[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

The Effect of Coördination on Some Imidazole Analogs[1]

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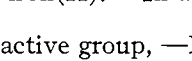
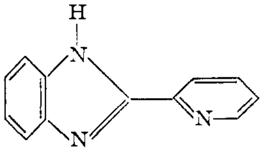
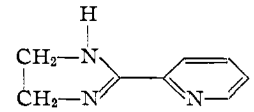
The coördination of 2-(2-pyridyl)-benzimidazole and 2-(2-pyridyl)-imidazoline with various divalent metal ions has been described. Coördination with a metal ion tends to increase the acidity of the imino hydrogen of the imidazole group, the acidity increase being a function of the individual metal.

2-(2-Pyridyl)-benzimidazole (A) and 2-(2-pyridyl)-imidazoline (B) have been recommended as suitable reagents for the photometric determination of iron(II).[2] In addition to the characteristic iron reactive group,  $-N{=}C-C{=}N-$, the biologically important imidazole grouping is also present


A


B

in (A), and its hydrogenated analog in (B). A potentiometric study of the coördination of these reagents with various metal ions should possibly be of help in evaluating the effects of trace metals on more complex and physiologically important imidazole-containing compounds.

Experimental

The titration apparatus is the same as that previously described.[3] Measurements were made using a Beckman model G pH meter equipped with a glass-saturated calomel electrode pair. All titrations were run at 25 ± 0.1°.

The purification of dioxane, standardization of sodium hydroxide and perchloric acid,[3] and preparation of reagents[2] also have been previously described.

Stock solutions of approximately 0.01 M metal ions were prepared by dissolving their reagent grade perchlorates (G. Frederick Smith Chemical Co.) in water. The copper(II) and cobalt(II) solutions were standardized by electrodeposition. The nickel(II) solution was standardized by precipitation with dimethylglyoxime. The zinc(II) and manganese(II) were standardized gravimetrically as $ZnNH_4PO_4$ and $Mn_2P_2O_7$, respectively.

The procedure is essentially the same as that described previously.[3] Fifty-five ml. of dioxane, 50 ml. of 0.01 N perchloric acid and 5 ml. of 0.01 M metal perchlorate were added to a weighed quantity of the reagent. The low solubility of the 2-(2-pyridyl)-benzimidazole in water necessitated the use of the 50% by volume dioxane–water solvent medium. However, an advantage in working in a solvent such as the 50% by volume dioxane–water mixture employed in this study is that a cationic acid, such as the imidazolium ion, becomes appreciably stronger.[4] The $pK_{a_{H^+}}$ for 2,2′-bipyridine, for example, was measured to be 4.33 in water and 3.33 in 50% dioxane–water (25°, μ 0.005).

The standard base (0.1 N) was added in small increments to the stirred solution. For the titration of reagent alone 5 ml. of water was substituted for the metal perchlorate solution.

Since one does not get a true reading of pH in the 50% by volume dioxane solution, the observed readings were corrected. A correction factor, obtained by comparing calculated values of pH for the titration of $HClO_4$ with experimentally determined values, was added to the observed pH readings.

Typical curves obtained in 50% by volume dioxane are shown in Figs. 1, 2 and 3.

Discussion

2-(2-Pyridyl)-imidazoline.—From Fig. 1 it may be observed that the Ni(II) and Co(II) combine with three reagent molecules per metal ion while the Cu(II) combines with only two. This is in contrast to the corresponding compound 2-(o-hydroxyphenyl)-imidazoline, which forms a complex with nickel(II), having the composition NiR_2.[5] The stoichiometry of three moles of reagent per mole of nickel ion, *i.e.*, $NiR_3{}^{2+}$, would explain an apparent stability order of Ni(II) > Cu(II) observed in a series of competition reactions.[2]

Some of the complex ions were isolated, using various anions to form insoluble salts. The results of their microanalysis are shown in Table I. Precipitates isolated at higher pH values were clearly different from these, but could not be sufficiently purified for analysis.

