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STUDIES OF THE EFFECT OF ANIONS ON THE ANTIBACTERIAL ACTIVITY OF NICKEL (II), COBALT (II) AND COPPER (II) COMPLEXES WITH AMPICILLIN

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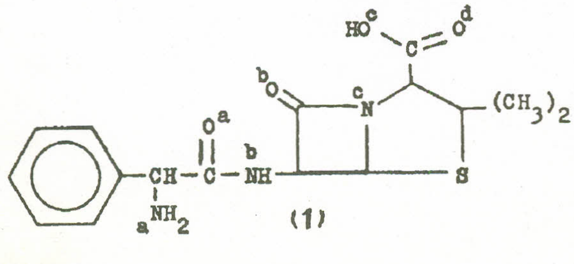
A number of cobalt (II), copper (II) and nickel (II) complexes with different counter anions such as sulphate, chloride, nitrate and acetate of the antibacterial drug ampicillin have been synthesised and characterised on the basis of molar conductance, magnetic moment, elemental analysis, infrared and electronic spectral data. In order to understand the possible role of anions on the antibacterial activity of ampicillin and their metal complexes, the synthesised metal complexes have been subjected for screening against bacterial species *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* The role of the counter anions have been found to be significant in increasing the antibacterial activity of ampicillin and their metal complexes.

Key words: Anions, Antibacterial activity, Metal complexes, Ampicillin.

Introduction

The use of metal chelation in biology and medicine is believed to have just begun [1-4]. It has been observed that metal chelation apparently play definite role in antibacterial [5-9], antitumour [10-12] and anticancer [13-16] activities. There are many indications [17-18] that metal chelates of biologically active ligands are more bacteriostatic or carcinostatic than the free ligand. To enlighten this role of metal ions and their possible mode of action we have commenced a research program in this laboratory to prepare and study various transition metal complexes of chelating agents which are known to have biological function or possess antibacterial properties. Our previous studies [19-22], so far done, confirm the idea that generally the role of metal atom is one of catalysis in the active centres, or as being, to lock the geometry of the active sites to activate enzymes or substrate bonds through coordination. In order to gain more information, the present studies have been performed in which the biological role of anions on the antibacterial activity is investigated and reported. For this purpose, metal complexes, having the same metal atom (cation) but different counter anions, with the antibacterial agent ampicillin (1) have been synthesised and characterised on the basis of molar conductance, magnetic moment, elemental analysis, infrared and their electronic spectral data. These complexes of the type $[M(L)_2(X)_2]y$ where M=Co(II), Cu(II) and Ni(II), L=ampicillin, $X=OH_2$ and $Y=Cl_2$, SO_4, NO_3 and CH_3CO_2 have been then subjected for their antibacterial activity against the bacterial species *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* It is interesting to note the results of these studies that anions also, significantly effect the antibacterial action of these metal chelates against all organism tested.



(1)

Materials and Methods

All solvents and chemicals used were analytical reagent grade. Cobalt (II), copper (II) and nickel (II) were used as their chlorides, sulphates, nitrates and acetates in the preparation of all metal complexes.

Ampicillin trihydrate was obtained from Beecham Pharmaceutical Company Ltd. and used without further purification.

Infrared spectra in nujol were recorded on an A-10 Hitachi spectrophotometer and electronic spectra were recorded on a Hitachi double - beam U-2000 model spectrophotometer using glass cell of 1 cm thickness. Magnetic measurements were done on solid complexes using a Gouys balance. Elemental analysis of C, H, and N was carried out on a Coleman automatic analyser. Conductance was measured on a conductance meter YSI model-32. All melting point were taken on a Gallenkamp melting point apparatus and are uncorrected.

Metal contents were determined by reported methods. Cobalt was determined by the pyridine method [23], copper by the salicyldioxime [24] and nickel by the dimenthylgloxime method [25].

General method for preparation of metal complexes. An ethanolic solution of ampicillin (15 ml, 2m mol) was added to a stirred aqueous solutions (20 ml) of respective metal salt (1m mol) and the mixture refluxed for 2 hrs. The resulting solution was then 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 solid product thus formed was recrystallised from water to yield 1 (30%); 2 (36%); 3 (45%); 4 (38%); 5 (52%); 6 (45%); 7 (35%); 8 (48%); 9 (52%); 10 (35%); 11 (45%) and 12 (52%).

