Hydrogen-1 Nuclear Magnetic Resonance Study of the Self-association of 1,10-Phenanthroline, 2,2′-Bipyridyl, and their Zinc(II) Complexes

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The ^{1}H n.m.r. spectra of 1,10-phenanthroline (phen) and of 2,2 -bipyridyl (bipy) are very concentration dependent in dilute aqueous solution. The concentration dependence of the chemical shifts is consistent with the isodesmic model of indefinite non-co-operative stacking; for phenanthroline $K = 23.6 \pm 1.8$, and for bipyridyl $K = 7.4 \pm 1.6$ dm^3 mol^{-1}. In solution in 25% v/v methanol in water self-association of phenanthroline is much weaker ($K = 5.2 \pm 1.0$ dm^3 mol^{-1}). No self-association is observed in organic solvents such as dioxan and chloroform. The ^{1}H n.m.r. spectra of $[\mathrm{Zn(phen)}]^{2+}$ and of $[\mathrm{Zn(bipy)}]^{2+}$ vary much less with concentration than do the spectra of the free ligands; the stability constants for the self-association of $[\mathrm{Zn(phen)}]^{2+}$ ($K = 1.1 \pm 0.2$ dm^3 mol^{-1}) and of $[\mathrm{Zn(bipy)}]^{2+}$ (*K ca.* 0.3 dm^3 mol^{-1}) are much lower than for phenanthroline and bipyridyl.

THE proton n.m.r. spectrum of 1,10-phenanthroline (phen) in non-aqueous solvents has been measured and analysed by several authors.[1–5] However owing to the low solubility of phenanthroline itself only the spectrum of the protonated species has been reported [6,7] in aqueous solution. The spectrum of aqueous phenanthroline has been extrapolated [6] from the measurements in dilute acid but another report [7] claims that in weakly acidic solutions the species $[\mathrm{H(phen)_2}]^+$ is formed; there is also some evidence from potentiometric titrations [8] and from calorimetric measurements [9] for this species.

The variation of the proton n.m.r. spectrum of 2,2′-bipyridyl (bipy) in a wide range of organic solvents has been reported [10,11] and variously attributed to a changing dihedral angle between the two skewed aromatic rings,[10] or to hydrogen bonding with the solvent.[11] Only small dilution shifts were observed in most organic solvents,[10] although somewhat greater (up to 0.12 p.p.m.) concentration-dependent shifts were found with 2-chloroethanol as solvent.[10] The spectrum of bipyridyl in saturated solution in D_2O has also been published.[11]

In the course of studies [12,13] on the association between phenanthroline or bipyridyl and the nucleotides † atp^{4-} or utp^{4-} in binary systems such as phenanthroline–atp^{4-} and in ternary systems such as Mg^{2+}–phenanthroline–atp^{4-} and Zn^{2+}–bipyridyl–utp^{4-} it was necessary to investigate the self-association of the nucleotides themselves [14] and of phenanthroline and bipyridyl, by proton n.m.r. The self-association of many aromatic species has been observed by a variety of spectroscopic methods including u.v.,[15] circular dichroism,[16] and n.m.r.[14,17,18] and appropriate mathematical methods have been developed, but surprisingly self-association of the widely used ligands phenanthroline [19] and bipyridyl has not been reported. However an enhanced rate of complex formation between $[\mathrm{Ni(phen)}]^{2+}$ and a heterocyclic aromatic ligand such as bipyridyl has been explained by 'stacking' between the co-ordinated aromatic ligand and the incoming aromatic ligand.[20]

EXPERIMENTAL

All chemicals were of the best purity available from Merck AG, Darmstadt, Germany.

Hydrogen-1 n.m.r. spectra were recorded using a Varian Anaspect EM-360 spectrometer (60 MHz) at 35 °C, or a Bruker WM-90 FT spectrometer (90.025 MHz) at 27 °C, using the centre peak of the triplet resonance of the tetramethylammonium ion (usually 0.005 to 0.02 mol dm^{-3}) as reference ($\delta = 3.188$ p.p.m.). The pD of the solutions was obtained by adding 0.40 to the pH meter reading.[21]

Analysis of second-order n.m.r. spectra and calculation of the stability constants for self-stacking were performed using a Hewlett–Packard 9821A calculator connected to a 9862A calculator plotter.

Choice of N.M.R. Reference.—Variations in the magnetic susceptibility of the strong solutions needed for the measurements on the Zn^{2+} complexes prevent the use of an external reference, as precise susceptibility corrections would be complicated. Of the internal references commonly used in aqueous solution, trimethylsilylpropanesulphonate and trimethylsilylpropanoate are known [22,23] to give ternary complexes with $[\mathrm{Zn(phen)}]^{2+}$ and $[\mathrm{Zn(bipy)}]^{2+}$ which result in considerable upfield shifts of the reference. Moreover this upfield shift is known [14,24] to completely mask the upfield shift due to self-association of atp^{4-}, and no concentration-dependent upfield shift of $[\mathrm{Zn(phen)}]^{2+}$ could be observed in preliminary experiments using trimethylsilylpropanesulphonate as reference. Acetonitrile has been found [23] to be shifted upfield by $[\mathrm{Zn(phen)}]^{2+}$ and by $[\mathrm{Zn(bipy)}]^{2+}$ due to the formation of the ternary complexes $[\mathrm{Zn(phen)(NCCH_3)}]^{2+}$ and $[\mathrm{Zn(bipy)(NCCH_3)}]^{2+}$, and in preliminary experiments using dioxan similar upfield shifts were observed, presumably also due to the formation of ternary complexes. t-Butyl alcohol is also known to be shifted upfield in the presence of high concentrations of aromatic solutes owing to hydrophobic interactions.[14,25] The suggested [26] use of the resonance of residual HOD, combined with determination of the position of this relative to an external reference in a separate experiment, seems to have no advantages, but rather to be extremely liable to considerable errors as the resonance of HOD is so variable,[27] changing with temperature, pH, and the concentration of hydrogen-bonding solutes.

