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Mixed Isonicotinylhydrazide – Pyridoxine Metal Complexes: Synthesis, Characterisation and Antimicrobial Activity

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Abstract

Some mixed metal complexes of isonicotinylhydrazide – pyridoxine have been synthesised and characterised by physical and spectroscopic studies such as melting point, conductivity measurement, TLC, FT-IR and UV-visible spectroscopy. The antimicrobial activities of the metal complexes were investigated against some clinical isolates of *Eschericha Coli, Klebsiella Pneumonia, Pseudomonas aureginosa* and *Stapylococcus species* using the agar well diffusion method. The result of the spectroscopic studies revealed that both isoniazid and pyridoxine behave as neutral bidentate ligands. The Isonicotinylhydrazide ligand coordinated through the carboxyl oxygen and amine nitrogen while pyridoxine coordinated through the oxygen of the hydroxyl group and nitrogen of the pyridine group. The result of antimicrobial activity shows that the metal complexes displayed higher inhibitory activity against the tested organisms than the parent ligands.

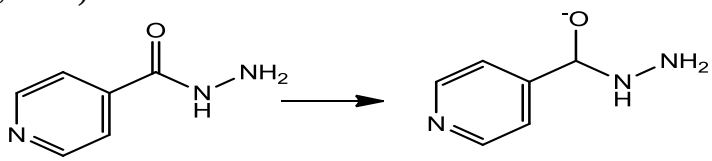
Keyword: isonicotinylhydrazide, pyridoxine, synthesis, metal complexes, antimicrobial activity.

Introduction

Tuberculosis, MTB or TB (*tubercle bacillus*) is a widespread infectious disease caused by various strains of mycobacterium, usually mycobacterium tuberculosis (Kumar *et al*., 2007). It is well known that

metal ions present in complexes accelerate the drug action and the efficacy of the organic therapeutic agents (Siddiqi *et al*., 2010). The pharmacological efficiencies of metal complexes depend on the nature of the metal ions and the ligands (Delaney *et al*., 2010). It is declared in literatures that different ligands and different complexes synthesised from same ligands with different metal ions possess different biological properties (Serbest *et al*., 2001 & Vogel, 1989). Transition metals play some important roles in the biological system, they are electron deficient and some biological molecules are electron rich such as proteins and DNA (Pattan *et al.*, 2012). Development of highly potent metal drug complexes are based on the ability of the coordinating metals to enhance the inhibitory potential of chemotherapeutic agents (Abd El *et al.*, 2004).

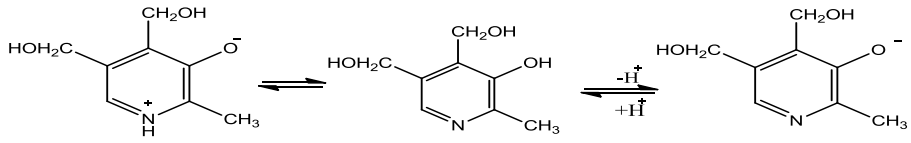
Isonicotinylhydrazide is one of the primary drugs for chemotherapy of tuberculosis. In order to form metal complexes, isoniazid must first form the anion (shown below) which is the chelating species (Albert, 1956).



isonicotinylhydrazide Anion of isonicotinylhydrazide

Pyridoxine (Vitamin B_6), (3-hydroxyl- 4,5-bis(hydroxymethyl)-2-methylpyridine) are essential for the metabolism of amino acids and the maintenance of body cells. (Theoharides,1999).

Pyridoxine molecule has several potential donor groups, such as the pyridine nitrogen and oxygen atoms of the phenolic and alcoholic groups by means of which its coordination to a metal ion can be realized. In aqueous solution, pyridoxine exists in two tautomeric forms and is partially ionized according to the scheme below (Llor & Asensio., 1995).



II pH7.0 1 pH9.0 III

There is an increasing requirement for the discovery of new compounds having antimicrobial, and antitubercular activities. Thus, this paper reports

the synthesis, characterisation and antimicrobial activities of mixed complexes of Isonicotinylhydrazide and pyridoxine.

Materials and Methods

The metal salts used for this research work were obtained from British House Chemical Limited, England. Pyridoxine was a gift from Peace Pharmaceutical Company, Ilorin, Kwara State Nigeria. Isoniazid (isonicotinylhydrazide) was commercially sourced from Sigma Aldrich. USA. Clinical isolates of the organisms were obtained from Department of Microbiology, University of Ilorin, University of Ilorin Teaching Hospital.

Synthesis of the mixed metal complexes

The method described by (Obaleye & Orjiekwe), (Abd El- Wahab & El-Sarrag), were used in the synthesis of the mixed metal complexes. Isonicotinylhydrazide (0.137g, 1 mmol) was dissolved in 20 mL ethanol, pyridoxine (0.169g, 1 mmol) was separately dissolved in 20 mL distilled water. 20 mL of aqueous solution of 1 mmol of metal salts was added to the solution of isoniazid and pyridoxine. There was instant formation of precipitate which was filtered, washed and dried for further analysis.

