Correlation of Resonances of Strongly Coupled Spin Systems *via* Responses Due to Strong Coupling in Homonuclear Two-Dimensional J-Resolved Spectra: Total Assignment of the 1H-NMR Spectrum of 2-(2'-Pyridyl)-1,8-naphthyridine

Edward L. Ezell #, Randolph P. Thummel # and Gary E. Martin* [1]

Department of Chemistry, University of Houston,
Houston, Texas 77004

*Department of Medicinal Chemistry, College of Pharmacy,
University of Houston, Houston, Texas 77004
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Homonuclear two-dimensional J-resolved (2DJ) spectra of molecules containing strongly coupled spin systems contain additional responses and thus additional information, the responses arising from the mixing effects of the 180° pulse employed to create the spin echo upon which the experiment is based. Although additional responses due to strong coupling are generally considered to be an undesirable complication inherent to the experiment, it is shown that they can serve a useful correlation function. Total assignment of the 1H-nmr spectrum of 2-(2'-pyridyl)-1,8-naphthyridine at 100 MHz is reported, and members of the three strongly coupled spin systems of the molecule are identified *via* the additional responses due to strong coupling.

Homonuclear two-dimensional J-resolved (2DJ) spectra [2,3] are particularly useful in the examination of the proton nmr spectra of complex molecules when scalar coupling information is to be extracted from the spectrum. The utility of the experiment arises from its ability to sort chemical shift information along one frequency axis (F_2) while scalar coupling information (J) is partitioned into the second frequency dimension (F_1). Individual spin multiplets are oriented at an angle of 45° relative to the F_2 axis. When homonuclear 2DJ data matrices are "tilted" [4], the proton multiplet structure is aligned orthogonally to the F_2 axis, thereby permitting the direct examination of individual proton spin multiplets in a fashion analogous to the use of selective excitation techniques [5-7] in 13C-nmr spectroscopy. In contrast to the heteronuclear 2DJ experiment [8] in which strong coupling effects are seldom a complication or concern [9,10], the homonuclear variant of the experiment is frequently complicated by the effects of strong coupling. Specifically, as a direct consequence of the 180° refocusing pulse employed in the experiment's pulse sequence, additional resonances may appear in the homonuclear 2DJ spectra of strongly coupled systems. These responses have been analyzed for a variety of spin systems [11-13], and although there has been interest in the changes in intensity and F_1 frequency of the responses as a function of observation frequency [14,15], they have generally been considered to be an undesirable complication inherent to the experiment. Recently, however, we have shown the utility of such responses in the identification of numbers of strongly coupled spin systems in the highly congested 1H-nmr spectrum of benzo[2,3]phenanthro[4,5-*bcd*]thiophene [16]. In an extension of our previous work, we would now like to illustrate the utilization of re-

sponses due to strong coupling as a useful criterion for the identification of resonances of strongly coupled spin systems which might otherwise be difficult from either COSY [2,17,18] or SECSY [19,20] auto-correlated two-dimensional nmr experiments in which the off-diagonal responses necessary for the correlation may reside too close to the diagonal to have utility. Additionally, if a 2DJ spectrum is to be acquired to facilitate the examination of long range homonuclear spin couplings, the utilization of strong coupling responses may be sufficient in itself to obviate the acquisition of an autocorrelated spectrum. Thus, we now wish to report the assignment of the proton nmr spectrum of 2-(2'-pyridyl)-1,8-naphthyridine (1) at 100 MHz, illustrating the utility of responses due to strong coupling as a correlation mechanism.
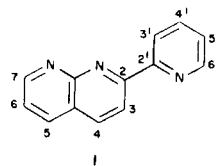
Consideration of the structure of 2-(2'-pyridyl)-1,8-naphthyridine (1) leads to the expectation of three discrete spin systems in the 1H-nmr spectrum of the molecule. Specifically, the H3 and H4 resonances would be expected to comprise an AB spin system while the remaining protons of the naphthyridine nucleus, H5, H6 and H7, would constitute an ABX spin system. Both of these spin systems have been analyzed for the general homonuclear 2DJ case [11,12] and have also been examined in previous work from this laboratory as well [14-16]. In contrast, the four spin system comprised of the H3', H4', H5' and H6' reso-

