Combined Application of Auto-Correlated (COSY) and Homonuclear J-Resolved Two-Dimensional NMR Spectra for the Assignment of Congested, Non-First Order Spectra of Polycyclic Aromatic Systems. Assignment of the ^{1}H-NMR Spectrum of Benzo[2,3]phenanthro-[4,5-*bcd*]thiophene and an Investigation of Long Range ^{1}H-^{1}H Spin-Coupling Constants

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Total assignment of the proton nmr spectrum of benzo[2,3]phenanthro[4,5-*bcd*]thiophene at 200 MHz is reported. Preliminary identification of the resonances comprising the individual spin systems was accomplished by the use of auto-correlated two-dimensional proton nmr spectroscopy (COSY). Individual assignments were made on the basis of spin-coupling information which was obtained from the "titled" two-dimensional J-resolved (2DJ) proton nmr spectrum of the title compound. Because of the greater resolving power of the latter experiment relative to conventional proton nmr spectroscopy, a number of long range inter-ring spin-couplings were observed. The observation of these long range couplings was crucial to the final assignment of several of the resonances in the spectrum. Resonances due to strong coupling in the 2DJ spectrum were also shown to be a useful means of correlating strongly coupled spins which provides an alternative to the COSY spectrum for this purpose.

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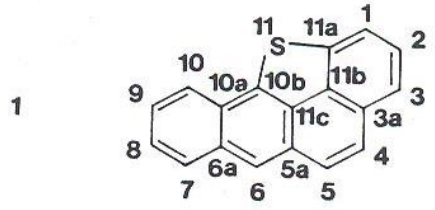
Assignments of complex nmr spectra may be greatly facilitated by the availability of the second frequency domain offered by two-dimensional nmr techniques. As an example, the complete assignments of the ^{1}H- and ^{13}C-nmr spectra of phenanthro[1,2-*b*]thiophene have been reported in a recent study which employed the concerted application of a variety of two-dimensional nmr experiments [4]. Despite the significant advantages offered by two-dimensional nmr techniques, the proton nmr spectra of polycyclic aromatic hydrocarbons (PAH) and their heterocyclic analogs still represent a very substantial assignment problem because of the frequently non-first order character of their nmr spectra even at moderate observation frequencies. For this reason, we were interested in exploring the concerted utilization of auto-correlated (COSY) [5-7] and homonuclear J-resolved [8] (2DJ) two-dimensional nmr spectra as a means of assigning the congested ^{1}H-nmr spectra of these complex polycyclic heterocycles. In particular, we were interested in supplementing the correlation ability and inherently higher resolution of these experiments with the information contained in the resonances due to strong coupling in the 2DJ spectra, the specific intent being to utilize this information as an additional assignment criterion. Thus, we now wish to report the total assignment of the ^{1}H-nmr spectrum of the highly mutagenic benzo[2,3]phenanthro[4,5-*bcd*]thiophene (**1**) [9].

Although the assignment of complex spectra, such as that of benzo[2,3]phenanthro[4,5-*bcd*]thiophene (**1**) which is shown in Figure 1, can be undertaken by resorting to very high field spectra, we were interested in developing methodologies applicable to the assignment of such spectra which are based on the more widely available medium field superconducting nmr spectrometers. For this reason, we elected to approach the assignment of the ^{1}H-nmr spectrum of **1** at a proton observation frequency of 200 MHz.



As a point of departure for the spectral assignment of **1**, the assignment of the ^{1}H-nmr spectrum of benzo[2,3]phenanthrene (benz[*a*]anthracene) has been reported at 220 MHz by Haigh and Mallion [10], as have a number of studies on long range inter-ring ^{1}H-^{1}H spin-coupling [11-17]. Because of structural similarities to benzo[2,3]phenanthrene, the proton nmr spectrum of benzo[2,3]phenanthro[4,5-*bcd*]thiophene (**1**) would be expected to contain proton resonances which may be sub-grouped into simpler spin systems when long range inter-ring couplings are neglected. Thus, the H1-H3 spin system would be expected to constitute an ABX spin system; the H4-H5 spin system an AB pair; the H6 resonance would be expected to appear as an isolated spin; and finally, the H7-H10 spin system could appear as any thing from an ABCD to an ABXY spin system. Upon inspection of the conventional ^{1}H-nmr spectrum of **1** which is shown in Figure 1, the assumption that the H6 resonance would appear as a single isolated spin is verified by the presence of the somewhat broadened singlet resonating furthest downfield at $\delta = 8.549$, thus providing an unequivocal starting point for the assignment.

Auto-Correlated ^{1}H-NMR Spectroscopy (COSY).

Beyond the assignment of the H6 resonance, little can be done directly with the conventional proton spectrum insofar as specific assignments are concerned. It was next necessary to obtain the auto-correlated two-dimensional ^{1}H-nmr spectrum (COSY) of **1**. Advantages inherent in the COSY spectrum are the off-diagonal responses which serve to correlate individual resonances which are spin-coupled to one another *via* the scalar coupling constant, J. The interpretation of this spectrum (Figure 2) is analogous to the utilization of homonuclear spin-decoupling information. Thus, in the auto-correlated spectrum, an off-diagonal response consisting of a square pattern of four resonances will appear when two doublets in the normal spectrum are coupled to one-another. Importantly, however, correlations may be made in many cases even when the resonances are in close proximity to one another, unlike conventional homonuclear decoupling experiments which can often lead to ambiguous results when the resonance to be

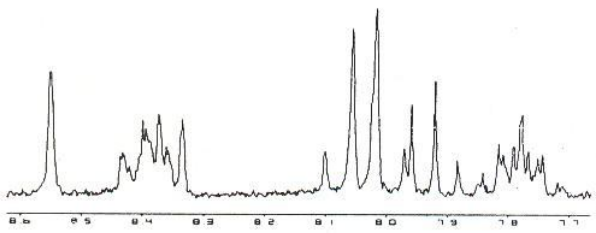


Figure 1. 200 MHz ^{1}H-nmr spectrum of benzo[2,3]phenanthro[4,5-*bcd*]-thiophene (**1**) in hexadeuteriodimethylsulfoxide at 25°.

