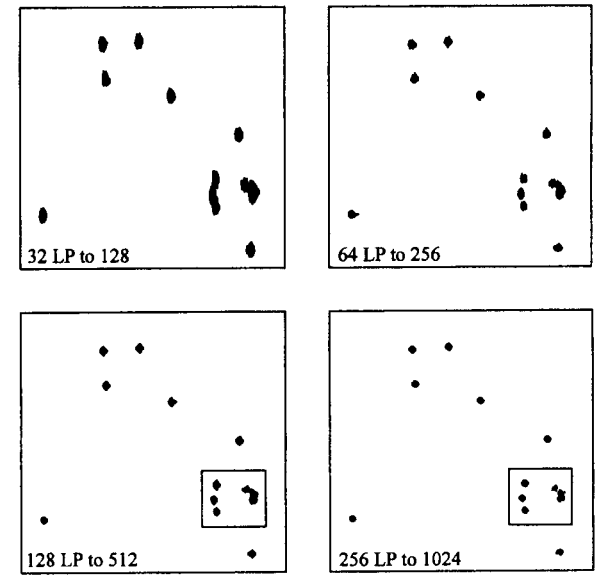


Figure 4. GHMQC spectra described in Figure 2, with linear predicion of the complex data points in F_1 to 128, 256, 512, and 1024, clockwise from top left. Gaussian multiplication was applied prior to both transformations.

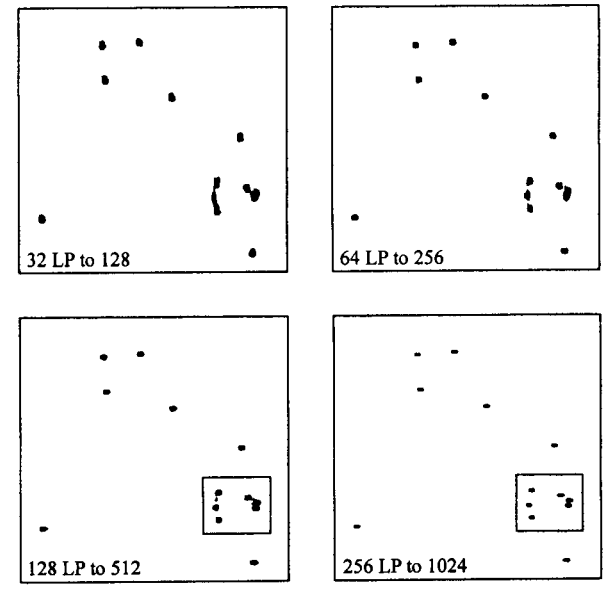


Figure 5. GHSQC spectra described in Figure 3, with linear predicion of the complex data points in F_1 to 128, 256, 512, and 1024, clockwise from top left. Gaussian multiplication was applied prior to both transformations.

whereas two of the resonances in the spectra from Figure 4 are still overlapped.

Carrying the linear prediction to an extreme, Figure 6 contains spectra from the GHMQC and GHSQC data acquired with 64 and 256 files, both linear predicted to 512 points. The 64 file GHSQC data, top left, not only exhibits

better F_1 resolution than the comparable GHMQC data, bottom left, but also exhibits a substantial gain in F_1 resolution over the non-linear predicted 256 file GHMQC data shown in Figure 2. The 256 file, linear predicted to 512 points, GHSQC spectrum is obviously the best resolved of the spectra shown in Figure 6.

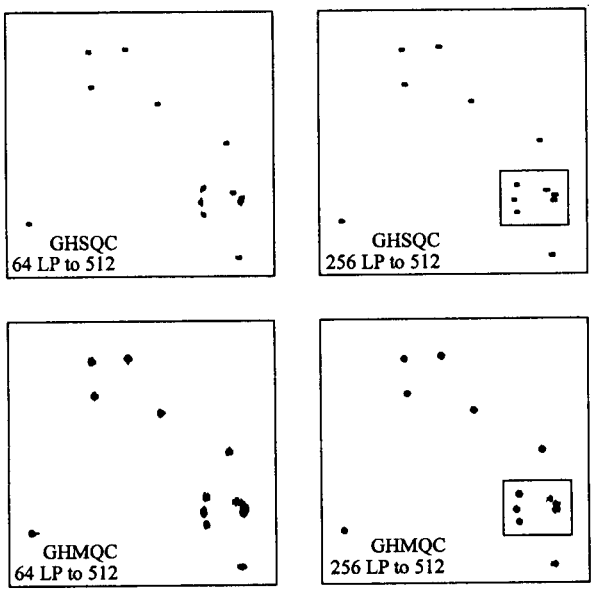


Figure 6. Comparison of excessive linear predicion in F_1. Top row: GHSQC spectra (described in Figure 3) with 64 files linear predicted to 512, left, and 256 files linear predicted to 512, right. Bottom row: GHMQC spectra (described in Figure 2) with 64 files linear predicted to 512, left and 256 files linear predicted to 512, right. Gaussian multiplication was applied prior to both transformations.

