Hemes and Hemoproteins. Part 10.[1] Co-ordination of Imidazole and other Azoles by the Iron(III) Porphyrin Microperoxidase-8

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Equilibrium constants K for the substitution of co-ordinated H_2O in the iron(III) porphyrin microperoxidase-8 (MP-8) by various azoles have been determined by UV/VIS spectrophotometry in 20% aqueous MeOH at 25 °C and I *ca.* 0.1 mol dm^{-3}. The results establish that the azoles obey (subject to a larger error at higher pK values) the relationship $\log K = a\cdot pK + b$, where $a = 0.34$ and $b = 2.1$, over 12 pK units from 1,2,4-triazole (pK_b 2.3) to imidazolate (pK_a 14.3). Comparison with previous results on the co-ordination of nitrogen-containing bases to MP-8 shows that the five-membered heterocycles form a group distinct from the six-membered heterocycles as well as the alkylamines, and establishes the order of importance of factors which determine the preference of iron(III) porphyrins for imidazole (as His) over pyridine and amines (as Lys) as 'group-specific factors' > basicity > π bonding. A further comparison of these linear relationships for the three groups as ligands with those published for the three groups as acceptors in hydrogen bonding shows significant parallels which demonstrate that the factors which distinguish five- from six-membered heterocycles are intrinsic to the bases (ligands) and independent of the nature of the Lewis acid, when referred to the pK for protonation in aqueous solution as the standard.

We have initiated a systematic study of the co-ordination of nitrogen-containing bases (N-bases) in aqueous solution by the water-soluble iron(III) porphyrin microperoxidase-8 (MP-8)[1-4] and, in parallel studies, by several cobalt(III) corrinoids.[5] Our aim is to further our understanding of the factors which determine the magnitude of the equilibrium constant $K = [Fe–B]/[Fe–OH_2][B]$ for the substitution of co-ordinated H_2O by the N-base B in MP-8, where the second axial co-ordination site is occupied by the imidazole group of His present in the octapeptide side-chain.[6]

Particular emphasis has been placed on establishing the well-known relationship (1)[7] between the log K for co-ordination

$$\log K = a\cdot pK + b \qquad (1)$$

and the pK for protonation of the free base, and determining the values of a and b, for the various groups of N-bases (*e.g.* with sp^2- or sp^3-hybridised N). Extension to other metal complexes such as the cobalt(III) corrinoids offers the possibility of comparing the effect of varying the electronic configuration (*cf.* d^5 Fe^{III} with d^6 Co^{III}) and varying the *trans* ligand (*cf.* the known *trans* effect in Co^{III} corrinoids).[8,9]

We have reported results[4] on the co-ordination by MP-8 of amines possessing sp^3-hybridised N and of six-membered heterocycles with sp^2-hybridised N (pyridines and diazines, collectively termed azines).[1] For both families of N-bases we have established the existence of the linear relationship (1) and shown that the values of log K can be decreased by steric hindrance (as expected) or increased by operation of the so-called α effect[10] (see below) in the case of NH_2NH_2, NH_2OH and the 1,2-diazine pyridazine (apparently the first established examples of the α effect in any metal complexes). In this paper we report results on the third family of N-bases, *viz.* five-membered heterocycles with two or more N atoms in the ring (azoles), including imidazole itself (Him) and 1,2,4-triazole (Htz). Parallel studies with the cobalt(III) corrinoid cyano-aquacobinamide are reported in the accompanying paper.[5]