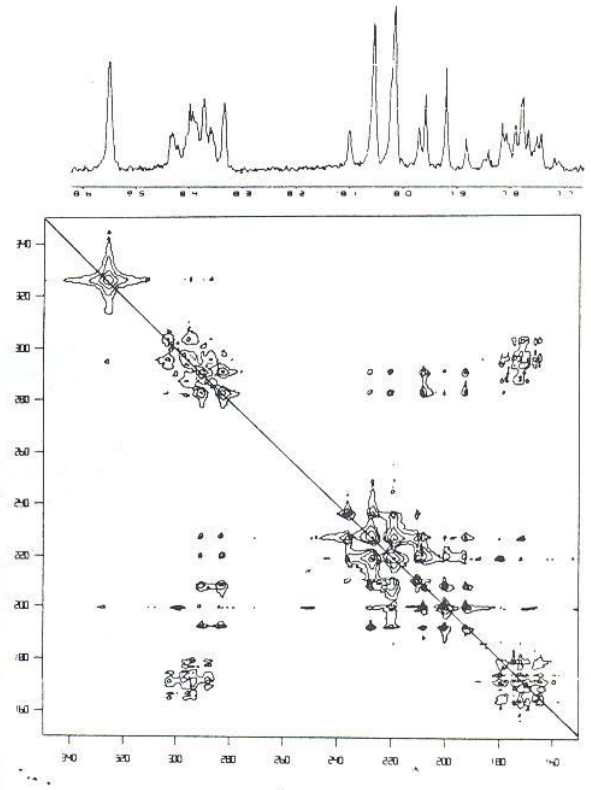


Figure 2. Autocorrelated two-dimensional ^{1}H-nmr (COSY) spectrum of **1** in hexadeuteriodimethylsulfoxide at 25°. The spectrum is plotted as a four level contour plot (axes are arbitrarily labeled in Hz - see text) in which normal responses from the one-dimensional spectrum appear along the diagonal (Figure 1). Off-diagonal responses correlate protons which are spin coupled.

decoupled is in close proximity to another resonance. Beginning with the H6 resonance which appears as the prominent four pointed star-shaped response in the upper left corner of Figure 2 ($\sim$325 using the arbitrary scale on the figure), the line shape a characteristic result of the double Fourier transformation process [18], we observe pairs of off-diagonal elements which correlate this resonance with the center resonance ($\delta = 8.379$) of the three resonances clustered at approximately δ 8.38 ($\sim$290) (Figure 3). Initially, several assignments are plausible for the resonance to which H6 is correlated. Logical possibilities include assigning this resonance as the H5 or H7 resonance (correlation *via* peri coupling) or as H10 (correlation *via* an epi zig zag coupling). Ultimately, we shall see that this resonance is correctly assigned as H10, the identity of the resonance confirmed by long range inter-ring couplings to other numbers of the four spin system. Continuing from the center of the three downfield resonances, a cluster of off-diagonal responses correlates this resonance with a member of tightly clustered pair of resonances, the first at $\delta =$

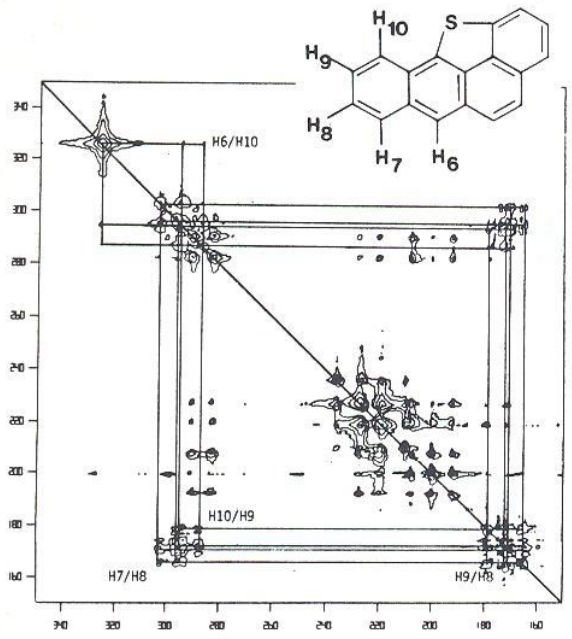


Figure 3. Autocorrelated two-dimensional ^{1}H-nmr (COSY) spectrum of 1 showing the correlation of the H6 resonance with the H7-H10 ABXY spin system. Off-diagonal clusters are labeled with the resonance downfield/upfield.