The results we have obtained in comparing the GHMQC and GHSQC spectra are consistent with those recently reported by Reynolds and co-workers [6]. Although the F_1 spectral width in the present study was considerably narrower than that used in Reynold's studies and was experimentally digitized more heavily, the F_1 spectral congestion of **1** would make lower levels of F_1 digitization with correspondingly higher linear prediction less desirable as suggested by Reynold's study.

Comparison of GHMQC-TOCSY and GHSQC-TOCSY.

Spectra with Inverted Direct Responses.

A viable alternative approach to the homonuclear TOCSY experiment when dealing with highly congested spectra involves the use of either GHMQC- or GHSQC-TOCSY [8,9]. These are hyphenated inverse-detected 2D nmr experiments in much the same sense as lc/ms, for example. The experiment first establishes the direct correlation between a proton and its directly bound carbon either *via* multiple quantum (GHMQC) or single quantum (GHSQC) coherences in the same sense that the liquid chromatograph first separates the peaks of interest in an lc/ms experiment. After "labeling" each proton with the chemical shift of its respective, directly-attached carbon, magnetization is refocused and then proton magnetization is propagated to the vicinal neighbor protons during an isotropic mixing period analogous to that in a homonuclear TOCSY experiment. The latter portion of the experiment allows the analysis. The analogy to the lc/ms experiment is fairly clear; coupled proton resonances are sorted by the chemical shift of the directly-bound carbon during the GHMQC- or GHSQC- portion of the experiment, and then the vicinal connectivity network of each proton is analyzed in the -TOCSY portion of the experiment. The difference relative to conventional homonuclear experiments, obviously, is that the proton-proton connectivity information is sorted in the second frequency domain as a function of carbon chemical shift. Since the spectral dispersion of carbon is generally much better than that of proton (distribution over ~200 ppm vs. ~10 ppm, respectively), the likelihood of establishing the needed connectivities is correspondingly much higher in the heteronuclear experiment than in its homonuclear counterpart since the possibility of inopportune overlap is correspondingly much lower.

Having shown the advantage to using the directly bound GHSQC experiment in terms of F_1 resolution, we next focused on a comparison of the GHMQC- and GHSQC-TOCSY experiments. Our initial expectation that the latter would prove to be superior in terms of F_1 resolution was subsequently supported by the data. Benefits of using linear prediction with the single-quantum based experiment were also consistent with the results presented above.

Clearly, unequivocal determination of the ^1H-^1H connectivity network is beyond the capabilities afforded by a homonuclear TOCSY experiment; the utilization of a hyphenated heteronuclear experiment is required if a total spectral assignment is necessary. The results with the GHMQC- and GHSQC-TOCSY are shown in Figures 7-10, with the single-quantum data plotted in the top panel and the multiple-quantum data plotted in the bottom panel. These data were acquired with 128 files in F_1, zero-filled to 256, and processed and plotted with identical parameters.

First, we will consider the region of the spectrum defined by 7.7-9.2 ppm in F_2 and 118-131 ppm in F_1 as above. Then we will focus our attention on the more congested region, defined by the range of 7.7-8.1 ppm in F_2 and 126-131 ppm in F_1, (expansions shown in Figures 8 and 10). The latter spectral expansions contain seven direct correlation resonances and four relayed responses. In the IDR-GHSQC-TOCSY experiments, the direct responses are inverted and displayed as the red contours; the relay correlations are displayed as the black contours. As is shown in Figures 7-10, using the GHSQC-TOCSY experiment, it is possible to differentiate the ^1H-^1H connectivity networks associated with these carbons despite a chemical shift difference of only 0.03 ppm.

