STUDIES ON SOME HETEROCYCLIC LIGANDS

SHOWING

LINKED IONIZATION EFFECTS

BY

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ABSTRACT

This work deals with the effect of coordination to a metallic cation on the ionization of acidic groups in the ligand bonded to the metal.

For the study of this problem, model systems with appropriate weakly acidic side groups were selected and some of these were completely synthesized. In each case the ionization pattern in the free ligand was obtained from the ultraviolet absorption spectra of the ligand at various pH values ranging from 2N alkali to 6N acid. Each ligand was next tested for its chelating properties and for any color changes on changing pH of the solution, with a view to obtaining the ionization pattern in the complex. Most of the work done in this phase was of a qualitative nature.

Analysis of available quantitative results leads to the conclusion that the effect of coordination is very large exceeding by far the effect of substitution on ionization of functional groups in organic compounds; and that it is essentially that of charge, conjugation, and steric factors. Moreover, the first two factors are additive when they operate concurrently.

The results are discussed in relation to heme-linked effects to which they are probably similar in nature. The significance of the results lies in the fact that, in view of the large effect of coordination, one cannot preclude the participation of very weakly acidic groups like -OH, -SH, =NH, etc.. in heme-linked phenomena.

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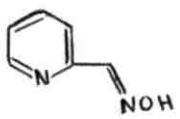
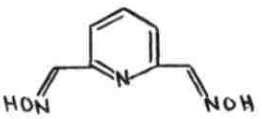
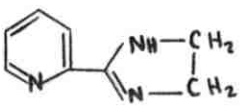
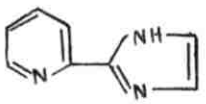
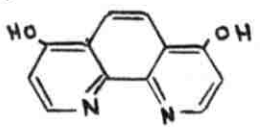
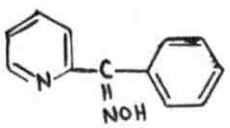
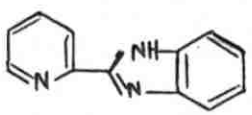
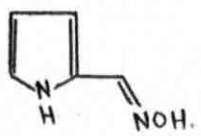
Formula	M.P.(°C.)	Chemical Name	Abbreviated name used in this thesis
	182-183	Imidazole-4(5)-aldoxime	IMAL
	113	Pyridine-2-aldoxime	PAL
	207-208	Pyridine-2,6-dialdoxime	DIPAL
	95-97	2,2'-pyridyl-imidazoline	PIM I
	74-75	2,2'-pyridyl imidazole (A)	PIM II (A)
	126-127	2,2'-pyridyl imidazole (B)	PIM II (B)
	300 (HCl salt)	4,7-dihydroxy-1,10-phenanthroline	
	152-154	phenyl-2-pyridyl ketoxime	
	222-224	2-(2-pyridyl)benzimidazole	
	164.5 (viii)	Pyrrole-2-aldoxime	PYRAL

I. INTRODUCTION

A. Object and Significance

The direct aim of this research was to prepare a number of nitrogen heterocyclic compounds containing certain weak acidic groups. These compounds were to be studied as possible ligands in model coordination compounds. In such compounds metal-linked ionizations of the acidic groups would be present, and a study of acid-base equilibria in the free ligand compared with a study of the corresponding equilibria in the metal complex would give a measure of the effect of coordination to metallic cations on the ionization of acidic groups in the ligand attached to the metal.

Hitherto, the extent of this effect has not been measured. However, recent work carried out by Hanania and coworkers at the American University of Beirut[1] has shown that the effect is large, exceeding by far the effect of substitution on the ionization of acidic functional groups encountered in organic chemistry. Since hemoproteins and metalloenzymes are complexes where metal-linked ionizations probably occur, the study of this phenomenon will clearly be of considerable biological significance, at least so in two respects:

1) Data obtained for simple model systems may be used to give an idea about the extent of the effect of coordination on ionization expected in large complex molecules like hemoproteins.

2) Comparative studies of analogous model systems can be used to give informations on the relation of properties to structural and other factors.

The subject of coordination compounds has been developing very rapidly in recent years, mainly along three lines: industrial, analytical, and biological.

Industrial significance: An increasing number of industrial and commercial problems which arise from contamination by traces of metal ions are being tackled through chelation of the ions by sequestering agents.

In the oil industry, for instance, traces of metals cause many harmful effects among which may be sited the catalyzing effect on resin formation in oil, a problem which has not yet been resolved. However, one way of eliminating these traces of metals is by chelating them with suitable sequestering agents giving rise to oil-soluble complexes.

Also, water softening processes have long been major consideration in industrial chemistry. Addition of water-soluble sequestering agents is the most widely used method for softening water. More recently, organic complexing agents such as alkali metal salts of hydroxy acids and aminopolycarboxylic acids, such as EDTA, have become more important[2a].

Analytical significance: Analytical reagents which are highly selective for certain metal ions are being sought among heterocyclic polydentate ligands[3,4,5,6]. In the closing years of the nineteenth century the Austrian chemist Fritz Blau[7] synthesized 2,2'-bipyridine and 1,10-phenanthroline. He discovered that they react with ferrous salts to produce soluble compounds having intense color $\left[Fe(bipy.)_3\right]^{++}$ and $\left[Fe(1,10\text{-phen.})_3\right]^{++}$ which can be oxidized with strong oxidizing agents to the corresponding ferric ions $\left[Fe(bipy.)_3\right]^{+3}$ and $\left[Fe(1,10\text{-Phen.})_3\right]^{+3}$,

intensely blue in color, and he observed that the oxidation-reduction couples were reversible. The uses of 2,2'-bipyridine and 1,10-phenanthroline in analytical chemistry began about 1930 with the application of 2,2'-bipyridine as a reagent for the colorimetric determination of iron and the use of the couple $\left[Fe(1,10\text{-phen.})_3\right]^{+3} + e^- = \left[Fe(1,10\text{-phen.})_3\right]^{+2}$ as a high potential oxidation-reduction indicator. Such chelating agents contain the feroin group, =N-C-C-N=, a term first proposed by Karl Gleu[8] for organic compounds containing this group which form coloured complexes with Fe(II) and Cu(I) and which have been known for over seventy years.

Chelating agents of the feroin type were then continuously improvised and studied from 1933 to the present. In the Skraup method[5] for the synthesis of substituted quinoline, a substituted aniline is condensed with glycerol in hot concentrated sulfuric acid in the presence of an oxidizing agent such as arsenic acid. Under these conditions the glycerol is dehydrated to acrolein, $H_2C=CH-CHO$, to which the aniline is believed to add in the 1,4 manner with subsequent ring closure by elimination of water. Finally removal of two hydrogen atoms (in the 1 and 2 positions), by the oxidizing agent, yields a quinoline substituted in the homocyclic ring in a position depending on which substituted aniline was used.

If o-nitroaniline is used in a Skraup reaction with glycerol, the resulting product is 8-nitroquinoline. Reduction of 8-nitroquinoline to 8-aminoquinoline followed by a second Skraup reaction with glycerol yields 1,10-phenanthroline.

Most of these analytical reagents, as a literature survey shows, were prepared with a view to making highly selective and sensitive reagents.

It is perhaps not surprising that very few available compounds of this type contain appropriate acidic groups to serve as model systems for the present study.

Biological significance: Metal ions in biological systems serve as catalysts for chemical reactions and many of these metal ions would hydrolyze and precipitate at biological pH if they were not carried as metal chelates[2b,9]. Even the most basic metals, such as calcium and magnesium which would ordinarily be soluble under physiological conditions are probably bound to a considerable extent to multidentate ligands.

Trace metals in biological systems are highly essential for the formation and functioning of many enzymes. Many enzyme systems are inhibited by reagents such as cyanide ion and carbon monoxide which are also known as metal inhibitors. More recently, specific studies of metal enzyme systems has indicated that the site of reactivity of enzyme and substrate is the metal itself through chelate groups of one kind or another. However, two recent pharmacological applications of metal chelates may be sited:

The exchange reaction between the Ca-EDTA chelate and a more strongly complexed metal ion has become the basis for a new and apparently successful treatment for acute lead poisoning. The Pb-EDTA chelate is roughly 10^8 times more stable than the calcium chelate, and as a result not only is the exchange of lead for calcium in the chelate complete, but also the resulting lead chelate is stable enough to be physiologically inert and non-toxic and its chemical properties are such that it is rapidly eliminated through the kidney.

Another case is the report that platinum and palladium complexes of 6-mercaptopurines are more potent than uncomplexed 6-mercaptopurines as anticancer chemicals[10]. The idea is that viruses may be the cause of the tumor, and since they contain proteins, nucleic-acids, and other sites that can coordinate with the metal, their chelation inactivates the virus.