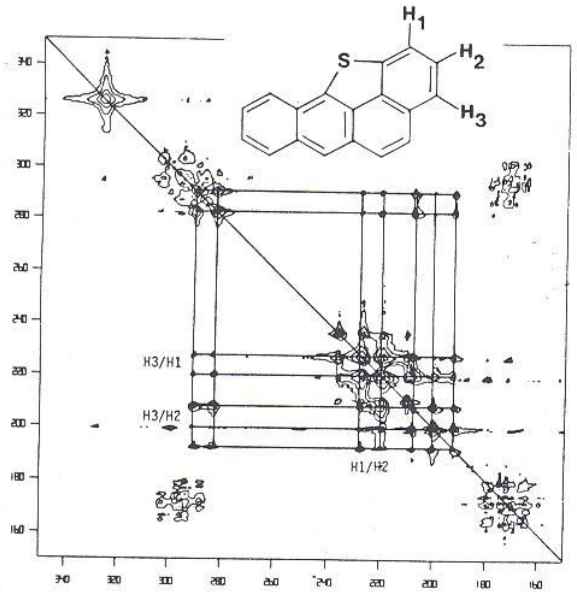


Figure 4. Autocorrelated two-dimensional ^{1}H-nmr (COSY) spectrum of 1 showing the spin-couplings of the H1-H3 ABX spin system. The H2 resonance which is a doublet of doublets with equivalent couplings appears as a 3 × 3 cluster astride the diagonal. Correlation of a "triplet" with doublets leads to the 2 × 3 clusters shown. The relatively small $^4J_{H_3H_1}$ = 1.21 Hz coupling also produces off-diagonal responses (labeled H3/H1).

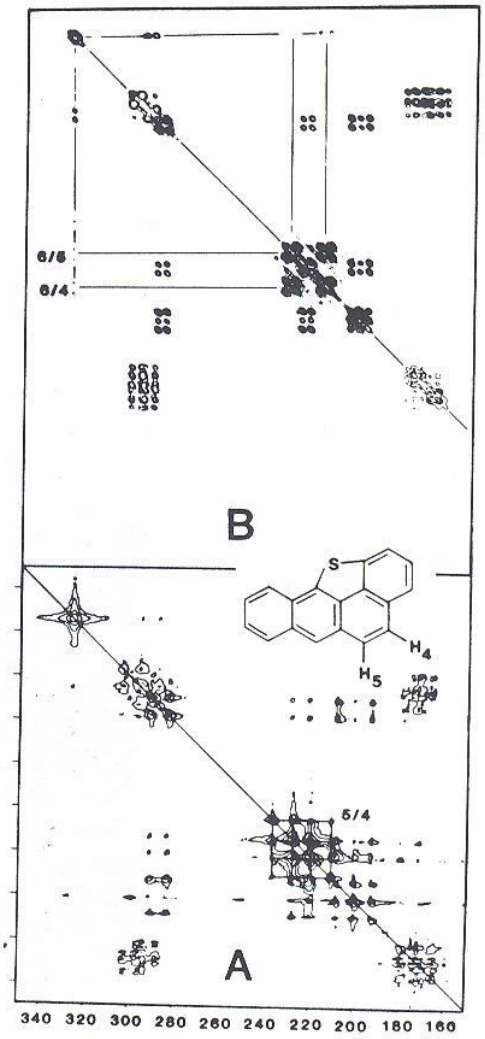


Figure 5. A) Autocorrelated two-dimensional 200 MHz ^{1}H-nmr (COSY) spectrum of 1 showing the spin-coupling of the strongly coupled H4-H5 AB spin system; B) 400 MHz COSY spectrum showing long range couplings of the H4-H5 AB resonances to the H6 resonance.

7.808 (~175) which is in turn coupled to the upfield member of the pair at δ = 7.757 (~168). Finally, the upfield resonance at δ = 7.757 is correlated with the furthest downfield of the resonances (δ = 8.409; ~300) in the cluster of three resonances at ~δ = 8.38 mentioned above, establishing this as an ABXY spin system and thus ruling out the possibility that H6 is correlated with the H5 resonance *via* an off-diagonal response. Within the spin system, however, unequivocal assignment of the resonance to which is coupled cannot be made as either H7 or H10 solely on the basis of the COSY connectivities. It should also be noted that as a consequence of the strong coupling contained in ABXY spin systems, additional responses are to be expected in the region between the A and B spins in the

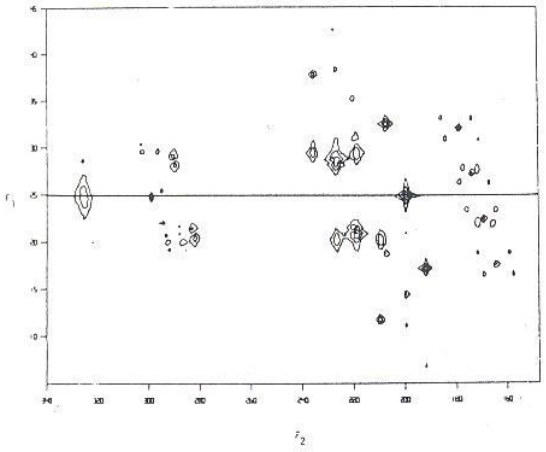


Figure 6. Homonuclear two-dimensional J-resolved (2DJ) spectrum of **1**. Multiplet structures are symmetric about the axis $F_1 = 0$ Hz (shown), the multiplet oriented on a diagonal centered at the chemical shift of the resonance.

