Establishing Vicinal Proton-Proton Connectivities Using Two-Dimensional Proton Zero Quantum Coherence NMR

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Phenanthro[3,4:3′,4′]phenanthro[2,1-b]thiophene (1) served as the model system to evaluate two-dimensional proton zero quantum coherence nmr in order to establish the vicinal proton-proton connectivities. The utility of the two-dimensional proton zero quantum nmr experiment has been compared with the utility of the traditional autocorrelated proton-proton (COSY) experiment. In the case of a molecule such as 1, where the proton chemical shifts are so highly congested, the zero quantum coherence experiment provides data not obtainable from the COSY experiment.

Vicinal proton-proton connectivities may now be established by various two-dimensional (2D) nmr methods. The autocorrelated homonuclear or COSY nmr experiment is probably the most commonly employed for this purpose [1,2]. In the case of polynuclear aromatic systems, molecules quickly attain a sufficient degree of complexity to preclude the successful utilization of the COSY experiment. In several instances, we have demonstrated that the combined utilization of heteronuclear chemical shift correlation and heteronuclear relayed coherence transfer may be successfully employed to establish the proton connectivity network [3,9]. Unfortunately, however, heteronuclear relayed coherence transfer suffers from diminished sensitivity relative to the conventional heteronuclear chemical shift correlation experiment [10,11]. In addition, the intensity of the "relay" response is highly dependent upon the optimization of the experiment. Thus, these limitations combine to potentialy restrict the general applicability of the heteronuclear relayed coherence transfer technique. Thus, there clearly exists a need for a highly sensitive means of elucidating proton-proton vicinal connectivity networks which can be applied to highly congested spectra. Homonuclear proton zero quantum coherence (HZQC) [12] fulfills these requirements and further can be performed in a minimal period of time with excellent sensitivity.

Since zero quantum coherence (ZQC) is probably a concept foreign to most, a definition of zero quantum coherence and a brief discussion of some of the properties of zero quantum coherence is warranted. The zero quantum transition between energy levels 2 and 3 for a simple AX spin system is illustrated in the energy level diagram shown in Figure 1. The zero quantum transition is defined
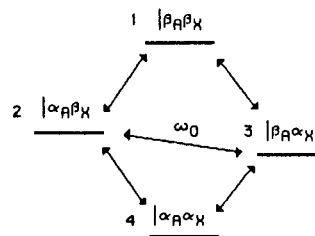

Figure 1. Engery level diagram for an AX spin system. Normal single quantum connectivities connect adjacent energy levels. The zero quantum connectivity is denoted by ω_o and connects levels 2 and 3. The double quantum transition between levels 1 and 4 has been omitted for clarity.

by ω_o where the population $\alpha_A\beta_X$ is interchanged with $\beta_A\alpha_X$. The zero quantum transition will have a characteristic frequency as do the single and the double quantum transitions of an AX spin system. Like double quantum coherence, the zero quantum coherence is not directly observable. Hence, zero quantum coherence will be created, allowed to evolve and then converted back into single quantum coherence (SQC) for observation. It should also be noted that the zero quantum transition does not necessarily represent a zero energy process.

Zero quantum coherence techniques suffered early on from a dependence on both the chemical shifts and spin coupling constants of the coupled nuclei [1,13]. These limitations made it impossible to select an appropriate τ value without a prior knowledge of the spectrum which would result, effectively reducing zero quantum coherence to the status of a quantum mechanical nuisance. These difficulties are, however, circumvented by the pulse sequence devised by Müller [12] which is shown in Figure 2. Thus,
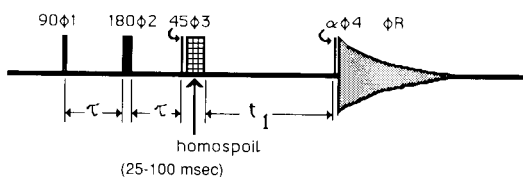
Figure 2. Homonuclear proton zero quantum coherence (HZQC) pulse sequence. The α pulse may be set to 90 or 45° although the latter was preferentially used in this work to simplify the spectra as described by Müller [12]. The homogeniety spoiling pulse is optional. When used, all coherences other than ZQC are destroyed in 100 ms or less [14] and a minimal four step phase cycle may be employed. Phases ($\phi 1$-ϕR) are given in [12]. When the homogeneity spoiling pulse is omitted, a more comprehensive 16 or 64 step phase cycle must be employed to eliminate residual single and double quantum responses.