Antibacterial studies. This part of the work was done in the Microbiology Laboratory, Department of Pathology, Quaid-e-Azam Medical College, Bahawalpur.

Preparation of disc. The metal complex/ligand (30 µg) in dimethylformamide (DMF) (0.01 ml) was applied on a paper disc prepared from blotting paper (3 mm size) with the help of a micropipette. The discs were left in an incubator for 48 hrs. at 40° and then applied on bacteria grown agar plates.

Preparation of agar plates. Minimal agar was used for the growth of the specific bacterial species. Blood agar base with low pH and Mac Conkey agar obtained from Merck Chemical Company were used for *Staphylococcus aureus and Escherichia coli* respectively.

Procedure of inoculation. Inoculation was done with the help of platinum wire loope which was firstly made red hot on a flame, allowed to cool in air and then used for the application of wild type pathogenic strains obtained from urine and sputum samples of different patients admitted in the local Bahawal Victoria Hospital, Bahawalpur, carrying these bacteria to the specific agar plates. The same agar plates were incubated for 24 hrs. at 40°.

Application of disc. A sterilised forecep was used for the application of paper disc on already inoculated agar plates. When the discs were applied, these were then incubated at 40° for 24 hrs. After then the diameter of the zone of inhibition/ growth around the disc was measured (mm).

Results and Discussion

All the metal complexes 1-12 formed are crystalline compounds with different colours (Table 1). All of them are stable at room temperature and decompose without melting. All the reported complexes are soluble in water and dimethylformamide (DMF), sparingly soluble in benzene and insoluble in other organic solvents.

The molar conductance of the complexes in aqueous solution show that all the complexes conduct electricity and their higher value thus show their ionic nature [26].

The stiochiometries of the synthesised complexes shown by the results of elemental analysis confirm that one metal atom is assumed to be coordinated to two ampicillin molecules and two water molecules (Table 2).

The ampicillin molecule apparently contains a number of potential donor groups such as nitrogen, oxygen and sulphur which act as coordinating sites for metal chelation. Model studies of the ampicillin molecule show that it can only act as a bidentate ligand and preferably coordinates itself to the metal atom through the nitrogen atoms of the amine and amide groups (N^a, N^b) or though the nitrogen of the B-lactam ring and the oxygen of the hydroxyl group (N^c, O^c).

The scrutiny of infrared spectra of uncomplexed ampicillin and the metal complexes show that vibrations corresponding to its M-S and M-O bonds are not detected in the spectra which indicate that the oxygen and the sulphur atoms are not coordinated with metal in the complex. However, on the basis of the new bands which appeared in the spectra of the metal complexes at 700-736 cm^{-1} due to (M-N) vibrations, a clue is given that ampicillin is coordinated to metal through N^a, N^b atoms forming a 5-membered stable chelate ring.

The room temperature effective magnetic moment (μ_{eff}) (Table- 1) for all the metal complexes lie well within the range for their observed geometries. The μ_{eff} for Co(II) complexes fall in the range (4.42-4.55 B.M) expected [27] to contain odd number of electrons (d^7-system). The μ_{eff} value for the Ni(II) complexes (3.23-3.28 B.M) is also expected [28] to contain odd number of electrons indicative of the six coordinated octahedral geometry. The μ_{eff} for Cu(II) complexes (1.76-1.84 B.M) suggested [29] distorted octahedral geometry having d^9-system with one unpaired electron.

The electronic spectra in DMF, exhibited bands due to d-d transitions. A strong band at 27000-30,000 cm^{-1} in all complexes was labelled as a charge transfer band probably due to transitions of electrons from non-bonding metal d-orbitals to an antibonding ligand π^* orbitals. The nickle (II) complexes exhibited three typical bands at 26500-30000, 15300-17200

TABLE 1. M. P., CONDUCTANCE AND MAGNETIC MEASUREMENTS OF SOME AMPICILLIN METAL COMPLEXES.