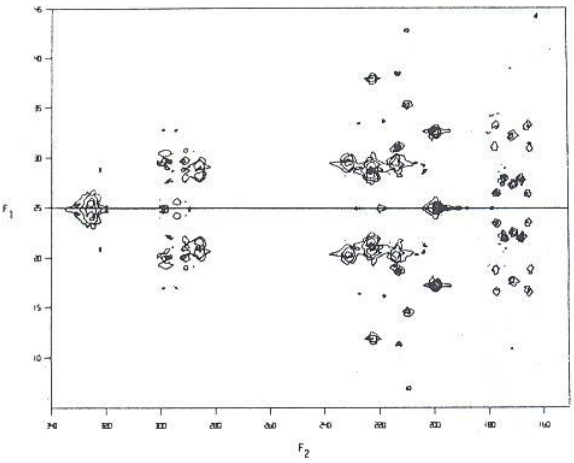


Figure 7. Homonuclear two-dimensional J-resolved (2DJ) spectrum of **1** after subjecting the data matrix to the tilt algorithm. Multiplet structures are contained in single files at the chemical shift of the resonance, the multiplet oriented orthogonally to the F_2 (chemical shift) axis.

homonuclear two-dimensional J-resolved (2DJ) spectrum and furthermore, additional responses are also possible in the region occupied by the X and Y spins as well, although these may be of low intensity [19-21].

Having established the identities of the H7-H10 resonances of the ABXY spin system, it was next necessary to begin to establish assignment criteria for the two remaining spin systems in the spectrum. Of the five proton resonances remaining to be assigned, one exhibited a distinguishing characteristic in the COSY spectrum. Resonating at $\delta = 7.925$ (Figure 4) (~ 200) is a cluster of nine responses centered on the diagonal. This pattern can arise for

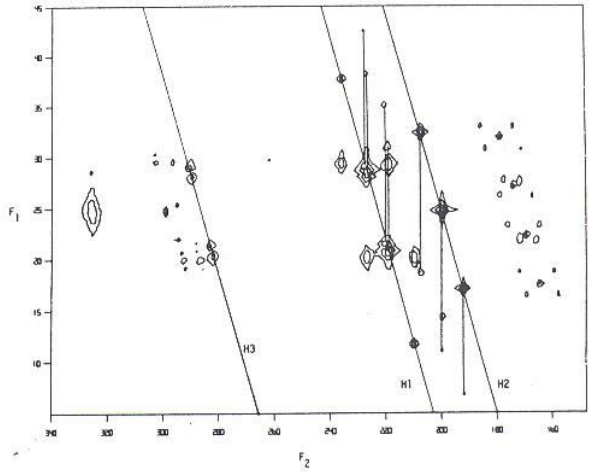


Figure 8. Contour plot of the homonuclear two-dimensional J-resolved (2DJ) spectrum of **1** showing the multiplets of th H1-H3 ABX spin system on diagonals centered at the chemical shift of the resonance in F_2. Responses due to strong coupling appear at the F_2 frequency of the normal multiplet response are correlated *via* vertical lines. Note that the H2 resonance, since it is really a doublet of doublets, shows two responses due to strong coupling at the F_2 frequency of the center response of the "triplet".

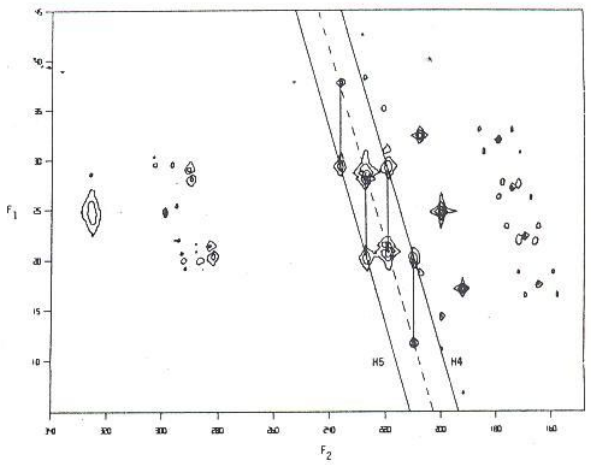


Figure 9. Contour plot of the homonuclear two-dimensional J-resolved (2DJ) spectrum of **1** showing the multiplets of the H4-H5 AB spin system (solid diagonal lines) and the H1 resonance of the H1-H3 ABX spin system (dashed line). Responses due to strong couplings appear at F_2 frequencies of the components of the AB responses correlated *via* vertical lines. Since the H1 resonance is degenerate with the H4-H5 AB spin system, strong coupling responses appear in the H1 multiplet (see Figure 10).

either a conventional triplet or for a doublet of doublets in which both of the couplings are identical. The only remaining unassigned resonance in the structure of **1** which can account for this type of multiplet structure is the H2 resonance which can have equal couplings to both H1 and

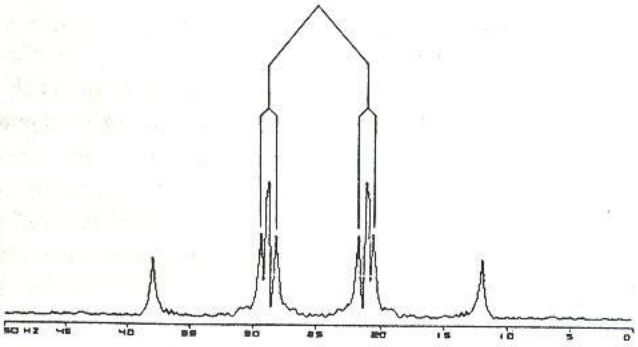


Figure 10. Spin multiplet of the H1 resonance obtained by ploting the appropriate "slice" of the tilted 2DJ spectrum. The doublet of doublets indicated by the spin-splitting diagram is the H1 resonance. The other responses contained in this "slice" are responses due to strong coupling of the H4-H5 AB spin system.

