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Full Length Research Paper

Synthesis, characterization, antimicrobial activity and toxicology study of some metal complexes of mixed antibiotics

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Mixed ligand metal complexes of ampicillin and chloramphenicol prepared by using Ni(II), Co(II) and Fe(III) metal chloride hexahydrate were reported and characterized based on some physical properties and spectroscopic analysis such as AAS, UV, and IR spectroscopy. The complexes were proposed to have the formulae $[ML^1L^2](Cl)_n$ (where M= Ni(II), Co(II), Fe(III); L_1 =ampicillin, L_2 = chloramphenicol , and n=2-3). IR spectra suggested that both L_1 and L_2 coordinated to the metal ions in a terdentate manner with v(O-H), v(C=O) and v(N-H) as donor sites in each of the ligands. From analytical and spectroscopic data obtained, the complexes were proposed to be of octahedral. The synthesized complexes, in compares to their ligands, were also screened for their antibacterial activity against isolated strains of *Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia* by using agar diffusion method. The activity data showed the metal complexes to be more potent antibacterial than the parent drugs against the three bacteria species. However, toxicology tests against some tissues of albino rat (*Rattus novergicuss*) revealed toxicity of the complexes as compared to the parent drugs because the complexes were found to significantly increase ($P<0.05$) alkaline phosphatase from homogenates of liver and kidney tissues of the tested doses. However, there was no significant difference ($P>0.05$) in ALP of rat serum. The results generally indicated that more potent compounds with better physical properties and enhanced antimicrobial activities upon complexation have been prepared.

Key words: Metal complexes, complexation, antibiotics, antimicrobial properties, alkaline phosphatase

INTRODUCTION

Chemotherapy is one of the most important tools for the management of diseases since the ninetieth century. However, emergence and spread of parasites resistance to almost all available drugs is of great concern. The situation is critical in Africa as a result of the spread of resistance to the inexpensive drugs widely used for treatment of diseases. As an alternative, a number of combinations are being recommended and implemented, but the questions about cost and adequacy of the supply necessitate the need to identify novel agents (Mohamed et al, 2006). The discovery of new metal based drugs has been largely based on ability of metals to increase inhibittory potential of chemotherapy agents. Efficacy of some therapeutic agents has been reported to have increased upon coordination to transition metals (Abd El and El-Sariag, 2004). The development of more potent metal based drugs has been under investigations over the last three decades, and it has been discovered that inorganic compounds have enormous impart in medicine. Literally, thousands of compounds have been prepared based on well conceived ideas of improving their efficacy and have been subsequently screened but few of them have successfully passed the clinical tests (Paul and Giann,

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2006). Some metal-based antibiotics such as bleomycin, streptonigrin, and bactracin have gained recognition and are more effective than pure drugs (Li-june, 2003). Ampicillin, a betalactam antibiotic, has been used extensively to treat bacterial infections. Chloramphenicol is a bacteriostatic antimicrobial agent that was effective against wide variety of micro-organisms. It was a frontline antibiotic for the treatment of meningitis and urinary tract infections (Mayne, 1999).

Metal-based ampicillin and metal based chloramphenicol have been investigated in our previous work (Ogunniran et al., 2007). The study was aimed at isolation of transition metal complexes, structural elucidation using various physicochemical techniques and their biological screening against human pathogenic micro-organisms. In continuation of this work, we intend to incorporate two different ligands (ampicillin and chloramphenicol) into a metal complex with the aim of possible availability of more potent dual character antibiotics. Thus, we report synthesis, some physico-chemical properties, micro-organisms inhibition and toxicity study of some metal complexes of ampicillin mixed with chloramphenicol.

