Mixed Ligand Complexes of Cobalt (II) Barbitone in Aqueous Medium and their Biological Activities

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Abstract

Complexes of cobalt (II) barbitone and thiocyanate ion mixed ligands have been synthesized and characterized by a direct mixing method on the basis of their solubilities, melting points, elemental analyses as well as the spectroscopic analyses. Biological evaluations of the solid metal complexes and the free ligand were screened against the selected bacteria pathogens viz: Pseudomonas aeruginosa and bacillus subtitis and fungi species which include *Sclerotiania trifoliorun, Fusarium oxyporium, Stemania paradoxa* and *Botrytis cinerea.* The results of the metal complexes analyses have revealed that cobalt ions coordinated bidentately with the ligand through the ring carbonyl oxygen and nitrogen atom of the amide group, and also found to be soluble in ethanol and dimethylsulphuroxide and non-electrolytic in nature/character. The biological activities of metal complexes revealed that they were toxic to the selected fungi but not effective as antibacterial agents except in the mixed ligand metal complexes. However, complexes were more potent than the free ligand.

Keywords: Barbitone, mixed ligand, cobalt and biological activities.

1.0 Introduction

The derivatives of barbituric acid (2,4,6- trioxopyrimidine) also known as barbitone in which sodium barbitone is one the barbiturates. They are a class of drugs that have diverse applications such as hypnotics, sedatives and anesthesia because of the wide range of medical applications of barbiturates and their ability to coordinate with transition metals through one or both deprotonated nitrogen and carbonyl oxygen atoms (Block & Beale, 2004). Barbitone was prepared by condensing diethylmalonic ester with urea in the presence of sodium ethanoxide and by adding at least two molar equivalent of ethyl iodide to the silver salt of malonylurea or possibly of the acid. The result was an odourless slightly bitter, white crystalline powder (Enghag, 2004).

Structural properties of barbiturates have received much attention due to their importance in medicine and therefore, the crystal structure of barbitone or barbital and its sodium and calcium salts were studied. The relationship between structure physical and chemical properties and pharmacological activity of a large amount of barbiturates were received (Doran, 1985). Most of the reported barbital metal complexes are of general formular $M(II)[Barbitone]_2L_2$ where M can be either of Zn, Cd, Pt or Cu and L = ammonia or have been studied and characterized (Dombrowski *et al.,* 1998). The molecular structure of these two complexes showed that the donor atom in the barbiturate atom is a deprotonated nitrogen atom.

A series of cobalt mixed ligand complexes of types $[Co(en)_2L]^{3+}$, $[Co(en)_2L]^{2+}$, where L can be bipyridine, phenanthroline, imidazole, methylimidazole, ethylimidazole, urea, acetamide, thioacetamide, semicarbazide, thiosemicarbazide or pyrazole have been employed by using absorption infrared and ‘HNMR spectra methods. All these complexes were found to be potent antimicrobial agents.

2.0 Materials and Methods

All the reagents, sodium barbitone, cobalt (II)chloride hexahydrate (sigma) and potassium thiocyanate Isigma) were of good analytical grade (AR) and were used without further purification.

2.1 Analytical Methods

Chloride and sulphate compounds were analyzed by gravimetric method; metal content was determined by complexometric titration. The molar conductance of solid complexes in DMSO was measured by using a model WPA CM35 conductivity meter in the Department of Chemistry, Federal University of Technology, Akure, Nigeria.

The solid state FTIR spectra of the metal complexes and the ligand were recorded on a Shimadzu FTIR spectrophotometer using KBr pellets in the range of 400-4000 cm^{-1}

2.2 Synthesis of the metal complexes

A general method was adopted for the preparation of the metal complexes. Co(II) complexes of barbitone were synthesized by direct mixing methods which involved the addition of 0.476 g of the metal salt($CoCl_2.6H_2O$) to 20 ml H_2O. The reaction mixture was stirred continuously for 3 h. The precipitate formed was filtered and washed properly with distilled water followed by diethyl ether and dried in a dessicator. Mixed ligand metal complexes were also synthesized using potassium thiocyanate by adding 0.197 g in 20 ml distilled water to the metal/barbitone mixture. The processes of stirring, filtration, drying and weighing were also carried out as above stated.

2.3 Antimicrobial evaluations of the complexes are as follows

2.3.1 Bacteria

Bacteria used for this experiment include *Xanthomonas oxynopoides, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis*. All bacteria were cultured aerobically at 37°C for 24 h in peptone water and antimicrobial tests were carried out on the nutrient Agar plates. These were seeded on the sterile nutrient Agar (NA) plates containing 8 mm Wells. 0.2 g/ml concentration of each of the metal complexes and the control (streptomycin sulphate at 0.05 g/ml concentration) prepared and 0.5 ml each sample solution was introduced into the bore Agar Wells and incubated for 24 h at 37°C. Zone of inhibition around wells was measured and results were quoted as the radii (mm) of the zone of inhibition around the Wells (Onifade, 1998; Mishra *et al.,* 1995).