However, the work presented in this thesis is primarily connected with only one aspect of the biological significance of coordination compounds, namely the "linked-effect" concept. This effect was first observed and studied in the case of hemoglobin where the metal ion is linked to four nitrogen atoms of the porphyrin ring and probably a fifth nitrogen of the imidazole ring in histidine (the sixth bond of the octahedral complex being free to react). Consequently it was decided to limit the present study to organic nitrogen heterocyclic compounds which could serve as model systems for the hemoproteins. A number of N,N-heterocyclic bidentate ligands have, therefore, been selected for this special study.

B. Problem of Heme-Linked Ionizations

In general a "metal-linked" effect may be regarded as the effect of coordination of a metal to a ligand on the free energy of ionization of ionizable side group on the ligand, and the concomitant effect of this ionization on the free energy of reaction between the metal ion and the ligand (stability of the complex).

Considerable theoretical and experimental work has been done on the problem of interaction between overlapping ionizations in diprotic acids and polyelectrolytes[11], and on the problem of the effect of

substitution in organic compounds on the acid strength of functional groups. However, very little attention seems to have been given to another aspect of the problem, namely the extent to which the strength of acidic side groups in a ligand can be modified as a result of coordination of the ligand to a metal.

The first observation of the "heme-linked" effect, called the Bohr effect[12], indicated that upon oxygenation, hemoglobin becomes a stronger acid, and that approximately 0.8 equivalent of H^+ ion is liberated per molecule of oxygen combined. Conversely, it was later found that the strength of the iron-oxygen bond in oxyhemoglobin is affected by the pH of the solution[13,14].

In 1933, Conant[15] made the suggestion that in hemoglobin the iron atom is not only linked to the protein through the imidazole part of histidine, but is actually linked on both sides of the heme disc to histidines, one bond being strong and the other weak. It is at this weak link that oxygen and other groups can form bonds with the iron atom. This idea came to be known as the "Imidazole Hypothesis".

The work of Wyman et al[16] gave one of the most important lines of evidence in favor of the imidazole hypothesis. In view of the relevance of Wyman's findings to the present work, his results are summerized here:

1. Differential acid-base titrations of ferrohemeglobin and oxyhemoglobin indicated that between pH 4.5 and 6.1 oxyhemoglobin is a weaker acid than ferrohemoglobin, while between pH 6.1 and 9.0 it is a stronger acid. Outside this limit the acidity of the two proteins is the same.

2. The effect of temperature on the differential acid-base titra-

tion led to a value about 7 Kcal./mole for the enthalpy of ionization of the titrable acidic groups in hemoglobin which appear between pH 6 and 8. Both facts support the view that such an ionization belongs to the dissociation of the imidazole (equation 1).

Based on Wyman's findings, Coryell and Pauling[17] gave an extremely interesting explanation for the change in acidity on the introduction of an oxygen molecule into hemoglobin. Their argument was based on changes in bond type and the stability of resonance hybrids in hemoglobin and oxyhemoglobin. They attributed one ionization to the proximal heme-linked group, and the other (and weaker) effect to the distal heme-linked group. Resonance effects account for the change in the acid strength of the proximal group, and steric factors for the change in the acid strenght of the distal group.

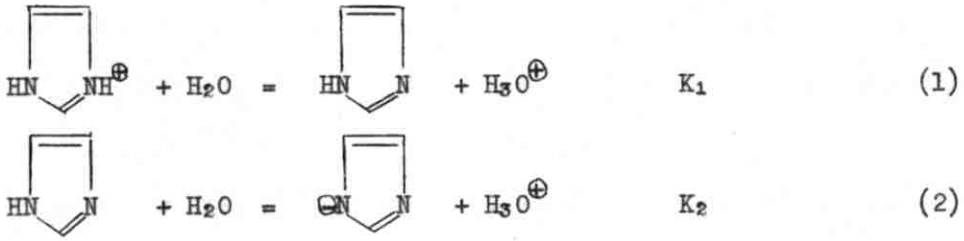
More recently, the direct X-ray crystallographic work of Kendrew[13] on myoglobin, and of Perutz[14] on hemoglobin has confirmed the picture of the molecule with iron in the heme plane bonded to the protein globin through histidine as predicted, and to the other side to water. Thus the explanation of the heme-linked effect in hemoglobin appeared to be satisfactory.

Further examination of the physical chemistry of hemoproteins, however, has shown that the problem of heme-linked ionizations is by no means solved[18,32]. The main difficulty concerns the imidazole-ferri-hemoglobin complex. Russell and Pauling[19] concluded from a magnetic study of the ferrihemoglobin-imidazole reaction that the resulting complex has an ionizing heme-linked group with $pK_{\sim}9.5$, but they did not record precise experimental conditions, nor did they identify this

acidic group, presumably because no comparable ionization was known to occur in that pH region.

The recent work of Hanania et al[32] on this problem has shown the following:

1. In imidazole two acid ionizations are possible:



(1)

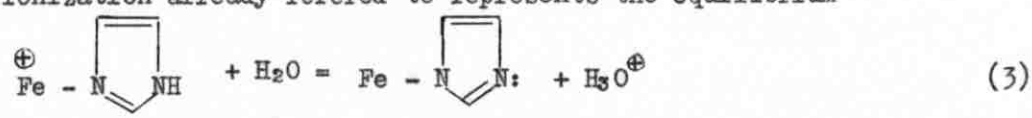
(2)

For the first ionization (eq. 1), that of the imidazolinium $\geq NH^+$ group, the thermodynamic data are well known. At 25°C. $pK^0 = 6.95$, $\Delta H^0 = 7700$ cal.mole^{-1}, $\Delta S^0 = -6$ e.u.[11].

For the second ionization, which is that of an extremely weak acid (eq. 2) Hanania and Irvine[32] obtained the following results at 25°C, $pK^0 = 14.45 \pm .03$, $\Delta H^0 = 17.8 \pm 1.8$ Kcal./mole, $\Delta S^0 = -6 \pm 5$ e.u.

2. In the ferrimyoglobin-imidazole complex, the ionization reported by Russel and Pauling[19] was studied and the corresponding data were found to be: $pK^0 = 10.12$, $\Delta H^0 = 11.2$ Kcal./mole, $\Delta S^0 = -9$ e.u.

From the above data, Hanania and his coworkers suggest that the ionization already refered to represents the equilibrium



(3)

It is interesting in this connection to note that N-methyl-imidazole, which forms a suitable complex with ferrihemoproteins but does not have H on the imino N of imidazole does not show a heme-linked

effect in the same pH range[20]. This supports the view that the heme-linked ionization with pK $\sim$ 10 obtained above, is probably that of the imino =NH group of imidazole as defined in the above equation (eq. 3).

The significance of the results obtained by Hanania et al[32] is threefold:

1. The acid strength of the imino =NH group in imidazole was shown to have been increased by a factor of 10^4 as a result of coordination of imidazole to the Fe(II) ion in ferrimyoglobin.

2. This effect was shown to be largely reflected in enthalpy rather than entropy.

3. The effect is far greater than the usual effects of substitution in aromatic rings on the ionization of acidic functional side groups.

Now, if the above interpretation is correct, then the imino group of imidazole in histidine on the protein side of the heme in hemoproteins should also ionize with a pK $\sim$ 10. B ut there is no evidence at all for any such ionization in ferrimyoglobin or ferrihemoglobin. Clearly the problem is not yet resolved although Abu-Isa[18] in his thesis discusses some possible explanations.

C. Model ligands

For the purpose of the work described in this thesis it was decided to select a number of suitable ligands that can serve as simple model systems on which the effect of coordination can be measured, and where the interpretation of results is definitive.

Several conditions were considered in the selection of model ligands for this study:

1. Nitrogen heterocyclic ligands were to be used, for the following

reasons:

a) Metal chelates of nitrogen heterocyclic ligands resemble those of hemoproteins in structure.

b) Complexes of nitrogen heterocyclic ligands are well colored and quite stable (nitrogen is considered to be a good donor atom).

2. These selected ligands were to have suitable weak acidic groups which can become metal-linked in the complex ion.

3. The resulting metal chelates should be stable over the pH range required to enable study of the ionization of the acidic groups.

4. Ligands that are soluble in aqueous media would be preferred in order to simplify the interpretation of results.

These conditions, combined, are very difficult to meet, especially the question of stability of the complex. Metal chelates, in general, dissociate in strongly acidic solution, consequently the acidic groups on the ligand have to be very weak that in the complexes the ionization occurs in not too acidic a medium. This, however, rules out carboxyl, sulfonic, phosphoric, and similar groups. Instead, the following three types of acid groups were chosen:

Imidazolinium	$\geqslant NH^+$	$pK \sim 7$
Oxime	$=NOH$	$pK \sim 10 - 12$
Imino	$>NH$	$pK \sim 14$

and the selected compounds containing these groups were the following:

Imidazole-4(5)-aldoxime	(Imal)
2,2'-pyridyl imidazoline	(Pim I)
2,2'-pyridyl imidazole	(Pim II)

Pyrrole-2-aldoxime (Pyral)

Pyridine-2-aldoxime (Pal)

Pyridine-2,6-dialdoxime (Dipal)

4,7-dihydroxy-1,10-phenanthroline

Phenyl-2-pyridyl ketoxime

2-(2-pyridyl)benzimidazole

D. Proposed method of study

For examining the effect of coordination to a metal ion on the ionization of acidic side groups in the ligand a method of study may be summarized in the following:

1. The preparation and purification of the ligands.

2. Study of the ionization pattern in each ligand obtained from spectrophotometric measurements over a suitable pH range.

