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Full Length Research Paper

Complexation of cobalt (II) with nicotinohydroxamic acid and its microbial sensitivity

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Complex of cobalt (II) with nicotinohydroxamic acid (NHA) has been investigated by spectroscopic method at 25 ± 0.1°C in aqueous solution of 0.1mol dm^{-3} ionic strength. The results reveal the sole formation of 1:2 complex at equilibrium. Spectra and magnetic studies of the isolated complex indicate octahedral mixed coordination mode (N.O). Microbial sensitivity test on eight micro-organism (that is, four gram +ve and four gram - ve bacterial strains) showed activity.

Key words: Complex, hydroxamic acid, spectroscopic investigations, microbial activity, cobalt (II).

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 (Celina 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, 1996). Hydroxamic acids have been shown to possess diverse biological activities, many of which are due to their complexing properties towards transition metal iron (Raymond, 1990; Crunblis, 1991; Raymond, 1994). 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, 1983).

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Definition of terms

$NaNO_3$ = Sodium nitrate; KOH = Potassium Hydroxide; HNO_3 = Nitric Acid; MeOH = Methanol; Na = Sodium; NaCl = Sodium Chloride; HCl = Hydrochloric Acid; Et_2O = Diethylether; $CoCl_2.6H_2O$ = Cobalt (II) chloride; NHA = Nicotinohydroxamic Acid; $NaHCO_3$ = Sodium bicarbonate; Co^{2+}= Cobalt (II) ion.

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, 1996). Monohydroxamic acid (such as acetohydroxamic acids, $CH_3CONHOH$ = Aha) after deprotonation acts as bidentate ligands forming octahedral complexes with a series 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, (Agnieszka 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 (Nigoric 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 et al., 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.

This paper is therefore a report on the work carried out on the complexation of cobalt (II) with nicotinohydroxamic acid with special emphasis on 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 report.

EXPERIMENTAL

MATERIALS

Ethylnicotinate was obtained from Aldrich. 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 potassium hydrogen phthalate and tris(hydroxymethyl), methylamine, respectively. The pH measurements were made using a radiometer Copenhangen Research pH meter calibrated with standard buffer tables (2, 4 and 9).

Electronic spectra were recorded on ATI Maltson Genesis series. FTIR^{TM} machine as Nujol mull in the 4000 - 200cm^{-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

Nicotinohydroxamic acid was prepared by method described by (Nwabueze, 1996). 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 ethylnicotinate (15.12 g, 0.1 mol) was added. The mixture was stirred for 40min 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

$[Co(NHA)_2.2H_2O]$ was prepared as follows:- $CoCl_2.6H_2O$ (0.48 g, 0.002 mol) in cold water was added with stirring to NHA(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 (1971) using boric acid and borax of ionic strength 0.1 moldm^{-3} and 0.025 moldm^{-3} buffer for NHA ligand. In each case, the ligand stock solution was 5.0 x 10^{-4} mol dm^{-3} diluted five folds in the buffer solution for NHA. 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 moldm^{-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 x 10^{-3} mol dm^{-3}. The same solution was used for all dilutions. In all case, the solution was thermostated at 25^{o}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 gm 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 (NARHK Nigerian Army Reference Hospital, Kaduna).

The paper disc diffusion method (Nuhu et al., 2002) was used to assess the antimicrobial activity. Sterilized paper disc were impregnated with various concentration of the ligand and the complex dried at 37^{o}C before use. The microbes were inoculated into the nutrient broth and incubated for 24 h at 37^{o}C. The inoculum was 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^{o}C for 24 h after incubation.

Observation comprising, diameter of disc, zone of inhibition and minimum inhibitory concentration (MIC) were made for paper evaluation. Two other sterile blank discs were impregnated with water to serve as negative controls.

RESULTS AND DISCUSSION

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

$$2Na + 2MeOH \longrightarrow 2MeONa + H_2$$

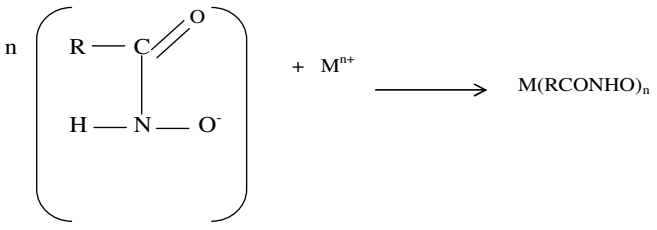
$$NH_2OH.HCl + MeONa \longrightarrow NH_2OH + NaCl \downarrow + MeOH$$

$$NH_2OH + RCO_2Et \longrightarrow RCON(H)OH + EtOH$$

$$RCON(H)OH + MeONa \longrightarrow RCON(H)O^-Na^+ + MeOH$$

$$RCON(H)O^- - Na^+ + HCl \longrightarrow RCON(H)OH + NaCl \downarrow$$

Where R = Pyridine ring for the complex of NHA model.



Where n is a neutral monodentate ligand.