Of these three families of N-bases the five-membered azoles have been the least studied by co-ordination chemists; they are, however, of greatest interest to bioinorganic chemists for several reasons. Firstly, the imidazole group of His occurs as a ligand in most of the known hemoproteins; by contrast, the amine group of Lys appears to occur as a ligand only in cytochrome f[11] and the alkaline form of cytochrome c,[12] although neither is proven. It has long been recognised[13] that identifying the key features of imidazole as a ligand, including the origin of the apparently higher binding constant of Him compared to other N-bases such as pyridine (py) or NH_3, will require the ability to separate the effects of basicity, π bonding and perhaps other factors. Secondly, interest in five-membered heterocycles has been further stimulated by the success of many agricultural and medicinal fungicides which inhibit steroid synthesis by acting as ligands to the iron(III) porphyrin in certain P-450 enzymes; although these fungicides include examples of alkylamines and substituted pyridines, the most successful are all based on imidazole and 1,2,4-triazole.[14-16] Thirdly, cobalt corrinoids usually possess a nucleotide side-chain terminating in either a benzimidazole (as in the cobalamins required by humans)[8] or a purine which, at least in the case of Factor A, has been shown by X-ray analysis to be co-ordinated to the Co *via* the imidazole N.[17] The five-membered azoles can also serve to link studies on the metal-binding properties of N-bases more closely with the rapidly accumulating information (both experimental determinations and theoretical analysis) on their proton affinities in the gas phase[18-20] and in solution[21-24] and on their hydrogen-bonding capacities.[25]

Establishing equation (1) for the imidazoles poses several problems because, as pointed out by others,[26] there are few readily available imidazole derivatives which offer both a sufficient range of pK and the absence of steric hindrance to co-ordination. The only attempts to compare a range of substituted imidazoles with pyridines and alkylamines appear to be those of Walker and co-workers[27] with porphyrins of Fe and Co and Nakatsuji *et al.*[28] with Ag^I. If we exclude *N*-acetylimidazole (pK 3.6), which may not be directly comparable (see Discussion), Walker was restricted to the narrow range of *ca.* 2 pK units from 5-chloro-*N*-methylimidazole (5Cl-mim) (pK *ca.* 5) to *N*-methylimidazole (mim) (pK 7.2). The readily available 4-nitroimidazole ($4NO_2$-Him) is potentially of interest, but equilibrium studies are difficult because of its limited solubility in

water.[29] The electron-withdrawing nitro substituent significantly decreases the pK [pK_b −0.1 and pK_a 9.2 in H_2O–dimethylformamide (dmf)][30,31] and increases its π-acceptor capacity towards metal ions in low valencies; complexes have been reported with the iron(III) porphyrin metmyoglobin,[32] cobalt(III) pentaammine[33] and iron pentacyanides[34] with iron in the oxidation states III–I. To test whether five-membered heterocycles obey equation (1) and form a class distinct from the six-membered heterocycles there is clearly a need to determine values of log K_1 in aqueous solution with bases offering a wider range of pK values.

Sufficient evidence is now available on pyridines, azoles and azolate anions from the analysis of proton affinities in the gas phase and in aqueous solution[21–23] and of catalytic activity in ester hydrolysis[35] to suggest that, to a first approximation, the azoles can be treated as a single family with the additional N atoms considered as substituents and the azolate anions as bases of similar nature whose strength and catalytic activity are independent of charge and related simply to the pK; *cf.* our findings (see Fig. 3) that the 1,3- and 1,4-diazines (the two points with the lowest pK values) behave as substituted pyridines. Considering only the two diazoles and two triazoles, the 1,2,4-triazole (Htz) appears to behave most like a substituted imidazole, while pyrazole and the 1,2,3-triazole behave slightly differently.[19,21–24] We have therefore selected Htz and the im^- and tz^- anions as well as Him, mim and 5Cl-mim (and redetermined its pK) as a set of 6 bases with pK values ranging over 12 units from 2.3 to 14.3 (see Table 1 below) with which to test whether the azoles obey the relationship (1) and to determine the values of a and b. We have also included *N*-acetylimidazole for comparison with Walker's results,[27] even though it shows somewhat anomalous behaviour (see Discussion). 4-Nitroimidazole proved too insoluble to study (see Results section). Other azoles will be considered later.