H3. Thus, the H2 resonance may be unequivocally assigned to the "triplet" observed at $\delta = 7.925$. Having established the identity of the H2 resonance, we can readily determine the locations of the two remaining resonances of this spin system (Figure 4) which, as initially anticipated, is an ABX spin system. Before discussing the locations of the H1 and H3 resonance, however, the nature of the off-diagonal clusters associated with the H2 resonance are worthy of comment. Specifically, triplets are not generally encountered in the proton nmr spectra of aromatic or hetero-aromatic compounds. The correlation of one resonance which appears as a triplet with another which is a doublet (fine couplings, because of the levels of digital resolution generally employed in COSY spectra, do not complicate the argument) leads to a cluster of six off-diagonal responses which appear in the COSY spectrum as a 2×3 grouping. Thus, as shown in Figure 4, the H2 resonance is correlated with both the H1 and H3 resonances by 2×3 clusters of off-diagonal responses. The set of off-diagonal resonances nearer the diagonal correlates the H2 resonance with a doublet resonating at $\delta = 8.039$ (~ 225), which may be tentatively asigned to H1 on the basis of the assignments of Haigh and Mallion [10]. It should also be noted that the components of the H1 multiplets are degenerate with the two central resonances of the remaining H4-H5 AB pair. Referring to the conventional spectrum shown in Figure 1, integration of the AB quartet just downfield of the H2 triplet gives an integral corresponding to three rather than two protons, thereby establishing the location of H1. Further confirmation of the H1 resonance is also obtained from the homonuclear 2DJ experiment discussed below (see also Figure 8). Due to this unfortuitous overlap and the strong coupling which arises in AB spin systems, considerable care must be exercised when examining the individual spin-multiplet slices plotted [22] from the "tilted" [23] 2DJ spectrum, this consideration discussed in detail below. Finally, the 2×3 cluster of off-diagonal responses from the diagonal correlates the H2 resonance with the H3 resonance ($\delta = 8.353$ or ~ 285) which constitutes the remaining resonance contained in the cluster of three resonances observed downfield in the conventional proton spectrum (Figure 1). It will also be noted that since thre is an appreciable coupling between the H1 and H3 resonances ($J_{H_1H_3} = 1.22$ Hz) that these are also correlated with each other *via* a 2×2 cluster of off-diagonal resonances (Figure 4).

Remaining to be assigned are the resonances of the H4-H5 AB spin system ($\delta = 8.072$ and 7.999; ~ 235 and ~ 215). This is a strongly coupled system which is correla-

Table I

Calculated and Observed Frequencies and Calculated Intensities for the H1-H3 ABX Spin System of **1** at 200 MHz

Resonances from the AB Region of the Spectrum

Transition Line	Calcd. F_1	Obs. F_1	Calcd. F_2	Obs. F_2[a]	Calcd. I
1368 A	−7.7	−7.78	1576.1	1576.68	0.93
2547 B	−0.1	0.00	1583.7	1584.30	0.89
1458 C	−4.6	4.44	1603.3	1603.45	0.89
2637 D	−3.2	−3.22	1604.7	1603.45	0.89
1358 E	5.9	5.97	1603.3	1603.45	0.31
2537 F	10.4	10.41	1604.7	1603.45	0.31
2647 G	−13.7	−13.60	1583.7	1584.30	−0.22
1468 H	−18.2	−18.05	1576.1	1576.68	−0.22
6813 I	7.7	7.78	1591.5	1591.95	0.93
4725 J	0.1	0.00	1583.9	1584.30	0.89
5814 K	4.6	4.44	1612.4	1611.17	0.89
3726 L	3.2	3.23	1611.0	1611.17	0.89
5813 M	−5.9	5.97	1591.5	1591.95	0.31
3725 N	−10.4	−10.41	1583.9	1584.30	0.31
4726 O	13.7	13.61	1611.0	1611.17	−0.22
6814 P	18.2	18.05	1612.4	1611.17	−0.22

Resonances from the X Region of the Spectrum

Transition Line	Calcd. F_1	Obs. F_1	Calcd. F_2	Obs. F_2 [b]	Calcd. I
7812 A	4.5	4.31	1675.64	1674.47	1.00
4635 C	−3.1	−3.23	1668.04	1666.82	0.89
1278 H	−4.5	−4.31	1666.63	1666.82	1.00
3546 J	3.1	3.23	1674.24	1674.47	0.89
3535 O	0.0	0.00	1668.04	1666.82	0.11
4646 P	0.0	0.00	1674.24	1674.47	0.11

$\nu_A = 1608.20$, $\nu_B = 1585.43$, $\nu_X = 1671.14$, $\nu_A - \nu_B = 22.77$, $\nu_A + \nu_B/2 = 1596.82$, $J_{AB} = 7.78$, $J_{AX} = 1.22$, $J_{BX} = 7.78$, $D_+ = 10.491$, $D_- = 13.592$, $\theta_+ = 10.882°$, $\theta_- = 8.316°$, $\Sigma = 19.198°$, $\Delta = 2.567°$.

[a] Discrepancies between calculated and observed F_2 values arise because of total obscurement of the A resonance (H1) of the ABX spin system of the H4-H5 AB spin system. Values reported in the Obs. F_2 column are those of the observable responses in the conventional spectrum, hence the disparity. [b] The fine coupling (J_{AX}) was unresolved for the H3 X resonance. Reported values for Obs. F_2 are those determined by J_{BX}.