nances of the pendant pyridyl-substituent could range from an ABKL spin system to an ABMX or ABXY spin system, none of which have been analyzed for the 2DJ case. Regardless of the exact nature of the four spin system, all three of the spin systems of **1** would be expected to exhibit additional responses due to strong coupling in the 2DJ spectrum which may be exploited for correlation purposes.
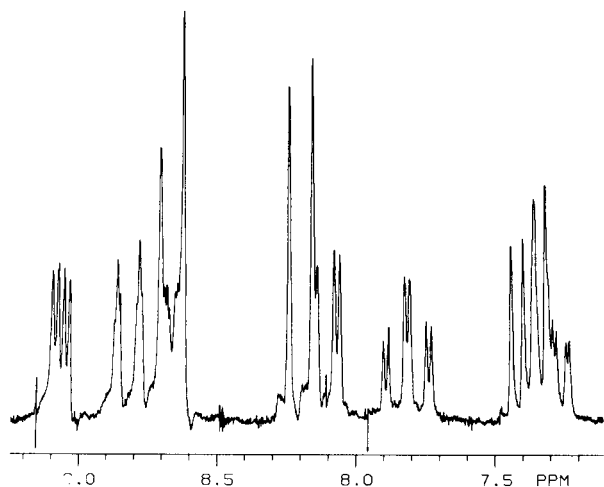

Figure 1. Conventional 100 MHz ^{1}H-nmr spectrum of 2-(2'-pyridyl)-1,8-naphthyridine (**1**) in deuteriochloroform at 33°. The spectrum was obtained using 4K data points to digitize the spectrum with the sweep width set to ±200 Hz.

The conventional 100 MHz ^{1}H-nmr spectrum of **1** is shown in Figure 1 and is reasonably well dispersed even at this low observation frequency. Despite the spectral dispersion, additional responses due to strong coupling may be expected. In accord with these expectations, the four level contour plot of the ^{1}H-2DJ spectrum (S(F$_1$,F$_2$) data matrix is shown) in Figure 2 (bounded by a 0° projection below, a 90° projection (J-spectrum) to the right, and a 45° projection (equivalent to homonuclear broad band decoupling), obtained with an F$_1$ spectral width of ±62.5 Hz, shows the presence of numerous groupings of responses due to strong coupling. Rather than representing a hindrance to the utilization of the experiment, we would now like to demonstrate the utility of these responses as a correlation mechanism.

Beginning with the H3/H4 AB spin system of the 1,8-naphthyridine nucleus, a doublet is readily located in the contour plot at δ 8.20 (see also 45° projection above the contour plot shown in Figure 2). Clearly, this doublet must arise from either H3 or H4 although unequivocal assignment cannot yet be made. Furthermore, even a very cursory inspection of the contour plot reveals that the counterpart to the doublet just cited is not readily visible and must therefore be obscured by overlap with another resonance in the spectrum. By recalling, however, that the strong coupling of the AB spin system produces four additional responses in the 2DJ experiment [11,12,14-16], we are provided with a convenient means of correlating the

Table 1

Assigned ^{1}H Chemical Shifts and Homonuclear Spin-Coupling Constants Obtained From the Homonuclear 2DJ Spectrum of 2-(2'-Pyridyl)-1,8-naphthyridine (**1**) in Deuteriochloroform at 33°

			Coupling Constant (Hz) [a]		
Assignment	Chemical Shift	$^3J_{HH}$	$^4J_{HH}$	$^5J_{HH}$	$^7J_{HH}$
7	9.07	$^3J_{76} = 4.2$	$^4J_{75} = 1.9$	—	$^7J_{73} = 0.4$
6'	8.82	$^3J_{6'5'} = 8.0$	$^4J_{6'4'} = 1.3$	$^5J_{6'3'} = 0.8$	—
3'	8.67	$^3J_{3'4'} = 4.7$	$^4J_{3'5'} = 1.8$	$^5J_{6'3'} = 0.9$	—
3	8.67	$^3J_{34} = 8.6$	—	—	$^7J_{37} = 0.4$
4	8.20	$^3J_{43} = 8.7$	$^4J_{45} = 0.7$	—	—
5	8.12	$^3J_{56} = 7.8$	$^4J_{54} = 0.7$	—	—
		—	$^4J_{57} = 1.8$	—	—
5'	7.72	$^3J_{5'4'} = 7.5$	$^4J_{5'3'} = 1.8$	—	—
		$^3J_{5'6'} = 8.0$	—	—	—
6	7.38	$^3J_{65} = 7.8$	—	—	—
		$^3J_{67} = 4.2$	—	—	—
4'	7.29	$^3J_{4'3'} = 4.8$	$^4J_{4'6'} = 1.3$	—	—
		$^3J_{4'5'} = 7.5$	—	—	—