MATERIALS AND METHODS

Sources of materials

All the solutions were prepared with analytical grade reagents (Sigma) and distilled/deionised water. (Milli-Q-ystem by Milli-Pore Inc.;water resistance 18 MΩ). Ampicillin trihydrate and oxytetracycline HCl were obtained from Rajrab pharmaceutical Company, Ilorin, Kwara state, Nigeria, which are product of Sigma Company, London. Metal salts used for complexation (iron (III) chloride hexahydrate, nickel (II) chloride hexahydrate, and cobalt(II) chloride hexahydrate) were obtained from British Drug House Chemical Limited Co. Poole, England. Alkaline phosphatase assay kit was obtained from Randox Laboratories Limited Co. Antrim, United Kingdom, through Biochemistry Department, University of Ilorin, Nigeria. Isolates of *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* were obtained from the Department of Microbiology, University of Ilorin, Nigeria. Albino rats (*Rattus novergicuss*) were obtained from the department of Biochemistry, University of Ilorin, Nigeria.

Synthesis of the metal complexes

A mixture of 0.01mole (4.085g) of ampicillin trihydrate dissolved in 10mls of distilled water and 0.01mole (3.231g) of oxytetracycline in 10mls of distilled water was heated on a steam bath until homogeneous solution was formed. The solution formed was mixed with the solution of each metal salts (0.01mole in 10ml of distilled water) in a round bottom flask fitted with a condenser. The reaction mixture was refluxed for 6 hrs after which it was cooled using ice. The crystalline precipitates that separated were filtered, washed thoroughly with distilled water and dried in a desiccator for one week.

Determination of physical properties of the complexes

The melting points were recorded on a Gallenkamp melting point apparatus and were uncorrected. The metal content of the metal complexes were determined using an SP Pye Unicam Atomic Absorption Spectrophotometer. Infra-red spectra (KBr) were measured using Perkin Elmer spectrophotometer. UV spectra (MeOH) were obtained on a LKB 4053 spectrophotometer. Conductivity (MeOH at 25^{o}C) was determined using WTW Conductometer Bridge with 0.82cm^{-1} as cell constant. Molecular weights of the compounds were determined by using Rast's camphor method (Vogel, 1989). Purity of the compounds was confirmed by using Thin Layer Chromatography (TLC).

Antibacterial screening

Antibacterial activities of the antibiotics and the metal complexes were screened against three human pathogenic bacterial viz: *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus*. For the detection of the antibacterial activities, the filter paper disc agar diffusion method was used. Pure ampicillin and oxytetracycline was used separately as standard for antibacterial activities test. Nutrient agar (NA) was used as basal medium for the cultured bacteria. 0.1cm3 of each of the compounds was applied to the agar media on which 1.0 cm diameter wells were punched and incubated at 37oC for one to three days. 1.0% w/v of the sterile filtered solutions of the ligands and the metal complexes were made using methanol. Discs with only methanol were used as control. Inhibitory activities were measured (in mm) as the diameter of the observed inhibition zone formed around the wall of the seeded agar plates. The antibacterial activities were based on percentage inhibition calculated by using the average diameter of bacterial colony on the growth medium compared with their respective control as follows:

$$\% \text{ inhibition} = \frac{(A-B) \times 100}{A}$$

Where A=Average diameter of growth of organisms in the control
B=Average diameter of growth of organisms in the test plates

Treatment of animals

A total of thirty albino rats of wistar strain weighing between 160-180 g, housed in clean metabolic cages contained in well-ventilated house conditions (Temp.28-31^{o}C); photoperiod: 12hrs natural light and 12hrs dark; humidity:50-55%, were allowed free access to rat pellets (Bendel Feeds and Flour Mill, Ewu ,Nigeria) and tap water. They were randomly categorised into six groups consisting of five animals each. Animals in group A serve as the control and received distilled water, whereas groups B and C were respectively administered with ampicillin and chloramphenicol only, while groups D, E, and F were administered accordingly with Co(AMP)(CHL)Cl_2, Ni(AMP)(CHL)Cl_2 and Fe(AMP)(CHL)Cl_3. The distilled water and solution of metal complexes (1cm^3) were administered orally to the rats in the various groups three times daily for 5 days at the dose level of 3.33 mgkg^{-1} body weight. All the rats were sacrificed after five days of treatment and blood samples were collected in dry and clean tubes.

