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STUDIES OF THE EFFECT OF ANIONS (CHLORIDE, SULPHATE, NITRATE, OXALATE AND ACETATE) ON THE PHARMACOLOGICAL PROPERTIES OF COBALT (II) COMPLEXES WITH DITHIOOXAMIDE-DERIVED ANTIBACTERIAL AGENTS

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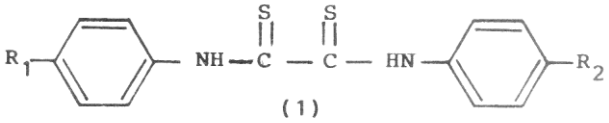
ABSTRACT

A number of cobalt (11) complexes with dithioovmide derived antibacterial agents having different anions i.e., chloride, sulphate, nitrate, oxalate and acetate have been synthesised, and the effect of anions on antibacterial properties of these complexes against bacterial species *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Escherichia coli* has been determined.

INTRODUCTION

We have previously reported (Chohan and Rauf 1992) some biologically active derivatives of dithiooxamide (1) and their potential cobalt (II) and nickel (11) complexes. By virtue of the bactericidal properties these compounds possess, they have been further investigated in context to the effect of different anions (chloride, sulphate, nitrate, oxalate and acetate) on their pharmacological properties. For this purpose, we have synthesised various cobalt complexes with these dithiooxamide-derived ligands (I-V) having stoichiometry $M(L)_2)C$, where M = cobalt (II), L = I, II, III, IV and V, and X = chloride, sulphate, nitrate, oxalate and acetate. These complexes have been screened against bacterial species *Staphylococcus anreus* (a), *Streptococcus* pneumoniae (b) and *Eschenchia coli* (c) in order to evaluate the effect of different anions on their antibacterial activity.



(1)

I $R_1 = R_2 = CN$

II $R_1 = R_2 = SO_2NH_2$

III $R_1 = R_2 = CHO$

IV $R_1 = R_2 = COOH$

V $R_1 = R_2 = OH$

MATERIAL AND METHODS

All solvents and chemicals used were Analar grade. Ligands (I-V) were prepared according to the same method as reported earlier (Chohan and Rauf 1992). Antibacterial studies were carried out with the help of the Department of Pathology, Quaid-e-Azam Medical College, Bahawalpur.

Preparation of Metal Complexes

A hot solution of ligand (0.002 mol) in ethanol (30 ml) was added to an aqueous solution (20 ml) of the respective metal salt (0.001 mol) with stirring. The mixture was then refuxed for 2h. The resulting solution was cooled, filtered and reduced to a small volume (20 ml). The concentrated solution was left overnight at room temperature which resulted in the formation of a solid product. The product thus formed was filtered, washed with ethanol and crystallised from aqueous ethanol (60% yield).

Antibacterial Activity

The synthesised metal complexes were screened for their antibacterial activity against bacterial species (a), (b) and (c) obtained from sputum and urine samples of different infected patients admitted in Bahawal Victoria Hospital, Bahawalpur, carrying these bacteria. The paper disc diffusion method devised and reported earlier (Chohan 1991, Chohan 1990, Chohan and Rauf 1990) was used for the determination of antibacterial activity.

Preparation of Disc

A metal complex/ligand (30 µg) in dimethylformamide (DMF) (0.01 ml) was applied on blotting paper disc with the help of a micropipette. The discs were then incubated for 48h at 37°C and applied on specific bacteria grown agar plates.

Preparation of Agar Plates

Minimal agar was used for the growth of bacterial species. *Staphylococcus* and Streptococcus species were grown in blood agar base. For this purpose, blood agar base (40 g) was suspended in cold water (1L) and heated to boiling. It was sterilized at 120°C for 15 minutes and cooled at 50°C. Then 5% sterile defibrinated cow blood was added to it and poured into washed and sterilized petri dishes. These were then stored at 4°C for inoculation.