TABLE I

COMPOSITION OF CHELATES[a]

	Analyses, % Calcd.	Found
2-(2-Pyridyl)-benzimidazole[b]		
$Co(C_{12}H_9N_3)_3I_2$	N, 14.03	13.83
$Cu(C_{12}H_9N_3)Br_2$	N, 13.69	13.85
2-(2-Pyridyl)-imidazoline[c]		
$Ni(C_8H_9N_3)_3I_2$	N, 16.72	16.41
$Co(C_8H_9N_3)_3I_2$	N, 16.71	16.72
$Cu(C_8H_9N_3)_2(ClO_4)_2$	C, 34.51	34.45
	H, 3.26	3.47

[a] Microanalysis by Mr. O. E. Harris of the University of Pittsburgh's Microlab. [b] Chelates isolated at a pH of 4.5. [c] Chelates isolated at a pH of 7.0.

The Co(II) titrations were slow to reach equilibrium above a pH of about 8. The pH decreased with time, possibly because of an oxidation of Co(II) to Co(III).

Because of an apparently weak complex formed between the manganese and reagent at a low pH, the departure of the manganese curve from that of the reagent in the basic region probably corresponds to the formation of the hydroxide, $Mn(OH)_2$.

Stability Constants.—The optimum pH range for an accurate evaluation of chelate stability constants by the Calvin–Bjerrum technique is between 3 and 6 for the solvent medium employed in this study.[3] The upper limit could be somewhat higher for metals that have less tendency to hydrolyze. At the lower pH limit the amount of hydro-

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(1) This work was supported by a grant from the United States Public Health Service.

(2) J. L. Walter and H. Freiser, *Anal. Chem.*, **26**, 217 (1954).

(3) H. Freiser, R. G. Charles and W. D. Johnston, THIS JOURNAL, **74**, 1383 (1952).

(4) J. C. James and J. G. Knox, *Trans. Faraday Soc.*, **46**, 254 (1950).

(5) W. D. Johnston, Ph.D. Thesis, University of Pittsburgh, 1953.

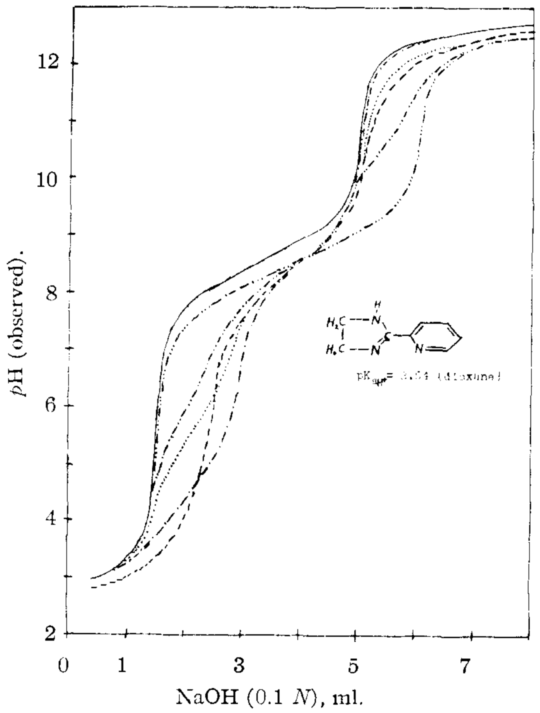

Fig. 1.—Titration of 2-(2-pyridyl)-imidazoline (B) in the presence of various metals at 25°: 0.353 mmole of reagent + 55 ml. of dioxane + 50 ml. of 0.01 N perchloric acid + 5 ml. of 0.01 M metal perchlorate: no metal, ——; copper(II), - - - -; nickel(II), —·—·—; cobalt(II), ...; zinc(II), —··—; and manganese(II), —···—.