Table II

Calculated and Observed Frequencies and Calculated Intensities for the H4-H5 AB Spin System of **1** at 200 MHz

Transition Line	Calcd. F_1	Obs. F_1	Calcd. F_2	Obs. F_2	Calcd. I
1334 A	−13.24	−13.09	1594.3	1594.17	−0.25
3413 B	13.24	12.91	1620.8	1620.20	−0.25
1324 C	−4.57	−4.67	1611.7	1611.17	0.72
2413 D	4.57	4.47	1620.8	1620.20	0.72
1224 E	4.10	3.88	1611.7	1611.17	0.81
2412 F	−4.10	−3.99	1603.5	1603.45	0.81
1234 G	−4.57	−4.67	1594.3	1594.17	0.72
3412 H	4.57	−4.47	1603.5	1603.45	0.72

$\nu_A = 1614.94$, $\nu_B = 1600.22$, $\nu_A - \nu_B = 14.72$, $\nu_A + \nu_B/2 = 1607.58$, C = 8.665, $\theta = 15.915°$, $J_{AB} = 9.14$.

ted by 2 × 2 clusters of off-diagonal responses which appear very near the diagonal (Figure 5A). Assignment of the two resonances of this spin system cannot be accomplished on the basis of the COSY spectrum and instead must rely on the observation of long range couplings contained in the 2DJ spectrum.

Interestingly, when a 400 MHz COSY spectrum was obtained as an additional confirmation of the location of the H1 resonance, several additional off-diagonal responses were observed. Although the responses in this case were quite weak and defined by only one contour level, they usefully correlated the H4 and H5 resonances with the H6 resonance downfield (Figure 5B). Usefully, these off-diagonal responses allow the fine couplings of the H6 resonance to be accounted for and furthermore, they also allow the final orientation of the individual spin systems relative to one another to be completed.

Despite the inherent complexity of the conventional ^{1}H-nmr spectrum of benzo[2,3]phenanthro[4,5-*bcd*]thiophene (**1**) it it possible to establish the locations of the resonances of each of the spin systems contained in the molecule although total assignments cannot be made solely on the basis of the COSY spectrum. To complete the assignments unequivocally, it is necessary to next consider the long range couplings which are readily apparent in the homonuclear 2DJ spectrum because of the T_2 determined line widths in the second dimension [18].

Homonuclear Two-Dimensional J-Resolved NMR Spectroscopy (2DJ).

Homonuclear two-dimensional J-resolved (2DJ) nmr spectra are among the oldest of the presently available two-dimensional nmr experiments, these reported [8] shortly following the introduction of the heteronuclear variant of the experiment in 1976 by Ernst and co-workers [24] 2DJ spectra are of considerable utility in the investigation of complex proton nmr spectra, such as that of **1**, since chemical shift (δ) is displayed along the F_2 axis while homonuclear spin-coupling information is displayed in the second dimension, orthogonal to F_2 after the data set is subjected to the tilt algorithm [23]. As an example, Hall and Sanders [25] have reported the complete assignment of the ^{1}H-nmr spectrum of 1-dehydrotestosterone which was in part based on the utilization of proton 2DJ spectra. Particularly noteworthy in their report was the observation that there were numerous long range couplings in the

Table III

Chemical Shift Assignments and Homonuclear Spin-Coupling Constants of Benzo[2,3]phenanthro[4,5-*bcd*]thiophene (**1**) in Hexadeuteriodimethylsulfoxide

Position	Spin Label	δ ^{1}H	Coupling Constants: $^3J_{HH}$ (ortho)	$^4J_{HH}$ (meta)	$^5J_{HH}$ (para or long range interring)	$^7J_{HH}$
H1	A	8.039	$^3J_{H_1H_2} = 7.78$	$^4J_{H_1H_2} = 1.22$	—	—
H2	B	7.925	$^3J_{H_2H_1} = 7.78$	—	—	—
			$^3J_{H_2H_3} = 7.78$	—	—	—
H3	X	8.353	$^3J_{H_3H_2} = 7.74$	$^4J_{H_3H_1} = 1.21$	$^5J_{H_3H_5} = 0.43$	—
H4	A	8.072	$^3J_{H_4H_5} = 9.14$	—	$^5J_{H_4H_6}$ [a]	—
H5	B	7.999	$^3J_{H_5H_4} = 9.14$	$^4J_{H_5H_6} = 0.44$	$^5J_{H_5H_8} = 0.44$	—
H6	—	8.549	—	$^4J_{H_6H_5} = 0.44$	$^5J_{H_6H_{10}} = 0.95$	—
					$^5J_{H_6H_4}$ [a]	
H7	Y	8.409	$^3J_{H_7H_8} = 9.75$	$^4J_{H_7H_9} = 2.13$	$^5J_{H_7H_{10}} = 0.63$	—
H8	B	7.757	$^3J_{H_8H_7} = 9.89$	$^4J_{H_8H_{10}} = 1.73$	—	—
			$^3J_{H_8H_9} = 6.86$			
H9	A	7.808	$^3J_{H_9H_8} = 6.84$	$^4J_{H_1H_2} = 2.13$	—	—
			$^3J_{H_9H_{10}} = 9.72$	—	—	—
H10	X	8.379	$^3J_{H_{10}H_9} = 9.61$	$^4J_{H_{10}H_8} = 1.79$	$^5J_{H_{10}H_7} = 0.52$	—
					$^5J_{H_{10}H_6} = 0.93$	—

[a] The existence of this coupling was shown by the 400 MHz COSY spectrum (Figure 5B) but was not resolved.

range of 0.3-0.6 Hz which were resolved in the two-dimensional experiment as a result of T_2 determined line widths in the second dimension. Thus, as a consequence of the considerably greater resolution of 2DJ spectra relative to conventional proton nmr spectra, long range inter-ring couplings, which have been studied thus far with some considerable difficulty [11-17], may be conveniently studied, and further, may be of considerable utility in assigning resonances unequivocally once their tentative identities have been established by other means such as the COSY spectrum of **1** discussed above.