The neutral Htz molecule exists as the 1*H*-tautomer in the crystalline lattice at −155 °C.[36] This also forms the most abundant tautomer in the gas phase;[37] in the apparent absence of any experimental evidence it seems reasonable to assume the same for its aqueous solution. X-Ray analysis of the solids has also shown that Htz is co-ordinated *via* the isolated N^4 in the three complexes studied.[38–40] There appears to be no direct evidence for the sites of protonation or co-ordination of Htz in solution and no evidence for the site of co-ordination of the tz^- anion, but theoretical calculations[22] suggest that Htz is protonated (as well as co-ordinated) on N^4. A further complication is that the introduction of a non-co-ordinated N or NH into a co-ordinated heterocycle opens up the possibility of hydrogen bonding with, *e.g.* the terminal cysteinyl NH_3^+ group of the peptide side-chain of MP-8 (see Discussion).

The aims of this paper are (*i*) to establish the validity of equation (1) and determine the values of a and b for the azoles, thereby testing whether the five-membered azoles form a group distinct from the six-membered azines and providing a set of ligands which may be used to determine basicity effects in complexes of azoles with other metal ions, (*ii*) to compare the values of a and b in equation (1) as found for co-ordination of the three families by MP-8 with those found when acting as hydrogen-bond acceptors,[25] in order to identify those factors which are intrinsic to the ligands, hence (*iii*) to identify at least some of the features which may influence the selection of His (*i.e.* Him) over Lys (*cf.* NH_3 or $MeNH_2$) as the ligand of preference for hemoproteins.

Microperoxidase-8 can be studied as the monomeric species at the concentrations required for UV/VIS spectrophotometry in 20% aqueous MeOH which suppresses dimerisation.[41] It exists primarily as the high-spin H_2O–Fe–Him(His) complex in the neutral pH range with pK 4.4 for protonation and displacement of the co-ordinated Him, pK 8.9 for formation of the hydroxo complex, pK 11–12 (depending on the co-ordinated base) for formation of the im^-(His) complex and pK *ca.* 10 for deprotonation of the terminal cysteinyl NH_3^+ group.[3] Values of K for neutral bases (HB) can be determined directly from binding studies in the range of pH 5–9 (with correction, where needed, for protonation of the base or deprotonation of the co-ordinated H_2O) and those for the anionic im^- and tz^- indirectly by determining the pK_c for deprotonation of co-ordinated HB (see Results section).

Experimental

Materials.—Microperoxidase-8 was prepared from cytochrome c (Sigma, Type III) as described.[42] 1,2,4-Triazole was obtained from Sigma, the other heterocycles from Aldrich; all were used as received.

Methods.—UV/VIS spectra were recorded and spectrophotometric titrations (following changes in absorbance at a fixed wavelength) were carried out on a Philips PU 8740 or 8720 spectrophotometer and, except where otherwise stated, in cells of 1 cm pathlength thermostatted at 25 °C with *ca.* 5 μmol dm^{-3} MP-8 in 20% (v/v) aqueous MeOH and for the quantitative determination of K, with 0.2 mol dm^{-3} phosphate buffers and I *ca.* 0.1 mol dm^{-3}. The pH measurements were made with a Hanna HI B417 pH meter and appropriate glass electrode. The pK_b of 5Cl-mim was determined potentiometrically in aqueous solution at 25 °C by titrating 50 cm^3 of 5×10^{-3} mol dm^{-3} solution of the base in 0.1 mol dm^{-3} $NaClO_4$ with 0.1 mol dm^{-3} NaOH (standardised against standard HCl) using an autoburette interfaced to the microprocessor model Hanna HI B417 in conjunction with a pH M82 potentiometer and pH combination electrode (standardised with buffers pH 4.00 and 7.00); the plot of pH *versus* NaOH added revealed a single inflection point and analysis of the data gave pK_b = 5.12 and 5.16 in duplicate experiments, average 5.14 ± 0.02.

Results

The heterocyclic ligands, both conjugate acids and bases, which have been studied here are listed in Table 1, together with the values of pK_b (for neutral azoles) and pK_a (for im^- and tz^- anions) which have been used for making any correction required in calculating the value of K as well as for testing (see Discussion) the validity of equation (1). All experiments were carried out in 20% aqueous MeOH at 25 °C.