The tetramethylammonium ion has been found in earlier related work [14,23] to be unaffected by the presence of high concentrations of aromatic species, and since no variation in the chemical shift could be observed at concentrations of Zn^{2+} or Na$^+$ up to *ca.* 2 mol dm^{-3} it is presumed that changes in ionic strength have no effect.‡ The centre resonance

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† atp^{4-} = Adenosine triphosphate; utp^{4-} = uridine triphosphate.

‡ In extremely strong solutions containing large amounts of stacked aggregates, *e.g.* 2—3.5 mol dm^{-3} phenanthroline at pD 5.4, no internal reference is reliable; all are shifted *downfield* by the ring current of the large stack.

of the triplet of the tetramethylammonium ion was used as reference throughout this work, but for each system its validity was checked by measuring the chemical shift of a proton that is strongly concentration dependent (*e.g.* H_δ) with respect to a proton that is less concentration dependent (*e.g.* H_α or H_β), *i.e.* an ' intramolecular n.m.r. reference '; the concentration dependence of this chemical-shift difference gave the same stability constant, within experimental error, as the concentration dependence of the individual chemical shifts. This use of the resonance of one proton in a molecule as reference for another proton in the molecule has been recommended [28] for the determination of acidity constants by n.m.r.

Measurement of Stability Constants for Self-association.—Owing to the low solubility of phenanthroline (*ca.* 0.015 mol dm^{-3}) the strongest solutions used were slightly supersaturated. However, no systematic errors were found for the chemical shifts of the phenanthroline resonances in such solutions, and the same stability constant was obtained if the results on the most concentrated solutions were omitted from the calculations (the standard deviation of the resulting constant was however larger when fewer concentrations were used).

If 1 : 1 ratios of Zn^{2+} : phenanthroline are used for the measurements on $[Zn(phen)]^{2+}$ the resonances of H_α and H_β are considerably broadened and precise measurements are impossible. With a 1 : 1 ratio of Zn^{2+} : phenanthroline the proportions of Zn^{2+}, $[Zn(phen)]^{2+}$, $[Zn(phen)_2]^{2+}$, and $[Zn(phen)_3]^{2+}$ are 24.6, 53.0, 20.2, and 2.2% respectively. (Calculated using literature values [29] for the stability constants.) Exchange of phenanthroline between these three complexes broadens the resonances, particularly those of H_α and H_β. However with a 5 : 1 ratio of Zn^{2+}: phenanthroline the proportions of $[Zn(phen)]^{2+}$, $[Zn(phen)_2]^{2+}$, and $[Zn(phen)_3]^{2+}$ are 96.1, *ca.* 3.9, and 0.04%: sharp resonances for all protons are then obtained. Zn^{2+}–Bipyridyl mixtures show a similar effect.

However as a result the ionic strength in some solutions was very high. Varying the ionic strength by adding a large excess of $Na[NO_3]$ did not alter the n.m.r. spectrum of $[Zn(phen)]^{2+}$, although crystallization then occurred more readily. Moreover, omitting the more concentrated solutions from the calculations gave about the same stability constant, although with a much higher standard deviation.

Calculation of Stability Constants for Self-association.—The theory developed by Heyn and Bretz [15] for the indefinite non-co-operative association of atp^{4-}, studied by u.v. absorption spectra and circular dichroism, was adapted for n.m.r. The equilibrium constants for the equilibria (1)

$$(phen)_n + phen \rightleftharpoons (phen)_{n+1} \qquad (1)$$
$$K = [(phen)_{n+1}]/[(phen)_n][phen]$$

are all assumed to be equal, and the relationship (2) between the observed chemical shift (δ) in a solution of total concentration C and the chemical shifts at infinite dilution, δ_0, of a molecule at the end of a stack δ_e, of a molecule within a stack δ_i, and the stability constant, K, can be derived.

As the distance between stacked phenanthroline or bipyridyl molecules is expected * to be *ca.* 0.35 nm, and as the upfield shifts due to a ring current fall off very rapidly with increasing distance from the ring,[33,34] only the ring current in adjacent molecules in the stack is expected to have a significant † effect on the upfield shift.

$$\delta = \delta_0 + (\delta_e - \delta_0)\left[\left(\frac{1}{KC} + \frac{1}{K^2C^2}\right)(4\,KC + 1)^{\frac{1}{2}} - \frac{3}{KC} - \frac{1}{K^2C^2}\right] + (\delta_i - \delta_0)\left[1 + \frac{2}{KC} + \frac{1}{2K^2C^2} - \left(\frac{1}{KC} + \frac{1}{2K^2C^2}\right)(4\,KC + 1)^{\frac{1}{2}}\right] \qquad (2)$$

Moreover the upfield shifts caused by the two adjacent molecules within a vertical [Figure 1, (*a*) and (*b*)] or staggered [Figure 1, (*c*), (*d*), and (*e*)] stack are expected to be additive, *i.e.* $(\delta_i - \delta_0)/(\delta_e - \delta_0) = 2$, and the shift of an infinitely long stack δ_∞ is then the same as the shift of a molecule within a shorter stack (δ_i). Expression (2) then simplifies to (3).