[a] For improved accuracy in the measurement of the homonuclear spin coupling constants, a second homonuclear 2DJ spectrum was obtained with a spectral width of ±12.5 Hz in F$_1$ which was digitized with 256 points during data collection and zero filled to 512 points prior to the execution of the second Fourier transformation to provide an effective digital resolution of 0.048 Hz.
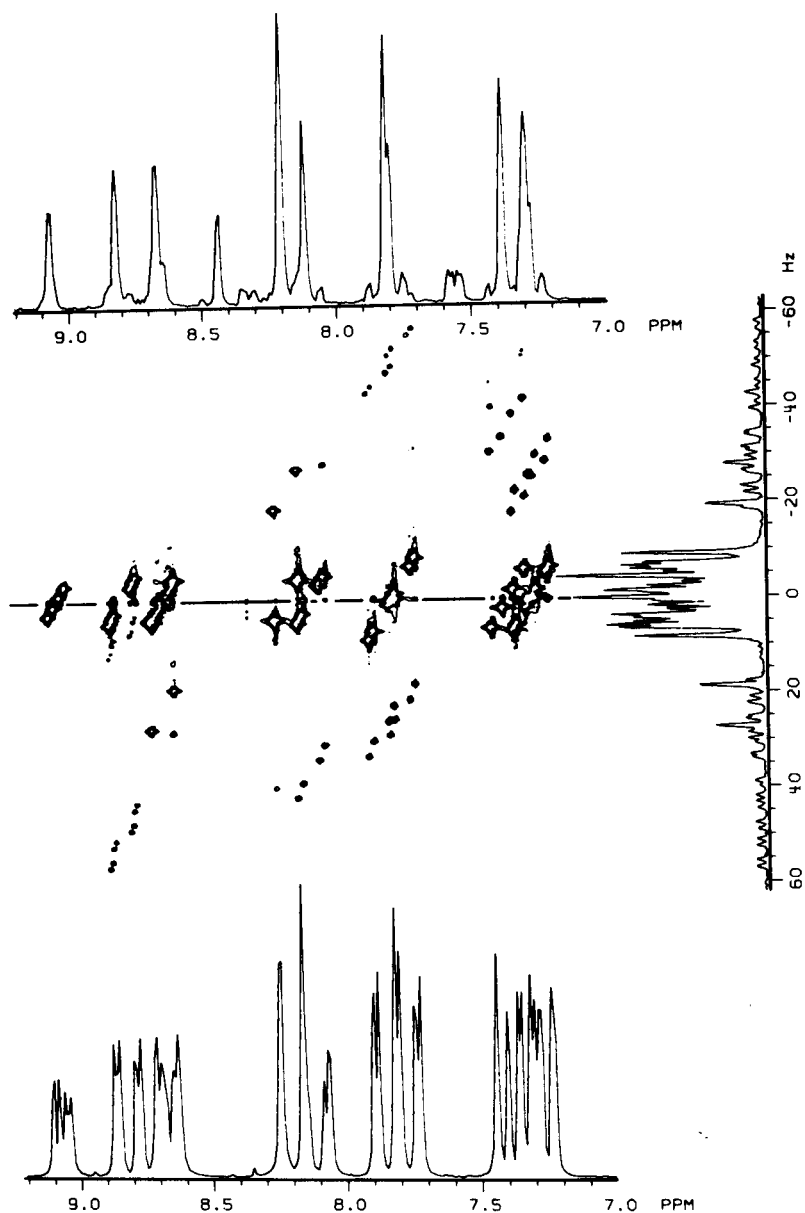
Figure 2. Four level contour plot of the homonuclear 2DJ spectrum of 2-(2'-pyridyl)-1,8-naphthyridine (1) obtained using 1K data points in F_2 × 256 data points in F_1 which was zero filled to 512 points prior to the second Fourier transformation to afford a $S(F_1,F_2)$ data matrix of 512 × 512 points. The spectrum is bounded by a 0° projection shown