Table 1 Equilibrium constants K for the substitution of co-ordinated H_2O in MP-8 by azoles and azolate ions

Ligand	pK of free base[a]	log K/dm^3 mol^{-1}
1,2,4-Triazole	2.3[b]	2.9 ± 0.1
N-Acetylimidazole	3.6[c]	3.8 ± 0.1
5-Chloro-*N*-methylimidazole	5.1[d]	3.9 ± 0.05
Imidazole	7.1[e]	4.38 ± 0.05[f]
N-Methylimidazole	7.2[g]	4.55 ± 0.05
1,2,4-Triazolate	10.1[h]	5.3 ± 0.5
Imidazolate	14.3[i]	6.75 ± 0.5

[a] Errors and experimental conditions (especially ionic strength) not always given. [b] *cf.* pK Values of 2.27 in water,[43] 2.28 in 28.5% aqueous ethanol[35] and 2.45 in water (I = 0).[44] [c] Ref. 45. [d] This paper (see Experimental section); *cf.* reported pK values of 4.75,[31] 6.23 (after reassignment of the isomer involved)[31] and 5.45.[27] [e] *cf.* pK values of 6.99, 7.03 and 7.31 with I = 0, 0.16 and 1.0 mol dm^{-3} respectively recommended by Smith and Martell[46] in their critical review of data published before 1975, also later value of 7.11 for I = 0.1 mol dm^{-3}.[19] [f] *cf.* Previous log K = 4.45.[6] [g] *cf.* Reported values of 7.25,[19] 7.20,[47] 7.18,[48] 7.20[49] and 7.21[50] for I = 0.1, 0.15, 0.5, 1.0 and 1.0 mol dm^{-3} respectively which, unlike the pK values of imidazole, appear to be relatively insensitive to ionic strength. [h] *cf.* pK Values of 10.1 (water),[51] 10.26 (water),[43] 10.10 (28.5% aqueous ethanol)[35] and 10.04 (water, I = 0).[44] [i] *cf.* pK Values of 14.44 and 14.29 for I = 0 and 0.5 mol dm^{-3} recommended by Smith and Martell.[46]

Preliminary experiments, scanning the spectrum over the range 300–600 nm, showed that at pH 6–8 all the neutral bases listed (except $4NO_2$-Him) reacted rapidly with MP-8 and without any obvious complications or anomalies which might reflect side-reactions or overlapping equilibria. The initial aqua complex of MP-8 with a Soret band at 397 nm was converted, with good to reasonable isosbestic points, into a species with a slightly weaker Soret band at 404–405 nm (*cf.* Fig. 2 of ref. 3); all equilibria were established instaneously. In the case of $4NO_2$-Him allowing a solution of MP-8 at pH 5–6 to stand in contact with an excess of the relatively insoluble solid base produced no detectable formation of any new complex even after 4 h, but at pH 9.5 (*i.e.* above its pK_a) sufficient of the base dissolved to produce a complex characterised by a prominent maximum at 530 nm with a shoulder *ca.* 555 nm (*cf.* the band at 526 and shoulder at *ca.* 555 nm in the complex with NH_3), which probably represents the complex with the anionic form of the ligand; the Soret band region was obscured by background absorption due to the ligand anion. It proved difficult to obtain any reliable value of the binding constant K.

Quantitative determination of the equilibrium constants K was carried out by spectrophotometric titration in at least duplicate experiments in 0.2 mol dm^{-3} phosphate buffer at pH 8.0 (for Him and mim) or 6.5 (all others) and I *ca.* 0.1 mol dm^{-3}, following the fall in absorbance at 397 nm. Analysis of the data (*cf.* ref. 3) confirmed a stoichiometry of 1.0 ± 0.1 base per Fe and the results were used (after correction for protonation of the base in the case of Him and mim) to derive the values of log K listed in Table 1.