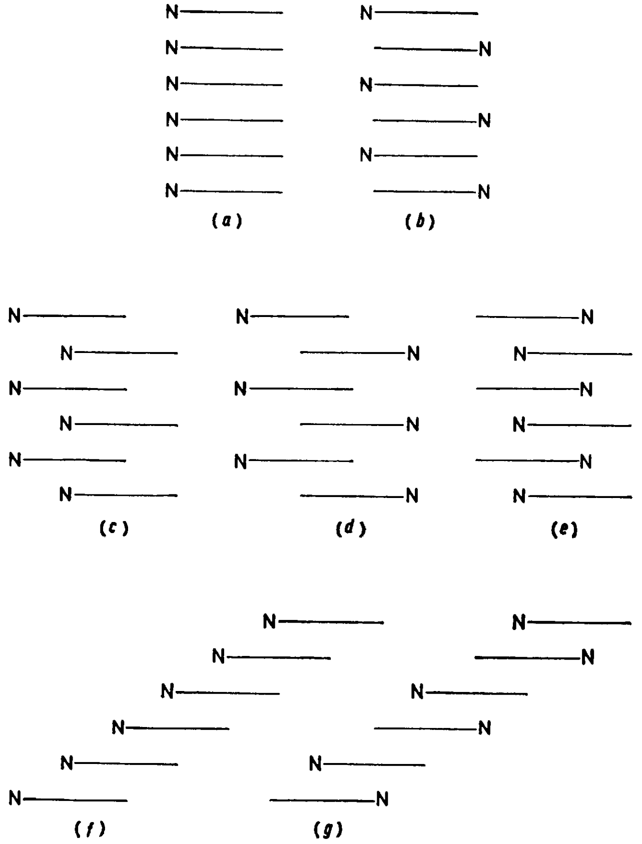

FIGURE 1 Schematic representation of the possible relative orientations of molecules in a stack: (*a*) vertical, head-to-head; (*b*) vertical, head-to-tail; (*c*) staggered, head-to-head; (*d*) and (*e*) staggered, head-to-tail; (*f*) stepped, head-to-head; (*g*) stepped, head-to-tail

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* No stacking occurs in crystalline phenanthroline hydrate,[30] in which a hydrogen-bonded chain is dominant, and in anhydrous phenanthroline [31] the nearest neighbours are not parallel but inclined. However in the crystal structures of two copper bipyridyl nucleotide complexes,[32] stacking between bipyridyl molecules occurs, with interplanar distances of 0.34 and 0.355 nm.

† Extrapolation of published results [33] suggests that the contribution of more remote molecules increases the upfield shift by ⩽6% in a tetramer, ⩽9% in a pentamer, ⩽11% in a hexamer, and ⩽20% in an infinitely long stack. Moreover, only in the experiment with phenanthroline in CD_3OD–D_2O is the concentration of species larger than trimer significant (see Discussion and Figure 4).

However, if the stack is ' stepped ' [Figure 1 (*f*) or (*g*)] then the successive molecules within the stack no longer have two equivalent nearest neighbours and $(\delta_e - \delta_0) \leqslant (\delta_i - \delta_0) \leqslant 2\,(\delta_e - \delta_0)$ might be expected.

$$\delta = \delta_\infty + (\delta_\infty - \delta_0)[1 - (4\,KC + 1)^{\frac{1}{2}}]/2\,KC \qquad (3)\ *$$

If species larger than dimers are ignored, the relationship between the observed upfield shift and the total concentration becomes (4). Expressions (3) and (4) are identical

$$\delta = \delta_d + (\delta_d - \delta_0)[1 - (8\,K_dC + 1)^{\frac{1}{2}}]/4\,K_dC \qquad (4)$$

except that δ_∞ is replaced by δ_d, the upfield shift in a dimer, and K is replaced by $2\,K_d$, twice the equilibrium constant for the dimerization (5).

$$\text{phen} + \text{phen} \rightleftharpoons (\text{phen})_2 \qquad (5)$$

$$K_d = [(\text{phen})_2]/[\text{phen}]^2$$

However the upfield shifts, particularly for H_γ and H_δ of phenanthroline, and of $[Zn(phen)]^{2+}$ (Table 1) are much larger than is possible for the shift due to a single adjacent molecule, as in a dimer. It is therefore clear that for phenanthroline and $[Zn(phen)]^{2+}$, indefinite stacking occurs, and this is presumably also true for bipyridyl and $[Zn(bipy)]^{2+}$.

TABLE 1

Equilibrium constants ($dm^3\ mol^{-1}$) for stacking and chemical shifts (p.p.m.) of monomeric and stacked phenanthroline and $[Zn(phen)]^{2+}$

Solvent		H_α	H_β	H_γ	H_δ	Average K
D_2O						
	K	20.8	20.3	21.8	24.5	23.6 ± 1.8
	δ_0	9.087	7.847	8.547	8.021	
	δ_∞	8.23	6.79	6.72	5.69	
	$\delta_0 - \delta_\infty$	0.86	1.06	1.82	2.35	
25% v/v $CD_3OD–D_2O$						
	K	5.2	4.4	5.9	7.6	6.2 ± 2.3
	δ_0	9.075	7.835	8.509	7.957	
	δ_∞	8.08	6.48	6.40	5.40	
	$\delta_0 - \delta_\infty$	0.99	1.36	2.11	2.55	
CD_3OD						
	δ_0	9.070	7.761	8.465	7.947	
$CDCl_3$						
	δ_0	9.196	7.644	8.262	7.804	
$[Zn(phen)]^{2+}$						
	K	*ca.* 1	0.96	1.06	0.99	$1.0_6 \pm 0.1_7$
	δ_0	9.142	8.072	8.800	8.183	
	δ_∞	8.77	6.94	6.14	4.28	
	$\delta_0 - \delta_\infty$	0.37	1.13	2.66	3.90	
Change in δ_0 on co-ordination of Zn^{2+}		0.055	0.224	0.253	0.162	

The functions (2) and (3) were fitted to the observed variation of chemical shift with concentration using a Newton–Gauss non-linear least-squares method.