Figure 7 contains most of the aromatic region for the resonances in **1**; the correlation for H8/C8 (9.79/144.4 ppm, further removed from the remaining resonances) was again

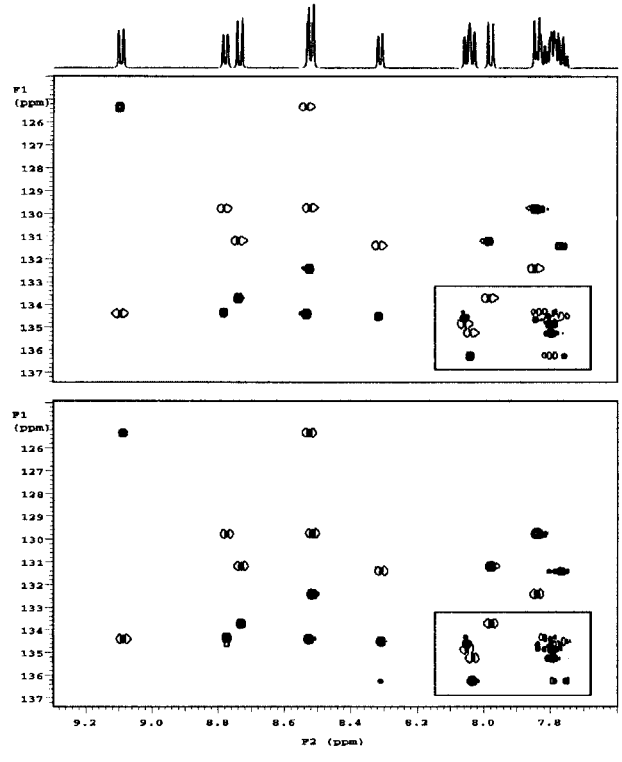


Figure 7. GHXQC-TOCSY spectra of **1**, with the single quantum variant on top an the multiple quantum variant on bottom. Experiments were acquired with identical parameters: spectral windows of 1860 Hz in F_2 and 4525 Hz in F_1, 16 transients per 128 increments, 1024 complex points in F_2 a recycle delay of 1.2 sec, and an acquisition time of 0.275 sec. The temperature was held constant at 35°C, and 32 steady state scans were pulsed prior to acquision. Data were sero-filled to 256 points in F_1, and a gaussian multiplication was applied prior to both transformations.

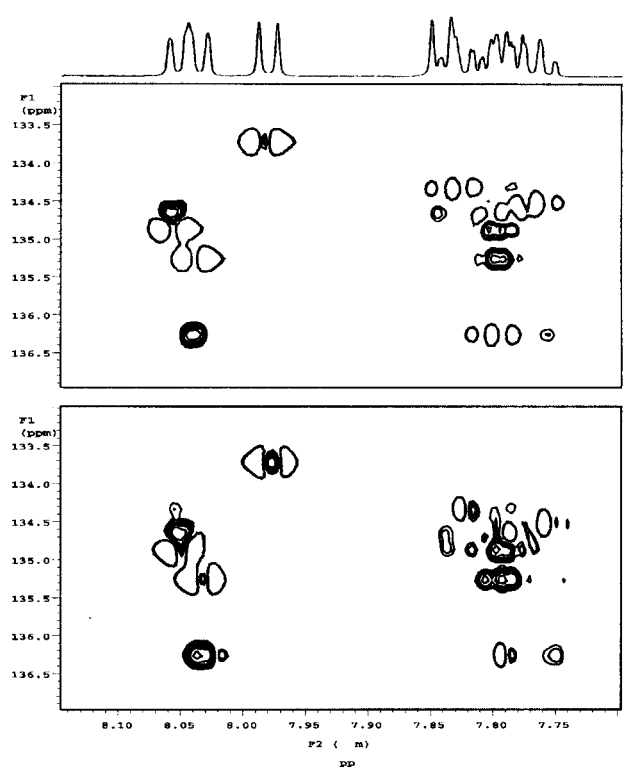


Figure 8. Expansions of the congested region of the GHXQC-TOCSY spectra described in Figure 7. The single quantum data are plotted in the top panel, and the multple quantum data is plotted in the bottom panel.

omitted from these plots to better illustrate the differences between the experiments. The GHSQC-TOCSY data, shown on top, exhibits a modest improvement in F_1 resolution over the GHMQC-TOCSY data. An expansion of the congested region is shown in Figure 8, more clearly highlighting the improved resolution of the single-quantum correlations, in which one can easily differentiate the direct and relayed responses in the heavily congested region of the spectrum relative to the multiple quantum data in the bottom panel.