The contour plot of the homonuclear 2DJ spectrum of **1** is shown in Figure 6. Shifts in Hz along the F_2 axis in this figure are arbitrary, resonances observed further downfield in the conventional spectrum appearing at higher shifts (numerically) in this spectrum as well. Multiplet structures in the second dimension are symmetric about the axis $F_1 = 0$ Hz (25 Hz on the axis shown in Figure 6) which is drawn through the spectrum. Total frequency width in the second dimension was ± 25 Hz although only ± 20 Hz is plotted in the contour plots shown. The results of tilting the two-dimensional 2DJ data matrix are shown by Figure 7 and it will be noted that all of the spin-multiplets are now aligned orthogonally to the F_2 axis, which allows their examination in the same fashion as a selective excitation carbon nmr experiment by selection of the proper data files from the $S(F_2,F_1)$ matrix.

Utilization of spin-coupling information contained in the homonuclear 2DJ spectrum, under most circumstances, is conveniently undertaken by subjecting the data matrix to "tilting" [23] (Figure 7) and then examining individual slices [22]. In the case of strongly coupled spin systems such as the H1-H3 ABX and the H4-H5 AB spin systems, however, there are responses which appear due to strong coupling [19-21] which provide a convenient means of correlating resonances which are spin-coupled to one another. Since resonances due to strong coupling are not shifted by the tilt algorithm in the same sense as normal responses, it is advantageous to extract the correlations provided by these responses from the two-dimensional data matrix prior to examining the tilted spectrum.

Beginning with the H2 resonance which we were able to assign as the "triplet" resonating at $\delta = 7.925$ (~ 200), it is possible to draw an axis through the spin multiplet. Coincident with the responses of the multiplet itself in the F_2 dimension, we would expect to find responses due to strong coupling [19-21], these shifted from the $F_1 = 0$ Hz axis by increasing spectrometer operating frequencies [21,26] accompanied by a corresponding decrease in the intensity of the responses. Since the H2 resonance is in reality a doublet of doublets, we would thus expect to observe four responses due to strong coupling for this resonance, two appearing at the F_2 frequency of the center resonance of the multiplet but shifted to different extents from the $F_1 = 0$ Hz axis. In accord with this expectation, four such responses are indeed seen to correlate with the H2 spin-multiplet, these shown in Figure 8. Further, since the H2 resonance is the A spin of the ABX spin system, we would expect four responses at identical frequencies (relative to $F_1 = 0$) in F_1 to appear coincident with the F_2 frequencies of the B resonance of the ABX spin system, these also shown in Figure 8. Thus, as a consequence of the complication of the 2DJ spectrum by resonances due to strong coupling, we also derive the benefit of an additional means of conveniently correlating spin-coupled resonances which would be useful if the correlation could not be made from the COSY spectrum for one reason or another.

Strong coupling is also expected to be prominent in the H4-H5 AB spin system. Additionally, since the H1 resonance is also degeneratively coincident with two of the resonances of the H4-H5 spin system, we would expect the appearance of its spin-multiplet, on extraction from the tilted data matrix, to be complicated by the inclusion of responses due to strong coupling from the H4-H5 spin system, a point which will be treated in more detail below. Identifying the two doublets of the H4-H5 spin system, we may again construct axes (~ 235 and ~ 215) through these spin multiplets (Figure 9) analogous to those for the ABX spin system (Figure 8). Once again, due to strong coupling, additional responses will also be oberved for these spins at the frequencies of the normal responses in the F_2 dimension and symmetrically disposed about the $F_1 = 0$ Hz axis. Responses due to strong coupling in the H4-H5 AB spin system are connected to their counterpart normal spectral responses by the vertical lines shown in Figure 9. In addition, a dashed axis is drawn through the responses for the H1 resonance and it will be noted that two of the responses due to strong coupling are contained within the normal responses for the H1 resonance, the two remaining responses also residing on the dashed axis for the H1 spin multiplet. Thus when tilted, the H1 spin-multiplet will contain, in addition to the normal components of the resonance, the four responses due to strong coupling of the H4-H5 AB spin system, these shown in the plot of the H1 spin multiplet illustrated by Figure 10. This observation highlights very clearly the care which must be taken in the interpretation of the spin-multiplets extracted from the tilted 2DJ spectra of complex molecules.

While discussing the presence of resonances due to strong coupling, it should be noted that responses due to strong coupling are also expected for the ABXY spin system. In support of thie expectation, there are a number of intense resonances clustered between the H8 an H9 resonances which are attributable to strong coupling. Since the expressions for the calculation of the position and intensity of resonances due to strong coupling in the ABXY spin system have not been derived, no analysis of responses due to strong coupling in the ABXY spin system will be

presented in this work. Work is, however, underway in these laboratories to analyze this behavior and will be forthcoming. Calculated *vs.* observed frequencies and intensities for the resonances due to strong coupling in the H1-H3 ABX and the H4-H5 AB spin systems are, however, presented in Tables I and II and show excellent agreement between the observed and anticipated behavior.

