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Studies on the Complexation of Cobalt (II) with Isonicotinohydroxamic Acid and It's Microbial Sensitivity

[1]A.O. Aliyu and [2]J.N. Nwabueze
[1]Department of Chemistry, Nigerian Defence Academy, P.M.B. 2109, Kaduna
[2]Research Laboratory, Department of Chemistry, Abuja University,
P.M.B. 117, FCT, Abuja, Nigeria

Abstract: A spectroscopic investigation of the reaction of Co^{2+} with isonicotinohydroxamic acid (INHAH) in aqueous solution of I = 0.1 moL dm^{-3} at 25°C reveals the sole formation of 1: 2 complex, spectral and magnetic studies on the isolated complex indicate an octahedral geometry with an O, N coordination mode for the ligand. Microbial sensitivity test on eight microorganism shown an enhanced antimicrobial activity for the complex relative to the free ligand.

Key words: Hydroxamic acid, spectroscopic investigations, microbial activity, cobalt (II), octhedral geometry

INTRODUCTION

Hydroxamic acids having one or more -CONHOH-groups have been extensively studied as a consequence of their biological importance, which is related to their ability to form metal ion complexes (Fernandes *et al*., 1997). Hydroxamic acids and other compound containing the hydroxamate group are ubiquitous in nature and are ultimately associated with iron transport in bacteria (Aliyu and Nwabueze, 2007; Nwabueze, 1996a, b). Hydroxamic acids have been shown to possess diverse biological activities, many, of which are due to their complexing properties towards the transition metal iron (Raymond, 1990, 1994; Crumblis, 1991). Data have been published on the inhibitory activity of hydroxamic acid derivatives of amino acids and peptides on metalloproteinases (Yatabe *et al*., 1998; Mock and Cherg, 2000). The mechanism of inhibition appears to involve chelation of metals at their active sites. Some amino hydroxamic acids have been investigated with the aim of designing metal chelates as suitable sources of various trace elements essential in animal (Brown and Roche, 1993).

Monohydroxamic acids form typical octahedral complexes with transition metals via coordination through the oxygen atoms and formation of reasonably ionic metal oxygen bonds (Aliyu and Nwabueze, 2008; Nwabueze, 1996a, b). Monohydroxamic acid (such as acetohydroxamic acids, $CH_3CONHOH$ = Aha) after deprotonation acts as bidentate ligands forming octahedral complexes with a series of metal ions via co-ordination through the two oxygen atoms of the-CONHON-group. This type of coordination has been characterized with Fe^{III}, Cr^{III}, Ni^{II} Co^{II} and Zn^{II} ions, which indicated the formation of octahedral complexes both in solid state and in solution (Kurzak *et al*., 1992).

Asides from the (O, O) bonding mode that has been reported both structurally and in solution, only in few cases has (N, O) bonding mode been suggested in solution studies with amino hydroxamic acids (Dobosz *et al*., 1999) and has also not been structurally established in the solid state. However, N, O bonding mode has been reported in dinuclear complex of copper II (Nigovic *et al*., 2002). Hydroxamic acids have been used as therapeutic agents in chelation theraphy and as metalloenzyme inhibitors (Nishino and Powers, 1979; Petrillo and Ondilti, 1982; Rockwell *et al*., 1996). Other medicinal applications of hydroxamates, which utilize their affinity for high charge density metal ions include the possible use of their complexes as imaging agent (Miller and Lin, 1999).

With regards to the strong ability of hydroxamic acids to form chelates, clarification of their interaction with metal ions is of particular importance in terms of biological effects. However, the affinity of the tittled ligand for other transition metal ions such as Fe^{3+}, Ni^{2+}, $Cu^{2+}$and VO^{IV+} have already been reported (Aliyu and Nwabueze, 2008, 2007; Aliyu *et al*., 2008).

This study is therefore, a report on the research carried out on the complexation of cobalt (II) with isonicotinohydroxamic acid with special emphasis on identifying the number and the nature of species formed in aqueous solution and the structure and nature of bonding involved. In addition, some physico-chemical properties were investigated and microbial sensitivity test carried out, which are also included in this research.

Corresponding Author: A.O. Aliyu, Department of Chemistry, Nigerian Defence Academy, P.M.B 2109, Kaduna

MATERIALS AND METHODS

Ethylisonicotinate was obtained from Aldrich Chemical Company Ltd. All other reagents were of Analar R-grade. $NaNO_3$ was used for the preparation of the background electrolyte and stock solutions. Water was doubly distilled, degassed using purified N_2 and stored in glass stoppered flasks. KOH and HNO_3 used for adjusting pH were stored in glass ampoules and were standardized with potassiumhydrogenphthalate and tris (hydroxymethyl) methylamine, respectively. The pH measurements were made using a radiometer Copenhangen Research pH meter calibrated with standard buffer tables.