To determine the resolution benefits of linear prediction of these data, the identical data in Figures 7 and 8 are linear predicted from 128 files to 512 points in F_1, and displayed in Figure 9, with the relevant expansion of the boxed region of the spectrum shown in Figure 10. The GHSQC-TOSCY data offers a considerably superior enhancement in resolution over that afforded by the GHMQCC-TOSCY data. The expansion of these data, Figure 10, more clearly illustrates the benefits of the single quantum experiment; the resonances are completely resolved and a complete assignment is now possible. The GHMQC-TOCSY data offer only poor resolution of the congested region, and compares unfavorably to the GHSQC-TOCSY data. The synthesis and complete assignment of the ^1H and ^{13}C spectra of naphtho[2',1':5,6]naphtho[2',1':4,5]thieno[2,3-*c*]quinoline will be reported separately [10].

Conclusion.

Early work from the author's laboratory [7] suggested that single quantum-based HSQC sequences were capable of giving somewhat better F_1 resolution than their multiple quantum-based HMQC counterparts for a polynuclear aromatic alkaloid. This subject has been treated much more rigorously, specifically for methylene carbons of natural products, in the more recent report of Reynolds and co-workers [6]. Under most circumstances, as Reynolds and colleagues aptly note, the advantages are generally not obvious since the experiments are seldom performed with sufficient F_1 spectral resolution for the inherent advantage of single quantum-based sequences to be observed. However, as Reyonlds and co-workers further note, with F_1 linear prediction, or as in the present case where relatively high levels of F_1 digital resolution are mandatory because of congestion in the carbon spectrum coupled with relatively narrow F_1 spectral windows, the advantages inherent to HSQC-based experiments become more obvious.

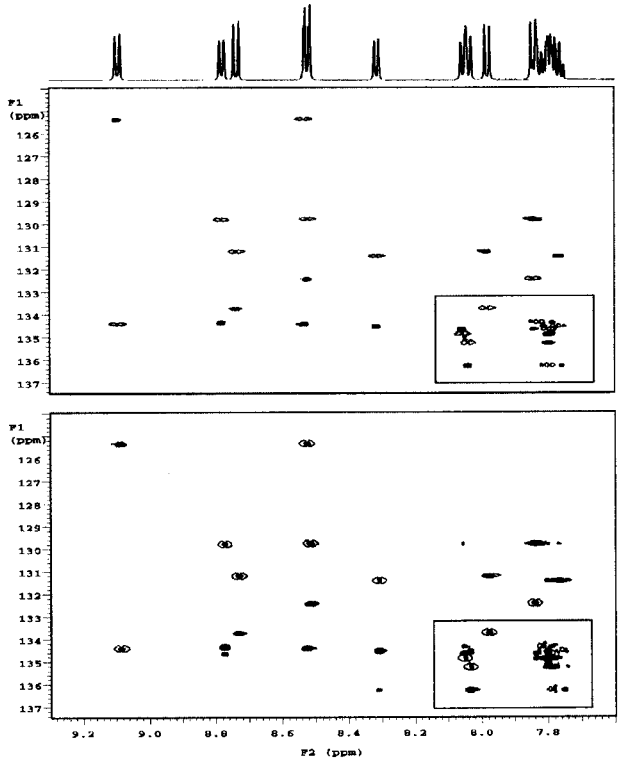


Figure 9. Linear prediced GHXQC-TOCSY data described in Figure 7. The 128 acquired F_1 files were linear predicted to 512 points, and gaussian multiplied prior to both transformations.