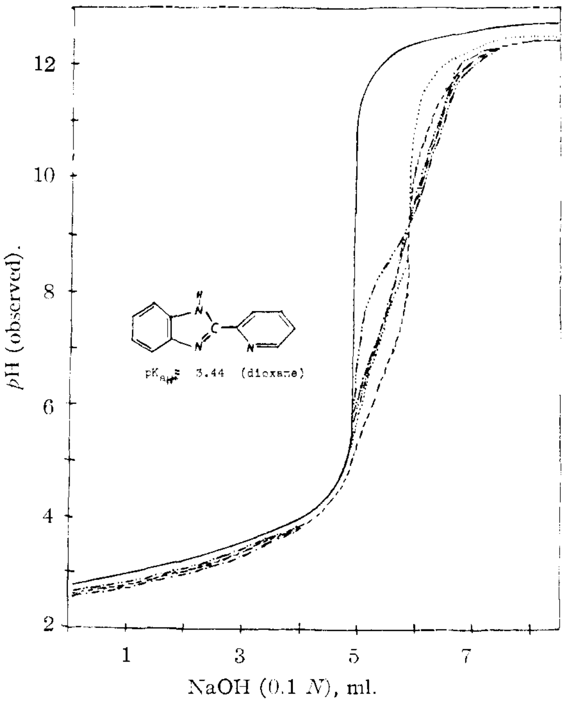

Fig. 2.—Titration of 2-(2-pyridyl)-benzimidazole in the presence of various metals at 25°: 0.404 mmoles of reagent + 55 ml. of dioxane + 50 ml. of 0.01 N perchloric acid + 5 ml. of 0.01 M metal perchlorate: no metal, ——; copper(II), - - -; nickel(II), —·—·—; cobalt(II), ...; zinc(II), —··—; manganese(II), —···—.

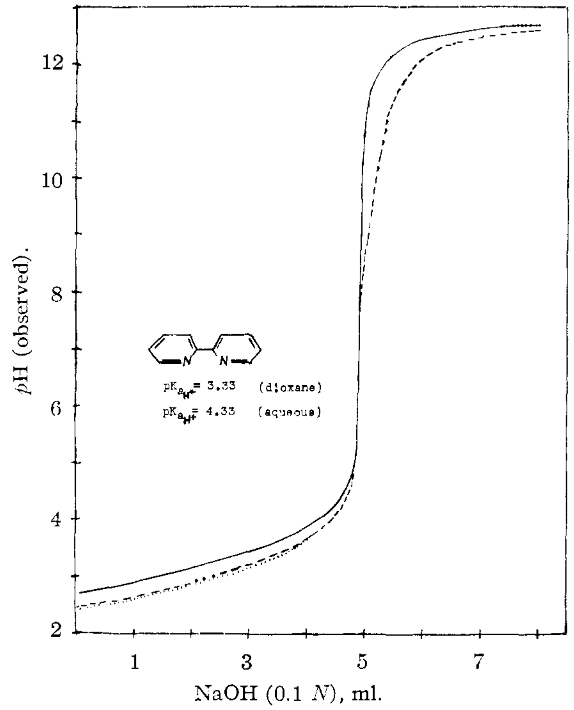

Fig. 3.—Titration of 2,2′-bipyridine in the presence of various metals at 25°: 0.400 mmole of reagent + 55 ml. of dioxane + 50 ml. of 0.01 N perchloric acid + 5 ml. of 0.01 M metal perchlorate: no metal, ——; nickel (II), ...; cobalt(II), - - -.

gen ions released because of chelate formation is small when compared with the amount of hydrogen ions present in solution. The upper limit is fixed by the hydrolysis of the metal ion. Even at this lower pH limit complete chelation had taken place in the case of the 2-(2-pyridyl)-benzimidazole. Values of $\bar{n}$, the average number of reagent molecules bound to a single metal ion, equalled 2 or 3 at the start of the titration. 2-(2-Pyridyl)-imidazoline has the disadvantage of having most of its chelation reactions occurring in the metal hydrolysis region, the upper limit of the optimum pH range. Any calculated values here would be maximum rather than exact quantities.