The fully form of protonated nicotinohydroxamic 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 moldm^{-3} boric/borax buffer is 8.68 $\pm$ 0.05. Comparison of this data with the analogous

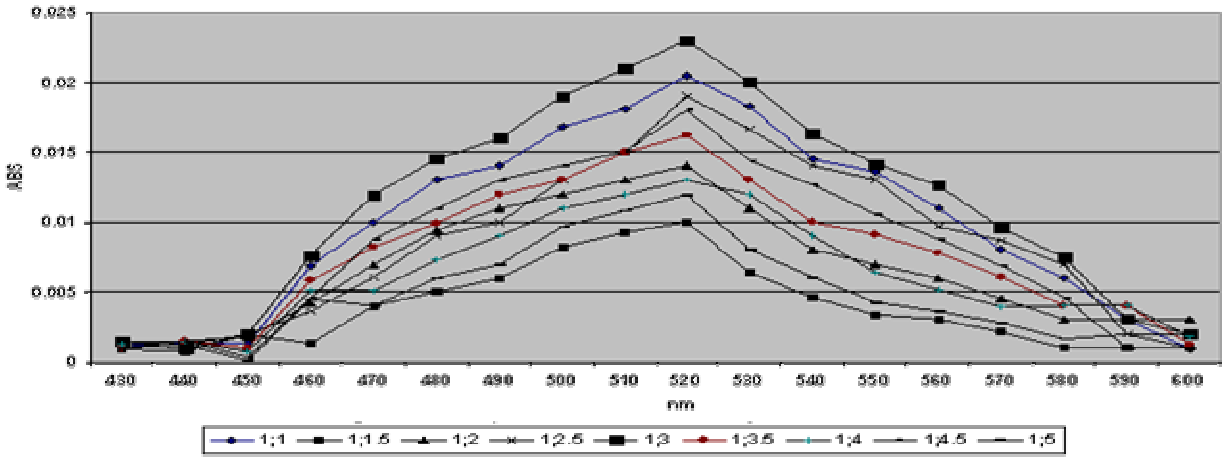


Figure 1. Isosbestic point search for Co".NHA system.

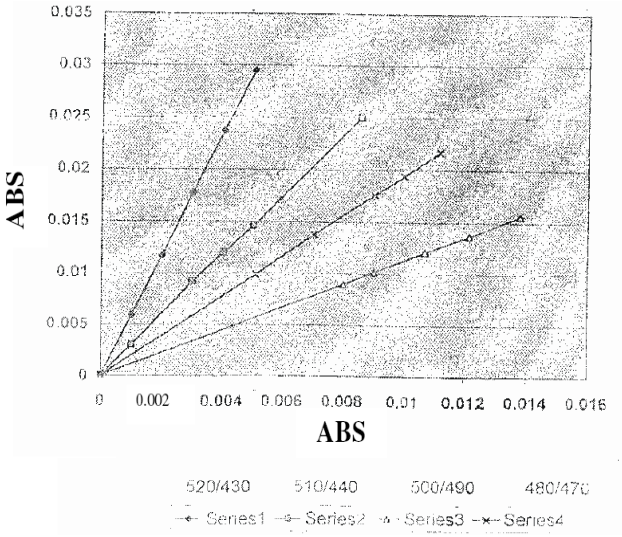


Figure 2. Graphical rank matrix analysis for Co"-NHA system (one specie test).

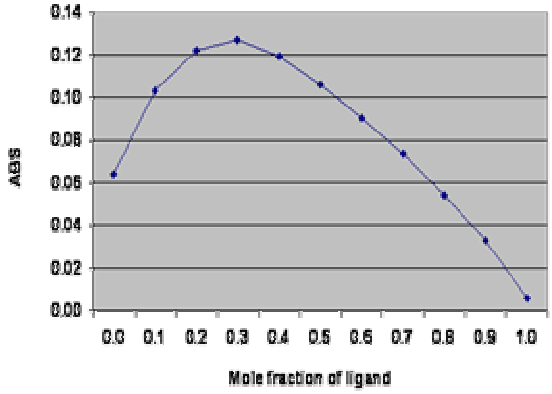
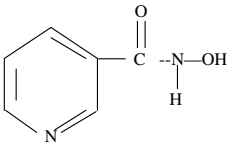


Figure 3. Continuous variations (Job's plot) method for Co"-NHA system.3.



Scheme 1. Structural formular of nicotinohydroxamic acid (NHA).

analogous values of benzhydroxamic acid, pKa = 8.79, and acetohydroxamic acid, pKa = 9.37, (Schwarzenbach and Schwarzenbach, 1963) shows an increase of acidity in the sequence acetohydroxamic acids < benzhydroxamic acid. 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 NHA system, while Figure 2 shows Graphical Matrix Rank analysis of the absorbance data generated from similar solutions for NHA system. The absence of an isosbestic point and the shape of the graph are typical of systems containing only one complex species. In this regard, the system shows similar behavior (Hartley et al., 1980).