3. Corresponding study of the ionization pattern in the iron(II) chelates of a number of these ligands. This is done by forming the complex in aqueous solution, and following the effect of pH on the color of the complex and other properties.

4. To get the charge types involved, comparative electrophoretic mobilities are measured as a function of pH for the ligand as well as the complex. The results here would help in interpreting the ionization pattern of the ligand as well as the corresponding complex.

5. The next stage, for this work to be complete, is the thermodynamic study of these ionizations, and the calculation of pK^0, $\triangle H^0$, $\triangle S^0$ and probably $\triangle C_p^0$ values for each ligand and its complex. However, this aspect of the work would obviously need special research which is the subject of a whole thesis by itself.

In this work it was not possible to cover all these aspects. Instead, a number of ligands were prepared, characterized, and purified; their spectra recorded; and their Fe(II) complexes studied in a qualitative manner over a wide pH range. The information obtained should therefore be regarded merely as an introduction to the detailed study of the problem at hand.

II. EXPERIMENTAL

The ligands listed above may be divided into two groups: one group of ligands was synthesized, and the other group was purchased and further purified. The first group consists of the following ligands:

1. Imidazole-4(5)-aldoxime (Imal)
2. 2,2'-pyridylimidazole (Pim II)
3. 2,2'-pyridylimidazoline (Pim I)
4. Pyrrole-2-aldoxime (Pyral)

The second group consists of the following ligands:

1. Pyridine-2-aldoxime (Pal)
2. Pyridine-2,6-dialdoxime (Dipal)
3. 4,7-dihydroxy-1,10-phenanthroline
4. Phenyl-2-pyridyl ketoxime
5. 2-(2-pyridyl) benzimidazole

It was originally intended to characterize carefully each new substance obtained. However, in view of the preliminary nature of the work presented in this thesis and because of the pressure of time, this has not yet been done.

The following is an account of the details of synthetic methods used in obtaining crystals of the four ligands that were prepared.

A. Preparation of Ligands

1. Preparation of Imidazole-4(5)-aldoxime

This compound was prepared from fructose as a starting material in three stages:

i) Conversion of fructose into 4(5)-hydroxymethyl imidazole following the classical method described in Organic Syntheses[21,22,23].

ii) Oxidation of 4(5)-hydroxymethyl imidazole to imidazole-4(5)-aldehyde using activated manganese dioxide as selective oxidizing agent following a method suggested by Dr. Issidorides (Department of Chemistry, A.U.B.)[24].

iii) Oximation of imidazole-4(5)-aldehyde in alkaline solution.

i) Preparation of 4(5)-hydroxymethyl imidazole

a) Conversion to picrate

To 222 g (1 Mole) of basic copper carbonate in 5 liter flask was added 1.5 liter of distilled water and 720 g (800 ml.) of 28% ammonia. The bulk of the copper carbonate was brought into solution by swirling; 112 g (100 ml.) of 37 - 40% formaldehyde and 90 g (0.475 mole) of commercial 95% fructose were added. The solution was mixed well and placed on a steam-bath under a hood. After 30 minutes of heating with occasional shaking, a moderate current of air was bubbled through the solution and heating was continued for 2 hours longer. The reaction mixture was chilled in an ice bath for 3 hours and the olive-brown precipitate of the copper complex of the imidazole derivative was filtered off and washed with 500 ml. of cold water, then suspended while moist in 1 liter water and made slightly acidic by addition of concentrated hydrochloric acid (about 40 ml.). H_2S gas was passed through the solution with

frequent shaking until copper sulfide precipitated out (2-3 hours). The precipitate was filtered and extracted with 500 ml. of hot water in two or three portions. The filtrate, light-brown to reddish-brown, was boiled for 15 minutes, and then 60 g (0.26 mole) of picric acid was added with stirring: heating was continued until solution was complete.

The greenish yellow plates, which separate as the solution is cooled to room temperature, were filtered, washed 3 times with 150-200 ml. portions of water, and air-dried. The filtrate and first washings were combined and heated, 10 g of picric acid were added, and the mixture was cooled and filtered. This process was repeated until the air-dried picrate fraction so obtained melted below 195°. All fractions melting above 200°C. were combined and recrystallized from water by adding 700 ml. of water for each 30 g of crystals and treating with charcoal and filtering through a warm funnel. The crystals deposited upon the slightest cooling, the yellow needles were filtered, washed, and air-dried. The yield of crude picrate obtained was 93.1 g which makes 60% yield (reported yield 61-64%)[21].

b) Conversion of the picrate derivative of 4(5)-hydroxy methyl imidazole to the chloride salt.

66.5 ml. concentrated hydrochloric acid, 166 ml. of water, and 332.5 ml. of benzene were placed in a 2 liter round-bottomed flask immersed in a water bath maintained at 80°C. 66.5 g of pure picrate were added to this mixture with stirring until the picrate dissolved. The benzene layer was decanted and the aqueous layer extracted 5 times with 220 ml. portions of benzene, treated with about 3 g of charcoal and filtered. The clear, pale yellow filtrate was evaporated to dryness

at 60-70°C. by first using an aspirator pump and then the vacuum pump. The resulting crystals were dissolved in a minimum quantity of hot absolute ethanol. Colorless needles deposited on cooling. Three to four volumes of ethyl ether were added, and the mixture was kept in the refrigerator overnight. The crystals were filtered, washed with little ether and dried in a vacuum desiccator. 21 g of the chloride salt were obtained representing a yield of 80% (reported yield 90-95%)[21].

c) Isolation of 4(5)-hydroxymethyl imidazole from its chloride salt

21 g of 4(5)-hydroxymethyl imidazole hydrochloride were dissolved in the minimum amount of water and excess sodium carbonate was added. The solution was evaporated to dryness under reduced pressure and the residue extracted several times with absolute ethanol and the extract was also evaporated nearly to dryness whereupon crystals began to deposit. After cooling in the refrigerator, they were collected and recrystallized from isopropyl alcohol. The melting point of the product is 91-92° (reported 92-94°C.)[23].

ii) Oxidation of 4(5)-hydroxymethyl imidazole to imidazole-4(5)-aldehyde

The classical methods of oxidation, namely, the chromic acid and nitric acid oxidations reported by Pyman[25,26,27] were not satisfactory and gave rather low yields. Also, the classical methods of oxidation often carry the oxidation one step further to the imidazole-4(5)-carboxylic acid which is difficult to separate from the desired product. However, upon the suggestion of Dr. Issidorides (Department of Chemistry, A.U.B.)[24] it was decided to try the selective oxidation method with

especially prepared manganese dioxide which was suitable for the oxidation of allylic alcohols to allylic aldehydes[28].

a) Preparation of manganese dioxide[24,28]

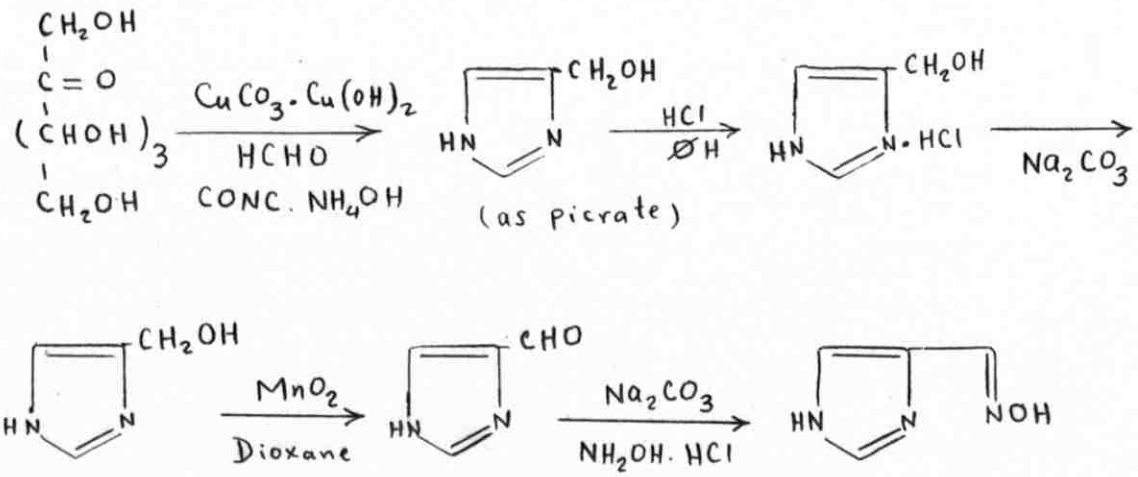
A solution of $MnSO_4.4H_2O$ (70 g) in water (1959 ml.) approximately 50% saturated was heated to about 90°C over a water bath. To the hot solution was added a concentrated solution of potassium permanganate (38 g) with sufficient mechanical stirring. Stirring was continued for 15 minutes at about 90°C. Filtration, followed by thorough washing with hot water and drying to constant weight gave a brown solid which could be powdered easily. A black hard solid MnO_2 was found to be unsuitable as selective oxidizing agent.