Electronic spectra were recorded on ATI Maltson Genesis series. FTIR™ machine as Nujol mull in the 4000-200 cm^{-1} spectra region. Room temperature magnetic susceptibility measurements were made on MSB auto magnetic susceptibility balance. The isolates were obtained from NITR (National Institute for Triphasonomiasis, Kaduna). ABUTH (Ahmadu Bello Teaching Hospital Kaduna) and NARHK (Nigerian Army Reference Hospital, Kaduna). They were cultured on chocolate broth.

Preparation of the ligand: Isonicotinohydroxamic Acid (INHA) was prepared by Nwabueze (1996a, b). Na metal (2.3 g, 0.1 moL) in MeOH (50 cm^3) was added to $NH_2OH.HCl$ (6.9 g, 0.1 moL) in MeOH (100 cm^3). The mixture was cooled to room temperature and ethylisonicotinate (15.12 g, 0.1 moL) was added. The mixture was stirred for 40 min and a further solution of Na (2.3 g, 0.1 moL) in MeOH (50 cm^3) added and stirring continued for a further 10 min. The mixture was filtered to remove the precipitated NaCl and the filtrate acidified with concentrated HCl and the precipitated NaCl removed by filtration. The filtrate was concentrated using rotary evaporator (without heating) and left in a refrigerator overnight. The crystals were removed by filtration and recrystallized from EtOH. The Yield was 58%.

Preparation of the complex: Co $(INHA)_2$ $2H_2O$ was prepared as follows: -$CoCl_2.6H_2O$ (0.48 g, 0.002 moL) in cold water was added with stirring to INHA (0.556 g, 0.004 moL) in EtOH (20 cm^3). To this mixture 10% solution of $NaHCO_3$ was added until a pink precipitate appeared. The precipitate was filtered, washed with small aliquots of Et_2O and dried over silica gel in a vacuum desiccators. Yield =72%.

Equilibrium studies: The pKa value for the ligand was determined spectrophotometrically by the method of Albert and Sergent using boric acid and borax of ionic strength 0.1 and 0.025 moL dm^{-3} buffer for INHA ligand. In each case, the ligand stock solution was 5.0×10^{-4} moL dm^{-3} diluted five folds in the buffer solution for INHA. Measurements were made in eight boric/borax buffer solutions at 215 nm on a UNICAM SP800 spectrophotometer.

The number of complexes present in solution at equilibrium was determined by the isosbestic point method and Graphical Matrix Rank analysis using nine solutions containing 1:1-1:5 metal:ligand ratio (ligand concentration increasing in unit of 0.5). A solution of I = 0.1 moL dm^{-3} made up of 0.01 moL dm^{-3} HNO_3 and 0.09 moL dm^{-3} $NaNO_3$ was used to prepare equimolar stock solution of Co^{2+} and the ligand of 2.5×10^{-3} moL dm^{-3}. The same solution was used for all dilutions. In all case, the solution were thermostated at 25°C for 2 h an ultrasonic bath.

Antimicrobial screening test: The nutrient agar was used as the growth medium for the microbes. The nutrient agar medium was prepared by dissolving 7.0 g of the agar 250 mL of distilled water. The solution was sterilized in an autoclave for 15 min, poured into Petri dishes and kept in refrigerator for 24 h. After 24 h the plates were retrieved and assessed (Nuhu *et al*., 2002). Standard strains of the microbes were obtained from (Nigerian Army Reference Hospital, Kaduna (NARHK)).

The study disc diffusion method (Nuhu *et al*., 2002) was used to assess the antimicrobial activity. Sterilized study disc were impregnated with various concentration of the ligand and the complex dried at 37°C before use. The microbes were inoculated into the nutrient broth and incubated for 24 h at 37°C. The inoculums were allowed to dry and the discs were then placed evenly on the surface of the inoculation and gently pressed down to ensure contact. The plates were incubated at 37°C for 24 h after incubation.

Observation comprising, diameter of disc, zone of inhibition and Minimum Inhibitory Concentration (MIC) were made for study evaluation. Two other sterile blank discs were impregnated with water to serve as negative controls (Table 1).