the $90°_x$ - τ - $180°_y$ - τ - $45°_y$ sequence creates the ZQC which is then allowed to evolve during the evolution period, t_1; chemical shift terms are suppressed by the $180°_y$ pulse midway through the excitation sequence. At this point, two alternatives are available: utilization of a homogeneity spoiling pulse with a minimal four step phase cycle; more extensive phase cycling (16 or 64 steps) without the application of a spoiling pulse. When employed, the homogeneity spoiling pulse at the beginning of the evolution period serves to destroy higher quantum coherences as suggested by Wokaun and Ernst [14]. Following evolution, ZQC is converted back into observable SQC with the α pulse. Shortening the α pulse from 90° has several beneficial effects [12,15,16]: first, transfer of ZQC to passive spins is suppressed; second, quadrature phase detection is made possible; and third, the number of responses contained in the data matrix is halved. Finally, it should also be noted that ZQC is insensitive to magnetic field inhomogeneities [14] thereby affording, on a comparative basis, higher effective resolution than can be obtained with competing techniques such as COSY, an important point when dealing with highly congested proton nmr spectra.

To illustrate the capabilities of the HZQC nmr experiment, phenanthro[3,4:3′,4′]phenanthro[2,1-b]thiophene (1) was selected as a model system because of the numerous pairs of protons with highly similar chemical shifts. Proton resonance assignments for 1 were previously established indirectly using a long range heteronuclear chemical shift correlation experiment (LROCSCM) [8]. Before examining the proton zero quantum spectrum of 1 it is appropriate to consider the problems encountered in attempting to establish vicinal proton-proton connectivities using the more routinely employed and familiar COSY spectrum which is shown in Figure 3. As would be expected from the structure of 1, the proton spectrum contains six discrete spin
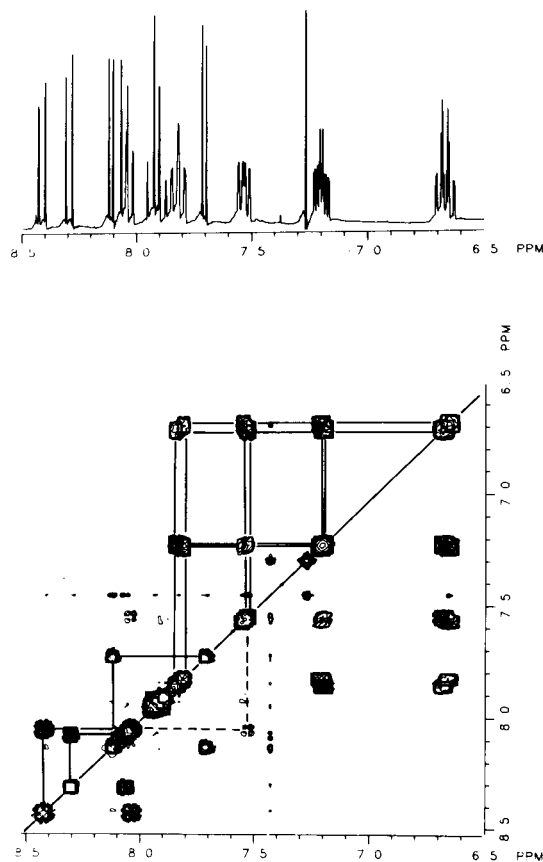
systems, two four spin systems and four two spin systems.

Figure 3. Autocorrelated proton (COSY) 2D-nmr spectrum of phenanthro[3,4:3′,4′]phenanthro[1,2-b]thiophene (1) in deuteriochloroform recorded at 300.068 MHz and 20°. The spectrum was acquired as a 256 x 512 complex point data matrix and was zero filled and subjected to sinusoidal multiplication prior to both Fourier transformations to give digital resolution comparable to that obtained with the proton zero quantum spectrum shown in Figure 5. The data was also symmetrized prior to plotting. A high resolution (8K points) resolution enhanced reference spectrum is shown above the contour plot. Individual spin systems are traced above the diagonal. The dashed connectivity shown below the diagonal corresponds to a 5-bond epi zig-zag coupling between H14 and H1. No other long range couplings were discernible.