RESULTS AND DISCUSSION

The n.m.r. spectrum of aqueous phenanthroline changes dramatically as the concentration is increased from 0.002 to 0.026 mol dm^{-3} [Figure 2, (*a*) and (*b*)]; the resonances of H_α, H_β, H_γ, and H_δ shift upfield by 0.220, 0.276, 0.471, and 0.612 p.p.m. respectively. In solution in $CD_3OD–D_2O$ (25% v/v CD_3OD) in which phenanthroline is much more soluble, the changes are even larger: 0.467, 0.638, 0.976, and 1.180 p.p.m. as the concentration is increased from 0.007 to 0.3 mol dm^{-3} [Figure 2(*d*)]. In methanolic solution all of the resonances occur at lower field than in aqueous solution or in aqueous methanolic solution [Figure 1(*c*)]. No significant concentration-dependent change of any of the coupling constants of phenanthroline was observed.

Self-association in Aqueous Solution.—Computer-calculated least-squares fits of the variation of the upfield shifts of each of the protons (Figure 3) with increasing concentration gave, using equation (3), the same stability constant for each of the protons, within experimental error; the values are given in Table 1. The mean value of the stability constant for the self-stacking of phenanthroline (23.6 ± 1.8) is much lower than values found for several dyes [36,37] which have similar sized aromatic systems but which differ in that they have alkyl substituents which are also hydrophobic. The value is comparable to that found for methylated adenine derivatives [26] and considerably higher than those found for adenine nucleoside and nucleotides [14] in which the aromatic system is smaller. Notable is that the same stability constant is obtained using the shifts of each of the protons and also using the difference in the shift between pairs of protons (*i.e.* using an intramolecular n.m.r. reference). This is in contrast to the work on methylated adenine derivatives in which the stability constants obtained using the shifts of the different protons varied by factors of up to seven. The absence of such a variation with phenanthroline can be taken to indicate that the assumption made about the relative size of the shift of a molecule within the stack, and at the end of the stack, is valid.

The stability constants found for phenanthroline and bipyridyl are similar to those which can be inferred from measurements of the enhancement of the rate of sub-

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* The simplified expression (3) is equivalent to the expression used by Dimicoli and Hélène.[35] However, the non-linear least-squares regression method used here has considerable advantages over rearrangement of the expression to give a linear relationship.

stitution of one aromatic ligand (*e.g.* bipyridyl or terpyridyl) with $[Ni(phen)]^{2+}$ or $[Ni(bipy)]^{2+}$. The enhancement of rate is *ca.* seven-fold for one pair of stacked rings, and *ca.* 23-fold for two pairs of stacked rings.[20]

The chemical shifts of the protons in monomeric phenanthroline and in completely stacked phenanthroline are also given in Table 1. The upfield shift on stacking ($\delta_0 - \delta_\infty$) is too high for the shift to be due to a single adjacent molecule, *i.e.* stacking proceeds beyond the dimer stage as is to be expected, and as has been found for numerous other molecules.[14-18,35-37] The size of the upfield shifts is naturally larger than the known upfield shifts caused by smaller aromatic molecules,[33] and is also larger than values found for some