below which recovers the normal spectrum; by a 90° projection plotted to the right (F_1 = ±62.5 Hz); and by a 45° projection plotted above which produces the equivalent of a proton decoupled proton spectrum which has no analogy in conventional spectroscopy.
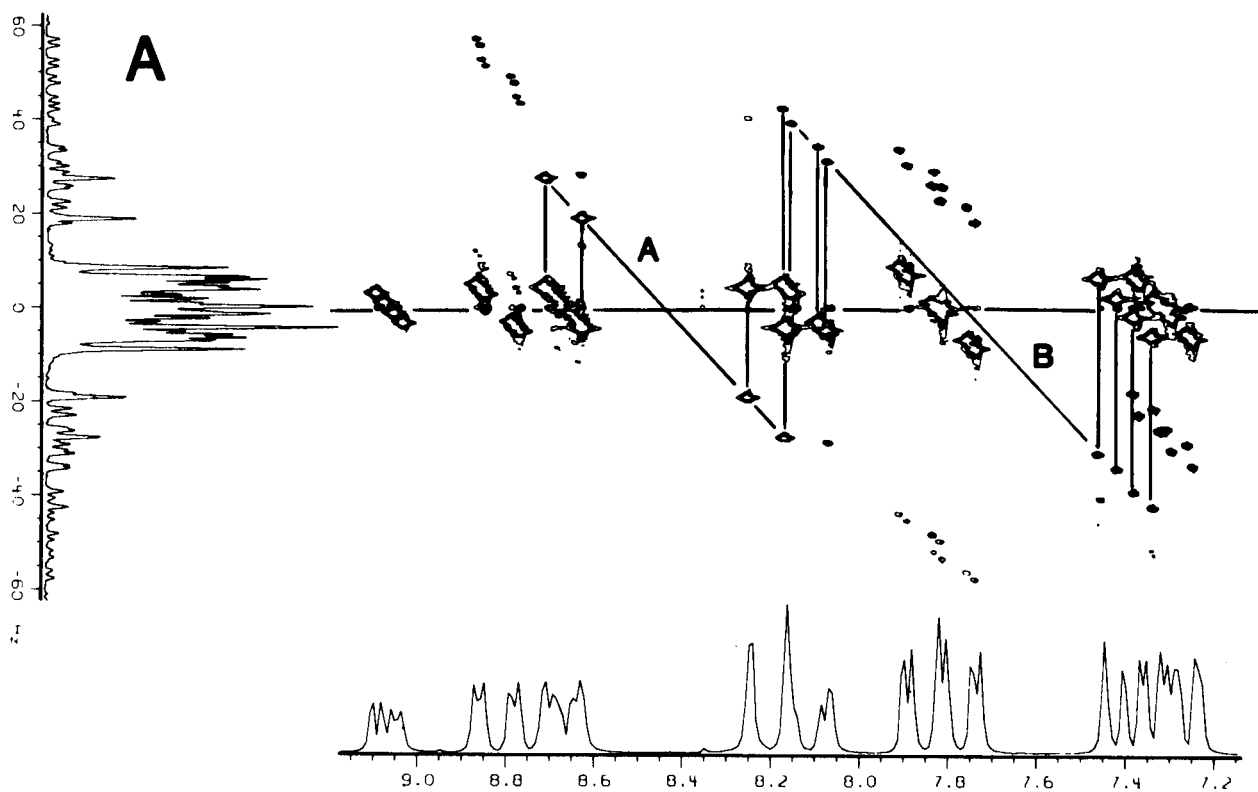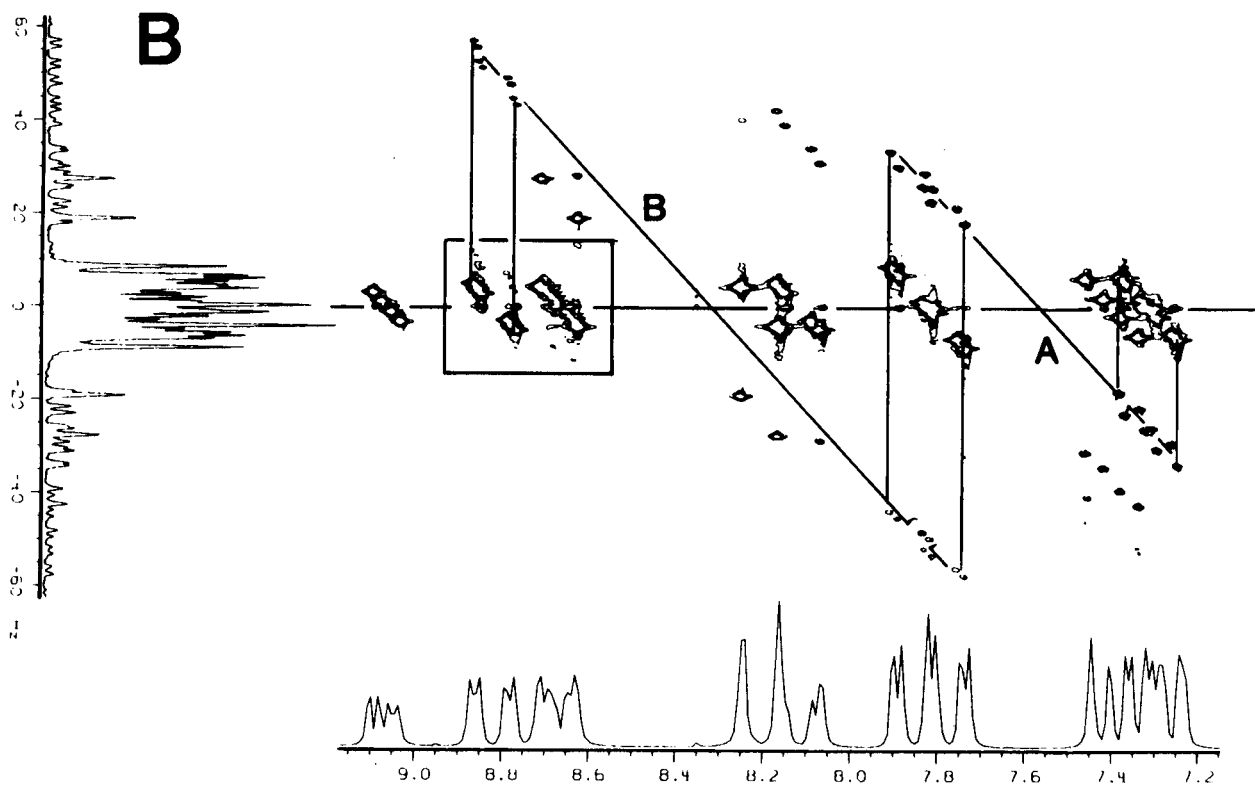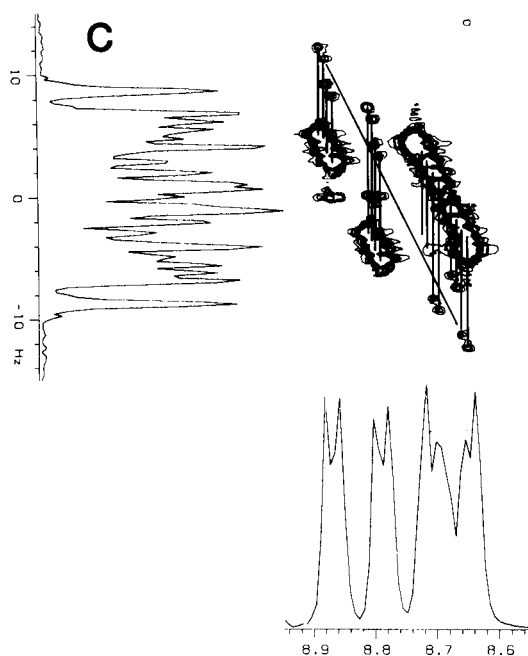
Figure 3