In the COSY spectrum, three of the two spin systems are straightforward, AX in character; the fourth two spin system, in contrast, is AB and is thus considerably more difficult to locate and identify because of the diagonal responses. Comparatively, the two four spin systems are considerably more difficult to disentangle. From the previous assignments made using long range optimized heteronuclear chemical shift correlation [8], the two multiplets furthest upfield each correspond to two proton resonances which are highly overlapped. The multiplet centered at δ_H = 6.69 corresponds to the resonances for H12 and H15 (δ_H = 6.68 and 6.70, respectively) while that located at δ_H = 7.21 contains the H11 and H16 resonances (δ_H = 7.21 and

7.22, respectively). The severe overlap of these resonances makes it difficult to unequivocally track the connectivities of these resonances to their respective vicinal neighbors in the COSY spectrum. Thus, the COSY experiment has shortcomings which make the availability of an alternative experiment such as proton zero quantum coherence (HZ-QC) highly desireable.

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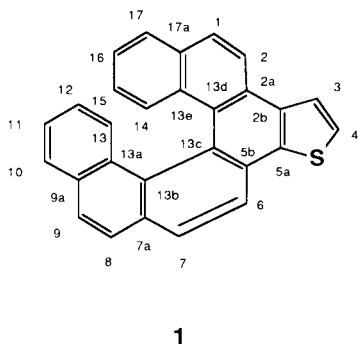
Before examining the zero quantum spectrum of **1**, it is appropriate to briefly describe the correlation pattern of the zero quantum spectrum. To begin, zero quantum responses appear in the second frequency domain, F_1, at the algebraic difference of the offsets of the coupled resonances relative to the transmitter. Zero quantum transition (ZQT's) correlating vicinally coupled spins are related by parallel lines with slopes of $2F_2 = +F_1$. Acquisition of the
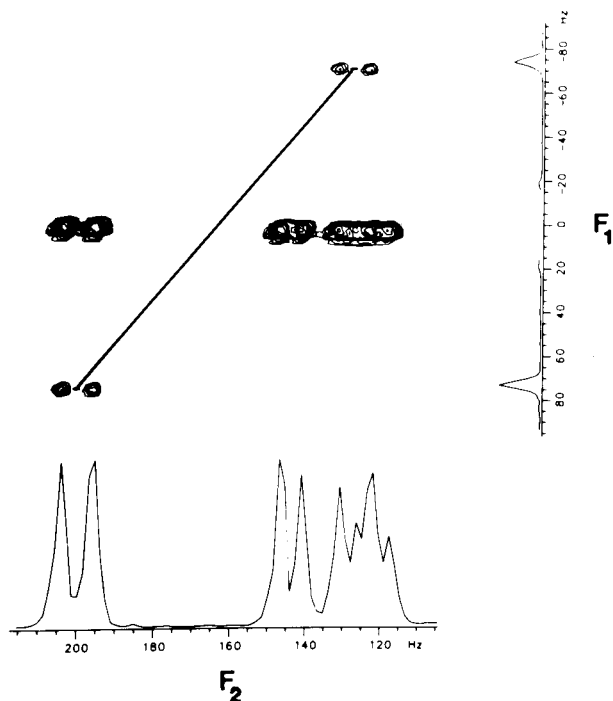

Figure 4. Contour plot showing the correlation of an AX pair of resonances using zero quantum coherence. The data was acquired using a 45° read pulse to suppress responses along the axis $2F_2 = -F_1$. The location of responses is discussed in the text.

spectrum using $\alpha = 90°$ would produce responses at both F_2 frequencies at each of the $\pm F_1$ frequencies possible. By shortening the duration of the "read" pulse, α, to 45°, the pair of responses located along the $2F_2 = -F_1$ axis are largely suppressed, their intensities a function of (tan $\alpha/2)^3$. This simplification of the data matrix makes the interpretation of the spectrum a more facile process hence making the pulse sequence with $\alpha = 45°$ the preferred verison of the experiment.