Table 1: Key diameter of zone of symbol comment inhibition (mm)

Diameter	Symbols	Activity
12-15	+	Insignificant
16-20	++	Minimum
21-25	+++	Moderate
26-35	++++	Maximum

Diameter of disc (mean) = 6×10^{-2} mm

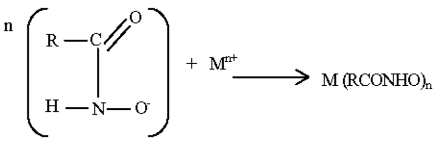
RESULTS AND DISCUSSION

The various stages in the preparation of INHA are as represented by the reactions:

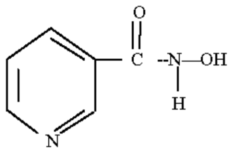
$$2Na + 2MeOH\ 2MeONa + H_2$$
$$NH_2OH.HCl + MeONa\ NH_2OH + NaCl + MeOH$$
$$NH_2OH + RCO_2Et\ RCON\ (H)OH + EtOH$$
$$RCON\ (H)OH + MeONa\ RCON\ (H)O^{-}Na^{+} + MeOH$$
$$RCON\ (H)O^{-} - Na^{+} + HCl\ RCON\ (H)OH + NaCl$$

Where:
R = Pyridine ring for the complex of INHA model

The complexation reaction is represented by the equation:



Where:
n = The number of molecules of the ligand involved in ligation



Scheme 1: Structural formular of Isonicotinohydroxamic Acid (INHAH).

The structure of isonicotinohydroxamic acid is shown in scheme 1.

The ligand can release only one proton in the pH range 1.5- 11.4, which may be attributed to the hydroxamic group. The determined proton dissociation constant at 1 = 0.1 moL dm^{-3} boric/borax buffer is 8.68±0.05. Comparison with the values for benzhydroxamic acid, pKa = 8.79 and acetohydroxamic acid, pKa = 9.37, respectively shows an increase of acidity (Schwarzenbach and Schwarzenbach, 1963). This is in accordance with the fact that pyridine ring has a lower electron donating ability than the phenyl and the methyl groups (i.e., the pair of electrons that gives pyridine its basicity occupies a sp^2 orbital and is less readily available).

Figure 1 shows, the absorption spectra of solutions containing a constant metal but variable ligand molar concentration for Co^{2+}/INHA system, while Fig. 2 shows graphical matrix rank analysis of the absorbance data generated from similar solutions. The absence of an isosbestic point in Fig. 1 and the shape of the graph in Fig. 2 indicates the presence of only one complex specie the complex in solution was determined as a 1:2 M:L in the system (Hartley *et al.*, 1980). The composition of species by Job's plot as shown in Fig. 3 (Hartley *et al.*, 1980).

The colour of the cobalt (II) complex is pink. The analytical data and some physical constant for the isolated solid complex are shown in Table 2. The spectrum of the complex (Co (INHA)$_2$.2H$_2$O) shows a band located at about 520 nm (19.230 cm^{-1}) with a shoulder at, 610 nm (16.393 cm^{-1}); there is a third band at 690 nm (14.492.75 cm^{-1}). The position of the band together with observed room temperature magnetic moment of 5.10 BM are in consistent with octerhedral coordination around the cobalt (II) ion (Nicholls, 1974; Nwabueze, 1996a, b)

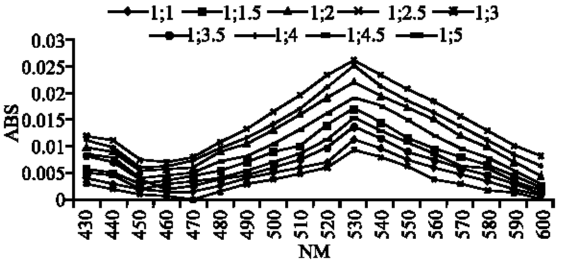


Fig. 1: Isosbestic point search for cobalt (II) INHA

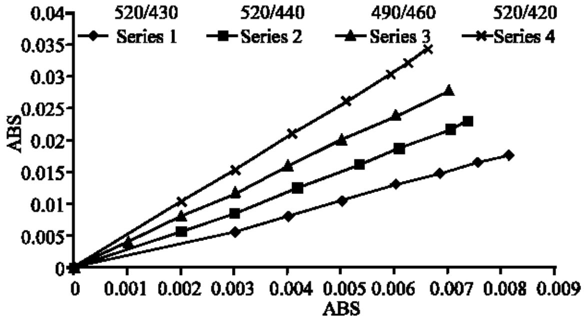


Fig. 2: Graphical rank matrix analysis for co-INHA system (one specie test)

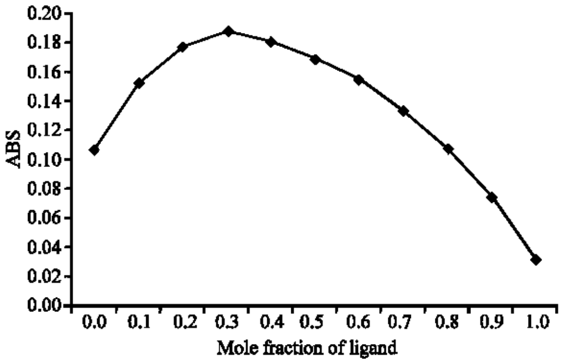


Fig. 3: Continuous variation Job's plot method for co (II) -INHA system

Table 2: Analytical data and some physicochemical properties of the isolated complex