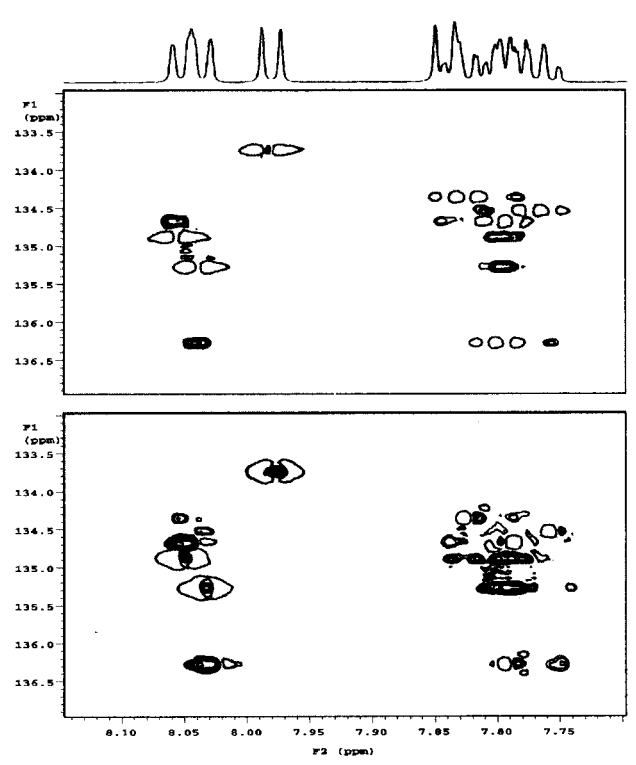


Figure 10. Expansions of the congested regions of the GHXQC-TOCSY spectra described in Figure 9. The single quantum variant is shown in the top panel, and the multiple quantum variant in the bottom panel. With this expansion, the advatages with the single quantum variant is obvious.

Quite simply, the short-coming of multiple quantum-based sequences is a result of ^1H-^1H multiplet structure being present in both the F_2 and F_1 frequency domains, while it is present only in the F_2 frequency domain of single quantum based experiments. This leads to inherently better F_1 resolution in the latter relative to the former. With the removal of the proton multiplet structure from the second frequency domain, the single quantum variants of the GHSQC and GHSQC-TOCSY experiments affords noticeable enhancements in F_1 resolution over that of the multiple quantum variants.

EXPERMENTAL

Experimental acquisition parameters can be found in the captions of the Figures. All nmr data were acquired on a Varian INOVA-600 NMR spectrometer operating at a ^1H frequency of 599.75 MHz, equipped with a Nalorac Z•SPEC™ MIDTG micro inverse detection triple resonance gradient probe. The samples were prepared in a dry box under argon gas, dissolved in ~ 150 µl deuteriochloroform (99.996%, Isotec), and transferred to a Wilmad 3 mm nmr tube. The sample was relatively insoluble, which was countered by the addition of a drop of trifluoroacetic acid to the deuteriochloroform solvent prior to sample preparation. The initial sample for the acquisition of the GHSQC and GHMQC data contained a higher level of trifluoroacetic acid than that of the second sample used for the acquisition of the GHSQC- and GHMQC-TOCSY data. After the acquisition of the GHSQC and GHMQC data, the sample was set aside for a protracted period of time, during which, the solvent evaporated. Upon redissolving, less trifluoroacetic acid was used to solubilize the sample, creating minor shift differences in several resonances.

REFERENCES AND NOTES

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[1] A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).

[2] G. Bodenhausen and D. J. Ruben, *Chem. Phys. Letters.*, **69**, 185 (1980).

[3] R. E. Hurd and B. K. John, *J. Magn. Reson.*, **91**, 648 (1991).

[4] J. Ruiz-Cabello, G. W. Buister, C. T. W. Moonen, P. V. Geldern, J. S. Cohen, and P. C. M. van Zilj, *J. Magn. Reson.*, **100**, 282 (1992).

[5] A. Bax, M. Ikura, L. E. Kay, D. A. Torchia, and R. Tschudi, *J. Magn. Reson.*, **68**, 304 (1990).

[6] W. F. Reynolds, S. McLean, L.-L. Tay, M. Yu, R. G. Enriquez, D. M. Estwick, and K. O. Pascoe, *Magn. Reson. Chem.*, **35**, 455 (1997).

[7] G. E. Martin and R. C. Crouch, Modem Methods in Plant Analysis, Alkaloids, Vol **15**, H. F. Linskens and J. F. Jackson, eds, Springer, New York, 1994, p 25.

[8] R. C. Crouch, A. O. Davis, and G. E. Martin, *Magn. Reson. Chem.*, **33**, 889, (1995).

[9] T. Domke, *J. Magn. Reson.*, **95**, 174, (1991); the non-gradient version described in this reference can be converted to a gradient experiment in a fashion similar to that in the preceding reference and in the GHSQC experiment as described in reference 4.

[10] J.-K. Luo, R. N. Castle, C. E. Hadden, and G. E. Martin, *J. Heterocyclic Chem.*, submitted (1999).