The correlation of a pair of coupled spins which was just described may be illustrated conveniently with the zero quantum connectivity of the AX spin system shown in Figure 4. The resonances whose correlation is shown in Figure 4 are the H5/H6 protons resonating at 8.28/8.04 ppm, respectively, with offsets relative to the transmitter ν = +200 and +126 Hz, respectively. The data shown in this contour plot was acquired using a read pulse, $\alpha = 45°$ to suppress half of the responses as described above. Zero quantum transition responses for the H5/H6 pair will appear in F_1 at the algebraic difference of the offsets of the spins relative to the transmitter, $F_1 = \pm 74$ Hz. The responses observed appear at $F_2 = +200$, $F_1 = +74$ Hz and $F_2 = +126$, $F_1 = -74$ Hz and are correlated by $2F_2 = +F_1$. The responses suppressed by using a 45° read pulse would have been located at $F_2 = +200$, $F_1 = -74$ Hz and $F_2 = +126$, $F_1 = +74$ Hz, correlated by $2F_2 = -F_1$.

In addition to the desired ZQT's, the ZQC spectrum may also contain some residual single and double quantum coherence when the duration of the homogeneity spoiling pulse is set too short. Single quantum coherence will produce a pair of diagonal response axes where $F_2 = \pm F_1$. Only the most minimal trace of the single quantum axis is visible in Figure 5 denoted by the dashed lines intersecting at the transmitter position in both frequency domains. The spectrum shown in Figure 5 was recorded with a 25 msec homospoil pulse with the power applied during the pulse intentionally minimized to allow artifacts to appear. There are also relatively strong double quantum responses which warrant comment. Double quantum responses will be symmetrically disposed about axes $2F_2 = \pm F_1$ which parallel or run perpendicular to the slope of the normal zero quantum transition correlation axes. Double quantum responses are quite easy to identify. First, the axes correlating double quantum respones will pass through the origin in both frequency domains (see Figure 5). Second, double quantum responses will appear in the F_1 frequency domain at the algebraic sum of the offsets of the coupled resonances relative to the transmitter and furthermore will be symmetrically disposed about the correlation axis. In the case of the zero quantum spectrum of **1** shown in Figure 5 the AX spin systems gave strong double quantum responses which are observed at frequencies of $\pm F_1 = 165$, 322 and 356 Hz. In general, it has been our
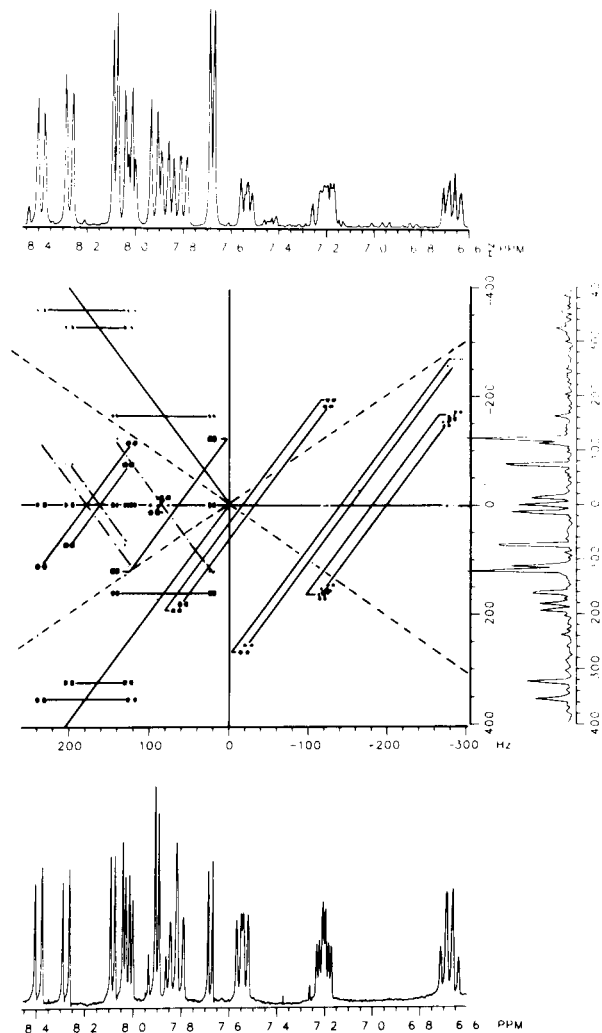
zero quantum spectrum of **1** and other polynuclear hetero-aromatic systems when the degree of homospoiling is insufficient. The prudent user of the zero quantum technique should be aware of this possibility. Further, because of the nature of the double quantum correlation, it may also be possible for folding of the double quantum frequency axes to occur. Thus, if the H2 (ν = offset relative to the transmitter = +220 Hz) and H6 resonances (ν = +200 Hz) happened to constitute an AX pairing, the double quantum response for this pair would appear at F_1 = ±420 Hz. Since the digitized range of F_1 in Figure 5 was only ±400 Hz, the double quantum response which would normally appear at F_1 = +420 Hz would be aliased in F_1 and would actually appear at F_1 = -380 Hz. Conversely, the double quantum response which would appear at F_1 = -420 Hz would instead appear at F_1 = +380 Hz. Thus, folding of the double quantum axis is another feature