Compound	Formular (Wt)	Mp/Dec (°C)	Colour	Found C	H	N	M	μeff (B.M)	λMax ×10^3 cm^{-1}	Assignment
Co (INHA)$_2$.2H$_2$O	370.90	160	Pink	38.6	4.28	14.8	15.41	5.10	19.23	^{4}T1g (F)→^{4}T2g(p)
	-	-	-	(38.8)	(4.32)	(15.0)	(15.88)	-	16.39	^{4}T1g(F)→^{4}T2g(P)
	-	-	-	-	-	-	-	-	14.49	^{4}T1g(F)→^{4}A2g

Figures in parenthesis are calculated

Table 3: Diagnostic I.R data for the complex (cm^{-1})

Compound	□ (NH) cm^{-1}	Δ□ (NH) cm^{-1}	□ (C=O) cm^{-1}	Δ□ (C=O) cm^{-1}	□ (CN) cm^{-1}	Δ□ (CN) cm^{-1}
INHA	3418.00	-	1659.61	-	1321.00	-
Co (INHA)$_2$ 2H$_2$O	3318.26	-99.74	1610.76	-48.85	1377.00	+56.00

INHAH: Isonicotinohydroxamic Acid anion; Co = Cobalt

Table 4: Minimum inhibitory concentration

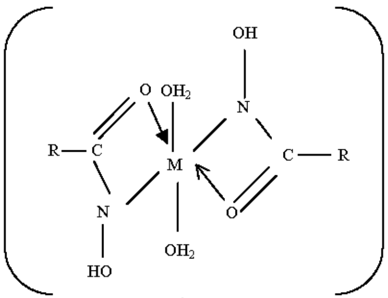
		Concentration (mm)					
Ligand	Organisms	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
INHA	*S. aureus*	+	+	+	-	-	-
	S. thyplium	+	+	-	-	-	-
	E.coli	+	+	-	-	-	-
	Klebsiella	+	+	-	-	-	-
	α-hemolytic strep	+	+	+	+	-	-
	Neisseria	+	+	-	-	-	-
	Pseudomonas	+	+	+	+	-	-
	Conynebacterium	+	+	-	-	-	-
Complex							
Co(INHA)$_2$.2H$_2$O	*S. aureus*	+++	++	++	-	-	-
	S. thyplium	+++	++	-	-	-	-
	E. coli	+++	++	-	-	-	-
	Klebsiella	+++	++	-	-	-	-
	α-hemolytic strep	+++	++	++	++	-	-
	Neisseria	+++	++	-	-	-	-
	Pseudomonas	+++	++	-	-	-	-
	Conynebacterium	+++	++	-	-	-	-

1996). The bands have been therefore, assigned to transition $^4T_{1g}$ (F) toT $_{1g}$(P), A $_{2g}$ and ^{4}T $_{2g}$ levels, respectively (Nicholls, 1974; Nwabueze, 1996a, b).

The diagnostic i.e., band for the ligand and the complex are compared in Table 3. The ν (C=O) band located at 1659.61 cm^{-1} in the in the spectrum of the ligand is lowered by 48 cm^{-1} in the spectrum of the complex; this is consistence with coordination via the carbonyl O (Nwabueze, 1996a, b). The ν (NH)vibration, which is located in the spectrum of the ligand at 3418 cm^{-1} in the ligand is lowered by 99.74 cm^{-1} in the spectrum of the complex indicating ligation via the imine N. The pyridine ring vibration observed in the spectrum of the ligand at 1580 cm^{-1} did not show any significant change in the spectrum of the complex as expected. There is no evidence that the pyridine ring N is involved in coordination. The ν (CN) frequency observed in the ligand 1132 cm^{-1} is increased by 56 cm^{-1}, which agrees with the postulates above (Suton, 1968; Hathaway and Billings, 1970). These together indicates mix bonding mode (N, O). Proposed structure for (N, O) bonding mode for octahedrally coordinated complex.

The results presented in Table 4 shows the antimicrobial activities of both the ligand and its isolated complex. It was observed that the ligand shown a moderate activity against all the microbes tested for while, the complex shown a high activity against the selected microbes. The inhibitory activity of the ligand and its isolated complex gives promise to their potential application in the treatment of microbial induced ailment or diseased conditions. Since many complexes have gained recognition as a source of curative agents for ailment. It is suggested that this complex should not be exceptional and scientific evaluation of its active constituents be given serious consideration.

Proposed structure for the cobalt (II) complex:



Scheme 2

Where, M = Co^{II}

CONCLUSION

It can be formulated as follow: Cobalt (II) hydroxamate complex favours mixed coordination mode (N, O) formation with isonicotinohydroxamic acid and the isolated complex shows significant activity against the selected microbes.

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