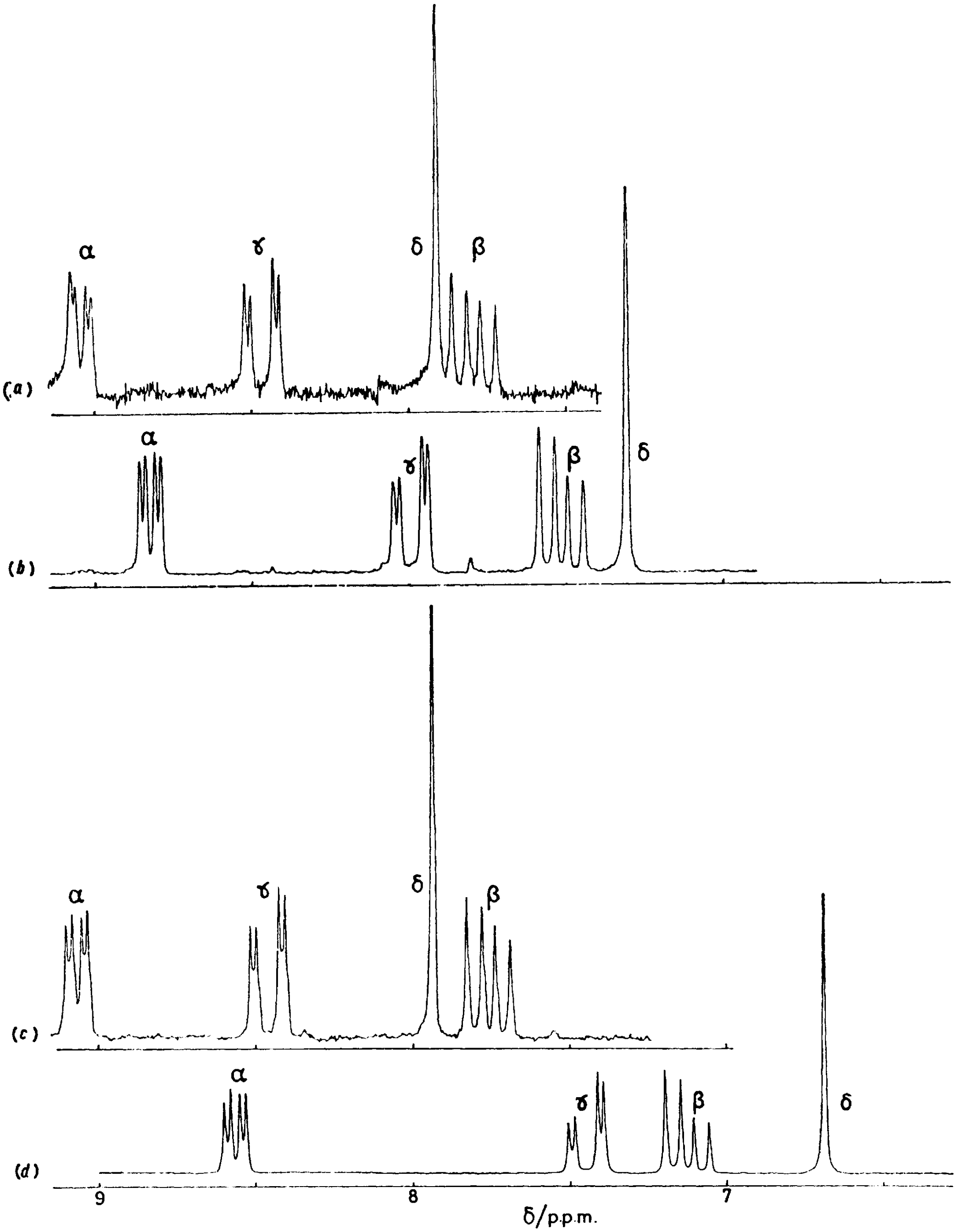

FIGURE 2 ^1H n.m.r. spectra of 1,10-phenanthroline (27 °C, $I = 0.1$ mol dm^{-3}, $Na[NO_3]$) measured relative to internal $[N(CH_3)_4][NO_3]$ and converted to p.p.m. relative to sodium 3-(trimethylsilyl)propane-1-sulphonate using the chemical shift of $[N(CH_3)_4][NO_3]$, $\delta = 3.188$ p.p.m. (*a*) 0.002 mol dm^{-3} in D_2O (predominantly monomer); (*b*) 0.026 mol dm^{-3} in D_2O (monomer : dimer : trimer : tetramer is 49 : 29 : 13 : 5%); (*c*) 0.020 mol dm^{-3} in CD_3OD (monomer); (*d*) 0.301 mol dm^{-3} in 25% v/v CD_3OD–D_2O (monomer : dimer : trimer : tetramer : pentamer is 26 : 26 : 19 : 12 : 7%)

acridine[36] and ellipticine[37] derivatives.* However the values found for $\delta_0 - \delta_\infty$ are consistent with shifts observed[12] in stacks formed by phenanthroline and adenosine or atp^{4-}. Moreover, the ring current of each phenanthroline molecule shifts the resonance of one of the H_α protons in the other phenanthroline *ca.* 2.2 p.p.m. upfield in bis(phenanthroline)metal complexes.[38]

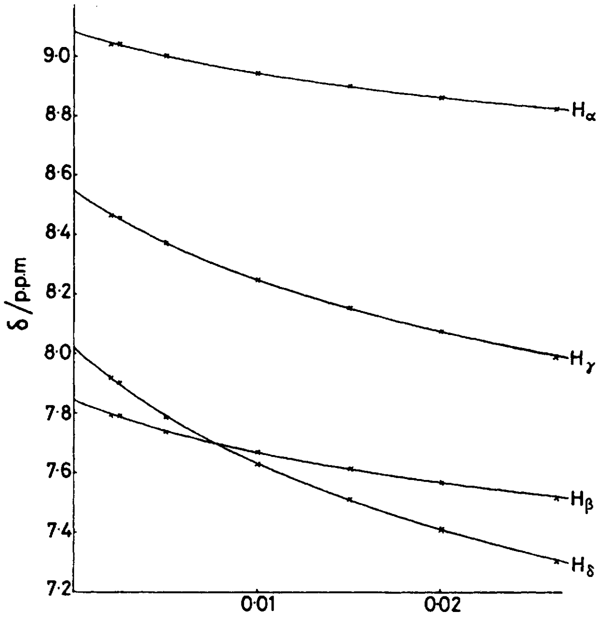

FIGURE 3 The chemical shifts of H_α, H_β, H_γ, and H_δ of phenanthroline with increasing concentration (90.025 MHz, 27 °C; $I = 0.1$ mol dm^{-3}, Na[NO$_3$] in D$_2$O, pD *ca.* 8). The curves shown are the computer-calculated best fit of the experimental points, $K = 23.6 \pm 1.8$ dm^3 mol^{-1}

TABLE 2

Equilibrium constants (dm^3 mol^{-1}) for stacking and chemical shifts (p.p.m.) of monomeric and stacked 2,2′-bipyridyl and [Zn(bipy)]$^{2+}$

		H(6)	H(5)	H(4)	H(3)	Average *K*
Bipyridyl						
	K	9.2	7.4	6.6	6.8	7.4 ± 1.0
	δ_0	8.68	7.57	8.07	8.10	
	δ_∞	8.15	7.11	7.49	7.39	
	$\delta_0 - \delta_\infty$	0.53	0.45	0.58	0.71	
[Zn(bipy)]$^{2+}$						
	K		*ca.* 0.3	*ca.* 0.3	*ca.* 0.3	0.3 ± 0.1
	δ_0	8.781	7.776	8.287	8.515	
	δ_∞	8.8	7.09	6.97	6.49	
	$\delta_0 - \delta_\infty$	*ca.* 0	0.69	1.31	2.03	
Change in δ_0 on co-ordination of Zn^{2+}		0.10	0.21	0.22	0.41	

The n.m.r. spectrum of molten anhydrous phenanthroline (*ca.* 140 °C) resembles that of unstacked phenanthroline, for which one characteristic is that the chemical shift of H_β is *less* than that of H_δ, whereas the n.m.r. spectrum of molten phenanthroline hydrate (*ca.* 90 °C, containing a trace of added water) is typical for that of stacked phenanthroline with the chemical shift of H_β *greater* than that of H_δ.