Where possible, however, approximate formation constants have been evaluated by the Calvin–Bjerrum technique and are presented in Table II. The constants k_1, k_2 and k_3 refer to the respective formation of the 1:1, 2:1 and 3:1 reagent–metal complexes.

TABLE II

FORMATION CONSTANTS OF 2-(2-PYRIDYL)-IMIDAZOLINE CHELATES IN 50% BY VOLUME DIOXANE–WATER AT 25.0°

$pK_{a_{H^+}}$ of Reagent = 8.54

Metal	log k_1	log k_2	log k_3
Copper(II)	. .	6.8	. .
Nickel(II)	7.5	6.6	5.9
Cobalt(II)	6.4	5.6	4.8
Zinc(II)	6.0	5.0	. .
Manganese(II)	3.9	. .	. .

2-(2-Pyridyl)-benzimidazole. Acidity of Imino Group.—Figure 2 shows a very interesting effect.

Complete neutralization of all the acid present in the system, either as the free acid or the protonated form of the reagent, occurs at about a volume of 4.9 ml. of base, at which point the chelate species is completely formed. As the *p*H begins to rise at this point, a further liberation of hydrogen ion occurs. This release of hydrogen ions may be due to either a hydrolysis reaction or a dissociation of the imino hydrogen of the reagent. In all cases, however, the *p*H at which hydrogen ion is released is less than the *p*H at which the respective metals begin to hydrolyze (Table III). 2,2′-Bipyridine, which has essentially the same pK_{aH^+} as 2-(2-pyridyl)-benzimidazole in 50% dioxane solution but does not possess an imino hydrogen, does not exhibit this effect (Fig. 3). It is worthwhile noting that the relative order of the "*p*H at half-neutralization of the complex" is the same as the usual stability order of these metals (with the exception of nickel-(II)). When the metal–reagent solutions had a slight color during the first portion of the titration, a color transition was observed at about the same time that the titration curve departed from the reagent curve (Table IV). The resultant colors were not characteristic of the simple metal hydroxide species. While similar conclusions might be reached for the 2-(2-pyridyl)-imidazoline titrations, these would be rendered ambiguous by the possibility of metal hydrolysis in the *p*H range of interest.

TABLE III

CHELATES OF 2-(2-PYRIDYL)-BENZIMIDAZOLE

Metal	*p*H of departure from reagent curve	*p*H of hydrolysis of metal ion in 50% dioxane[3]	*p*H at half-neutralization of complex
Cu(II)	4.5	5.2	6.4
Ni(II)	5.6	7.3	7.4
Co(II)	5.4	5.8	7.1
Zn(II)	5.5	6.4	7.3
Mn(II)	5.6		8.4

TABLE IV

COLOR CHANGE ACCOMPANYING TITRATION OF METAL IONS AND 2-(2-PYRIDYL)-BENZIMIDAZOLE[a]

Metal	From	To
Cu(II)	Green (2.8)	More intense and different shade of green (8.5)
Ni(II)	Slightly pink (2.8)	Slightly yellow (10.0)
Co(II)	Slightly yellow (2.8)	Golden yellow (8.0)
Zn(II)	Colorless throughout	
Mn(II)	Pink (2.8) Tan (4.0)	Yellow-tan (8.5)

[a] Numbers in parentheses indicate the *p*H at which color observations were made.

All these observations point to a dissociation of the imino hydrogen. Coördination of the metal to the pyridine nitrogen of the imidazole ring tends to decrease the electron availability on the imino nitrogen (through resonance interaction), thereby facilitating the dissociation.