Antimicrobial activity

The antimicrobial activities of the ligands and the corresponding metal complexes were investigated using the agar diffusion method described by Matangi and co-workers (Matangi *et al*., 2012). Stock cultures of the bacteria were maintained on nutrient agar slants and stored at $4^{o}C$ till when needed.

Preparation of bacterial inoculums

The overnight (18 hours) culture (0.2 ml) of each bacterium was dispersed into 20 ml of sterilized nutrient broth and incubated for about 3-5 hours to attain similar turbidity for all the organisms. The standardized cultures were used as inoculums in the antimicrobial assay. Sterile cotton swabs were used to apply inoculums of each bacterium on the surface of a sterilized cork borer (6 mm diameter). Aliquot (1 mg/ml) of the complexes were then administered into the holes. The petri dishes were then incubated for 24 hours at 37 ^{o}C. After incubation, the effect of the complexes were measured by calculating the zone of inhibition using a transparent ruler and the result recorded to the nearest millimeters.

Table 1: Analytical data of some mixed Isonicotinylhydrazide - Pyridoxine drug metal complexes:

Ligand/ Complexes	Color	Elemental Analysis C H N (calc)%	% Yield	Melting point (^oC)	Conductivity $\Omega^{-1}cm^2 mol^{-1}$	TLC (R_f)
(Isonicotinylhydrazide)	White	-	-	170-172	-	0.75
Pyridoxine	White	-	-	214-216	-	0.53
Cu(IND)(PYR)SO_4	Green	54.89(54.90) 5.92(5.88) 18.29(18.30)	65	264-266	4.67	0.90
Zn(IND)(PYR)SO_4	Cream	54.55(54.58) 5.90(5.86) 18.40(18.43)	72	300-302	3.78	0.81
CU(IND)(PYR)Cl_2	Brown	55.10(55.30) 5.73(5.96) 18.62(18.75)	68	302-304	3.85	0.40
Cd(IND)(PYR)SO_4	White	55.22(55.81) 5.46(5.87) 18.31(18.72)	60	360-362	2.43	0.70

Results and Discussion

The results of the analytical data, spectroscopic studies and antimicrobial activity are presented in Tables 1 to 4 respectively. The melting points of the complexes were generally higher than that of the corresponding parent ligands which suggests the formation of complexes indicating a strong bonding between the ligands and metal ion (Didarul *et al.*, 2010). There was also some observable colour change in all the complexes. The synthesized complexes showed a single spot on the thin layer chromatography. This indicates the coordination between the drug and metal ions and proved that the complexes were obtained in pure form. (Radha *et a*l.,2015). The conductivity measurements of the complexes were very low in the range of 2.43 – 4.67 $\Omega^{-1}cm^2mol^{-1}$ implying that they are non-electrolytes with some degree of polarity (Ogunniran, *et al.*, 2008). From the elemental analysis of the complexes, it was observed that

the data are in good agreement with the proposed structure.

Infrared study

The strong band at 3331 cm^{-1} in the IR spectrum of the pyridoxine is assigned to the vibration of the hydroxyl (O-H) group. This band was lowered to 3420 cm^{-1} for Cu(II), 3398 cm^{-1} for Cd(II) and 3468 cm^{-1} for the Zn(II) complexes indicating the involvement of the hydroxyl oxygen atom in coordination to the respective central metal atoms. The band at 1653 cm^{-1} in the spectrum of Isonicotinylhydrazide is characteristic of the C=O (Didarul et al., 2010). This band was shifted to 1650, 1660, 1588, and 1654 cm^{-1} in the IR spectra of Cu(II), Zn(II), Cu(II) and Cd(II) complexes respectively, indicating the participation of the carbonyl oxygen ion in coordination (Ogunniran, *et al.*, 2008). The shift of the band 3294 cm^{-1} to lower wave numbers suggests the involvement of the amino nitrogen in the coordination to the metal ions The same band was also observed in the spectra of the complexes ([Cd(IND)(PYR)SO_4], [Zn(IND)(PYR)SO_4] and [Cu(IND)(PYR)SO_4]) at higher wavelengths. The infrared spectra of Isonicotinylhydrazide and pyridoxine were compared with that of the complexes as shown in Table 2. Based on the infrared spectra data, the result of the studies supports the proposed bidentate coordination mode for Isonicotinylhydrazide and pyridoxine ligands with Isonicotinylhydrazide coordinating through the carbonyl oxygen and amine nitrogen while pyridoxine coordination occurs through the oxygen of the hydroxyl group and nitrogen of the pyridine group (Mosset *et al*., 1978).

Table 2: Selected infrared spectra for some Isonicotinylhydrazide - Pyridoxine drug metal complexes.

