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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF ISONICOTINIC ACID HYDRAZIDE COMPLEXES WITH Co(II), Cu(II), Mn(II) AND Zn(II) IONS

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ABSTRACT

The ligand isonicotinic acid hydrazide (INH) and its complexes with Mn(II), Co(II), Cu(II) and Zn(II) nitrates were successfully synthesized and characterized by elemental analysis, melting point, conductance, magnetic susceptibility, electronic and infrared studies. Infrared data suggest that the ligand is neutral bidentate donors ligating through the carbonyl oxygen and the azomethine nitrogen towards the metal ions. The nitrate ion is in the outer coordination sphere in the complexes. The magnetic and electronic spectral data indicate octahedral structures for the divalent M(II) ions. Octahedral geometry has been suggested for all the metal (II) complexes. Antibacterial activity of the ligand, INH and its metal complexes were studied against gram-positive bacteria: *Streptococccus pyogenes, Staphylococcus aureus;* and gram-negative bacteria: *Escherichia coli* and *Shigella species.* The results showed that the metal complexes have better antibacterial activities than isonicotinic acid hydrazide ligand.

Keywords: Antibacterial, Complexes, Hydrazides, Spectroscopy, Transition metals.

INTRODUCTION

Over the past three decades, intensive efforts have been made to design novel compounds to confront new strains of resistance micro-organisms. The ongoing intense search for novel and innovative drug delivery systems is predominantly a consequence of the well-established fact that the conventional dosage forms are not sufficiently effective in conveying the drug compound to its site of action and this has necessitated the search for more potent drugs. The recognition of metal complexes and chelates in therapeutic application provides useful outlet for basic research in transition metal chemistry [1].

The hydrazide properties are of major interest due to their biological activities. Hydrazides have been demonstrated to possess, amongst others, antibacterial, antifungal, and antitumoral activities. These properties are sometimes enhanced on coordination to certain transition metal ions. The formation of metallic complexes play an important role in the enhancement of their biological activity and therefore, many complexes of hydrazides have been synthesized and characterized. Some of these metal complexes also exhibit better fungicide, antitumoral and antibacterial activities [2].

Recent studies have shown several new compounds of INH being synthesized to evaluate the effects of carbonyl compounds on the biological activity of isonicotinic acid hydrazide and the effect of metals like Zn(II), Ni(II), Co(II) and Cu(II) on the antibacterial activity of these compounds. In this study, the synthesis, characterization and antibacterial activities of metal complexes derived from isonicotinic acid hydrazide were reported.

MATERIALS AND METHODS

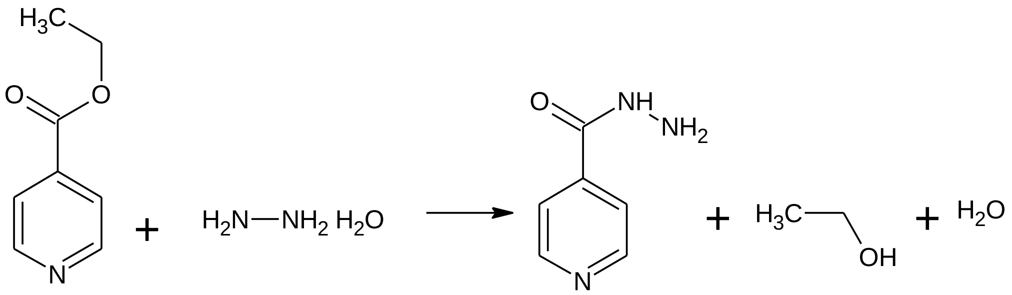
All chemicals used were reagent grade from Sigma-Aldrich or B.D.H companies and used as supplied.