Figure 3

Figure 3.   A) Correlation of the H3 and H4 AB resonances of the 1,8-naphthyridine nucleus *via* strong coupling responses (labeled A), the H3 resonance observed at δ 8.67 overlapped by the H3' resonance; correlation of the H5 and H6 resonances of the ABX spin system *via* the responses due to strong coupling (labeled B).   B) Correlation of the responses of the pyridyl substituent *via* responses due to strong coupling - the pathway labeled A correlates the H4' and H5' resonances, the pathway labeled B correlates the H5' and H6' resonances.   C) Expansion of the region containing the H6' and H3' resonances of Figure 3B to show the detail of the responses due to strong coupling which correlates these resonances.

H3 and H4 resonances. Thus, at the F_2 frequencies of the doublet observed at δ 8.20 we also observe two somewhat less intense but nonetheless significant responses at negative F_1 frequencies of −19.16 and −27.73 Hz. The AB spin system also exhibits responses at correspondingly displaced positive frequencies (in F_1) which correspond to the F_2 frequencies of the member of the AB pair to which the resonance at δ 8.20 is coupled (observed F_1 frequencies: +18.90 and +27.41 Hz). Correlation *via* the responses due to strong coupling (these responses originated by strong coupling also may potentially give rise to additional signals in excess of the total number of proton signals expected for the 45° projection of the $S(F_1,F_2)$ data matrix, *e.g.* that observed at δ 8.44) finally allows the remaining resonance of the AB pair to be located at δ 8.67, clearly overlapped by a disassociated resonance from one of the
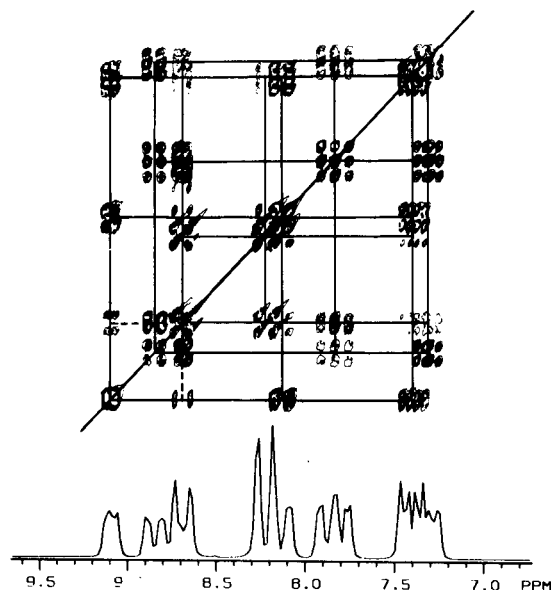

Figure 4.   COSY spectrum of **1** obtained using the technique described in the experimental section to provide a spectrum consisting of 512 × 512 data points. The "normal" spectrum resides along the diagonal while off-diagonal responses correlate coupled spins. Although all of the connectivities can be established with the spectrum, including some long range couplings, the homonuclear 2DJ spectrum may provide occasion to allow the same correlations to be made, circumventing the necessity of performing a second two-dimensional nmr experiment in those cases where the 2DJ spectrum is to be acquired in any case.

other spin systems in the spectrum as initially anticipated.

Schematically, the correlation process just described is illustrated in Figure 3A (the correlation network labeled A). Assignment of the individual resonances of the AB spin system to provide proper orientation relative to the resonances of the ABX spin system also contained in the 1,8-naphthyridine nucleus requires the utilization of long range couplings to the ABX protons and is discussed below.

Location and assignment of the H5, H6 and H7 resonances of the ABX spin system can also be accomplished in a fashion analogous to that just described for the AB spin system. Neglecting additional complication which might arise due to long range coupling, each of the resonances of the ABX spin system will appear as a doublet of doublets. Further, both the A and B resonances of the spin system will each have four additional responses associated with it due to strong coupling, thereby providing a means of unequivocally correlating these resonances. The X resonance of the ABX spin system, based upon our previous work [15], would only be expected to exhibit two additional re-

sponses of appreciable intensity as a result of strong coupling, both residing on the $F_1 = 0$ Hz axis. Based upon the arguments just presented, the doublet of doublets resonating at δ 7.38 may be correlated as shown in Figure 3A (the correlation network labeled B) with the doublet of doublets doubled which resonates at δ 8.12, these constituting the A and B components of the ABX spin system. Finally, the X member of the spin system, also a doublet of doublets (resolved into a doublet of doublets doubled (see Table I) at higher digital resolution), resonates at δ 9.07 exhibiting, as expected, two responses on the axis $F_1 = 0$ Hz.