Table 1

Antibacterial Activity Data of Ligands/Complexes

Ligands/Complexes	Microbial Species		
	a	b	c
L_I	+ +	+	+
L_{II}	+ +	+	+ +
L_{III}	+ +	+ +	-
L_{IV}	+	+ +	+
L_V	+	+	-
$^{1}Co(L_I)_2Cl_2$	+ + +	+ +	+ +
$^{2}CO(L_I)_2(SO_4)$	+ + +	+ +	+ +
$^{3}Co(L_I)_2(NO_3)_2$	+ + + +	+ + +	+ + +
$^{4}Co(L_I)_2C_2O_4$	+ + +	+ + +	+ + +
$^{5}Co(L_I)_2(CH_3CO_2)_2$	+ + +	+ +	+ + +
$^{6}Co(L_{II})_2Cl_2$	+ + +	+ +	+ +
$^{7}Co(L_{II})_2SO_4$	+ + +	+ + +	+ +
$^{8}Co(L_{II})_2(NO_3)_2$	+ + + +	+ + +	+ + +
$^{9}Co(L_{II})_2C_2O_4$	+ + + +	+ +	+ + +
$^{10}Co(L_{II})_2(CH_3CO_2)_2$	+ + +	+ +	+ + +
$^{11}Co(L_{III})_2Cl_2$	+ + +	+ +	+ +
$^{12}Co(L_{III})_2SO_4$	+ + +	+ +	+ +
$^{13}Co(L_{III})_2(NO_3)_2$	+ + + +	+ + +	+
$^{14}Co(L_{III})_2C_2O_4$	+ + +	+ + +	+ +
$^{15}Co(L_{III})_2(CH_3CO_2)_2$	+ + +	+ + +	+
$^{16}Co(L_{IV})_2Cl_2$	+ +	+ + +	+
$^{17}Co(L_{IV})_2SO_4$	+ +	+ +	+ +
$^{18}Co(L_{IV})_2(NO_3)_2$	+ + +	+ + +	+ + +
$^{19}Co(L_{IV})_2C_2O_4$	+ +	+ + +	+ +
$^{20}Co(L_{IV})_2(CH_3CO_2)_2$	+ +	+ +	+ +
$^{21}Co(L_V)_2Cl_2$	+ +	+ +	+
$^{22}Co(L_V)_2SO_4$	+ +	+ +	+ +
$^{23}Co(L_V)_2NO_3)_2$	+ + +	+ + +	+ + +
$^{24}Co(L_V)_2C_2O_4$	+ +	+ + +	+ + +
$^{25}Co(L_V)_2(CH_3CO_2)_2$	+ +	+ + +	+ +

a = *Staphylococcus aureus*, b = *Streptococcus pneumoniae,*
c = Escherichia coli
Inhibition zone in diameter; +, 6-10 mm; + +, 10-14 mm;
+ + +, 14-18 mm; + + + +, 18-20 mm.

For the preparation of agar plates for *Eschenchia coh,* Mac Conkey's agar (50 g) obtained from Merck Chemical Company, was suspended in water (1L). It was allowed to soak for 15 minutes and then boiled on waterbath with continuous shaking until the agar was completely dissolved. The mixture was autoclaved for 15 minutes at 120°C, poured into previously washed and sterilized petri dishes and stored at 4°C for inoculation.

Method of Inoculation

A platinum wire loop was used for inoculation. It was made red hot, cooled in air and then used for the application of bacterial strains. The preculture was prepared in 2 ml of nutrient broth by selecting a suitable colony of bacteria and transferring it to the nutrient broth which was incubated for 2h at 37°C. The 500µl of the culture was spread on the specific agar plate and incubated for 24h at 37°C.

Application of Disc

A sterilized forecep was used for the application of disc on already inoculated agar plates. When the disc was applied, it was then incubated at 37°C for 24h. The zone of inhibition/growth was measured in diameter.

RESULTS AND DISCUSSION

The structure of ligands and their metal complexes was established (Chohan and Rauf 1992) with the help of their spectral and analytical data. All the complexes are amorphous solids which are soluble in water and DMF, sparingly soluble in ethanol and insoluble in non-polar solvents.

Antibacterial activity of the complexes having different anions i.e., chloride, sulphate, nitrate, oxalate and acetate outside the coordination sphere, in comparison to the uncomplexed ligands against bacterial species (a), (b) and (c) was tested in DMF solution at concentration 30 µg/0.01 ml. The results of these studies reproduced in Table 1 show that beside the role of metal ions (cations) in chelation which increases the bactericidal activity of biologically active compounds (Chohan and Rauf, 1990; Chohan and Main, 1991; Chohan and Khaliq, 1989; Chohan and Moazzam, 1989; Chohan and Ansari, 1989; Chohan and Kausar, 1992; Chohan *a* al, 1992), anions also have a significant influence on this activity. The results reveal that cobalt complexes of the ligands having nitrate as counter anion are, generally, more antibacterial against all bacterial species than the ones having anions other than nitrate. The same complexes of cobalt having oxalate as counter anion showed less bactericidal activity than the complexes having nitrate anions but greater than the ones having the rest anions.

The order of activity in context to the presence of different anions in the complexes, in general, was found to be as:

nitrate > oxalate > acetate > sulphate — chloride.

It is interesting to note that uncomplexed ligands III and V were found to be inactive against bacterial species (c) but, when they were complexed with cobalt (II), they became active and their activity significantly varied from one complex to another due to the presence of different anions. These observations support our previous studies (Chohan and Pervez, 1992; Chohan and Sidiqui, 1992) in which we have reported that anions significantly influence the biological activity of metal chelates.

On the basis of above observations, we strongly claim that different anions affect the pharmacological behavior of metal chelates. However, at this stage, we are not aware of the actual mechanism. It is suspected that various factors such as solubility, conductivity, dipole moment and cell permeability mechanism (which are influenced by the presence of anions) could be the ones held responsible for affecting the activity/potency of these compounds.

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