The infrared spectra of the ligand and complexes were recorded as KBr pellets on a (4000-350cm^{-1}) FTIR-8400S Fourier Transform Infrared spectrophotometer (Germany). The UV-Vis spectra of the complexes were recorded at room temperature on UV-2500PC spectrophotometer (Japan), using absolute ethanol as solvent in 1cm quartz curettes. Molar conductance of the complexes was determined in absolute ethanol at room temperature using CON 6/TDS 6 Hand-held Conductivity Meter (USA). The melting point was determined using AAR 3235 Gallenkamp melting point apparatus and the C.H.N. elemental analysis was performed on AA-7000 Atomic Absorption Spectrophotometer (Turkey). These instruments were obtained from National Research Institute for Chemical Technology, Zaria, Kaduna State, Nigeria. Magnetic susceptibilities of the complexes were measured at room temperature using the HO-EM-08 magnetic susceptibility balance (India) at Chemistry department, Kwara State University, Molete, Kwara State, Nigeria. The necessary diamagnetic corrections for ligands were done using Pascal’s table.

The metal content of complexes was determined spectrophotometrically [Co(II), Cu(II), Mn(II), Zn(II)] using Varian AA240 Atomic Absorption Spectrophotometer (USA) and the nitrates content of complexes was also determined spectrophotometrically [3] using PD303 UV spectrophotometer (Japan) at Springboard Research Laboratories, Awka, Anambra State, Nigeria.

Preparation of the Ligand; isonicotinic acid hydrazide (INH)

The ligand INH was prepared by the reaction of ethyl isonicotinate and hydrazine hydrate in absolute ethanol using standard method [4]. 3.2 ml (3.3 g, 0.066 mol) of hydrazine hydrate was added to 10 g (0.066 mol) of ethyl isonicotinate in 50 ml of absolute ethanol. The mixture was refluxed for about 6 h on a magnetic stirrer, poured into a beaker and left overnight to crystallize. The resulting crystal was recrystallized in ethanol, dried over calcium chloride in a desiccator and weighed [6.2g, 68.51% yield]. The equation for the reaction is shown as Figure 1.



[Ethyl isonicotinate] [INH]

Figure 1: Schematic diagram showing the synthesis of Isonicotinic acid hydrazide (INH)

Preparation of metal nitrate complexes

The appropriate metal nitrates (0.007mol) [2.04g $Co(NO_3)_2.6H_2O$, 1.25g $Mn(NO_3)_{2(aq)}$, 1.7g $Cu(NO_3)_2$. $3H_2O$, 2.04g $Zn(NO_3)_2$] in (10 ml) absolute ethanol were heated under reflux and constant stirring with (1.0g, 0.007 mol) of the ligand INH in (10 ml) absolute ethanol for about 2 h. The solid complexes formed were then separated by filtration, washed with ethanol (5 ml) and finally dried and kept in desiccators.

Antibacterial activity

This was evaluated using agar diffusion method [5]. Gram-positive bacteria; *Streptococccus pyogenes*, *Staphylococcus aureus*, and Gram-negative bacteria; *Escherichia coli, Shigella species* were cultivated in nutrient agar on Petri dishes. The test solution was prepared by dissolving (10 mg) of each of the tested compound in (1 ml) acetone. A 6mm diameter filter paper discs were socked in the tested solutions. After 24 hours cultivation at 37°C, diameters of zones of inhibition were determined. Acetone was used as control and was inactive under applied conditions.

RESULTS AND DISCUSSION

The reaction of INH with the metal nitrates, [metal = Co(II), Mn(II), Cu(II), and Zn(II)] in (1:1) molar ratios gave the complexes (Table 1).

The physical properties and chemical analysis of the complexes are given in the Tables (1and 2) and it is in good agreement with the proposed formulas. All the complexes are non-hygroscopic solids and stable in air and are of higher temperatures than the ligand. They are insoluble in water, sparingly soluble in organic solvents like formalin but soluble in acetone and moderately soluble in ethanol and methanol.

The molar conductance values Table 1 of the metal complexes in ethanol are in the ranges (0.02-1.60) ohm^{-1} cm^{2} mol^{-1} indicating non-electrolyte nature of these complexes [6]. The molar conductance values reveal that the metal nitrate complexes Table 1 are non-electrolytic complexes [7].