b) Oxidation of 4(5)-hydroxymethyl imidazole to imidazole-4(5)-aldehyde with MnO_2

1.5 g of pure 4(5)-hydroxymethyl imidazole were dissolved in 80 ml. (1:53) of pure dioxane (spectral grade) at 60-70°C. over a water bath. To the hot solution which was mechanically stirred, 15 g of specially prepared MnO_2 were added and stirring continued for 4 hours at 80°C. The reaction mixture was filtered off and the precipitate thoroughly washed with hot dioxane (4 x 25 ml.). The filtrate was evaporated to dryness under reduced pressure and the solid residue washed with 80 ml. of ethyl ether to remove traces of dioxane. The yield of crude imidazole-4(5)-aldehyde was 0.8 g (53%) and melted at 172-174°. Crystallization of the product was effected from a mixture of a minimum hot absolute ethanol and excess petroleum ether (b.p. 40-70°C.) until cloudiness. The melting point of pure crystallized imidazole-4(5)-aldehyde was 173-174°C.

iii) Conversion to imidazole-4(5)-aldoxime

The pyridine method of oximation did not work out. As an alternative method, the oxime was prepared in alkaline carbonate solution. 0.48 g of imidazole-4(5)-aldehyde and 0.35 g of hydroxylamine hydrochloride (1:1 molar ratio) were dissolved in a minimum amount of water. 0.80 g of sodium carbonate (1:1½ molar ratio) was added to the solution and the resulting mixture was kept at room temperature for 2 days. The mixture was evaporated to dryness under reduced pressure and the residue extracted with 10 ml. of absolute ethanol 4 times and the ethanol extract was evaporated almost to dryness. Crystals deposited on cooling. The product was recrystallized from a minimum amount of absolute ethanol including treatment with charcoal to remove colored impurities. The crude product weighed 0.49 g (88% yield) and melted at 177-181°. After recrystallization, the melting point rose to 181-182° (reported value 183-184°)[25]. The reaction is written as follows:



2. Preparation of 2,2'-pyridyl imidazole

This compound was prepared from pyridine 2-aldehyde and tartaric acid dinitrate as starting materials in two stages:

i) Condensation of pyridine 2-aldehyde and tartaric acid dinitrate into pyridine 2-(imidazole-4,5-dicarboxylic acid) following a procedure similar to that reported by Fargher et al[29] for the preparation of 2-phenyl imidazole.

ii) Decarboxylation of pyridine 2-(imidazole-4,5-dicarboxylic acid) by dry distillation.

i) 20.6 g of powdered tartaric acid were dissolved in 89 ml. of fuming HNO_3 and then 103 ml. of conc. H_2SO_4 were added gradually during which process crystals started to deposit, then it was left in the refrigerator overnight, collected, and washed with about 60 ml. of 50% sulfuric acid. The tartaric acid dinitrate so formed was immediately stirred with 124 g of crushed ice and immersed in a mixture of ice and salt to dissolve the tartaric acid dinitrate, whereupon, it was poured into a round-bottom flask immersed in a freezing mixture and then 82.5 ml. of concentrated aqueous ammonia were added dropewise keeping the temperature always below zero. 16.5 g of pyridine-2-aldehyde (freshly redistilled to give b.p. 176-177°C) were added slowly. The mixture was mechanically stirred for 7 hours allowing the temperature to approach room temperature gradually. After keeping the mixture overnight, crystals were collected and air-dried then put in a vacuum desiccator. The solid-dried cake weighed 12 g (35% yield).

ii) In a 200 ml. round-bottom flask carrying an 18" Vigreux

Column and a condenser set for distillation was placed 12 g of pyridine 2-(imidazole-4,5-dicarboxylic acid) . The flask was heated with a small flame and decarboxylation took place smoothly. The product was distilled under vacuum to yield a gum-like distillate sticking to the side of the Vigreux column which was difficult to scrape out. The distillate was extracted several times with petroleum ether (b.p. 40-70°). The combined extracts were collected, treated with charcoal, filtered, and the filtrate left overnight in the refrigerator whereupon crystals deposited. The product was recrystallized from light petroleum ether (b.p. 40-70°) and the melting point was found to be (126-127°) which compound we called Pim II B.

(Note 1): When the above decarboxylation was carried out at atmospheric pressure a different product was obtained and melted at 74-75° which we called Pim II A. This was an unexpected result and an effort to investigate this problem further is being made. For the present purposes we may include the following observations:

1. Both products form red colored complexes with Fe(II) and they seem to have similar chelating properties.

2. Their ultraviolet spectra (200 - 350 mu) are quite distinct.

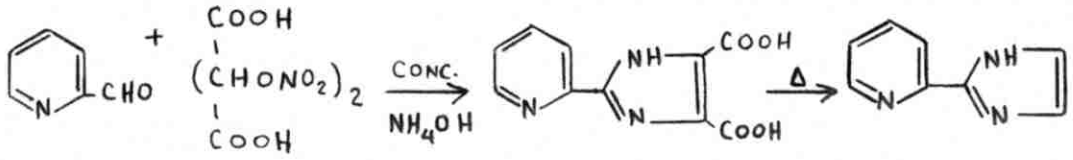
3. Their ionization patterns are also different.

4. The melting points of the two species differ widely.

However, it is intended to test their charges (by comparing their electrophoretic mobilities) and to run their infrared spectra in the hope of proving the presence or absence of carboxylic groups.

(Note 2): In this connection reference should be made to the work of Holmes et al[3]. In their recent paper, these authors report the synthesis of 4,2'-pyridyl imidazole (m.p.111.5°) following the method of Clemo et

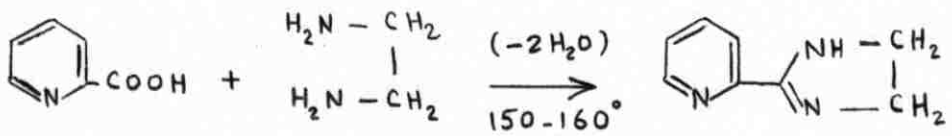
al[30]. This substance is an isomer of our compound 2,2'-pyridyl imidazole (m.p. 74-75° or 126-127°). In the same paper, however, the structure of the copper complex of 4,2'-pyridyl imidazole is shown as that of 2,2'-pyridyl imidazole which is probably an error the authors committed in preparing the manuscript.



3. Preparation of 2,2'-pyridyl imidazoline

This compound was prepared by condensing ∝ -picolinic acid with ethylene diamine at 150-160° according to the following reaction scheme:

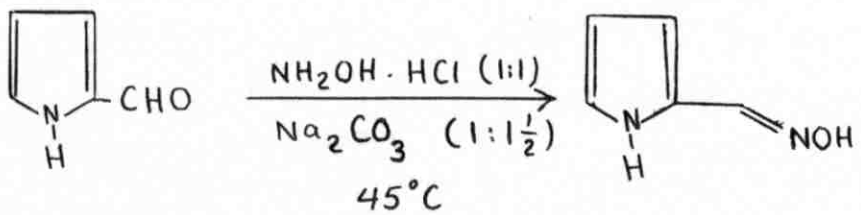
61.5 g (0.5 mole) of ∝ -picolinic acid and 30 g (0.5 mole) of ethylenediamine were allowed to react in a distilling flask immersed in an oil bath maintained at a temperature of 150-160°C for 4 hours. The flask was then stoppered and the product distilled under atmospheric pressure using direct heat. The resultant product was a thick, green oil which was induced, by scratching the side of its container, to crystallize as white solid crystals. The melting point of crude 2,2'-pyridyl imidazoline was 80-84°, and weighed 17 g (23% yield). The crude product was recrystallized twice from petroleum ether (b.p. 100-120°C.) giving a melting point of 95-97°C. (reported m.p. 96-97°C.).