Values of log K' for co-ordination of the anionic tz^- and im^- (here denoted by B^-) were obtained indirectly from an experimentally determined value of pK_c for ionisation of the co-ordinated neutral base HB to co-ordinated B^- by use of equation (2) which involves both log K for co-ordination of the

$$K' = \frac{[Fe\text{–}B^-]}{[Fe\text{–}OH_2][B^-]} = \frac{[Fe\text{–}BH]}{[Fe\text{–}OH_2][HB]} \cdot \frac{[HB]}{[H^-][B^+]} \cdot \frac{[Fe\text{–}B^-][H^+]}{[Fe\text{–}BH]} \quad (2)$$

parent HB and the pK for deprotonation of the free base. Equation (2) can be rewritten in terms of equation (3).

$$\log K' = \log K + pK_a - pK_c \quad (3)$$

Qualitative experiments showed that the complex with Htz (max. 404 nm) exhibits a reversible change in spectrum with good isosbestic points corresponding to pK_c *ca.* 7, *i.e.* well below the pK (8.9) for conversion of the aqua to the hydroxo complex, to a product with a slightly more intense Soret band at 407 nm. Varying the concentration of MP-8 over a 10-fold range of concentration (from 1×10^{-6} to 1×10^{-5} mol dm^{-3} in 4 and 1 cm pathlength cells respectively) has no obvious effect on the position of the Soret band at pH 5 or 9 or on the occurrence of the isosbestic points, indicating the absence of any monomer–dimer equilibrium involving a bridging Htz or tz^- ligand. The value of pK_c for the Htz complex was determined quantitatively by spectrophotometric titration in four experiments of a solution of MP-8 in 0.1–1 mol dm^{-3} Htz and 0.2 mol dm^{-3} phosphate from pH 5 to 10 with μl aliquots of concentrated NaOH, following the change in absorbance at 407 nm (see Fig. 1). Using graphical extrapolation to obtain the initial (A_0) and final (A_∞) values of A_{407}, analysis of the changes in A_{407} (corrected for dilution) with pH corresponded to an equilibrium involving one proton with pK = 7.2 ± 0.2 (see Fig. 2). The value of log K' = 2.9 + 10.1 − 7.2 = 5.8 was then obtained and corrected (see Discussion) for the likely stabilisation of the co-ordinated B^- over HB through coulombic interaction with the neighbouring Cys-NH_3^+ group by substracting 0.5 to give log K' = 5.3 with a relatively large error.

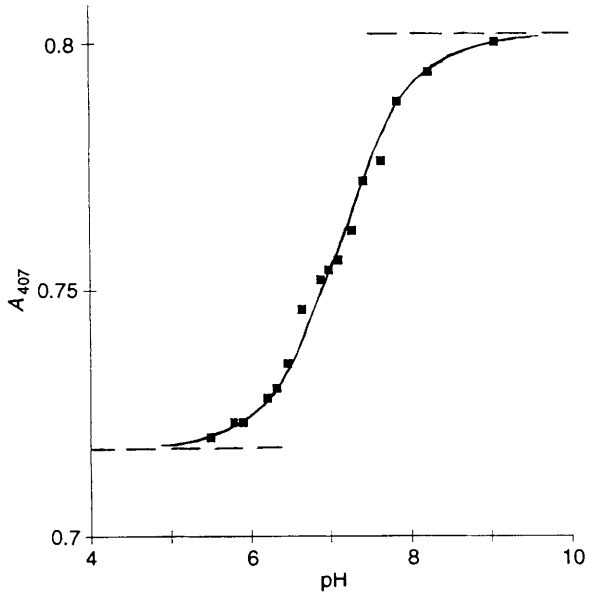

Fig. 1 Example of the pH titration of the 1,2,4-triazole complex of MP-8, showing the change in absorbance *vs.* pH and extrapolated (— — — —) initial and final values