Table 1: Physical properties and analysis data of the ligand and its complexes

Compound	**Formula**	**Formula weight (gmol^{-1})**	**Colour**	**M.P °C**	**%Yield**	**Molar conductivity (ohm^{-1} cm^{2} mol^{-1})**
INH	$C_6H_7N_3O$	137.0	Cream	170	68.5	0.02
Zn-INH	$C_{12}H_{18}N_8O_{10}Zn$	499.40	White	179	99.1	0.16
Cu-INH	$C_{12}H_{18}N_8O_{10}Cu$	497.50	Light green	187	96.6	0.16
Co-INH	$C_{12}H_{18}N_8O_{10}Co$	492.93	Pale pink	177	80.2	0.26
Mn-INH	$C_{12}H_{18}N_8O_{10}Mn$	488.93	Pale brown	181	92.3	1.60

Table 2: Elemental analyses of the ligand and complexes

Compound	**Molecular Formula**	**Mol. Wgt**	**Elemental analysis: Found (calculated) %**				
			Metal	C	H	N	NO_3^-
INH	$C_6H_7N_3O$	137.00	–	52.86 (52.56)	5.25 (5.11)	30.21 (30.66)	–
Zn-INH	$C_{12}H_{18}N_8O_{10}Zn$	499.40	13.52 (13.10)	28.22 (28.83)	3.52 (3.60)	22.60 (22.43)	24.43 (24.83)
Cu-INH	$C_{12}H_{18}N_8O_{10}Cu$	497.50	14.99 (12.76)	29.05 (28.95)	3.56 (3.62)	22.47 (22.51)	23.53 (24.93)
Co-INH	$C_{12}H_{18}N_8O_{10}Co$	492.93	12.31 (11.96)	29.16 (29.21)	3.22 (3.65)	22.16 (22.72)	24.91 (25.16)
Mn-INH	$C_{12}H_{18}N_8O_{10}Mn$	488.93	12.53 (11.24)	30.15 (29.45)	3.83 (3.68)	22.88 (22.91)	25.86 (25.36)

Infrared Spectra

The main frequencies of the infrared spectra of the ligand and its complexes and their tentative assignments are shown in Table (3). The spectrum of the free INH ligand showed characteristic absorption bands located at 3112cm^{-1}, 1655cm^{-1}, 1550cm^{-1}, 1139cm^{-1} and 993cm^{-1}which are due to ῡ(N–H), ῡ (C=O), ῡ(C=N),ῡ(C–N) and ῡ(N–N) respectively. The IR spectra of the complexes were compared with those of the free ligand in order to determine the involvement of coordination sites in the complexes. In the spectra of metal nitrate complexes, the ῡ(N–H) band is almost unaltered or shifted to higher frequencies indicating non-involvement of this group in coordination and a decrease in hydrogen bonding on complexation may occur. On the other hand, a considerable shift (3-10cm^{-1}) in ῡ(C=O) was observed indicating a decrease in the force constant of (C=O) bond as a consequence of coordination through the carbonyl oxygen atom in these complexes. The shift of the imino group i.e ῡ(C=N) bands to lower wave numbers in the complexes of the ligands by about 3-32cm^{-1}indicates the involvement of the azomethine nitrogen in bonding. The coordination through the azomethine nitrogen atom was further supported by the shift of the ῡ(N–N) vibration observed at 993cm^{-1} in the spectrum of the INH ligand to higher frequency in the complexes by 41-71 cm^{-1}. This is due to the reduction of ions pair repulsive forces in the adjacent nitrogen atoms [8].

The metal complexes also exhibited a very strong band at the region 1350-1380 cm^{-1}which are attributed to the ν_3 vibrations of uncoordinated nitrate ion of D_{3h} symmetry [9].

The appearance of broad band at 3408 – 3449cm^{-1}in the complexes have been assigned to ν(OH) which is associated with coordinated or solvated water molecules which is indicative of the presence of water molecules in the complexes and this implies that water molecules held by a crystal lattice is present in the complexes [10].