Coupling constants for all of the resonances of **1** are summarized in Table I. Having available the long range couplings for the X and B resonances of the ABX spin system and also long range couplings for the H3 and H4 resonances of the AB spin system, it is now possible to correctly assign the H3 and H4 resonances, thus establishing the proper orientation of the spin system relative to the ABX spin system. Beginning with the X spin observed at δ 9.07, we observe a long range coupling of 0.40 Hz which corresponds quite favorably with an 0.42 Hz coupling observed for the resonance of the AB pair at δ 8.67. This coupling we attribute as a seven bond coupling ($^7J_{37}$ or $^7J_{73}$), thereby allowing the assignment of the resonance at δ 8.67 as H3 *via* the alternative coupling networks shown by **2a** and **2b** in an extended *epi* zig-zag pathway. Support for the

2a   2b
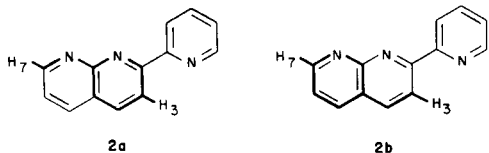
assignment just made is provided by the long range coupling of the resonance at δ 8.12 (the B spin of the ABX spin system) which exhibits a coupling of 0.67 Hz, corresponding to the 0.68 Hz coupling of the resonance at δ 8.20 of the AB spin system. Since this coupling can be accounted for by a four bond *peri* ($^4J_{45}$) coupling network as illustrated by **3**, the assignment of all five of the resonances of the 1,8-naphthyridine nucleus is now complete.

Remaining to be assigned at this point are the four resonances of the pendant pyridyl substituent attached to the 2-position of the naphthyridine nucleus. With the assignment of the resonances of the naphthyridine nucleus completed above, the resonances at δ 7.29, 7.82, 8.67 and 8.82 may now be unequivocally attributed to the pyridine system. Thus, the remaining four spin system may be classified as either an ABKL or an ABXY spin system, although

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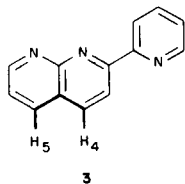
we favor assigning it as the former because of the extensive network of responses due to strong coupling.

Again, as in the cases of the preceding two spin systems, the resonances of the four spin system may be correlated using the responses due to strong coupling. Beginning with the resonance at δ 7.29 we observe a set of eight responses at negative F_1 frequencies which range from about -30 to -42 Hz (coupling network A in Figure 3B). These correlate with an analogous set of eight symmetrically displaced responses at positive F_1 frequencies, correlating the resonances at δ 7.29 and 7.82. Continuing, the resonance at δ 7.82 has asssociated with it an additional set of eight responses at negative F_1 frequencies ranging from -43 to -57 Hz which serve to correlate it with the resonance at δ 8.82 (coupling network B in Figure 3B). Finally, the resonance at δ 8.82 is correlated *via* a cluster of eight responses with positive F_1 frequencies ranging from approximately $+4$ to $+12$ Hz (see expanded region of Figure 3B shown in Figure 3C) with the resonance at δ 8.67.

Assignment of the resonances of the four spin system may now be undertaken based, in part, upon the behavior of the strong coupling resonances of the ABX spin system described above. Intuitively, we would expect that either the resonance observed at δ 8.82 or alternatively, that at δ 8.67, should be assigned as H6'. Comparison of the two resonances shown in Figure 3C reveals that the former exhibits two responses on the $F_1 = 0$ Hz which suggest that this resonance would be correctly assigned as H6'. From this starting point, based on homonuclear spin coupling constants extracted from the slices of the tilted $S(F_1,F_2)$ data matrix [21,22], the balance of the resonances of the pyridyl moiety were assigned as shown in Table I.