Several equilibrium models were tried but it was only with ML_2 model that convergence was achieved. The composition of the complex as determined by Job's Plot is shown in Figure 3. The ratio of Co^{II} to the ligand under investigation was ML_2 that is, Co^{II} –NHA. The colour of cobalt (II) complex is pink. The analytical data and some physico-chemical properties are shown in Table 1. The spectrum of Co $(NHA)_2.2H_2O$ shows three bands located at about 520 nm (19,230 cm^{-1})' 610 nm (16,393 cm^{-1}) and 690 nm (14,492.75 cm^{-1}). The ratio of this band was less than 1.8 and agrees with the ratio reported for octahedral

Table 1. Analytical data and some physicochemical properties of the isolated complex.() calculated (%).

Compound	Formular. wt	Mp/ Dec ^oC	Colour	Found				reef (B. M)	λMax X10^3 cm^{-1}	Assignment
				C	H	N	M			
$Co(NHA)_2.2H_2O$	370.90	160	Pink	38.6 (38.8)	4.28 (4.32)	14.8 (15.0)	15.41 (15.88)	5.10	19.23 16.39 14.49	$^4T1g(F) \rightarrow {}^4T2g(p)$ $^4T1g(F) \rightarrow {}^{4T2g(P)}$ $^4T1g(F) \rightarrow {}^4A2g$

Key: NHA = Nicotinohydroxamic acid.
M = metal.
Form.wt = Formular weight.
MP/Dec = Melting Point/Decomposition.
B.M = Borh Magneton.

Table 2. 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}
NHA	3418.00	-	1659.61	-	1321.00	-
Co $(NHA)_2.2H_2O$	3318.26	-99.74	1610.76	-48.85	1377.00	+56.00

Key: NHA = Nicotinohydroxamic acid
Co = Cobalt.

Table 3a. Determination of the minimum inhibitory concentration of the Ligand.

Ligand	Organisms	Concentration (mm)					
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
NHA	*Staph. Aureus*	+	+	+	-	-	-
	S. Thyplium	+	+	-	-	-	-
	E. Coli	+	+	-	-	-	-
	Klebsiella	+	+	-	-	-	-
	α -heamolytic strep	+	+	+	+	-	-
	Neisseria	+	+	-	-	-	-
	Pseudomonas	+	+	+	+	-	-
	Conynebacterium	+	+	-	-	-	-

cobalt (II) complex (Nicholls, 1974; Boadanov et al., 1972). These bands are thereforebeen assigned the transition $^4T_{1g}$ (F) $\longrightarrow$ $^4T_{2g}$ (P) and $^4T_{1g}$(F) $\longrightarrow$ $^4A_{2g}$ respectively (Nicholls, 1974; Nwabueze, 1996). The position of these bands and observed magnetic moment of the complex are consistent with octahedral geometry.

The diagnostic that is, band in the free ligand was compared with the complex reported in Table 2. The (C=O) vibration located at 1659.61 cm $^{-1}$ in the ligand is lowered by 48cm^{-1} in the complex likewise the (NH) vibration located at 3418 cm^{-1} in the ligand is lowered by 99.74 cm^{-1} in the complex. The observed decrease in the frequency in the carbonyl vibration is due to the carbonyl oxygen which serves as a donor centre in a metal chelate. As a consequence, there will be an electron withdrawal from carbonyl group, which in turn, will increase the electron density in the C-N band. Therefore a lowering of the carbonyl frequency and increase of ν̃(CN) frequency are expected. The ν̃ (CN) frequency observed in the ligand 1132 cm^{-1} is increased by 56 cm^{-1} which agrees with the postulates above (Sutton, 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 Tables 3(a) and (b) show the antimicrobial activities of both the ligand and its isolated complex. It was observed that the ligand showed a moderate activity against all the microbes tested for while the complex showed 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

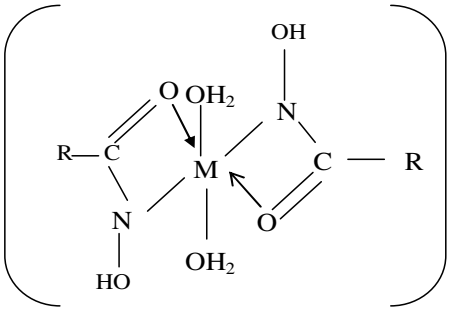
Table 3b. Determination of the minimum inhibitory concentration of the complex.

Complex	Organisms	Concentration (mm)					
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
$Co(NHA)_2.2H_2O$	*Staph. Aureus*	+++	++	++	-	-	-
	S. Thyplium	+++	++	-	-	-	-
	E. Coli	+++	++	-	-	-	-
	Klebsiella	+++	++	-	-	-	-
	α-heamolytic strep	+++	++	++	++	-	-
	Neisseria	+++	++	-	-	-	-
	Pseudomonas	+++	++	-	-	-	-
	Conynebacterium	+++	++	-	-	-	-

Table 4. Key diameter of zone of symbol comment inhibition (mm).

12-15	+	Insignificant activity
16-20	++	Minimum activity
21-25	+++	Moderate activity
26-35	++++	Maximum activity

Diameter of Disc (mean) = 6 x 10^{-2} mm



Scheme 2: The proposed structure of Cobalt(II)Complex of nicotinohydroxamic acid.
Key: M = Co^{II}

serious consideration.

Conclusion

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

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