Conclusive evidence of the bonding is also shown by the observation that new bands appear in the spectra of the complexes at about 578-605cm^{-1} and 392-477 cm^{-1} which are assigned to ν(M-O) and ν(M-N) stretching vibrations, respectively [11] [12] which are not observed in the spectra of the ligands, because there is no metal coordinated to nitrogen and to oxygen in the ligands [13] [14].

Table 3: Infrared spectra of the ligand and complexes

Compound	**ν(N-N)**	**ν(C=N)**	**ν(C=O)**	**ν(C-N)**	**ν(N–H)**	**ν(H_2O)**	**ν(NO_3^-)**	**ν(M-N)**	**ν(M-O)**
INH	993	1550	1655	1139	3031 3112	–	–	–	–
Cu-INH	1043	1547	1652	1225	3196	3449	1375 852	409	578
Zn-INH	1043	1562	1646	1122 1213	3069 3194	3388	1359 852	396	605
Co-INH	1034	1567	1645	1224	3186	3395	1378 851	477	578
Mn-INH	1064	1568	1645	1194	3248	3389	1345 849	403	598

Electronic Spectra and Magnetic Properties:

The room temperature (25°C) magnetic moments and the electronic spectral data of the complexes are listed in Table (4).The INH ligand spectrum in ethanol shows absorption band at 13,495cm^{-1} due to n $\rightarrow \pi^*$ transition. This band shifts to lower wave numbers in the electronic spectra of most of the complexes at the regions between 13,089cm^{-1} and 12,804cm^{-1} may indicate the coordination of the hydrazide to the metal ions.

The magnetic moment value of Co(II) complex was found to be 4.91 B.M for Co-INH, suggesting octahedral geometry for Co(II)ion [11]. The electronic spectra of Co- INH showed absorption band at the regions 20,921 cm^{-1} assigned to$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transition, corresponding to three unpaired electrons and favouring octahedral geometry around the metal ion[15] [16].

The electronic spectra of Cu –INH complex contain a broad band at 12,804 – 21,097cm^{-1}. This band is characteristic for Cu(II) ion with distorted octahedral stereochemistry and can be assigned to two or three of the transitions $^2B_{1g} \rightarrow {}^2E_g$, $^2B_{1g} \rightarrow {}^2B_{2g}$and $^2B_{1g} \rightarrow {}^2A_{2g}$ [17]. The magnetic moment of the complexes was found to be 1.85 B.M for Cu-INH corresponding to one unpaired electron [15].

The electronic spectra of manganese complex is consistent with spectra of Mn(II) ions with chelates, weak bands of low intensities. The spectrum show single band at 13459cm^{-1} for Mn-INH and is assigned to $^6A_{1g} \rightarrow {}^4T_{1g}(G)$ transition favoring octahedral geometry. The magnetic moment of the complex was found to be 6.07B.M corresponding to five unpaired electrons present in their atomic structures [15].

The diamagnetic Zn(II) complex show no prominent absorption in the visible region because of d^{10} and configuration for Zn(II) ions. Zn-INH complex showed absorption band at 13,477cm^{-1} with no significant shift from that of the ligand. This absorption band is assigned to n $\rightarrow \pi^*$ transition and the complex is expected to have an octahedral geometry as the magnetic data confirmed that the complexes essentially diamagnetic because all the *d* electrons are completely filled and it lies within the octahedral range [15].

Table 4: Electronic spectral and magnetic susceptibility data of the ligand and its complexes

Compound	**μeff B.M.**	**Electronic spectra cm^{-1}**	**Assignment**	**Geometry**
INH	–	13,495	$n \rightarrow \pi^*$	–
Zn-INH	Diamagnetic	13,477	$n \rightarrow \pi^*$	Octahedral
Cu-INH	1.85	12,804 13,089 14,566 16,529 19,084 21,O97	$^2B_{1g} \rightarrow {}^2E_g$ $^2B_{1g} \rightarrow {}^2E_g$ $^2B_{1g} \rightarrow {}^2A_{2g}$ $^2B_{1g} \rightarrow {}^2A_{2g}$ $^2B_{1g} \rightarrow {}^2B_{2g}$ $^2B_{1g} \rightarrow {}^2B_{2g}$	Octahedral
Co-INH	4.91	20,921	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$	Octahedral
Mn-INH	6.07	13,459	$^6A_{1g} \rightarrow {}^4T_{1g}(G)$	Octahedral