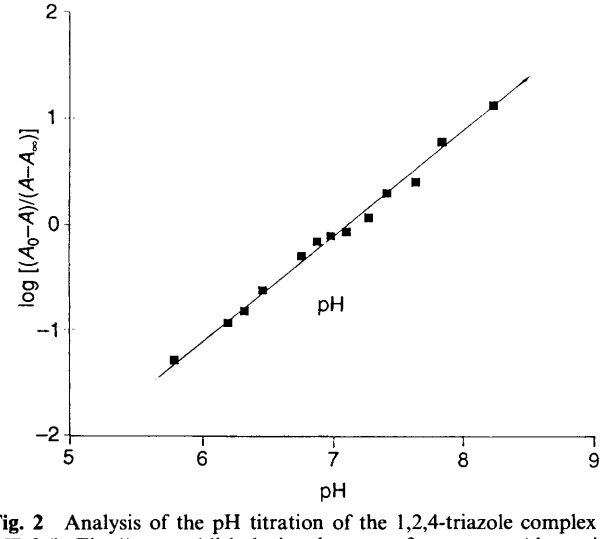

Fig. 2 Analysis of the pH titration of the 1,2,4-triazole complex of MP-8 (in Fig. 1) to establish the involvement of one proton (theoretical slope for n = 1.0 protons given by the solid line) and the value of pK_c (= pH at y = 0)

We have already reported a reversible shift in λ_{max} of the complex of MP-8 with Him from 404 to 410 nm, reflecting the loss of a proton both from the added Him and the His-bound Him with pK values in the range 12–13;[3] it seems reasonable to ascribe the higher pK value to the latter because of the inductive effect of the alkyl substituent (and absence of charge in the peptide-bound His) and to assume pK_c = 12 ± 0.5 with λ_{max} = 407 nm (*i.e.* half the shift from 404 to 410 nm) for the complex with co-ordinated im^- (*cf.* 407 nm for the complex with tz^-). The pK_c was converted to log K by use of equation (3). These values of log K' for the complexes with tz^- and im^- are listed in Table 1 as log K.

Discussion

The values of log K determined for the co-ordination of various uncharged azoles (by direct equilibrium studies) and the two azolate anions im^- and tz^- [indirectly from the pK_c for ionisation of the co-ordinated Him and Htz *via* equation (3)], together with the pK of the unco-ordinated ligand/base, are listed in Table 1. All the reactions of MP-8 with the neutral bases (and the pH-dependent deprotonation of the complex

with Htz) involve the rapid and reversible reaction with base (or H^+) in 1:1 stoichiometry and give reasonable to good isosbestic points. The possibility of the azole acting as a bridging ligand in a binuclear complex is clearly impossible in the case of the two *N*-methylated ligands and was experimentally excluded in the case of Htz and tz^-; because of the regular pattern observed, it is assumed not to occur with the other ligands. The proximal charge effect of the terminal Cys-NH_3^+ group (p*K ca.* 10) in the side-chain may act to stabilise any co-ordinated anion formed below pH 10,[3] *i.e.* tz^- ($pK_c = 8.0$) but not im^- (pK_c *ca.* 12), and should be discounted when comparing tz^- with the other ligands; this stabilisation is assumed to be less than the 0.9 p*K* units observed [52] for the histamine complex of the cobalt(III) cyanoaquacobinamide in aqueous solution (which appears to have an effective charge similar to that of MP-8) and is rather arbitrarily taken as 0.5. It proved impossible (see Results section) to obtain a value of log *K* for 4-nitroimidazole even as the anion since its solubility was too low.

It should be noted that none of the values of p*K* or log *K* used in Table 1 has been corrected for the so-called 'statistical factor' [53–55] which should be applied when one of the partners in the equilibrium has two equivalent N atoms (as in im^-, the neutral pyrazine or protonated species H_2im^+) and one wishes to compare them on the same basis as analogues with only one such N atom. Such a correction would increase the pK_b of Him, decrease the pK_a of Him and the pK_b of pyrazine and decrease log *K* for the co-ordination of Him and the diazines, all by *ca.* 0.3 (*i.e.* log 2). These corrections have not been applied because they are comparable in magnitude to the uncertainties in p*K* and log *K* and because of additional uncertainties over the site of protonation and co-ordination (hence number of equivalent sites) in Htz and its anion as well as in higher azoles and azolates.