Figure 5. Proton zero quantum coherence (HZQC) spectrum of **1** in deutriochloroform recorded at 300.068 MHz and 20°. The experiment was performed using a 25 msec homogeneity spoiling pulse with the spoiling power attenuated to allow the appearance of single and double quantum artifacts. The contour plot is bounded by a high resolution proton spectrum (4K complex points, below); a 90° projection through the zero quantum (F_1) frequency domain (vertical); and a 0° projection to illustrate the resolution afforded by the zero quantum technique (above). Single quantum correlation axes are shown by the dashed lines (- - -) where F_2 = ±F_1 and it should be noted that these responses have been suppressed quite effectively by the homogeneity spoiling pulse. Double quantum correlation axes are shown by the solid lines originating from the origin (F_2 = 0 Hz, F_1 = 0 Hz) where $2F_2$ = ±F_1. Double quantum responses correlating the three AX spin systems give rise to responses which are symmetrically disposed about the correlation axis at ±F_1 frequencies of 165, 322 and 356 Hz. Zero quantum connectivities between vicinally coupled resonances are traced by the parallel solid lines $2F_2$ = +F_1. In the case of the three AX pairs, the 45° read pulse incompletely suppressed the alternate pair and they are faintly visible along the parallel axes where $2F_2$ = -F_1 (denoted by - · - · -). The data was collected as 512 x 1K complex points and was processed with a sinusoidal multiplication prior to both Fourier transformations. Total acquisition time for the raw data was 23 minutes.

experience that AX spin systems almost routinely give rise to double quantum responses of the type identified in the
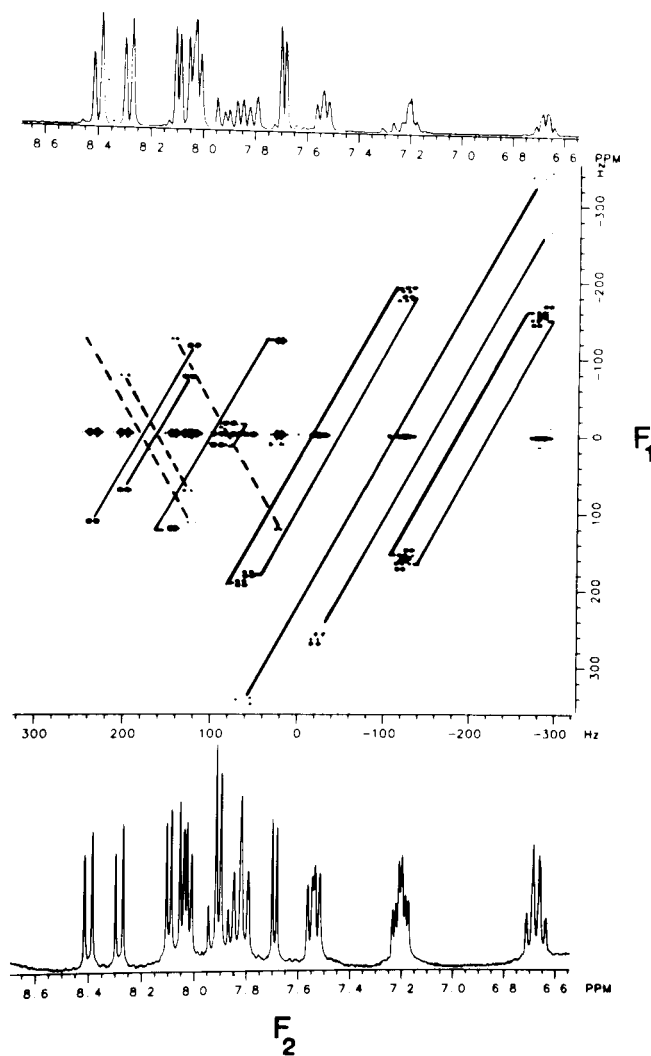