Antibacterial Activity

The ligand INH and its complexes were tested against two Gram positive bacteria: *S. aureus* and *S. pyogenes* and two Gram-negative bacteria*: E. coli* and *Shigella spp*. The results are shown in Table 5. From the results, it is clear that the metal complexes of the hydrazide ligand (INH) are more active towards the bacteria tested than the ligand. The results also show that complex formation of the ligand with metal ions increases its antibacterial activity since the complexes showed more activity than their corresponding ligands against the tested bacteria.

Table 5: Antibacterial effects of the investigated compound

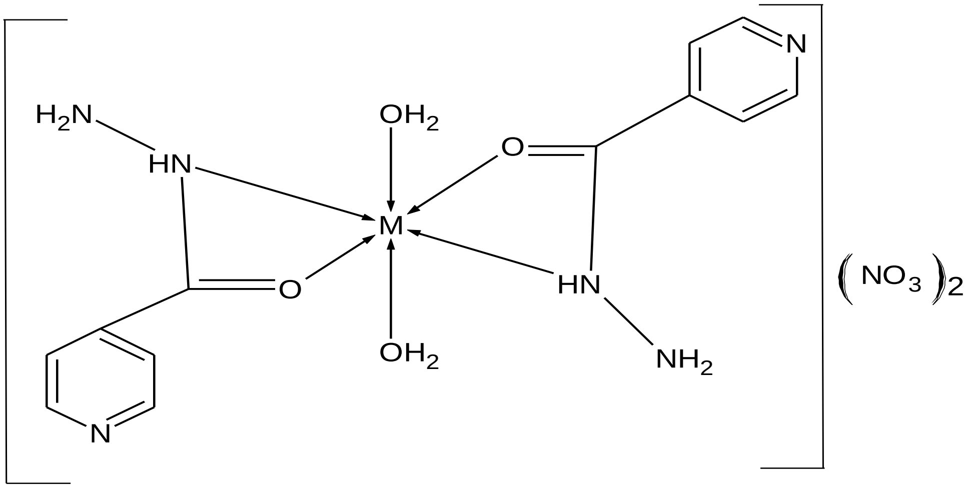
Compound	**Gram positive bacteria**		**Gram negative bacteria**	
	S. pyogenes	*S. aureus*	*E. coli*	*Shigella spp*
INH	+	+	+	+
Zn-INH	+++	++	++	++
Cu-INH	++	++++	++	++
Co-INH	+	++	+	++
Mn-INH	+	++	++	+

Note: ++++ = very high activity, +++ = high activity, ++ = moderate activity, + = some activity

CONCLUSION

The ligand isonicotinic acid hyrazide (INH) was successfully synthesized and formed complexes with Co(II), Cu(II), Mn(II) and Zn(II) nitrates. The ligand acted as neutral bidentate donor, coordinating via the carbonyl oxygen and the azomethine nitrogen. Metal-ligand complexes all show 1:1 molar ratios on complexation. Octahedral geometry is suggested for all the synthesized Co(II), Cu(II), Mn(II) and Zn(II) complexes. The anions (nitrate ions) appear to be in the outer coordination sphere of the complexes.

Generally, the ligand and complexes showed fairly comparable behavior towards the test organisms. INH showed some antibacterial activity and the antibacterial properties of the ligand were enhanced on coordination with transition metal (II) ions. On the basis of their physicochemical properties, the structure proposed for the complexes is presented in Figure 2.



M = Co^{2+}, Cu^{2+}, Mn^{2+} or Zn^{2+}

Figure 2: Proposed structure for the hydrazide (INH) complexes

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