All the products discussed here, like those with primary amines and azines, show spectra typical of a low-spin complex; the main (Soret) band is situated at 404–405 nm with the neutral azoles (*cf.* simple primary amines 403–404 nm,[4] most pyridines and diazines 403–405 nm) [1] and at 407 nm with the two azolate ions (*cf.* amines possessing a benzene or indole ring in the side-chain 405.5–406 nm,[4] $4NH_2$- and $4Me_2N$-py 406–407 nm[1]). The similarity in λ_{max} and in the general shape of the spectrum suggests a general similarity in the nature of the Fe–N bond in all three series; this allows comparisons to be made directly from the values of log *K*. Less attention was paid to the broader and far less intense bands in the αβ region (500–600 nm); the higher concentrations required for their characterisation would have promoted aggregation, at least of the starting aqua complex,[41] or required the use of a solvent with a lower water content.

The values of log *K* for co-ordination are plotted against the p*K* for protonation of the free base (both sets of data from Table 1) in Fig. 3. It is assumed that the presence of 20% MeOH used in determining log *K* does not invalidate any correlation with the p*K* values determined in purely aqueous solution. The experimental points exhibit a reasonably linear rise in log *K* with p*K*, except for *N*-acetylimidazole (acim) which lies *ca.* 0.5 above the line as drawn. The fact that acim, 5Cl-mim and Him occupy similar relative positions in the plots of equation (1) both for Walker's cobalt(II) porphyrin in toluene [27] and our iron(III) porphyrin in water suggests that the anomalously high log *K* for acim may reflect additional features of the NCOMe group which are not present in other substituents such as the NMe group, *e.g.* conjugation with the π electrons of the Him ring, addition of H_2O to form $NC(OH)_2Me$ or other little understood solvation effects, which appear to be more common with azoles than azines; [56] this ligand is therefore ignored for the purpose of establishing equation (1) for the azoles. The remaining six probe ligands show a good linear increase of log *K* with p*K*, which is not vitiated even by the errors and assumptions built into the determination of the two highest values of log *K* (for tz^- and im^-). We conclude (*i*) that the inclusion of Htz with imidazoles and azolate anions with the neutral azoles in the series of probe ligands and treating them all as a single family (see Introduction) is justified, and (*ii*) that the azoles as a group do obey the linear relationship (1) when appropriate ligands are selected. We suggest that these ligands could serve as useful probes for examining the basicity effects of azoles in other labile metal complexes.

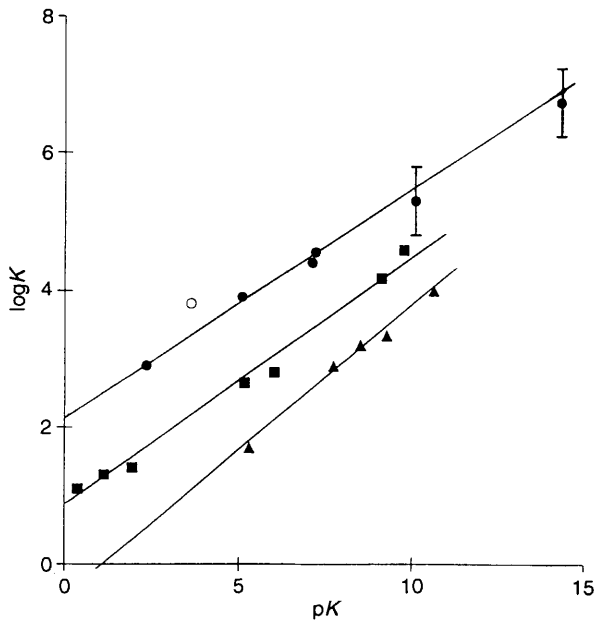

Fig. 3 Plot of log *K* (for co-ordination) *vs.* p*K* (for protonation of the free base) for (*a*) the five-membered azoles and azolate anions (●, except for *N*-acetylimidazole ○, data from Table 1), compared with (*b*) the six-membered pyridines and diazines (■, from ref. 1) and (*c*) primary amines (▲, from ref. 4). The solid lines correspond to the equation log $K = a \cdot pK + b$ with the values of *a* and *b* listed in Table 2. Note that different scales have been used for the *x*- and *y*-axes in order to separate the three groups more clearly