4. Preparation of pyrrole-2-aldoxime

This compound was prepared from pyrrole-2-aldehyde as a starting material by treatment with hydroxylamine hydrochloride (1:1 molar ratio) in alkaline carbonate medium (1:1½ molar ratio) according to the following reaction scheme:

2.85 g of pyrrole 2-aldehyde were dissolved in 30 ml of distilled water and 2.1 g of hydroxylamine hydrochloride were dissolved in 10 ml. of distilled water. The two solutions were mixed and warmed to 45°C. over a water bath, and then shaken thoroughly. 3.2 g of sodium carbonate were dissolved in 15 ml. of distilled water and added to the solution mixture already prepared. A precipitate immediately formed. The oximation mixture was left standing overnight at room temperature, put in the refrigerator for 4 hours, and then the solid crystals were filtered off. The crude product of pyrrole 2-aldoxime weighed 2.80 g (85%) and melted at 142-147°C. The compound was recrystallized twice from chloroform giving a melting point of 163° (reported m.p. 164.5°C.)[31].



B. Spectra

As a background to the measurement of ionization constants, the general ionization pattern in these ligands was established spectrophotometrically, that is from the measurement of absorption spectrum over a wide range of pH values.

First, the ligands were purified to a constant melting point and then stock solutions, about 10^{-3}M, were prepared using deionized redistilled water throughout. A series of buffer solutions as well as strong acid and base were prepared with no special attention to keeping constant ionic strength. The buffer solutions were usually as follows:

2N-NaOH
pH 12 (phosphate)
pH 11 (carbonate)
pH 10 (borate)
pH 9 (borate)
pH 8 (borate)
pH 7 (phosphate)
pH 2 (HCl-KCl)
2N-HCl
6N-HCl

In some cases, the original stock solution had to be diluted in order to keep the absorbancy within a measurable range namely from zero to one. All the measurements made were taken after putting both the solution and its blank in matched quartz cuvettes of 1.0 cm optical path length. The cuvettes were checked at the start of each experiment by running the spectrum of water against water, and the readings were adjusted accordingly.

For taking the spectra of the ligands, solutions in every case were made and blanked against the buffer as follows:

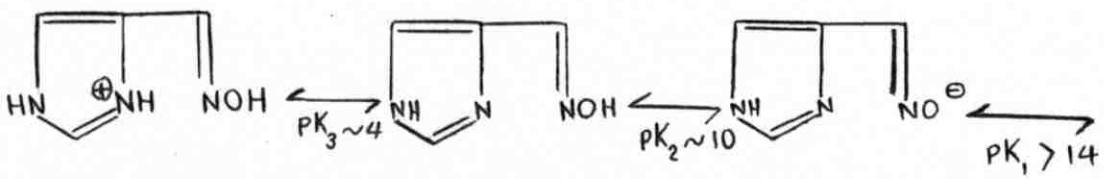
Solution: 1.0 ml. (stock solution) + 9.0 ml. (buffer).

Blank: 1.0 ml. (water) + 9.0 ml. (buffer).

The spectra were recorded automatically on a Bausch & Lomb 505 Spectrophotometer covering the wave length range from 200 to 350 mμ which is the region of interest. As a check, some of the spectra were also taken on Unicam S.P. 500 quartz spectrophotometer and on Zeiss PMQII Spectrophotometer. It is interesting to note, however, that small discrepancies were observed between the recording and non-recording spectrophotometers (absorbancy discrepancy about 4% higher on the non-recording than the recording, and positions of the bands about 1 mμ shorter on the recording instruments). Special care was exercised to get maximum precision and reproducibility of results. The spectra of duplicate solutions of some ligands were prepared under exactly the same conditions and the reproducibility of the results obtained was found to be about 0.6%. On the following pages are given the spectral characteristics of five ligands at various pH values showing the positions of the maxima, minima, and the shoulders (if any) with the corresponding values for the molar extinction coefficients. The ionization pattern with the probable pK values are also shown schematically in each case.

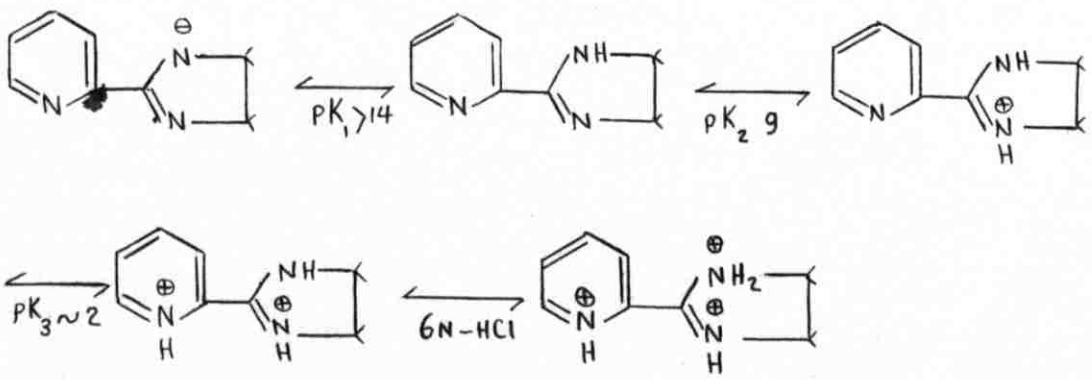
Imidazole-4(5)-aldoxime (IMAL)

pH	Maxima		Shoulder		Minima	
(approx.)	λ,mμ	ε ($M^{-1}cm^{-1}$)x10^{-4}	λ,mμ	ε ($M^{-1}cm^{-1}$)	λ,mμ	ε ($M^{-1}cm^{-1}$)x10^{-4}
2N-NaOH	273	1.225			237	0.374
12	265	1.225			228	0.374
9	255	1.165			215	0.367
7	254	1.206			215	0.360
W	253	1.165			214	0.388
2	245	1.210			202	0.338
2N-HCl	243	1.091			209	0.461
6N-HCl	243	1.070			212	0.461



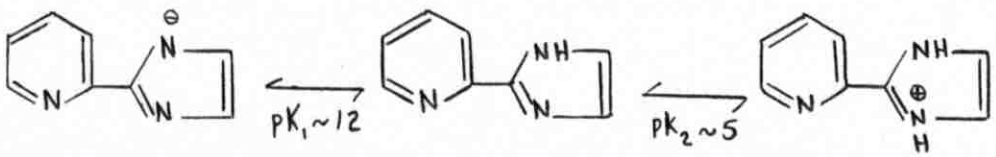
2,2'-pyridyl imidazoline (PIM I)

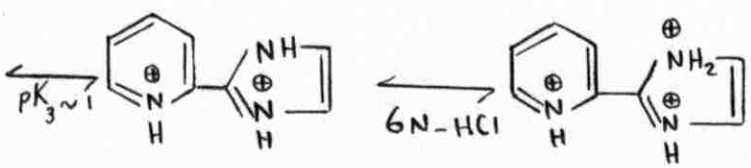
pH	Maxima		Shoulder		Minima	
(approx.)	λ, mμ	ε ($M^{-1}cm^{-1}$)x10^{-3}	λ, mμ	ε ($M^{-1}cm^{-1}$)	λ, mμ	ε ($M^{-1}cm^{-1}$)x10^{-3}
11	268	4.47			246	2.52
	222	7.30				
7	267	6.17			247	3.39
	228	8.17				
2	267	6.09			247	3.13
	228	7.90				
2N-HCl	267	6.78			247	3.44
	228	7.40				
6N-HCl	272	2.41			258	1.83
	230	3.18				



2,2'-pyridyl imidazole (PIM II A)

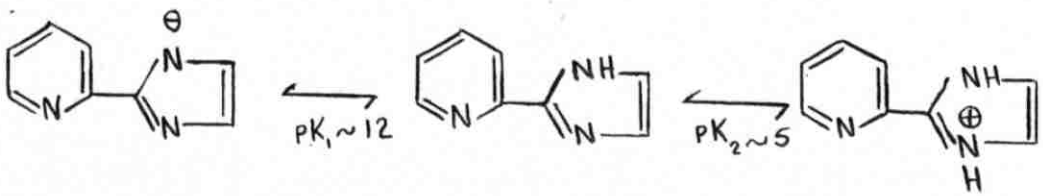
pH	Maxima		Shoulder		Minima	
(Approx.)	λ,mμ	$\epsilon(M^{-1}cm^{-1})x10^{-4}$	λ,mμ	$\epsilon(M^{-1}cm^{-1})x10^{-4}$	λ,mμ	$\epsilon(M^{-1}cm^{-1})x10^{-4}$
2N-NaOH	304	0.606			280	0.495
"	272	0.576				
"	266	0.562			239	0.222
"	223	0.465				
12	293	0.697	272	0.702	280	0.606
"	267	0.758			239	0.318
"	217	0.778				
10	293	0.707	272	0.702	280	0.606
"	267	0.758			239	0.318
"	217	0.748				
8	293	0.707	272	0.702	280	0.606
"	267	0.758			239	0.318
"	217	0.738				
6	288	0.662	272	0.702	281	0.635
"	267	0.758			238	0.390
"	218	0.707				
2	266	0.975	297 283 273	0.454 0.758 0.925	232	0.414
2N-HCl	267	0.920	285	0.658	233	0.328
"	209	0.596	272	0.880		
			297	0.526		
6N-HCl	302	0.611			285	0.494
"	268	0.757	273	0.718	238	0.344
"	213	0.672				