2.3.2 Fungi

Five fungi pathogens which include *Sclerotinia trifoliorum, Fusarium oxyporium, Stemania paradoxa* and *Botrytis cinerea* were used for the experiment. 0.02 g/ml of each of the samples were aseptically mixed with 15 ml of Potato Dextrose Agar (PDA) sterilized inside autoclave, the paired plates 0.25 g/ml of *Benlate* (a standard antifungal agent) was used as positive control. The paired plates were inoculated aseptically with 6 mm fungi and incubated at 27°C for 7 days. The fungi growth in each of the plates was measured at interval of 24 h and the percentage growth inhibition was calculated using the formular

$$\%\ \text{Growth inhibition} = \frac{(NTR-TR)}{NTR}\ x\ \frac{100}{1}$$

Where: **NTR =** average diameter of fungal colony in negative control sets (without treatment)
TR = average diameter of fungal colony in treatment sets.

3.0 Results and Discussion

Solid Co(II)barbitone complexes and the mixed ligand, thiocyanate ions gave appreciable yields and black residues, indicating that ligands have dominant effect on the colours of the (metal) cobalt(II) complexes (Lee, 1989; Wilkinson, 1998; Osowole, 1989). The sharp melting points achieved between 190 – 250°C, showing the metal complexes are pure while solubility of the complexes of cobalt(II) barbitone and its mixed ligand are soluble in ethanol and DMSO and molar conductance results revealed that the complexes are non-electrolyte.

The results of the elemental analysis showed a close relationship between the calculated and experimental, these assist in predicting the tetrahedral geometry for the metal complexes (Table 1).

Table 1: Physical analysis of barbitone cobalt complexes

Complexes	Ratio	M/Wt	%Yield	Colour	M.pt	S	Cl
$[CoLCl(H_2O)_2]H_2O$	1:1	353.67	22.5	Black	190	–	10.0(9.79)
$[CoL_2ClH_2O]H_2O$	1:2	541.84	47.6	Black	205	–	6.56(7.57)
$O[CoLClH_2OSCN]H_2O$	1:1:1	359,17	35.2	Black	195	9.7(10)	9.0(8.9)
$[CoL_2ClSCNH_2O]H2O$	1:2:1	582.84	22.2	Black	225	6.0(6.04)	6.90(7.20)

L = $C_8H_{11}N_2NaO_3$; **Mol/Wt. =** 206.17

IR-spectroscopic studies of the metal complexes demonstrated different bands/absorptions between free ligand and the metal complexes. The prominent band at 521 cm^{-1} in the ligand spectrum but with a shift to 508, 520, 621 and 520, 621.29 cm^{-1} in the metal complex spectra have shown the presence of chloride ions from the cobalt(II) salt found in the inner coordination sphere, a band found at 784 cm^{-1} in the spectra of the ligand with a shift bath chromic shift to 840.40, 820, 628 cm^{-1} in the metal complexes could be attributed to the coordination of cobalt ion to ligand (Kemp, 1989).

On the other hand, absorption band at 1116 cm^{-1} attributed to C=O, but with a characteristic shift to 1040, 1042 and 1119 and 1121.16 in the metal complexes, revealing the coordination of cobalt to the oxygen atom of the carbonyl group, C=O in the ligand (Co-L). also uncoordinated C=N, C=C stretching vibration coupled bands at 1560 but with conspicuous shifts to 1637, 1670.11 and lower shift to 1415 and 1400 cm^{-1} in the mixed ligand metal complexes showed the involvement of nitrogen donor atom in the amide group of the ligand. This implies that ligand is bidentately bonded to the metal ion through the ring carbonyl oxygen and nitrogen atom of the amide group. Another prominent band in the ligand spectrum of 3187 cm^{-1} which is attributed to NH but with the disappearance bands in the metal complexes show the coordination of cobalt ions to the ligand through the nitrogen donor atom of the ligand (Omuku *et al.,* 2010).

Table 2: Solubility tests of barbitone cobalt complexes

	Complexes/Compounds			
Solvent	$[CoLCl(H_2O)_2]H_2O$	$[CoL_2ClH_2O]H_2O$	$[CoLSCNClH_2O]H_2O$	$[CoL_2SCNCl]H_2O$
Water	Soluble	Soluble	Insoluble	Insoluble
Acetone	Soluble	Insoluble	Slightly	Slightly soluble
Ethanol	Soluble	Soluble	Soluble	Soluble
n-hexane	Insoluble	Insoluble	Soluble	Slightly Soluble
Chloroform	Soluble	Insoluble	Slightly	Slightly
Propan-2-ol	Insoluble	Insoluble	Slightly	Slightly
DMSO	Soluble	Soluble	Soluble	Soluble
Diethyl ether	Insoluble	Insoluble	Insoluble	Insoluble