Returning to the problem of unequivocally assigning the individual resonances in the spectrum of benzo[2,3]-phenanthro[4,5-*bcd*]thiophene (**1**), it is once again most convenient to begin with the H6 resonance. Thus, while the H6 resonance appears as a singlet in the conventional proton spectrum, the slice from the tilted 2DJ spectrum shows this resonance essentially as a triplet (which might be attributed as a doublet of doublets with equivalent couplings) with J = 0.95 Hz. Resolution enhancement of the conventional 400 MHz ^{1}H-nmr spectrum, however, shows the resonance as a four line pattern at a minimum, with a hint of other couplings also being present. The resolution enhanced multiplet is thus consistent with the expected couplings of the H6 resonance which may be inferred from the COSY spectrum. Thus, the large coupling observable in the 200 MHz 2DJ spectrum (J = 0.95 Hz) may be assigned as a five bond epi zig-zag coupling between H6 and H10. Five bond epi zig-zag couplings have been observed in naphthalene, anthracene, phenanthrene and in various substituted analogs of these systems and range from 0.4 to 1.1 Hz [11,13]. With regard to the other couplings of H6, the COSY spectrum shown in Figure 5B clearly demonstrates the existence of couplings between H6 and both members of the H4/H5 AB spin system.

Completion of the assignments of **1** requires the consideration of the long range couplings of the H4 and H5 resonances. Of these, H4 is coupled only to H5 and long range to H6, the coupling not resolved in either the 200 MHz 2DJ spectral slice corresponding to the H4 resonance or in the slice for H6. Thus, the sole evidence for the existence of this coupling is in the off-diagonal responses in Figure 5B and in the resolution enhanced 400 MHz spectrum of the H6 multiplet. The H5 resonance, in contrast, is richly detailed, exhibiting two long range couplings in the 200 MHz 2DJ spectrum. Thus, the H5 resonance exhibits a coupling to the H6 resonance, $^4J_{H_5H_6}$ = 0.44 Hz and an additional long range coupling to H3, $^5J_{H_5H_3}$ = 0.44, the H3 resonance also showing a correspondingly well resolved coupling of 0.43 Hz. Analogous couplings to that between the H3 and H5 resonances in the case at hand have also been reported. Specifically, Jarvis and Moritz have shown that five bond couplings in a series of coumarins between the H3 and H5 resonances of that system (see **2**) are in the range of 0.3 to 0.45 Hz [14], which is in excellent agreement with the case at hand. Final resonance chemical shift assignments and spin-spin coupling constants are collected in Table III.

Conclusions.

Through the use of auto-correlated homonuclear ^{1}H-spectroscopy, it is possible to conveniently and rapidly subgroup the individual protons of polycyclic aromatic heterocycles into simple spin systems. Strong coupling in the homonuclear 2DJ spectrum has been shown to serve as an alternative means of identifying coupled spins which may be useful in cases where there are numerous protons in close proximity, thereby complicating the COSY spectral interpretation. Finally, as a direct result of the T_2 determined line widths in the second dimension of homonuclear 2DJ spectra, it is possible to observe and utilize long range inter-ring spin-spin couplings to orient the individual spin systems. Further work on related ring systems is at present underway and will be reported.

EXPERIMENTAL

Spectra utilized in this study were predominantly acquired using a Varian XL-200 spectrometer operating at a proton observation frequency of 200.057 MHz, with the exception of that shown in Figure 5B which was acquired on a JEOL GX-400 spectrometer. All spectra were taken in hexadeuteriodimethylsulfoxide and were referenced to the central line of the residual solvent multiplet which was taken as 2.49 ppm downfield of tetramethylsilane. Because of the relatively limited solubility of **1**, the sample employed was prepared by dissolving approximately 100 mg of **1** in 2.0 ml of hexadeuteriodimethylsulfoxide heated to 50°. On cooling, approximately 0.5 ml of the clear supernatant solution was removed and transferred to a 5 mm nmr tube for the subsequent spectroscopic studies giving an effective concentration of ~0.19 *M*.

The reference spectrum of **1** was acquired using 15,600 data points with a sweep width of 2600 Hz and a pulse width of 5 μseconds (90° pulse = 6.5 μseconds). The interpulse delay was set equal to the acquisition time which was 3.00 seconds. A total of four transients were accumulated and processed without resorting to any digital filtering (see Figure 1).

The auto-correlated (COSY) spectrum of **1** was obtained using an initial time domain ($S(t_1,t_2)$) consisting of 1K × 512 data points for t_2 and t_1, respectively (at both 200 and 400 MHz). A total of 8 transients were accumulated for each value of t_1. The dwell time for the second dimension was set equal to the dwell time for the first dimension. A spectral window of 400 Hz (200 MHz) was thus employed in each dimension, giving an acquisition time of 1.280 seconds (200 MHz). Data was processed in both dimensions using a Gaussian apodization of 0.125. The final frequency domain data set was then plotted as a four level contour plot (see Figures 2-5). Total performanace time for the experiment was approximately 7 hours.

The homonuclear two-dimensional J-resolved spectrum was obtained using an initial time domain ($S(t_1,t_2)$) consisting of 1K × 256 data points for t_2 and t_1, respectively. An initial evolution time of 100 μseconds was employed as a minimum value of $t_1/2$ which was incremented by 5 msec for each of the 256 successive values of t_1 to give a final spectral width in F_1 of ±25 Hz. A spectral width of 400 Hz was employed in F_2, with 12 transients accumulated for each value of t_1, with an acquisition time of 1.280 seconds. Data were processed in both dimensions without any digital filtering. The final frequency domain data was plotted as a four level contour plot (see Figures 6-9). Total acquisition time for the experiment was approximately 6.5 hours. Inidividual spin multiplets were examined as single slices of the $S(F_2,F_1)$ data matrix after tilting [23].