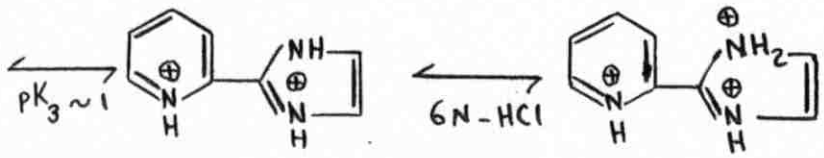




2,2'-pyridyl imidazole (PIM II B)

pH	Maxima		Shoulder		Minima	
(Approx.)	λ,mμ	ε ($M^{-1}cm^{-1}$)x10^{-4}	λ,mμ	ε ($M^{-1}cm^{-1}$)x10^{-4}	λ,mμ	ε ($M^{-1}cm^{-1}$)x10^{-4}
2N-NaOH	220	0.339			237	0.229
"	303	1.13				
12	210	1.143	265	0.888	230	0.242
"	293	1.273				
10	203	0.662	265	0.880	230	0.194
"	293	1.272				
8	203	0.674	267	0.888	230	0.215
"	293	1.261				
6	203	0.678	267	0.888	227	0.242
"	289	1.192				
2	255	0.98			219	0.169
"	285	1.290	297	0.780	267	0.910
2N-HCl	255	0.685	296	0.645	219	0.086
"	286	1.080	310	0.450	265	0.645
6N-HCl	211	0.580			236	0.396
"	252	0.468			266	0.404
"	302	1.14				





Pyrrole-2-aldoxime (PYRAL)

pH	Maxima		Shoulder		Minima	
(Approx.)	λ, mμ	ε ($M^{-1}cm^{-1}$)x10^{-4}	λ, mμ	ε ($M^{-1}cm^{-1}$)x10^{-4}	λ, mμ	ε ($M^{-1}cm^{-1}$)x10^{-4}
2N-NaOH	283	1.750	290	1.6		
12	280	1.712	310	0.152		
9	277	1.745	310	0.152		
7	277	1.720	310	0.152		
W	277	1.750	310	0.152		
2	279	1.17			295	0.875
2N-HCl	312	2.40	279	0.495		
6N-HCl	314	2.52	279	0.495		

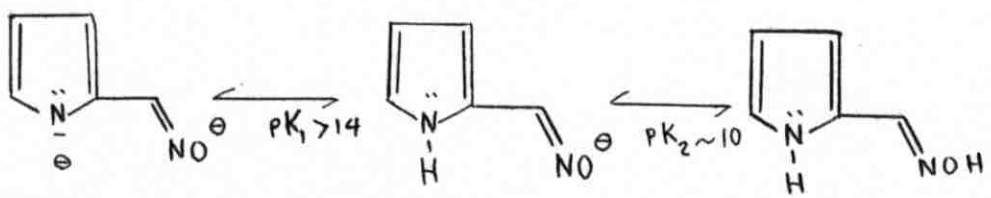


Table 1

Summary of Ionizations on Ligands

	$\geq$NH (imino)	=NOH (oxime)	$\overset{\oplus}{\geq}$NH (imidazolinium)	$\overset{\oplus}{\geq}$NH (pyridinium)	$\overset{\oplus}{}$NH$_2$
PIM I	14	-	9	2	2-6N HCl
PIM II A	12	-	5	1	2-6N HCl
PIM II B	12	-	5	1	2-6N HCl
IMAL	12	10	4	-	-
PYRAL	14	10	-	- (3)	-
PAL	-	10	-	3.5	-

C. Metal Chelates

Having examined the ionization patterns in the ligands, which are summarized in Table 1 above, the next stage was the study of the metal chelates of these ligands with a view to finding any ionization in the complex ion corresponding to that in the ligand.

In the present work, it was possible to do only a qualitative survey of some of the chelates, mainly with iron(II). There is an enormous amount of work left to be done on this aspect in the future.

In two cases, those of pyridine-2-aldoxime and 2,2'-pyridyl imidazoline, Hanania and Irvine[1,35] have described the Fe(II) complexes in detail. For the rest of the ligands, practically no information whatever was available. Iron(II) and (III), nickel(II), and Copper(II) metal ions were treated with some of the ligands in aqueous solution using deionized redistilled water. The color, ease of formation of complex, the speed of the reaction, and the stability of the colors formed were noted. The effect of acid and alkali was also examined and in case there was a color disappearance conditions were reversed to see if the color can be produced again (reversibility of ionic equilibria).

1. Pyridine-2-aldoxime (PAL)

Iron(II): forms well defined colored complex rather easily in aqueous solution. The ferrous complex is violet-red in alkaline solution, reddish-orange in slightly acidic solution, and golden yellow below pH 3. All color transformations were reversible with no sign of precipitation.

Iron(III): forms well defined colored complex very easily in dilute aqueous solution which is relatively stable. The ferric complex

is reddish-brown in alkaline, neutral and slightly acidic solution and yellow in more acidic solution. All color changes were reversible without any sign of precipitation.

Nickel(II)): forms a light-yellow colored complex with the addition of slight excess of the ligand. The Nickel(II) complex is orange in alkaline solution up to pH 12 and fades away in acidic solution (pH $<$ 3). Color transformations were not reversible although there was no precipitation.

Copper(II): forms a light-green colored complex upon addition of excess ligand which is not stable. The copper(II) complex is greenish in alkaline solution even in strong alkaline medium (2N-NaOH), light-green in slightly acidic solution (pH $\sim$ 4). All color changes were reversible without any precipitation.

2. Pyridine-2,6-dialdoxime (DIPAL)

Iron(II): forms orange-red colored complex easily in aqueous solution which is quite stable. The ferrous complex is brownish-red in alkaline solution up to pH $\sim$ 12, brownish in slightly acidic (pH $\sim$ 5) becoming rosey-violet in acidic (pH $>$ 2) solutions and the colors seem to be quite stable. Two changes in color were observed: one in the pH range 2-4, and the other in the pH range 6-8, without any sign of precipitation.

Iron(III): forms a yellow colored complex easily in aqueous solution which is fairly stable. The ferric complex is light yellow in alkaline solution up to pH 12 and light yellow in slightly acidic solution. Color changes were irreversible without any precipitation.

Nickel(II): forms a light yellow colored complex in aqueous

solution upon addition of slight excess of the ligand. The nickel(II) complex is yellowish in slightly alkaline solution up to pH $\sim$8, and dissociates completely in slightly acidic solution (pH$\sim$4). Color transformations were irreversible without any precipitation.

Copper(II): forms a light green colored complex quite easily but not stable enough. The copper(II) complex is greenish in alkaline solution. Color transformations were reversible without any sign of precipitation.

3. 2,2'-pyridyl imidazoline (PIM I)

Iron(II): forms a deep violet colored complex in aqueous solution quite readily and the colour is stable. The ferrous complex is violet in slightly alkaline (pH $\sim$8), becoming light violet in strong alkaline solutions (pH$\sim$12), turns violet upon addition of excess alkali and the color transformation was not reversible. In slightly acidic solution (pH$\sim$4) the color becomes light violet which turns violet upon addition of a base.

Iron(III): forms a fairly stable yellow colored complex readily in aqueous solution. The ferric complex fades away in alkaline and slightly acidi solution. Color changes were irreversible showing no sign of precipitation.

Nickel(II): forms a yellow colored complex in dilute aqueous solution very slowly and with addition of excess ligand. The nickel(II) complex is light yellow in alkaline solution, faintly yellowish in slightly acidic solution. Color transformations were not all reversible and showed no sign of precipitation.

Copper(II): forms a blue colored complex readily in dilute

aqueous solution. The copper(II) complex is light bluish in alkaline and acidic solutions. Color transformations were not reversible.

4. 2,2'-pyridyl imidazole (PIM II A)

Iron(II): forms a stable orange colored complex with excess ligand in aqueous solution becoming pale green in alkaline (pH 8-10). In slightly acidic solution (pH$\sim$6) the color changes probably to deep orange and dissociates into pale yellow, then turns very pale at around pH 9. Color transformations were irreversible with no sign of precipitation being observed.

Iron(III): forms a yellow colored complex very readily in aqueous solution. The ferric complex is light yellow in alkaline solution and dissociates completely in acidic solution. Color transformations were not reversible and showed no sign of precipitation.

Nickel(II): forms no colored complex even with the addition of excess ligand and metallic salts.

Copper(II): forms a light blue colored complex readily in dilute aqueous solution. The nickel(II) complex does not change color in alkaline or in slightly acidic solutions. Color transformations were not reversible, and no precipitation was observed.