The variation of the u.v. spectrum of an aromatic molecule with concentration has been used widely in studies of self-stacking, particularly of dyes.[39] However, the extinction coefficients of the main u.v. maxima of phenanthroline, at 229 nm and 264 nm, remain fairly constant, ±5% and ±1.5% respectively, as the concentration is increased from 10^{-5} to 2×10^{-3} mol dm^{-3}. The weaker maximum at 324 nm changes more (by 30% as the concentration is increased from 10^{-4} to 2×10^{-2} mol dm^{-3}) but no stability constant for self-stacking could be calculated from the variation. In dilute (10^{-4} mol dm^{-3}) aqueous solution the u.v. spectrum of phenanthroline is 229 ($\varepsilon = 43\,100$), 264 (29 460), 309(sh) (970), and 324 nm (700 dm^3 mol^{-1} cm^{-1}).

For aqueous 2,2′-bipyridyl the upfield shifts in the n.m.r. spectrum are much smaller; 0.088, 0.075, 0.104, and 0.106 p.p.m. for H(6), H(5), H(4), and H(3) as the concentration is increased from 0.012 to 0.058 mol dm^{-3}. The stability constant for self-stacking (7.4 ± 1.0, Table 2) is smaller than that for phenanthroline, due to the smaller hydrophobic entity. The upfield shifts in completely stacked bipyridyl ($\delta_0 - \delta_\infty$, see Table 2) are on average considerably smaller for bipyridyl than for phenanthroline (Table 1), due to the smaller ring current. It is not however clear why for bipyridyl the shifts of all four protons are fairly similar whereas for phenanthroline there is a clear trend $H_\alpha < H_\beta < H_\gamma < H_\delta$, implying that the stack is probably slightly staggered.

Effect of Solvent on Self-association.—In aqueous methanolic solution (25% v/v CD$_3$OD in D$_2$O) self-stacking of phenanthroline also occurs, but the stability constant (6.2 ± 2.3) is much smaller than in D$_2$O. The limiting upfield shifts are however similar to those in D$_2$O although, particularly for H_α and H_β, slightly higher (Table 1). A repeat experiment in solution in 25% v/v CH$_3$OH in H$_2$O at 35 °C gave $K = 4.6 \pm 0.7$ dm^3 mol^{-1} and, within experimental error, the same limiting upfield shifts as at 27 °C. Preliminary measurements on 50% v/v CH$_3$OH in H$_2$O solutions indicated $K \simeq 1$.

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* However, even if *K* were considerably higher, although $\delta_0 - \delta_\infty$ would be reduced somewhat, it would still be significantly larger than found for these other molecules and the variation of chemical shift with concentration given by the higher *K* would be totally inconsistent with the experimental results.

The decrease in K on changing from water to aqueous methanol parallels the decrease in the rate enhancement of the substitution of terpyridyl into $[Ni(phen)]^{2+}$:[20] in water the rate enhancement due to stacking of the incoming ligand with the co-ordinated phenanthroline is 65-fold, whereas in methanol–water (65 : 35 w/w) it is only *ca.* five-fold. The stability of stacked complexes of methyl cinnamate with theophylline was also found to decrease by a similar amount on addition of methanol to aqueous solution.[40]

In aqueous dioxan solution (25% v/v dioxan), self-stacking is very much less than in aqueous methanol, and K *ca.* 0.8 $dm^3\ mol^{-1}$; self-stacking is barely detectable ($K \leqslant 0.3$) in 50% v/v dioxan, and not detectable in solution in dioxan alone.

In solution in $CDCl_3$ a very slight variation of the spectrum with concentration is observed: δ_α, δ_β, δ_γ, and δ_δ shift upfield by 0.058, 0.094, 0.110, and 0.121 p.p.m. as the concentration is increased from 0.05 to 0.5 mol dm^{-3}. The upfield shift is proportional to concentration and is probably analogous to the dilution shift of the resonance of the aromatic component observed[41,42] when an aromatic solvent is diluted with an aliphatic solvent. The same is probably true of the equally small shift observed in solution in dry methanol. The shift caused by significant self-stacking of aromatic molecules in a hydrophilic solvent can be distinguished from this dilution shift in binary mixtures of an aromatic and an aliphatic solvent since: (*a*) the shift caused by self-stacking is five to 50 times larger than that observed in the dilution studies; (*b*) the shift is observed with much lower concentrations, considerable shifts being observed for 0.001 to 0.01 mol dm^{-3} solutions whereas the dilution shifts of aromatic molecules are only seen in solutions 50 times more concentrated; (*c*) the variation of the shift with concentration is curved, and fits equation (3) for self-stacking. However, naturally the borderline case of very weak self-stacking is harder to distinguish from a dilution shift.

It seems that authentic self-stacking, at least of phenanthroline, occurs only in aqueous solution and in mixtures in which water is the predominant component.

Self-association of $[Zn(phen)]^{2+}$ *and* $[Zn(bipy)]^{2+}$.—The change of the ^1H n.m.r. spectrum of $[Zn(phen)]^{2+}$ with concentration is smaller than that of phenanthroline itself; owing to the increased solubility stronger solutions (up to 0.2 mol dm^{-3}) can be used and again extremely large upfield shifts can be observed; 0.059, 0.168, 0.386, and 0.566 p.p.m. for H_α, H_β, H_γ, and H_δ respectively, between 0.005 and 0.2 mol dm^{-3} solutions. Computer-calculated least-squares fit of the variation again gave the stability constant, $K = 1.1 \pm 0.2$ $dm^3\ mol^{-1}$ (see Table 1). Owing to Coulombic repulsion between the charged species the stability constant is reduced 20-fold from that of phenanthroline itself. A similar, but smaller change in stability has been observed[43] for $[H(phen)]^+$ for which $K = 12.0 \pm 2.0$ $dm^3\ mol^{-1}$. Conversely however, co-ordination increases the self-stacking of atp^{4-} as the Coulombic repulsion is reduced.[14]

The concentration variation of the n.m.r. spectrum of $[Zn(bipy)]^{2+}$ is even smaller than that of $[Zn(phen)]^{2+}$, giving $K \simeq 0.3$ $dm^3\ mol^{-1}$ (Table 2), again a 20-fold stability decrease.