Complex No.	Complex	M.P. (decomp) (°C)	Conductance Siemens/ cm2/mol	B.M (µ)	Colour
1.	$[CoL_2(OH_2)_2]Cl_2$	176-178	87.5	4.54	Mustard
2.	$[CoL_2(OH_2)_2]SO_4$	180-182	91.0	4.45	Mustard
3.	$[CoL_2(OH_2)_2]NO_3)_2$	168-170	124.5	4.55	light mustard
4.	$[CoL_2(OH_2)_2](CH_3CO_2)_2$	189-190	123.0	4.42	Dark mustard
5.	$[NiL_2(OH_2)_2]Cl_2$	139-141	145.5	3.28	Mustard
6.	$[NiL_2(OH_2)_2]So_4$	155-157	81.0	3.26	Green
7.	$[NiL_2(OH_2)_2]No_3)_2$	140-142	77.5	3.25	Light green
8.	$[NiL_2(OH_2)_2](CH_3CO_2)_2$	158-160	82.5	3.23	Dark green
9	$[CuL_2(OH_2)_2]Cl_2$	155-156	91.0	1.84	Green
10.	$[CuL_2(OH_2)_2]SO_4$	164-165	77.5	1.78	Dark brown
11.	$[CuL_2(OH_2)_2]NO_3)_2$	180-182	135.5	1.81	Brown
12.	$[CuL_2(OH_2)_2](CH_3CO_2)_2$	150-152	70.5	1.76	Dark green

and 8500-10700 cm^{-1} corresponding to the transitions $^3A_{2g} \rightarrow {}^3T_{1g}(P)$, $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g} \rightarrow {}^3T_{2g}(F)$ in a octahedral field [30]. The cobalt (II) complexes showed absorption bands at 28000-31000, 172000-19000 and 8250-11000 cm^{-1}. The latter two bands assigned [31] to transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ and the first band is already assigned to metal → ligand charge transfer. Similarly, the copper (II) complexes showed typical charge transfer band at 28500-29500cm^{-1} and at 15000-17500cm^{-1} due to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition in a distorted octahedral geometry [32].

On the basis of the above observations it is proposed that all metal chelates show an octahedral geometry (Fig. 1) in which the two molecules of ampicillin acting as bidentate ligands accommodate themselves to bind with one metal atom. The two water molecules are also assumed to bind around the metal atom in a trans arrangement. Since the ampicillin molecules are quite bulky groups, they lie at large distance from each other, in such a way that a stable geometry of the 5-membered metal chelate is attained.

Antibacterial studies. The synthesised metal complexes

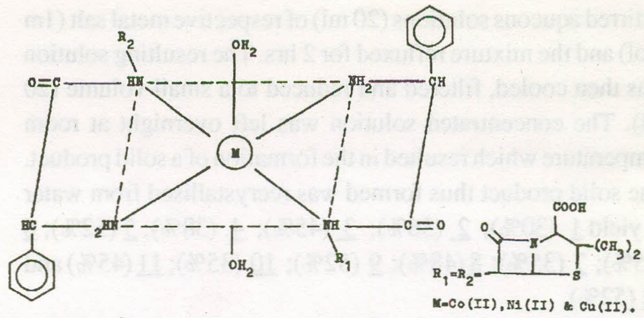


Fig. 1. Proposed structure for [M(ampicillin)$_2$(OH$_2$]$^{+2}$ ion.

1-12 have been tested to determine the effect of anions on the antibacterial activity against a number of bacterial species such as, *E. coli* (a), *S. aureus* (b) and *P. aeruginosa* (c). The disc diffusion method devised in this laboratory [33] was adopted for assessing the antibacterial activity. The results of antibacterial studies reproduced in Table 3 show that metal chelates are more antibacterial against all the bacterial species (a), (b) and (c) tested than the pure unchelated ampicillin. Moreover, the counter anions chloride, nitrate, sulphate and

TABLE 2. INFRARED, ELECTRONIC AND ELEMENTAL ANALYSIS DATA OF SOME AMPICILLIN METAL COMPLEXES.