Preparation of serum and tissue homogenates

The method described by Yakubu et al. (2005) was modified and used to prepare the serum. The rats under ether anesthesia were made to bleed and blood collected into clean, dry centrifuge tube after which they were left for 10 min at room temperature. The tubes were then centrifuged for 15 min using Uniscope Laboratory Centrifuge (Model SM 800B, Surgifriend Medicals Essex, England). The sera were thereafter aspirated using Pasteur pipettes into clean, dry sample bottles and kept at a temperature of -10^{o}C overnight. The rats were quickly dissected and the liver and kidney

Table 1. Some physical properties of the ligands/metal complexes

Compounds	Colour (Form)	Melting Point (^oC)	Conductivity($\Omega^{-1}cm^{-1}$) * Methanol (solvent)
Ni(AMP)(CHL)Cl_2	Shining green (crystal)	201 (Decomposed)	1.8 x 10^{-6}
Co(AMP)(CHL)Cl_2	Light Brown (crystalline powder)	158-159 (Decomposed)	5.9 x 10^{-6}
Fe(AMP)(CHL)Cl_3	Yellow (crystalline powder)	155 (Decomposed)	1.5 x 10^{-6}
Ampicillin(AMP)	White powder	204-206	9.9 x 10^{-7}
Chloramphenicol(CHL)	White powder	151-152	9.9 x 10^{-7}

Table2. % Yeild of the metal complex of Ampicilin(AMP) mixed with Chloramphenicol(CHL) and their proposed structural formulae

Ligands+Metal salt	% Yield	Molecular Mass [m.wt/g] Theoretical (Exp.)	Metal Content (%) Theoretical (Exp)	Proposed Structural formulae
$NiCl_2$ + AMP + CHL	59.3	802.25 (798.0 ± 1.01)	7.32 (7.40)	Ni(AMP)(CHL)Cl_2
$CoCl_2$ + AMP + CHL	50.8	802.47 (800.04 ± 0.22)	7.34 (7.37)	Co(AMP)(CHL)Cl_2
$FeCl_3$ + AMP + CHL	56.4	834.84 (835.10 ± 0.97)	6.69 (7.03)	Fe(AMP)(CHL)Cl_3

Table 3. Solubility of the ligands and metal complexes in some selected solvents

Ligands/complexes	Distilled water	Ethanol	Methanol	Acetone	Benzene	Petroleum ether
Ampicillin(AMP)	S	SS	SS	SS	NS	NS
Chloramphenicol(CHL)	SS	SS	SS	SS	NS	NS
Ni(AMP)(CHL)Cl_2	SS	S	S	S	NS	S
Co(AMP)(CHL)Cl_2	SS	S	S	S	NS	S
Fe(AMP)(CHL)Cl_3	S	S	S	S	NS	S

S-Soluble, SS-Slightly Soluble, NS-Not Soluble

organs removed. The kidneys were decapsulated after which the organs were blotted in tissue paper and weighed. The tissues were homogenized separately in 0.25 M sucrose solution (1:5 w/v). The homogenates were stored in a temperature of -10^{o}C for 24h before being used for the estimation of alkaline phosphatase activities.

Estimation of enzyme activity

The activities of alkaline phosphatase concentration in the serum and homogenate of both liver and kidney were estimated using the method described by Wright et al. (1972).

Statistical analysis

Statistical significance was determined using Duncan Multiple Range Test and values were considered statistically significant at $P < 0.005$.

RESULT AND DISCUSSION

The complexes are of various colours (Table I). The Ni(II) complex [Ni(AMP)(CHL)]Cl_2 is shining green, Co(II) complex [Co(AMP)(CHL)]Cl_2 is light brown while Fe(III) complex [Fe(AMP)(CHL)]Cl_3 is yellow in colour. The results obtained from micro analytical measurements and metal estimation data (Table I and II) confirm the stoichiometry of the complexes and suggest the formation of the complexes as per the equation 1. The yields (%) of the complexes are averagely commendable. Ni(II) complex has the highest % yield of 59.3 while Co(II) complex has the lowest yield of 50.8%. Theoretical metal content (%) and molecular weight (g) obtained were found to compete favourably to experimental values obtained. The complexes are non-hygroscopic, air and photo stable crystalline powder with different melting point ranging from 155-200^{o}C. The melting points of the complexes are higher than their respective antibiotics. The results of the conductivity measurements (Table I) in methanol revealed that the complexes are non-electrolyte. The solubility of the metal-complexes in various solvents (Table III) confirmed the diversity of the complexes as the ligands.