Having illustrated the utilization of responses due to strong coupling for the correlation of strongly coupled spins in the homonuclear 2DJ spectrum, it is useful to directly compare the 2DJ spectrum with the COSY spectrum of **1** which is shown in Figure 4. Here, the conventional spectrum appears along the diagonal while coupled resonances are correlated with one another *via* the off-diagonal responses. While the resonances can assuredly be correlated through the COSY spectrum (long range interring couplings are also visible in several cases), if the coupling constants are desired it may still be necessary to obtain the 2DJ spectrum as well. Thus, the correlation of resonances which are strongly coupled to one another *via* the additional responses as illustrated above, may provide a convenient alternative to running two two-dimensional experiments. This work is by no means, however, intended to suggest that there is any likelihood that the homonuclear 2DJ experiment will have any potential for supplanting the COSY experiment. Rather, we feel that the homonuclear 2DJ experiment has potentials which have not been exploited in many cases and we thus wish to illustrate the

utility of the responses due to strong coupling with the specific intent of dispelling the notion that they instead are an inherent hindrance to the use of the experiment.

EXPERIMENTAL

All of the nmr spectra recorded during the course of this study were obtained using a sample of 2-(2'-pyridyl)-1,8-naphthyridine (1) prepared by the Friedlander condensation of 2-aminonicotinaldehyde with 2-acetylpyridine [23]. The sample was prepared by dissolving approximately 100 mg of 1 in 0.4 ml of deuteriochloroform. Chemical shifts in the conventional reference spectrum shown in Figure 1 were referenced to internal TMS. All of the spectra were taken on a Varian XL-100-15 spectrometer operating at 100.060 MHz in the Fourier transform mode equipped with a Nicolet 1180 data system and a dual disc drive interfaced through a Model 293A' pulse programmer.

The conventional 'H-nmr spectrum (Figure 1) was obtained using 4K data points with a sweep width of ±200 Hz (quadrature). The data was baseline corrected and Fourier transformed directly.

The homonuclear two-dimensional J-resolved spectra shown in Figures 2-5 were obtained using the standard pulse sequence (90° − t½ − 180° − t½ − acquire) modified to deliver a composite 180° pulse [24-27]. The spectra shown as contour plots were acquired using 1K data points to digitize the ±200 Hz sweep width, a total of 256 blocks of data collected in each case with 80 acquisitions/block taken to provide adequate signal-to-noise to permit the facile examination of the responses due to strong coupling. Data were processed using a 0.1 Hz sinusoidal multiplication prior to the first Fourier transformation. Interferograms obtained from the transposition were zero filled to 512 points, subjected to a 0.1 Hz sinusoidal multiplication and then Fourier transformed the second time. Delays for the evolution period were selected to provide ±62.5 Hz spectral width in F_1. The contour plot shown in Figure 2 was generated as a four level contour plot after a second transposition to give the $S(F_1,F_2)$ data matrix so that it would be amenable to tilting [4]. A 0° projection is shown below the contour plot, a 90° projection ("J-spectrum") to the right and a 45° projection above (the equivalent of a broad band proton decoupled proton spectrum [3]). Individual slices plotted in Figures 4A-D were obtained by 90° projection of the $S(F_1,F_2)$ data matrix after tilting [4]. Coupling constants reported in Table I were obtained from a second 2DJ spectrum (not shown) obtained using 256 blocks of data to digitize an F_1 spectral width of ±12.5 Hz, the interferograms again filled to 512 points prior to the performance of the second Fourier transformation to provide significantly enhanced digital resolution.

Finally, the COSY spectrum shown for purposes of comparison in Figure 5 was obtained using the standard 90° − t_1 − 90° − acquire pulse sequence [2] data collected using single phase detection as a consequence of limitations of the 293A' pulse programmer [17,18]. The spectrum was acquired as 512 × 1K blocks of data using the same sweep width as the 2DJ experiment described above, the evolution period set equal to the dwell time in t_2. The data set was processed using 0.1 Hz sinusoidal multiplication prior to both Fourier transformations to minimize tailing of the responses which would have severely limited the utility of the spectrum. The data shown in Figure 5 is presented as a four level contour plot.

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