Synthesis, Characterization and Pharmacological Properties of a Cobalt (II) Complex of Antibiotic Ampicillin

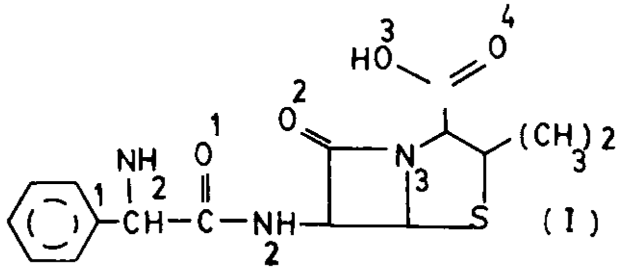
CHRISTY MUNIR, SHAHID MASOOD YOUSAF
AND NASIR AHMAD
Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan

(Received 29th February, 1984)

Summary: A new complex $[Co(amplicillin)_2(OH_2)_2]\ Cl_2.3H_2O$ has been synthesised and characterized by elemental analysis, magnetic and spectral studies. The pharmacological assay show that the complex is 2-3 times more active than ampicillin itself, against bacteria such as Salmonela arizona, Proteus vulgaris, Staphylococcus pyogeneus and Klebsiella pneumonie while inactive against Enterobector agrogenus.

Metal ions play an important role in biological systems. The role of iron as an oxygen carrier in hemoglobin has been well established. A number of biological reactions are also catalysed by enzymes containing coordinated metal ions such as Zn,Mo etc. [1,2]. In recent years extensive studies have been undertaken on syntheses and characterization of metal complexes of biologically important compounds such as amino acids, peptides, hydroxyacids etc. in an attempt to understand the role of metal ions in human body. Although reactions occuring inside the body are very complicated and have never been duplicated in a laboratory, one can have some insight by studying similar but simpler systems in the laboratory. Another aspect of the role of metal ions in biology which came to light in recent years,is the use of metal complexes as medicine in certain ailments. For instance cis-dichlorodiammineplatinum(II) has been found effective against some varieties of cancer [3-5]. Kirachner and coworkers [6] have found that metal complexes of some anticancer drugs (such as 6-mercaptopurine) are more effective than the drug itself against certain types of cancer. It is interesting to note that most of organic drugs contain common donor atoms such as nitrogen, oxygen, sulphur, phosphorous etc. which could easily coordinate with transition metal ions. The medicinal properties of drugs are expected to be altered drastically upon coordination with a metal ion. The present investigation was undertaken with an intention of exploring the relative changes in drug action of a medicine upon complexation. In this paper, synthesis, characterization and antibacterial properties of a cobalt(II) complex of ampicillin are reported.

Ampicillin (D-(-)α-aminobenzylpenicillin) (I) is a broad spectrum antibiotic which is quite active against many gram positive and gram negative microorganisms [7]. This ligand coordinates with cobalt (II) ion in aqueous medium to form a six coordinated octahedral complex in which two ampicillin molecules (acting as bidentate ligand) occupy four coordination sites while the remaining two are filled by water



(I)

molecules. Cobalt(II) ampicillin complex, as compared with ampicillin itself, is found to be two to three times more active aginst certain types of bacteria e.g. Salmonela arizona, Proteus vulgaris, Staphylococcus pyogeneus and Kebsiella pneumonie while it is inactive against Enterobector agrogenus.

Experimental

(i) Materials and Techniques

Cobalt(II) chloride hexahydrate and other chemicals used for synthesis and structure determination were of reagent grade and used as such. Ampicillin trihydrate was obtained from Beecham Company Ltd. Karachi and used without further purification.

For determining the metal content of the complex, the ampicillin complex was first treated with concentrated nitric acid, boiled for 15-20 minutes and then evaporated to near dryness in a fumehood. This process was repeated in order to destroy the organic matter completely. The residue was extracted with water and filtered. The filtrate was used for estimation of cobalt(II) by pyridine method (8).

A conductance bridge (YSI model 31) was used to determine the percentage of ionic chloride in the complex by a titration with silver nitrate. Magnetic moment of the solid complex was carried out by Guoy's method using $Hg[Co(SCN)_4]$ as a reference [9]. The absorption spectrum (in UV, visible and near I.R. regions) of aqueous solution of the complex was recorded on Hitachi model 323 spectrophotometer using water as reference. A matched set of two quartz cuvettes of 1cm thickness were used for obtaining the spectrum. Infrared spectrum were recorded on Beckman model IR-20A spectrophotometer using KBr pellet. Elemental analyses of carbon, hydrogen and nitrogen were obtained from Microanalytical Laboratories, University College, Dublin, Ireland.