Table 4. Infrared Spectroscopic and Electronic Spectra of the Ligand and Metal Complexes

Ligands/complexes	Infrared frequencies			Methanol
	v(*O-H*) cm^{-1}	v(*N-H*) cm^{-1} (amide)	v (*C=O*) cm^{-1}	$\lambda_{max}cm^{-1}$(nm)
Ampicillin(AMP)	3514.5 w,b 3454.5 m,b	3620.7 m 3610.6 m	1776.1 v,s	31250 (320)
Chloramphenicol(CHL)	3482.6 w 3346.58 b	3793.8 m,b 3705.5 w	1692.2 v,s	33333 (300)
Ni(AMP)(CHL)Cl_2	m,b 3256.7 m,b	3797.2 m,b 3722.9 w	1659.3 s	23810 (420)
Co(AMP)(CHL)Cl_2	3353.0 m 3259.5 m,b	3786.4 m,b 3718.7 m,b	1684.9 s	24390 (410)
Fe(AMP)(CHL)Cl_3	m 3262.3 m	3782.1 m,b 3712.3 m,b	1661.0 s	25315 (395)

The complexes were found to be soluble in ethanol, methanol, acetone and petroleum ether. Ni(II) complex [Ni(AMP)(CHL)]Cl_2 and Co(II) complex [Co(AMP)(CHL)]Cl_2 were found to be slightly soluble in distilled water while Fe(III) complex [Fe(AMP)(CHL)]Cl_3 was completely soluble. However, all the complexes were found to be non-soluble in benzene.

The presence of chloride ion outside the coordination sphere was confirmed by the presence of white precipitate of AgCl with the use of $AgNO_3$ solution. Hence, the proposed synthetic equation for the synthesized complexes could be represented as:

$$M_1X_2.6H_2O + L_1 + L_2 \rightarrow M_1L_1L_2X_2 + 6H_2O$$
$$M_2X_3.6H_2O + L_1 + L_2 \rightarrow M_2L_1L_2X_3 + 6H_2O$$

Where:

$M_1 = Co(II)\&Ni(II)$, $M_2 = Fe(III)$, $X = Cl$, $L_1 = Ampicillin$, $L_2 = Chloramphenicol$

The related infrared data of the complexes and the ligands have been collected (Table IV). The vibrations centred around 3454.5cm^{-1} and 3514.5cm^{-1} have been assigned to v(*O-H*) stretching frequency which upon complexation have undergone hypsochromic shift in the complexes with increased intensities. The two medium bands at 3620.7cm^{-1} and 3610.6cm^{-1} observed in ampicillin's spectrum were critically assigned to v(*N-H*) vibraion of amide group (Oladipo et al., 2005). The similar bands were observed at higher wavelengths in the spectrum of chloramphenicol with one medium/broad band at 3793.8cm^{-1} and one weak band at 3705.0cm^{-1}. However, related bands appeared in the metal complexes with bathochromic shift as compared to both ligands. The bands attributed to v(*C=O*) vibrational stretching were observed in the spectra of both ligands at 1776.1cm^{-1} in ampicillin and at 1692.2cm^{-1} in chloramphenicol. The bands were observed to be strong in intensity. The relevant bands were observed in the metal complexes with lower wavelength shift as compared to both ligands coupled reduction in their intensities. This suggests complexation through the v(*O-H*), v(*N-H*) and v(*C=O*) moieties of both ampicillin and chloramphenicol. The shift to lower frequencies for v(*O-H*) and v(*C=O*) vibrational bands suggest weak *O-H* and *C=O* bonds and stronger *M-O* bond in the complexes. While shift to higher frequency region suggests a v(*N-H*) moiety of complexes suggest stronger *N-H* bond and weak *M-N* bond being formed. The assignment and interpretation of vibrations below 1500cm^{-1} are difficult to make with certainty because they occur in the finger-print region where coupled vibrations occur. Other metal-ligand related vibrations were observed below 600cm^{-1}.