Table 2 Comparison of values of *a* and *b* in equation (1) for N-bases acting as hydrogen-bond acceptors towards 4-nitrophenol in 1,1,1-trichloroethane and as ligand to Fe^{III} in MP-8

	Hydrogen-bond acceptor[a]		Ligand to Fe^{III}	
	a	*b*	*a*	*b*
Five-membered heterocycles	0.29	+1.6	0.34[b]	+2.1[b]
Six-membered heterocycles	0.27	+1.1	0.36[c]	+0.8[c]
Amines NH_2R	0.37	−1.1	0.43[d]	−0.5[d]

[a] Data from ref. 25. [b] This paper. [c] Ref. 1. [d] Ref. 4.

We have already established similar 'baselines' of basicity effects for the six-membered heterocycles [1] and alkylamines; [4] the three series are shown graphically in Fig. 3 and the values of *a* and *b* in equation (1) are listed in Table 2. Comparison of this data confirms, as already suggested by other more limited data, [27,28] that the five-membered heterocycles form a family of ligands which are as distinct from the six-membered heterocycles as the latter are from the primary alkylamines and, secondly, shows that these three families exhibit broadly similar values of the slope *a* but differ significantly in their values of the intercept *b*.

Table 2 also compares the values of *a* and *b* for co-ordination of these three families to the Fe^{III} in MP-8 with those in the analogous equation relating the equilibrium constant *K* for hydrogen-bond formation to the N-base (as acceptor in an aprotic solvent) to the p*K*. In spite of the very different equilibria involved there are striking similarities; for both hydrogen bonding and co-ordination the values of *a* remain relatively

unchanged between the three families, while the values of b fall by significant amounts and in the same order. Since neither the hydrogen bonding of any group nor the co-ordination of the alkylamines can involve π bonding, the close parallels between all these series means that π bonding plays little if any role in the co-ordination of either azoles or azines to Fe^{III} and that differences in π bonding capacity cannot explain the differences between five- and six-membered heterocycles. Conversely, the most significant factors leading to the enhanced binding of Him (or His) over py and NH_3 (or Lys) are the 'group-specific factors' (of as yet unknown origin) which cause separation of the three lines in Fig. 3, while differences in basicity play an intermediate role. The higher values of log K (for a given pK) shown by azoles compared to azines and amines will also explain the predominance of fungicides (see Introduction) based on substituted imidazoles or triazoles.

Our results can be summarised as follows. (*i*) We have provided a series of ligands which may be used for investigating basicity effects in the five-membered azoles (including imidazoles) and hence the first clear-cut demonstration of the occurrence of the linear relationship (1) for the azoles with any metal ion. (*ii*) The results have highlighted group-specific differences between the five- and six-membered heterocycles which cannot be ascribed to differences in π bonding and have established the order of importance of factors which determine the preference of iron(III) porphyrins for imidazoles (or His) over pyridines or NH_3 (or Lys) as group-specific factors > basicity > π bonding. (*iii*) Our demonstration of the occurrence both of the α effect and of systematic differences between azoles and azines as ligands will serve to link studies on metal–ligand bonding to the very detailed and instructive material being accumulated on pK values, hydrogen bonding and gas-phase proton affinities.

Acknowledgements

We thank the University of Surrey for a research studentship and the Arab Student Aid International for financial support (to M. S. A. H.).

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Received 14*th October* 1993; *Paper* 3/06152C