Complexes/Ligands	υ_{N-H}	υ_{O-H}	$\upsilon_{C=N}$	$\upsilon_{C=O}$	υ_{C-N}	υ_{C-O}
Isonicotinylhydrazide	3294	-	1554	1653	-	-
Pyridoxine	3241	3331	1541	-	1217	-
Cu(IND)(PYR)SO_4	3251	3421	1583	1650	1377	1054
Zn(IND)(PYR)SO_4	3257	3468	1549	1661	1348	1116
Cu(IND)(PYR)Cl_2	3300	3420	1525	1588	1391	1045
Cd(IND)(PYR)SO_4	3261	3396	1549	1654	1340	1063

UV/Visible spectra analysis

The UV/ visible spectra of the ligands were compared with those of their corresponding mixed complexes. The spectrum of the free Isonicotinylhydrazide ligand shows two absorption bands at 211 and 295 nm while pyridoxine shows two absorption bands at 260 and 245 nm. These bands were assigned to (π - $\pi^{ж}$ and n- π), (π - $\pi^{ж}$ and π - $\pi^{ж}$) respectively. The spectrum of the mixed complex of Zn(IND)(PYR)SO_4 showed two absorption band at 214 and 265 nm which was assigned to π - $\pi^{ж}$ and n - $\pi^{ж}$. The spectrum of mixed complex of Cd(IND)(PYR)SO_4 showed absorption band at 330 nm assigned to n - $\pi^{ж}$. The bands in the ligands have been shifted to higher wavelengths in the mixed complexes due to complexation (Orgel, 1985).

Table 3: Selected UV-visible spectra for some Isonicotinylhydrazide - pyridoxine drug metal complexes.

Ligands/ complexes	Wavelength(nm)	Energies(cm^{-1})	Assignment
Isonicotinylhydrazide	211	47393	π - $\pi^{ж}$
	295	33898	n- $\pi^{ж}$
Pyridoxine	260	38461	π - $\pi^{ж}$
	245	40816	π - $\pi^{ж}$
Zn(IND)(PYR)SO_4	214	46728	π - $\pi^{ж}$
	265	37735	n- $\pi^{ж}$
Cd(IND)(PYR)SO_4	330	30303	n- $\pi^{ж}$
Cu(IND)(PYR)SO_4	479	20876	$^2Eg(D) \rightarrow {}^2T_2g(D)$
	230	43478	π-$\pi^{ж}$
Cu(IND)(PYR)Cl_2	467	21413	$^6A_{1g} \rightarrow {}^4T_1g(G)$
	310	32258	$^6A_{1g} \rightarrow {}^4T_{1g}(G)$

Antimicrobial activity studies

The antimicrobial activity studies of the ligands and it complexes against *Eschericha coli, Klebsiella pneumonia, Pseudomonas aureginosa, Stapylococcusaureus* have been evaluated. The diameter of the zone of inhibition for the complexes and their ligands are within the range of 1-14.5 mg/ml. The results showed that the mixed complex Cd(IND)(PYR)SO_4 has the highest activity against *Stapylococcusaureus* and *Klebsiella pneumonia* while others showed moderate activity under

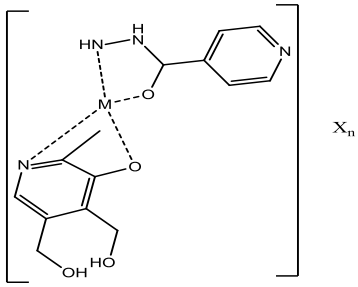
the same condition.

Table 4: Antimicrobial activities of some mixed isoniazid -pyridoxine metal drug complexes.

Complexes/Ligands (1mg/ ml)	ZONE OF INHIBITION			
	E. Coli	*S. Aureus*	*K. Pneumoniae*	*P. Aureginosa*
Isonicotinylhydrazide	1.0	0	13	1.0
Pyridoxine	NA	4.0	NA	NA
$Cu(IND)(PYR)Cl_2$	1.0	0	2.0	5.0
$Zn(IND)(PYR)SO_4$	0	2.0	5.0	0
$Cd(IND)(PYR)SO_4$	0	8.2	14.5	0
$Cu(IND)(PYR)SO_4$	4.2	8.0	0	NA

NA=No activity

Based on these data of structural investigation on the complexes prepared, the following structure is suggested.



where X= SO_4 or Cl_2

Conclusion

Some mixed metal complexes of Isonicotinylhydrazide and pyridoxine have been prepared and characterised by physical and spectroscopic methods. The results suggest that both ligands were bidentate in their mode of coordination with the metal ions. Isonicotinylhydrazide coordinated through the carbonyl oxygen and amine nitrogen while pyridoxine coordinated through the oxygen of the hydroxyl group and nitrogen of the pyridine group. The results of the antimicrobial studies showed that the mixed complexes had higher activity compared to the parent ligands.

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