Complex No./Mol.Formula	I.R(cm^{-1})	λ_{max} (cm^{-1})	% Calc. (%Found) C	H	N
1. $CoC_{32}H_{44}N_6O_{10}S_2Cl_2$	3432, 2830, 1648, 1380 1130 1021, 924, 700	28100, 17200 8500	44.36 (44.35)	5.07 (5.17)	9.69 (9.68)
2. $CoC_{32}H_{44}N_6O_{12}S_3$	3200, 2932, 2864, 1644, 1510 1466, 1130, 722, 698	29100, 17700 9500	43.71 (43.69)	5.11 (5.11)	9.77 (9.76)
3. $CoC_{32}H_{44}N_8O_{14}S_2$	3236, 2940, 2864, 2734, 1638 1550, 1380, 924, 710	29500,18500 9500	43.31 (43.29)	4.95 (4.94)	12.62 (12.61)
4. $CoC_{36}H_{50}N_6O_{14}S_2$	3304, 2932, 2860, 2734, 1647 1584, 1467, 1158, 723	29500, 18200 9200	47.33 (47.33)	5.47 (5.46)	9.19 (9.17)
5. $NiC_{32}H_{44}N_6O_{10}S_2Cl_2$	3436, 2932, 2864, 2336, 1664 1322, 1130, 896, 724	27500, 16200 8500	44.34 (44.38)	5.08 (5.11)	9.67 (9.67)
6. $NiC_{32}H_{44}N_6O_{12}S_3$	3452, 3336, 2936, 2800, 2060 1652, 1532, 1380, 872, 724	28100, 16200 9100	44.72 (44.73)	5.12 (5.11)	9.77 (9.75)
7. $NiC_{32}H_{44}N_8O_{14}S_2$	3332, 2932, 2864, 1660, 1530 1464, 1380, 1032, 944, 724	27200,17100 9500	43.32 (43.31)	4.95 (4.96)	12.62 (12.62)
8. $NiC_{36}H_{50}N_6O_{14}S_2$	3400, 2932, 2864, 2320, 1625 1464, 1380, 1022, 724, 702	30000, 17100 9500	47.34 (47.33)	5.47 (5.44)	9.19 (9.20)
9. $CuC_{32}H_{44}N_6O_{10}S_2Cl_2$	3380, 2932,1788, 1650, 1515 1467, 1275, 1134, 918, 753	29100, 15500	44.13 (44.12)	5.05 (5.15)	9.64 (9.64)
10. $CuC_{32}H_{44}N_6O_{12}S_3$	3328, 2932, 2860, 1872, 1648 1467, 723, 693	29500, 16550	44.47 (44.45)	5.09 (5.12)	9.72 (9.71)
11. $CuC_{32}H_{44}N_8O_{14}S_2$	3328, 2932, 2860, 1872, 1648 1467, 1382, 736, 696	29500, 16550	43.08 (43.12)	4.93 (4.93)	12.55 (12.54)
12. $CuC_{36}H_{50}N_6O_{14}S_2$	3352, 2932, 2320,1650, 1584 1467, 1122,730	29200, 16100	47.09 (47.07)	5.44 (5.46)	9.15 (9.13)

Same numbering as in Table-1.

TABLE 3. ANTIBACTERIAL ACTIVITY DATA OF SOME AMPICILLIN METAL COMPLEXES.

Complex. No/Ligand	Zone of Inhibition (mm) Microbial Species		
	a	b	c
Ampicillin	14	16	16
1.	20	20	18
2	16	18	18
3	22	20	18
4	18	22	16
5	18	20	20
6	16	18	18
7	22	22	22
8	22	22	22
9	16	16	16
10	18	18	18
11	20	20	20
12	20	20	20

* Same numbering as in Table 1 & 2; a = *E. coli*; b= *S. aureus*; c= *P. aeruginosa*.

acetate which stay outside the coordination sphere of the metal complex also take part in the mechanism of increasing the antibacterial activity of the metal complex. For example, the nickel (II) complex of ampicillin with nitrate as counter anion is shown to be more antibacterial than the nickel (II) complex of ampicillin with acetate, sulphate and chloride as counter anions. The order of activity against the bacterial species tested due to these anions is: nitrate> acetate ≥ chloride> sulphate against the bacterial species (a); acetate≥ nitrate> chloride> sulphate against (b) and acetate≥ nitrate> chloride> sulphate against (c).

In the case of cobalt (II) complexes this order of activity was found to be nitrate> chloride> acetate> sulphate against the bacterial organism (a) and acetate> nitrate≥ chloride> sulphate against (b). Similarly Cu(II) chelates were also tested against (a), (b) and (c). The order of sensitivity effected by anions was found as acetate ≥ nitrate> sulphate against (a), acetate ≥ nitrate ≥ sulphate against (b) and acetate ≥ nitrate against (c) respectively.

These studies clearly indicate that not only the metal ions (cations) which directly bind themselves with the ligand, increase the antibacterial activity, but also the anions which act as counter part of the metal chelate play a significant role in increasing the antibacterial activity. However, we are not definite about this mechanism of the antibacterial activity effected by anions in the complex but many of the considerations such as the formation constant, the solubility constant, the polarizibility and the cell permeability of the microorganism may be relevant factors. The key phenomenon may also be the membrane penetration concept through the lipoid layer of the microorganism, effected by these anions.

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