The ligands (Ampicillin and Chloramphenicol) showed single band at 31,250cm^{-1} (320nm) and 33,333cm^{-1} (300nm), characteristic λ_{max} obtained from numerical calculations based on Woodward-Fieser calculation respectively (Table IV). The complexes show similar band (sharp) at 23,810cm^{-1} (420nm), 24,390cm^{-1} (410nm) and at 25,317cm^{-1} (395nm) for Ni(AMP)(CHL)Cl_2, Co(AMP)(CHL)Cl_2 and Fe(AMP)(CHL)Cl_3 respectively. Shifting of the band to higher wavelength in the metal complexes confirmed the effective coordination of the ligands with metal ions and thereby, the formation of the complexes. The transition from ultraviolet region to the visible region observed in Ni(II) and Co(II) complexes was attributed to d-d transition which could be d_{xz} or $d_{yz} \rightarrow d_{x^2-y^2}$ electronic transition.

The inhibition results (Table V) of the metal complexes against some bacteria species showed significant differences in metal complexes sensitivity as compared to the parent drugs. The percentage inhibition values obtained for the metal complexes against the three bac-teria used were more significant than those of the parent drugs. They were almost double folds of those of both ligands and the values were higher than those of single-ligand complexes. Therefore, this observation concluded that the mixed ligands complexes were more active than both the single-ligand metal complexes and the pure ligands used. The effect of oral administration of the ligands and

Table 5. % Zone of inhibition (mm) of the drugs and the metal complexes at concentration of 1.0% w/v on the microbial population

Ligands/complexes	Bacteria		
	Escherichial coli	***Staphylococcus aureous***	***Klebsiella pneumonial***
Ampicillin(AMP)	48.67 ± 1.15 *	37.00 ± 1.00 *	33.00 ± 1.73 *
Chloramphenicol(CHL)	50.82 ± 0.82 *	51.00 ± 0.31 *	49.12 ± 0.97 *
Ni(AMP)(CHL)Cl_2	88.02 ± 1.20 **	85.32 ± 0.38 **	87.56 ± 2.31 **
Co(AMP)(CHL)Cl_2	82.80 ± 1.01 **	78.56 ± 0.56 **	76.62 ± 1.07 **
Fe(AMP)(CHL)Cl_3	88.51 ± 0.92 **	70.86 ± 1.21 **	92.72 ± 0.34 **

Values are mean ± S.D of 3 replicates.
Values carrying superscript different from their parent antibiotics for each micro-organism are significantly different (P<0.05)

Table 6. Results of toxicology test

Ligands/complexes	Kidney homogenate	Liver homogenate	Serum
Control	125.92 ± 11.20 *	10.03 ± 1.43 *	4.52 ± 0.07 *
Ampicillin(AMP)	262.70 ± 189.30 *	16.07 ± 1.26 *	4.00 ± 0.73 *
Chloramphenicol(CHL)	261.21 ± 17.53 *	15.05 ± 1.14 *	4.07 ± 0.05
Ni(AMP)(CHL)Cl_2	301.01 ± 15.02 **	23.99 ± 0.35 **	7.02 ± 0.04
Co(AMP)(CHL)Cl_2	292.11 ± 15.34 **	21.65 ± 0.76 **	7.00 ± 0.03
Fe(AMP)(CHL)Cl_3	298.37 ± 15.90 **	26.90 ± 0.63 **	6.92 ± 0.05

* P>0.05 **P<0.05

the mixed ligands metal complexes on the serum and homogenates of liver and kidney of albino rats are as reported (Table VI). Compared with the control, administration of the ligands and metal complexes at the dose of 3.33mg kg^{-1} body weight, all produced significant increase (P<0.05) in the alkaline phosphatase (ALP) activities of the serum, liver and kidney of albino rats. Ni(AMP)(CHL)Cl_2 produced about 1.2 and 1.4 folds increase in kidney and liver activity respectively. Co(AMP)(CHL)Cl_2 produced about 1.1 and 1.35 folds increase of the enzyme activity in the kidney and liver respectively. While Fe(AMP)(CHL)Cl_3 also produced about 1.1 and 1.7 folds increase in the activity of the kidney and liver respectively while. However, administration of the ligands and the complexes at 3.33 mg kg^{-1} body weight did not produce any significant change (P > 0.05) in the serum (ALP) activities.