On the addition of an excess of Zn^{2+} to a 0.02 mol dm^{-3} solution of phenanthroline, the resonances of H_α, H_β, H_γ, and H_δ shift downfield by 0.27, 0.48, 0.67, and 0.69 p.p.m. respectively. Although protonation or co-ordination normally causes a downfield shift of nearby protons, the shift on co-ordination of Zn^{2+} to phenanthroline is however mainly due to the greatly decreased self-stacking in $[Zn(phen)]^{2+}$ compared with that in phenanthroline. The downfield shifts calculated for the effect of co-ordination in infinitely dilute solution are only 0.055, 0.224, 0.253, and 0.162 p.p.m. respectively (Table 1). With bipyridyl the effect is similar but smaller.

Although the relative sizes of the upfield shifts of the resonances of phenanthroline change little as the solvent is changed from water to aqueous methanol, the relative sizes of the upfield shifts of the resonances of $[Zn(phen)]^{2+}$ on stacking are quite different from those of phenanthroline. In $[Zn(phen)]^{2+}$ the relative upfield shifts of H_α, H_β, H_γ, and H_δ are 0.10 : 0.29 : 0.68 : 1, whereas for phenanthroline the relative shifts are 0.37 : 0.45 : 0.78 : 1 in aqueous solution and 0.39 : 0.52 : 0.83 : 1 in CD_3OD–D_2O (25% v/v). Indeed, the upfield shift of H_α is three times larger in phenanthroline than in $[Zn(phen)]^{2+}$, the shift of H_β is about the same in both systems, and the shifts of H_γ and H_δ are much larger for $[Zn(phen)]^{2+}$ than for phenanthroline. It may be concluded from this, and from the much lower stability constant, that with $[Zn(phen)]^{2+}$ a staggered head-to-tail stack [Figure 1(*d*)] occurs owing to the strongly hydrophilic character of the zinc and the hydrophobic character of the aromatic rings.

Although in 2,2′-bipyridyl itself all protons are shifted to a fairly similar extent [0.74 : 0.63 : 0.82 : 1 for H(6), H(5), H(4), and H(3) respectively], in $[Zn(bipy)]^{2+}$ H(6) is not shifted at all, whereas H(4) and H(3) are strongly shifted [0 : 0.34 : 0.65 : 1 for H(6), H(5), H(4) and H(3) respectively]. The complex $[Zn(bipy)]^{2+}$ therefore probably also forms a staggered head-to-tail stack.

The Orientation of Molecules within the Stack.—These measurements do not lead to decisive information about the orientation of the stack for most of the systems studied: however as mentioned above $[Zn(phen)]^{2+}$ and $[Zn(bipy)]^{2+}$ probably form staggered head-to-tail stacks. For all of the systems studied (except phenanthroline in aqueous methanol, see below) no improvement of the fit of the experimental results could be obtained by varying the ratio * $(\delta_i - \delta_0)/(\delta_e - \delta_0)$ and indeed it seems that in general $(\delta_i - \delta_0)/(\delta_e - \delta_0) = 2$. This implies that the stacks of phenanthroline and bipyridyl are usually more or less vertical with perhaps slight staggering. The staggering cannot be considerable or

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* The value of the stability constant is very dependent on the value of this ratio. A similar relationship was found[26] in the self-stacking of dialkyladenines.

the change to a staggered head-to-tail stack on formation of the zinc(II) complex would not decrease the stability constant by the factor of 20 which was observed.