5. 2,2'-pyridyl imidazole (PIM II) B)

Iron(II): forms red colored complex in aqueous solution and is quite stable. The color turns dark red in neutral solution with precipitation; becoming reddish in alkaline solution (pH 12) but dissociates instantly. In slightly acidic solution the color dissociates, then forms orange color at pH$\sim$6 turning to pale violet in slightly alkaline solution but not stable enough. Color transformation were

partially reversible with no sign of precipitation.

6. Imidazole-4(5)-aldoxime (IMAL)

Iron(II): forms deep orange colored complex upon addition of excess ligand in aqueous solution. In slightly alkaline solution, the color becomes pale and cloudy. At pH 10 it becomes deeper with less cloudiness, and in strong alkaline solution (pH $>$11) the color becomes pale violet and dissociates instantly, then becoming clear rose orange at pH $>$ 12. In slightly acidic solution (pH 4) the color is the same, then turns to weak yellowish orange (pH 3) and the color disappears completely at pH 2.

7. Pyrrole-2-aldoxime (PYRAL)

Iron(II): forms dark violet colored complex upon addition of excess ligand and little dioxane because the ligand dissolves in water with difficulty. In neutral medium (pH 7) the color becomes brownish, turns to brownish red in alkaline (pH 10) giving a stable red color in strong alkali (pH $>$ 12). In slightly acidic solution (pH $\sim$ 4) the color turns pale becoming pale red when alkali is added to it which is stable for quite a while. Color transformations are partially reversible with no sign of precipitation.

Table 2

The Iron(II) Complexes of Some Ligands Examined

Ligand	Fe(II) Complex in water	Effect of Acid	Effect of Base
PAL	Red, stable complex	Yellow, dissociates pH $>$ 3	Violet
DIPAL	Orange red, stable complex	Rosy violet pH $>$ 2, stable; pH 5 brown	Brownish red, dissociates pH $>$ 12
PIM I	Deep violet, stable complex	Light violet pH 4	Light violet dissociates pH $>$ 12
PIM II A	Orange, stable complex with excess ligand	Deep orange, dissociates pH $<$ 6	Pale green (pH 8-10) dissociates
PIM II B	Red, stable complex, with precipitation in neutral solution	Dissociates in slightly acidic solution	Reddish pH $>$ 10 but dissociates
IMAL	Deep orange with excess ligand	Yellowish orange pH 3 but dissociates	Pale orange and cloudy (pH 8): pale violet (pH) 11) and dissociates rapidly
PYRAL	Dark violet with excess ligand and addition of little dioxane	Pale violet (pH 4)	Brown red (pH 10); reddish (pH $>$ 12) and is quite stable

III. DISCUSSION

The work described in this thesis was not so much concerned with the question of stability, specificity, or sensitivity of color tests for metal ions, important as these topics are. Rather, it may be regarded as an introductory work for the study of the effect of coordination to metallic cationson the ionization of appropriate acidic side groups in nitrogen heterocyclic ligands.

In the introduction (page 9) it was stated that model ligands for this study were selected satisfying certain, requirements, the most exacting of which was the choice of ligands with very weak acidic groups. It may be helpful to review the reasons for this choice of model systems.

1. Physiological pH is approximately neutral, and the main heme-linked phenomena of biological significance occur around the same neutral range of pH. If these phenomena represent metal-linked ionizations, it follows that the ionizing groups in the un-coordinated ligands would be expected to be very weak acids with very high pK's. Hence the choice of imino, oxime, and similar groups.

2. Fortunately, complex ions also tend to be relatively stable in neutral solutions, the region in which the ionizations of the complex ions were to be studied. If, however, one were to choose ligands having acidic groups with pK around 7 or less, then in the complex ion the corresponding ionization would have a pK value of 3 or less, a strongly acidic region in which complex ions usually dissociate. It would then be extremely difficult to measure pK values. Thus, sulfonic, carboxylic, and

similar groups were not considered.

Now, we are in a position to discuss the factors which contribute to the overall effect of coordination on ionization in the systems studied. In general, the stability of metal chelates is determined by a number of factors, and Martell[34,35] gives the following:

1. Basicity of the ligand.
2. Resonance effects.
3. Number of metal chelate rings per ligand.
4. Size of chelate ring.
5. Steric effects.
6. Specific effects.

The first two of these, basicity of the ligand and the resonance effects, may be grouped together as concerned with conjugation. This is because both relate to the availability of the lone pair of electrons on the nitrogen for coordination purposes. Factors 3 and 4 are constant in this study, where the ligands form octahedral five-membered chelate rings with the metal ion. The fifth factor is variable and is dealt with specifically in some cases. The only other effect, the sixth factor, which is also variable, may be attributed to the electrostatic charge on the complex ions being different in each case depending on the number and nature of the ionizable side groups on the ligand, and depending also on the charge on the metal ion used for chelation. Hence, only three main factors need be considered here:

1. Conjugation (resonance) effect.
2. Charge (electrostatic) effect.
3. Steric (structural) effect.

Charge and conjugation effects: Hanania et al[1] have considered these two factors in detail, using pyridine-2-aldoxime (PAL) as a model system for their study. They observed two series of spectral changes corresponding to two acid ionizations one with pK = 3.2 associated with the pyridin-

ium $\rangle NH^+$ nitrogen, and the other with pK = 10.2 which is probably the ionization of the oxime =NOH group. The normal acid pK value for pyridine nitrogen is 5.3 shifting to 5.8 - 6.0 in some pyridine-2-derivatives[36]. For the ionization of the oxime =NOH group in the free ligand they obtained the following thermodynamic constants at 25°C and zero ionic strength: $pK^0 = 10.22 \pm 0.02$, $\Delta H^0 = 6.8 \pm 0.8$ Kcal/mole, and $\Delta S^0 = -24 \pm 2.8$ e.u. For tris-(pyridine-2-aldoxime)-Iron(II) complex they obtained the following values for the ionization of the oxime =NOH group at 25°C and zero ionic strength: $pK^0 = 7.13 \pm 0.02$, $\Delta H^0 = 0.8 \pm 0.4$ Kcal/mole, and $\Delta S^0 = -30 \pm 2$ e.u.

Comparing the two sets of data, we find that the acid strength of the oxime =NOH group has increased about 10^3 times as a result of coordination to the metallic cation and that the heat of ionization has changed from 6.8 to 0.8 Kcal/mole, the entropy of ionization remaining unchanged.

A similar study was made by Hanania et al[1] on 2,2'-pyridyl imidazoline (PIM I). Theyobtained the following thermodynamic constants for the ionization of the imidazolinium $\rangle NH^+$ group in the free ligand at 25°C and zero ionic strength: $pK^0 = 8.92 \pm 0.02$, $\Delta H^0 = 13.7 \pm 0.9$ Kcal., $\Delta S^0 = -5 \pm 3$ e.u. For tris-(2,2'-pyridyl imidazoline)-iron(II) complex they obtained the following values for the ionization of the imidazolinium $\rangle NH^+$ group at 25°C and zero ionic strength: $pK^0 = 6.09 \pm 0.03$, $\Delta H^0 = 6.9 \pm 0.5$ Kcal/mole, $\Delta S^0 = -5 \pm 2$ e.u.

From the above data, it is obvious that the acid strength of the imidazolinium $\rangle NH^+$ group has increased about $10^{2.8}$ times as a result of coordination, and that the heat of ionization has decreased by 6.8 Kcal/

mole while the entropy of ionization remained practically constant. The above results are summarized in Table III.

Table 3

pK values and charge types of the conjugate acids referring to the ionization in the free ligand and in the corresponding iron(II) complex of two groups: a) oxime =NOH in PAL, b) imidazolinium $\rangle NH^+$ in PIM I (data from Hanania and Irvine[1]; for formulas refer to Index to Formulas page viii).

	Ligand		Fe(II) Complex					
			1st ioniz.		2nd ioniz.		3rd ioniz.	
	Charge	pK	Charge	pK	Charge	pK	Charge	pK
a) PAL	0 → -1	10	+2 → +1	< 1	+1 → 0	3.5	0 → -1	7
b) PIM I	+1 → 0	9	+2 → +1	6.8				

Comparing the pK value for (PAL) in the free state with that for the third ionization in the complex we observe that the charge types involved are the same and that the pK value became three units less. This can only be attributed to stabilization of the conjugate base in the complex through resonance.

In the case of (PIM I), the charge type in the free ligand is +1 → 0 while that of the complex is +2 → +1, hence, there is an increase of one positive charge. The pK value drops from 9 in the free ligand

to 6.8 in the complex. This change in the pK value by 2.8 units is probably an electrostatic charge effect on account of the fact that the conjugation factor is not favored in this case, since electronic mobility is blocked because of the aliphatic character of the imidazoline ring. Increase of positive charge on the complex tends to increase the acid strength of the ionizing imidazolinium $\rangle\!\!\rangle NH^+$ group probably through a direct electrostatic mechanism.