Figure 6. Proton zero quantum spectrum of **1** performed using a 64 step phase cycle. The high resolution proton spectrum is plotted below the contour plot; the projected proton spectrum is plotted above the contour plot.

which the new user of the zero quantum experiment should be acquainted with.

We find the use of the four step phase cycle with the homogeneity spoiling pulse to be convenient for the acquisition of quick survey spectra. In the case where high quality data is desired, it may be more practical to employ 16 or 64 step phase cycles rather than expending energy over the efficiency of the spoiling pulse. Indeed, the data shown in Figure 6 was acquired using a 64 step phase cycle [12] and will serve as the basis for the remainder of the discussion which follows.

Given the description of the ZQC experiment above, it is now appropriate to examine the zero quantum responses contained in the spectrum of **1** shown in Figure 6.
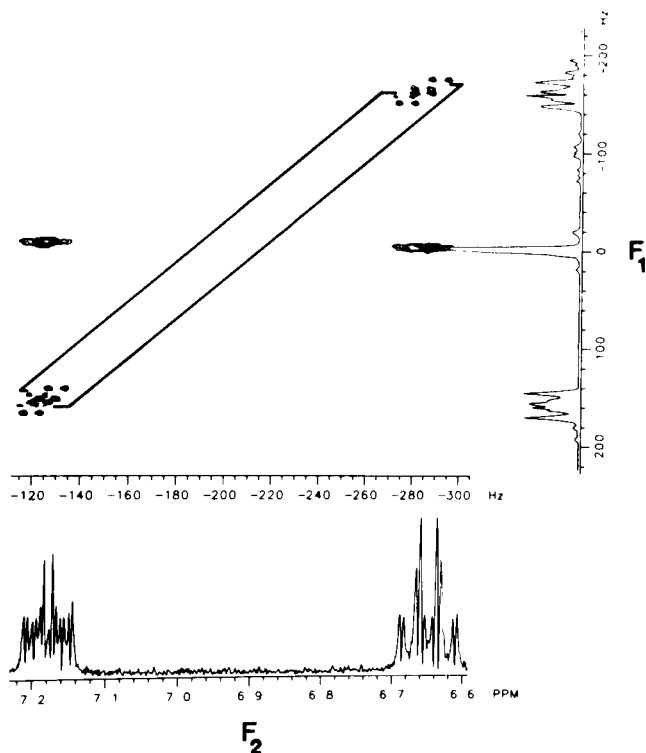

Figure 7. Expansion of a region of the spectrum shown in Figure 6 to enlarge response detail and illustrate the correlation of the H11/H12 and H15/H16 resonances.