More potent metal complexes were confirmed by analytical and spectroscopic data obtained. This was ascertained by inhibition results obtained (Table V). The colours exhibited by metal complexes (Table 1) are attributed to the dxz, dyz→dx^2-dy^2 electronic transition. Observation of the d-d transition suggests a tetragonally elongated octahedral geometry around metal ions in the complexes. The percentage yield (Table II) of the metal complexes showed that Ni(AMP)(CHL)Cl_2 with 59.3% yield could be produced experimentally than both Co(AMP)(CHL)Cl_2 and Fe(AMP)(CHL)Cl_3 with 50.8% and 56.4% yield respectively. Higher conductivity, indication of high degree of dissociation and solubility of the ions in solution, observed in the complexes as compared to the ligands confirmed the presence of metal ion in the coordination sphere of the complexes. The reduction in melting points (Table I) as compared to the ligands confirmed weak *C-O, N-H* and perhaps some *M-L* bonds in the metal complexes. The complexes are practically useful in the pharmacy because they are partially soluble in polar solvent and completely soluble in ethanol (Table III). The similarity in the spectra is due to the presence of the same ligands in the metal complexes. The strong bands attributed to carbonyl vibrational bands v(*C=O*) in the spectrum of the ligand [1776.1cm^{-1} (Ampicillin) and 1692.2cm^{-1} (Chloramphenicol)] have shifted to lower wavelength, coupled with reduction in intensity in the metal complexes, due to complexation. Coordination of the ligands to the central metal ions in the complexes was also proposed to be through v(*O-H*) and v(*N-H*) vibrational group due to hypsochromic shifts observed. Thus, both ligands were proposed to co-ordinated to the central metal ion, and therefore each of them acted as terdentate ligand. Therefore, complexes were proposed to be of octahedral structure.

The electronic spectral data further confirmed d-d transition in the metal complexes. It showed that there is π-π* transition of the ethylenic double band and n-π* of v(*C=O*), v(*N-H*) and v(*O-H*) vibrational group. The red shift observed in these bands in the metal complexes as compared to the ligands spectra critically confirmed com-

plexation (Zeinab, 2006). The features of the ligand field spectral bands in the complexes are typically of octahedral complexes (Brian, 1999).

The increased percentage inhibition property (Table V) of the metal complexes as compared to the ligands confirmed increased in efficacy of the complexes. Mixed ligands complexes are about 30% higher in activity than the single ligand metal complexes (Ogunniran et al, 2007). Thus, suggest that mixed antibiotics complexes could be more toxic to pathogenic bacteria than pure antibiotics and therefore are better potential antibacterial drugs. ALP is used as the marker enzymes for obstructive jaundice and intra-hepatic cholestasis. Serum alkaline phosphatase activity can be used to study bile duct obstruction and bile duct diseases such as primary biliary cirrhosis or primary sclerosing cholangitis (Davern and Scharschmidt, 2002). It is also a marker enzymes for kidney, placenta and bones (Mayne, 1999, Yakubu et al ,2005). The significant increase in the ALP activity of rat kidney and liver (Table VI) may be attributed to toxicity of the complexes to the enzymes of the organs and thereby increasing indiscriminately, hydrolysis of phosphate ester of the organs and other cells requiring these essential molecules. This indicate that the complexes may likely cause damages to the external boundary of the cells of liver and kidney. However, insignificant values obtained for the serum enzymes are an indication that, the complexes may not affect serum plasma (Yakubu, 2006).

This study has shown the feasibility and a justification for the synthesis of mixed antibiotics metal complexes. The complexes possessed better physical properties and are much more effective as chemotherapy agents than their parent antibiotics. However, the complexes may be toxic at the dose level used to the liver and kidney (Stanley, 2003) but can be consider as potential antibiotics drugs after reduction in the level of metal ion which is responsible for the toxicity.

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