Coryell and Pauling[6] have considered the influence of the iron atom on the increased acidity of the heme-linked histidine substituents of hemoglobin. They explain the effect in hemoglobin as resulting from the electrostatic repulsion between the ionically bound iron atom and the imidazole hydrogen atom. They view the greater effectiveness of the metal upon the increase in acidity of oxyhemoglobin as arising from the increased participation of iron, here covalently linked, in the electronic distribution of the oxyhemoglobin molecule. In the compounds studied here, similar explanations for the acidity increase would be in order.

It is noteworthy that the effect is dependent on the individual metal. Since these metals have almost the same ionic potential, a purely electrostatic reasoning for the effect would lead one to expect the increase in acidity to be independent of the metal. Therefore, one may conclude that the metal atoms are essentially covalently linked to the imidazole nitrogens. The differences observed in the acidity increase for the various metals can be considered to reflect differences in metal–nitrogen bond strengths. An attempt to correlate the two by plotting the formation constants of the related imidazoline chelates against the $p\text{H}_{1/2}$ (mid-point of neutralization of the metal–benzimidazole complex) values is illustrated in Fig. 4. The deviation of nickel from linearity might well reflect the extraordinary sensitivity of this metal to steric hindrance.[7] Thus, the adverse steric influence of the benz-portion of the benzimidazole would result in a weakening of the nickel–reagent bond and thereby lower the acidity of the imino hydrogen (in addition

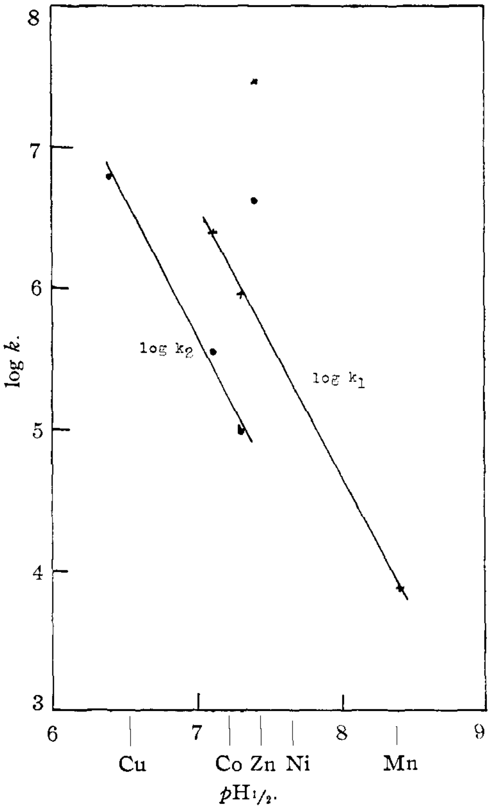

Fig. 4.—Log k_1 (X) and log k_2 (●) of the divalent metal–imidazoline complexes *vs.* $p\text{H}_{1/2}$ (mid-point of neutralization of the benzimidazole chelates).

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[6] C. D. Coryell and L. Pauling, *J. Biol. Chem.*, **132**, 769 (1940).

[7] W. D. Johnston and H. Freiser, *Anal. Chim. Acta*, **11**, 201 (1954).

to decreasing the relative formation constant of the complex). This would tend to place nickel in a more favorable position in Fig. 4.

Preliminary investigations by the authors of the titration of imidazole in the presence of metal ions in aqueous medium indicate that the acidity-increase effect is present in the parent compound as well. The pK_a values of the metal–imidazole complexes would appear to be in the vicinity of 8.5 to 9. This is not inconsistent with the findings of Edsall, *et al.*,[8] who, working at pH values below 7.5, did not find any appreciable increase in the acidity of imidazole in the presence of copper and zinc ions.

Where this "increase in acidity" effect is observed, it should be mentioned that the formation of a metal complex might possibly serve as a useful intermediate in the preparation of N-substituted imidazole derivatives.

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[8] J. T. Edsall, G. Felsenfeld, D. S. Goodman and F. R. N. Gurd, THIS JOURNAL, **76**, 3054 (1954).