The simplest place to begin this analysis is with the three AX spin systems of **1**. The AX spin systems are constituted by the H1/H2, H3/H4 and H5/H6 pairs of resonances which resonate at 8.03/8.40, 8.09/7.69 and 8.28/8.04 ppm, respectively. Relative to the transmitter in the F_2 frequency domain, these resonances are located at $\nu = 129/240$, 147/27 and 204/132 Hz, respectively. For the first pair of resonances, H1/H2, they will have a zero quantum frequency of $F_1 = \pm 111$ Hz. The zero quantum transition (ZQT) is represented by the responses located at (F_2, F_1) +240, -111 and +129, +111 Hz and connected by the

solid line. The pair of responses suppressed by the $\alpha = 45°$ read pulse is connected by the dashed line and is located at $F_2, F_1 = +240, +111$ and $+129, -111$ Hz. In similar fashion, the remaining two AX ZQT connectivities are also located. For purposes of comparison, the double quantum response associated with the H1/H2 AX spin system has responses which are located at (F_2, F_1) $+240$, ± 356 and $+129$, ± 356 Hz in Figure 5 and that they are symmetrically disposed about the double quantum correlation axis.

Resolution available with the HZQC nmr experiment is nicely illustrated by the H7 and H8 resonances which constitute an AB system. The component resonances are observed at $\delta_H = 7.93$ and 7.90, respectively. Relative to the transmitter, these resonances are observed at $\nu = 99$ and 90 Hz, respectively. In this case, despite a difference in chemical shifts of only 0.03 ppm, the connectivity between H7 and H8 is still readily established in the HZQC spectrum as a direct benefit of the minimal residual signal along the axis $F_1 = 0$ Hz. In contrast, the COSY spectrum of **1** (Figure 3) contains strong diagonal responses along the axis $F_1 = F_2$ which virtually obscure the H7/H8 AB connectivity. The HZQC spectrum shown in Figure 6 displays a similarly ability in resolving the connectivity pathways of the two four spin AKMX systems despite a chemical shift difference of 0.01 ppm between the H11 and H16 and 0.02 ppm between H12 and H15. These connectivities are illustrated in the expansion shown in Figure 7. The ability to establish the H11/H12 and H15/H16 connectivities is important because they could not be established using the heteronuclear relayed coherence transfer experiment.

Finally, only minimal long range coupling information is provided by the HZQC spectrum shown in Figure 6. Connectivities linking H10 and H17 (7.81 and 7.84 ppm, respectively) to their *meta* coupling partners, H12 and H15 (6.68 and 6.70 ppm, respectively) are only barely visible in the contour plot shown in Figure 6 at $F_1 = \pm 342$ Hz. No other long range connectivities could be observed in the 7 Hz optimized experiment.

Preliminary efforts directed toward the optimization of the HZQC experiment to probe longer range connectivities was disappointing. Recent work in these laboratories [17] has shown that the proton double quantum INADEQUATE experiment may be very beneficially optimized at 1.75 Hz (142.85 msec) which thus leads to the establishment of double quantum coherence with couplings ranging from <1 Hz to >15 Hz. An attempt to optimize the HZQC experiment in this fashion using the same strychnine model system [18] and **1** in the present study were both unsuccessful. However, because of the very minimal time requirements for the acquisition of a survey HZQC experiment, it may be worth further investigation in other systems.

Finally, in addition to the advantages in resolution offered by the HZQC experiment there is one further plus associated with the HZQC experiment. While the 256 x 512 complex point COSY spectrum shown in Figure 3 took 2 hours to acquire. The survey HZQC spectrum shown in Figure 5 which was acquired using a homospoil pulse required only 23 minutes for the acquisition of a 512 x 1K complex point spectrum. Thus, the HZQC experiment can be performed quickly. Quick data acquisition coupled with the facile establishment of vicinal proton-proton connectivities using the HZQC experiment makes it a potentially superior alternative to the COSY experiment for routine usage with polynuclear aromatic and heteroaromatic systems.

In conclusion, we feel that the proton zero quantum coherence nmr experiment offers considerable potential in the assignment of the proton nmr spectra of complex polynuclear aromatics. Our previous work with strychnine [18] and that of Hall and Norwood [19] have recently demonstrated the capability of the technique with complex natural products and amino acid spectra. The extension to other complex natural products and peptides should logically follow. At present we are utilizing the HZQC nmr experiment to probe the proton vicinal connectivity networks of a number of complex polynuclear aromatic systems and we anticipate that this work will form the basis for forthcoming reports.

Acknowledgements.

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