Let us now consider the case where both charge and resonance factors concur. Reference to table 3 shows that the second ionization in the (PAL) complex has a charge type of $+1 \rightarrow 0$ compared to $0 \rightarrow -1$ in the free ligand; an increase of one unit positive charge. The pK value for the ionization of the oxime group in the free ligand is 10 and the pK value for the second ionization in the complex is 3.5 with a decrease of 6.5 pK units. The tremendous increase in the acid strength of the oxime =NOH group in (PAL) as a result of coordination to a metal ion in this case is due to the cumulative effect of both the charge and conjugation. An increase of one positive charge unit in the complex stabilizes the conjugate anion because of electrostatic interaction and this stabilization is further enhanced as a result of resonance stabilization in the conjugate anionic base in the complex.

Steric factor: In general, steric factors are of two kinds, one related to the radius of the cation and one to structural features of the chelating agent. On the whole maximum stability for complexes results when the normal coordination position of the metal ion favours the positions consistent with the structural features of the chelating agent.

Courtauld atomic models were used in building the various

ligands used in this study, and then in testing the strain which results on forming octahedral complexes from iron(II) and each of the ligands in turn. The ligands could be divided into three groups on the basis of the ease of forming molecular models of the complex ion:

1. The feroin group of ligands which are not part of this work, represented by 2,2'-bipyridyl and 1,10-phenanthroline. Here the angles on the two nitrogen atoms were disposed nearly correctly for octahedral bonding with the central iron atom. These compounds are well known for their chelating properties toward iron[4].

2. IMAL and PYRAL in which the disposition of the nitrogen angles was far from that required for octahedral bonding. Considerable strain was needed to force the atomic models into the structure expected for the complex.

3. Most of the remaining ligands were in an intermediate position for this point of view, the angle between their nitrogens being not much greater than the 90^{o} required for octahedral bonding. The strain in the bonding was moderate.

It is interesting to note that IMAL and PYRAL were the two weakest ligands studied, which accords with the fact that their nitrogens are at too wide an angle for stable coordination. PYRAL, however, was very stable in highly alkaline solution. This is really a chemical not a structural effect, the stability arising from the shift of equilibrium in favor of the complex through the removal of H^{+}, one of the products of the reaction:

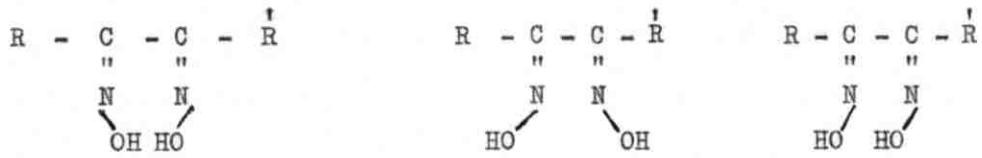
$$Fe^{+2} + 3(H\text{-}PYRAL) = \left[Fe(PYRAL)_3\right]^{-} + 3H^{+}$$

The majority of the other compounds were reasonably good ligands,

and it is possible that one of them may turn out to be a highly successful analytical reagent.

One of the questions which arise in connection with the discussion of structure for these complexes is that of bonding. Is the metal bonded to nitrogen or to oxygen or to both? There is ample evidence to substantiate the fact that the metal ion is linked to the ligand through two nitrogens and not through one nitrogen and one oxygen. The following are some of the lines of evidence in this regard:

1. Martell and Calvin[37] have indicated that of the three possible isomers of dialkylglyoxime, namely:



(Syn) (Anti) (Amphi)

only the anti isomer forms chelates with metals. By analogy one would expect that in the case of PAL, and the other ligands which form 5-membered rings, only the anti form will complex, chelation occuring between the metal ion and the two nitrogen atoms.

2. Krause and Busch[38] prepared the nickel(II) complex of PAL and compared it with the corresponding complexes of 2,2'-bipyridyl and dimethyl-glyoxime, and they argued for nitrogen bonding to nickel in the complex.

3. The red or violet color of the iron(II) complexes of most of the ligands studied is similar to that of ferrous complexes of oxime groups in which the metal-ligand bonding is known to be between two nitrogens[39,40].

4. The infrared spectrum of PAL in chloroform was examined

by Hanania and Irvine[33] and was found to have a sharp band at 2.77 μ which is characteristic of a free OH group. This favors the syn configuration and hence nitrogen bonding in the complex.

5. The syn form of pyridine-2-aldoxime-Iron(II) complex is less strained than the anti as can be shown by testing with atomic models.

Difficulties encountered

Throughout the work several inherent difficulties were met, mostly experimental in nature, and because of the pressure of time and the wide scope of the problem undertaken no effort could be made to minimize them. The preparation of IMAL was a long and tedious process and involved many stages the result of which was a very low yield. However, it was not worth the trouble of repeating the synthesis once more because, as it turned out, the compound formed a very weak complex.

Another difficulty encountered was in synthesizing 2,2'-pyridyl imidazole (PIM II). The difficulty arose at the decarboxylation stage because we obtained two kinds of crystals as the final product of decarboxylation, one with a sharp melting point of 74 - 75°C, and the other also with a sharp melting point of 126 - 127°C. The products had the same electrophoretic mobility around neutral pH. Therefore, they cannot be different in their charge types. The results are reported without further investigation at present; but it is planned to repeat the preparation in order to obtain greater yield and to check these results.

Significance of this work in relation to heme-linked effects

Perhaps the most significant result of this work is the light

it may throw on the problem of heme-linked effects in hemoproteins and possibly in metallo-enzymes in general. For, one can now place limits on the extent to which ionization of groups in large complex molecules, as in the case of hemoproteins, will be affected by conjugation to the metal ion although this effect cannot be measured directly. Thorough investigation of simple model systems paves the way to the interpretation of many complicated phenomena in large molecules.

Since the effect of linked ionization is profound one cannot depend on results obtained from titration or any other method in identifying acidic groups in large molecules without taking this effect into consideration. Reference to Wyman's work[16] again illustrates this point. Thus, Wyman reported pK $\sim$ 7 and $\Delta H \sim 7$ Kcal/mole for a heme-linked ionization in ferrihemoglobin and concluded that it corresponds to the ionization of the imidazolinium $\gt NH^+$ group since it had the thermodynamic properties characteristic of imidazole. However, since conjugation is possible in ferrihemoglobin, especially in the heme ring, and since iron(II) has a net positive charge of +1 in this complex molecule, then we should expect a decrease in the pK value for the ionization of the imidazolinium group by, say, 3 units at least with a corresponding decrease in the heat of ionization of about 6 Kcal/mole because of heme-linked effect. Therefore, there could be one of two alternatives: either Wyman's results refer to some other heme-linked group, or else there are other factors operative which require further investigation.

Similar criticism probably holds for all other cases where the study of heme-linked ionization was used as evidence for the identifi-

cation of the chemical nature of the metal-linked groups in large molecules.

In biochemical consideration, the imidazolinium $\rangle NH^+$ ionization seems to be the main factor which is used by investigators in accounting for such linked phenomena. The present work with model systems would suggest that any very weak acid could well become strong enough, through conjugation and electrostatic effects, to give linked ionization within the appropriate physiological significance. In particular, there is the imino $\rangle NH$ group, ordinarily an extremely weak acid, present in histidine, which may be involved. This is substantiated by the following evidence;

1. Abu-Isa[18] found that in ferrimyoglobin-imidazole complex, the pK value was about 10. This is attributed to the ionization of the imino $\rangle NH$ group which has a pK of around 14.5 in the free ligand.

2. Recent studies by Hanania et al[32] on vitamine B_{12} factor B imidazole complex showed that in cobalt-imidazole complex there is a pK of about 11. This may be attributed to the ionization of the imino $\rangle NH$ group.

Suggestions for future work

Owing to the preliminary nature of the present work, a number of suggestions are made indicating lines along which future research in this field may proceed.

1. It would be valuable to make quantitative studies of the various ionizations referred to in this thesis, both in the free ligand, and also in the metal chelates. Thermodynamic data should be carefully collected on these and perhaps other analogous systems. In each of

the cases to be studied, however, it would just be necessary to determine the composition of the complex and to prove the reversibility of the acid-base equilibria.

2. The interpretation of the electrostatic charge effects cannot be made without a thorough study of the charge types involved in every ionization whether on the ligand or in the chelate. One simple and elegant method of getting these charge types is that of electrophoretic mobility on paper, measured against standards and over a wide pH range. This phase of the work is necessary and easy to perform.

3. A special feature which is worth exploring is the effect of symmetry in the ligand molecule. Thus, one could compare PAL with DIPAL, PIM II with 2,2'-biimidazolyl, and monohydroxy with 4,7-dihydroxy-1,10-phenanthroline. In each case, one would be comparing an unsymmetrical with a symmetrical ligand. There is no information on the possible influence of symmetry in connection with linked ionizations.

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