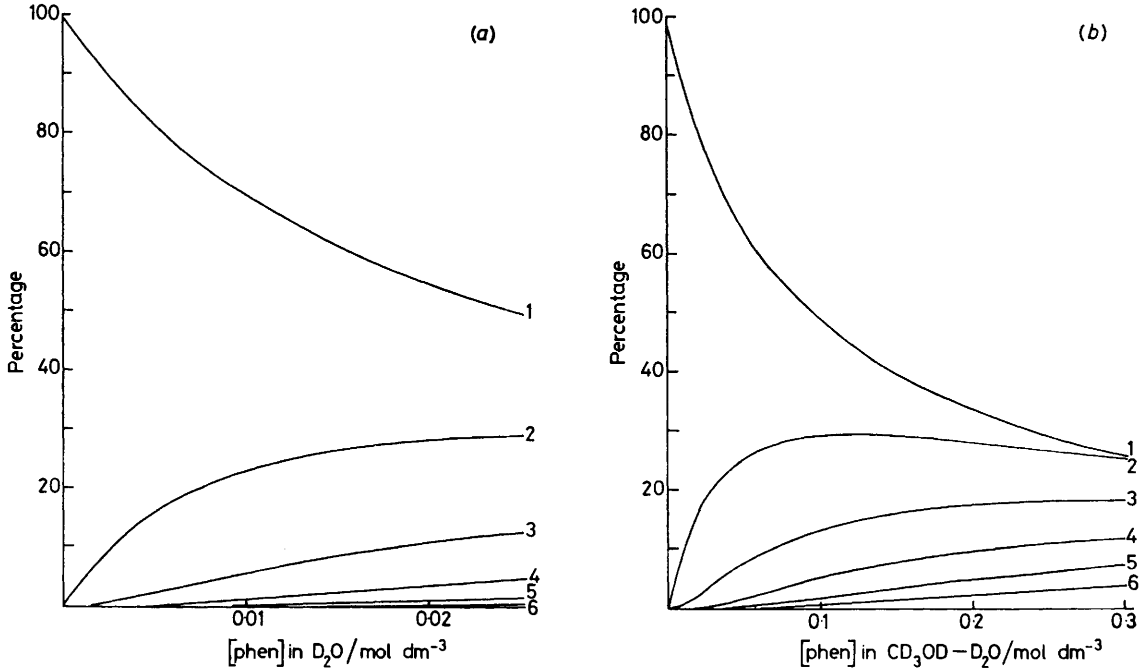

FIGURE 4 The variation of the proportions of monomer (1), dimer (2), trimer (3), tetramer (4), pentamer (5), and hexamer (6) in (*a*) an aqueous solution of phenanthroline at 27 °C, (*b*) a solution of phenanthroline in 25% v/v CD_3OD-D_2O at 27 °C

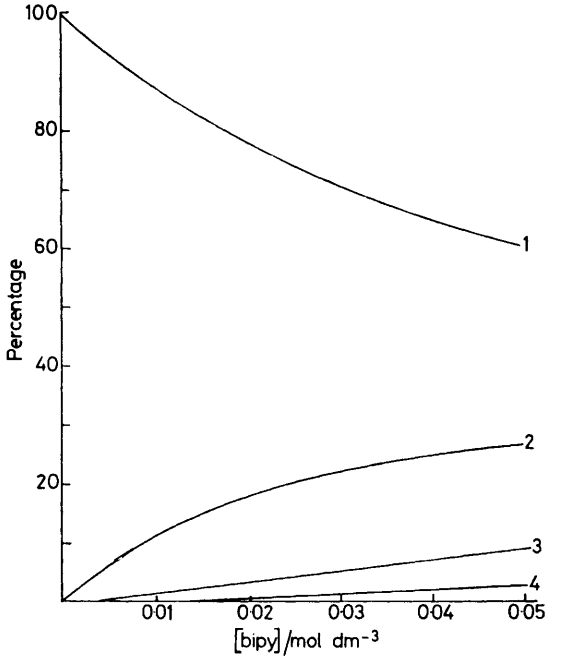

FIGURE 5 The variation of the proportions of monomer (1), dimer (2), trimer (3), and tetramer (4) in an aqueous bipyridyl solution at 27 °C

However, for the solution of phenanthroline in aqueous methanol there is some evidence that $(\delta_i - \delta_0)/(\delta_e - \delta_0) < 2$ and also that it is not the same for all four protons: for H_α, H_β, and H_γ $(\delta_i - \delta_0)/(\delta_e - \delta_0) \simeq 1.5$ whereas for H_δ it equals 1.1 ± 0.3. A stepped stack [Figure 1 (*f*) or (*g*)] would result in just this sort of size and variation of the ratio $(\delta_i - \delta_0)/(\delta_e - \delta_0)$ as the stacked molecules do not have two equivalent nearest neighbours. Thus for the system of phenanthroline in aqueous methanol, an alternative to the fit of the experimental data with equation (3) [*i.e.* $(\delta_i - \delta_0)/(\delta_e - \delta_0) = 2$], with $K = 4.6 \pm 0.6$ at 35 °C, would be a fit using equation (2) which gives $K = 3.3 \pm 0.4$ dm^3 mol^{-1} at 35 °C. The extrapolated values of the chemical shifts at zero concentration (δ_0) are the same within experimental error as those obtained with equation (3) and given in Table 1. However, using equation (2) gives the upfield shifts of a molecule at the end of a stack $(\delta_e - \delta_0)$ as 0.59, 0.83, 1.23, and 1.72 p.p.m. for H_α, H_β, H_γ, and H_δ respectively and those of a molecule within the stack $(\delta_i - \delta_0)$ as 0.90, 1.27, 2.01, and 1.89 p.p.m. respectively. However, it is not possible to say unambiguously whether or not a stepped stack does in fact occur in this system.

The Extent of Self-association.—Using the stability constants listed in Tables 1 and 2 it is possible to calculate the variation in the proportions of the various oligomers as the concentration is changed over the range used in the n.m.r. experiments. Such plots are shown for phenanthroline in solution in D_2O [Figure 4(*a*)] and in solution in 25% v/v CD_3OD-D_2O [Figure 4(*b*)]. In aqueous solution tetramers are present in up to 5%, and pentamers in up to 2%, of the total phenanthroline concentration: the average number of molecules in each stacked aggregate is 2.4 at 0.025 mol dm^{-3}. In 25%

v/v CD_3OD–D_2O, hexamers are present in up to 4.4%, and even octamers at up to 1.4%: the average number of molecules in each stack is *ca.* 3 at 0.3 mol dm^{-3}. The variation in the proportions of the various oligomers present, with changing concentration, is shown for bipyridyl in Figure 5: in 0.05 mol dm^{-3} solution the ratio of monomer, dimer, trimer, and tetramer is 60 : 27 : 9 : 3% and for [Zn(phen)]$^{2+}$ (0.2 mol dm^{-3}) the ratio is 72 : 22 : 5 : 1%.

The extent of stacking, although considerable, is very much less than that observed for phenanthroline in weakly acidic (pH *ca.* 5) solution:[43] in 0.6 mol dm^{-3} solution only 6.4% of the phenanthroline is present as the monomer and 44% is present in stacks containing more than five phenanthroline molecules: the average number of phenanthroline molecules in each stack is five.

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