However, the new bands at 3425, 3460, 3458, 3455 and 3760 cm^{-1} found in the spectra of the metal complexes but conspicuously absent in the ligand spectrum reflecting the presence of O-H from the water of crystallization in the cobalt(II) salt (William & Fleming, 1989; Omuku *et al.,* 2010) (Table 3). Also a strong band at 2371.42 cm^{-1} common to the mixed ligand metal complexes could be attributed to the presence of thiocyanate ions –S–C≡N which confirmed the coordination of cobalt(II) ions to sulphur atom in the thiocyanate ions (secondary ligand) (Kemp, 1989; William & Fleming, 1989; Olagboye *et al.,* 2013).

Table 3: Spectroscopic bands of barbitone cobalt complexes

	Na	**Co-L**	**C≡N**	**C=O**	**-SCN**	**NH**	**OH**
Ligand	521	784	1117	1560	–	3187	–
[CoLClH$_2$O)2]H2O	508	–	1041	1637	–	–	3425.60
$[CoL_2ClH_2O]H_2O$	520	840	1042	1670.11	–	3169.70	3460
$[CoLSCNCl]H_2O$	621	820	1119	1559	2371.42	–	3458
$[CoL_2SCNCl]$	520/621.29	828	1121	1582.0	2371	–	3455/3760

The solid metal complexes and parent or free ligand in the control were screened against the *Xanthomonas oxynopoides, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* bacteria and *Sclerotina trifoliorum, Fusarium oxyporium, Stamina paradoxa* and *Botrylis cinerea* fungi. The results of the bacteria analyses revealed that only the mixed ligand metal complexes were active against the tested bacteria pathogens/species whereas the free activity of the mixed ligand metal complexes could be explained on the basis of overtone concept of cell permeability whereby lipid membrane that surrounds the cell wall favours the passage of only lipid-soluble materials in which liposolubility is an important factor that controls the antibacterial activity (Ahmed *et al*., 2008; Srinivas *et al.,* 2005) as shown in Table 4.

Table 4: Zone of inhibition after 24 h incubation: Microbial Activities of metal complexes at 2.5% concentration

Complexes	*Xanthomonas Oxynopoides*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aureginosa*	*Bacillus Subtilis*
$[CoLCl](H_2O)_2]H_2O$	–	–	–	–	–
$[CoL_2Cl]H_2O]H_2O$	–	–	–	–	–
$[CoLClSCN]H_2O]H_2O$	–	2.5 mm	–	4 mm	–
$[CoL_2ClSCNH_2O]H_2O$	2.5 mm	3.0 mm	–	–	–
Ligand	–	–	–	–	–
Control (Streptomycin sulphate (0.05mg/L) conc.	10 mm	15 mm	10 mm	11 mm	11 mm

Furthermore, Table 5, explained the results of antifungal screening of the metal complexes, mixed ligand metal complexes and the free ligand as well as the control. The results demonstrate that the free ligand showed no sign of inhibition whereas, the respective complexes have shown moderate activities with high performance recorded in the mixed ligand complexes perhaps, may have been due to the presence of sulphur atom of thiocyanate ion (–S–C≡N) as explained by Iqbal *et al.* (2006), that the biological activity of metal complexes containing sulphur increases on complexation (Muhammed & Arjun, 2002; Olagboye *et al.,* 2013). It is evident rather, that cobalt(II) complexes of barbitone in aqueous medium showed activity of percentage inhibition between 31.17 – 71.14%. Fungi toxicity of the complexes observed to increase as the concentration of the ligand increases.

Table 5: Antifungal Evaluation (Assay), % incubation of the metal complexes against the selected pathogens

Complexes	***Sclerotina trifoliorum***	***Fusarium oxyporium***	***Stemania paradoxa***	***Botrytis Cineria***
$[CoLCl(H2O)_2]H_2O$	52.80%	53.13	31.17	39.08
$[CoL_2ClH_2O]H_2O$	60%	56.63	50.65	44.94
$[CoLClSCNH_2O]H_2O$	40%	56.25	59.09	46.09
$[CoL_2ClSCN]H_2O$	76.0	70.0	84.42	72.41
Ligand	10	12	11.5	11
Benlate (control) [0.05g/ml] conc.	80%	80	90	85%

4.0 Conclusion

Cobalt(II) complexes of barbitone and thiocyanate mixed ligand in aqueous medium have been synthesized and characterized. The results showed that ligand is bidendately coordinated to the cobalt(II)ions through the oxygen atom of the carbonyl group and nitrogen atom of the amide in the ring, while thiocyanate ions secondary ligand attached to the central Cobalt(II) ion through the sulphur atom. Also the biocidal evaluation results proved that mixed ligand metal complexes were suitable for bacteria whereas all complexes except the free ligands were fund to be good antifungal agents.

5.0 References

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