(ii) Synthesis of Diaquo-bis-(D-(-)α-aminobenzylpenicillin) cobalt(II) chloride trihydrate. $[Co(ampicillin)_2(OH_2)_2]Cl_2.3H_2O$

This complex was prepared by adding 7.5 ml of 0.1 M aqueous solution of cobalt (II) chloride 6-hydrate (7.5 x 10^{-4} moles) to 150 ml of a 0.01 M solution of the ligand (15.0 x 10^{-4} moles) with stirring and refluxing near the boiling point for about two hours. The resulting solution was cooled, filtered and later reduced to a small volume. The concentrated solution was then left overnight which resulted in the formation of yellowish green crystals of the complex. The product was recrystallized from water, m.p. 192°C (decomposed), magnetic moment (μ_{eff}) = 4.54 at 293°K.

Anal: Calcd. for $[Co(C_{16}H_{19}N_3O_4S)_2(OH_2)_2]Cl_2.3H_2O$. C 41.83, H 5.23, N 9.15, Cl 7.72, and Co 6.42%. Found: C 41.24, H 5.16, N 9.6, Cl 7.40, and Co 6.80%. Infared Bands: (All bands are reported in cm^{-1}) Ampicillin: 3440 (b), 3200 (b), 3230, 2960, 2640, 2080, 1770, 1705, 1602 (t), 1490, 1455, 1380, 1370, 1330, 1305, 1260, 1218, 1200, 1170, 1155, 1120, 1080, 1050, 1020, 1000, 990, 930, 875, 845, 800, 735, 693.

$[Co(ampcn)_2(OH_2)_2]Cl_2.3H_2O$: 3400 (b,m), 3030, 1630 (m), 1490, 1460, 1390, 1342, 1280, 1185, 1110, 1030, 1005, 880, 840, 805, 750, 695 595 cm^{-1}. (b broad, m multiplet and t triplet).

(iii) Antibacterial Studies

Experimental work on antibacterial activity of the complex was carried out in National Institute of health, Islamabad, Antibacterial activity of cobalt (II)-ampicillin complex against Staphylococcus pyogenus, Salmonila arizona, Proteus vulgaris, Klebsiella pneumonie and Enterobactor agrogenus was determined using turbidity measurement method in which standard opacity tubes were used as reference. The results were compared with studies done on the ligand (ampicillin) under similar conditions.

Discussion

The complex $[Co(ampicillin)_2(OH_2)_2]Cl_2.3H_2O$ is a yellowish green crystalline compound. It is soluble in water and slightly soluble in alcohol. It decomposes between 190-200°C. The complex is paramagnetic and its magnetic moment (μ = 4.54 B.M.) indicates three unpaired electrons in d orbitals. An aqueous solution of the complex conducts electricity showing its ionic nature. The observed molar conductance (265 ohm^{-1}/mole) of the complex is consistent with molar conductance of complex which dissociate in water to give three ions per mole. Further, conductometric titration of the complex with silver nitrate shows the presence of two anionic chloride ions outside the coordination sphere.

Elemental analysis of the complex shows that cobalt (II) ion is bonded with two ampicillin ligands and five water molecules. Although ampicillin contains a number of potential donor atoms such as nitrogen, oxygen and sulphur, models show that it can only act as a bidentate ligand and preferentially bond with the metal ion through its N_1,N_2 or N_3,O_3 atoms. A comparison of the infrared spectra of ampicillin and its cobalt(II)-complex reveals that strong carbonyl stretching vibration at 1602 cm^{-1} in the ligand is very little affected upon coordination. Similarly it was not possible to detect any vibration corresponding to M-S bond. These observations indicate that oxygen and sulphur atoms are not coordinated with metal in the complex. On the basis of infrared spectrum, it is proposed that the ligand is coordinated to the metal atom through N_1, N_2 atoms forming five membered chelates. The absorption spectrum of the complex consists of four bands with extinction coefficients in the range 40-70 cm^{-1} $mole^{-1}$ which are typical for octahedral species. These studies indicate that the complex consists of a six coordinated bivalent cation with formula $[Co(ampicillin)_2(OH_2)_2]Cl_2.\ 3H_2O$. In the complex, ampicillin acts as a bidentate ligand and other two coordination positions are occupied by water molecules shown in Fig. 1.

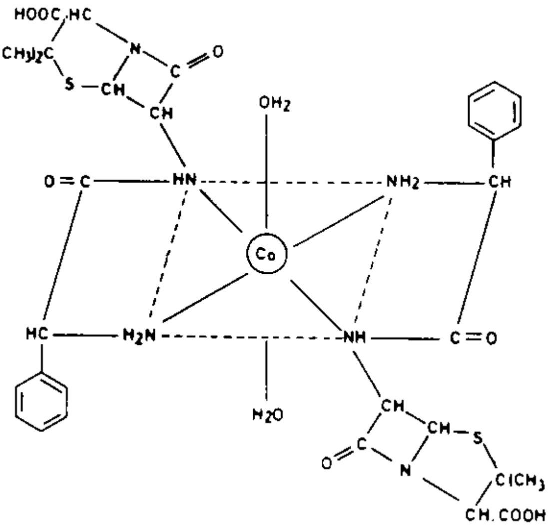


Fig.1: Proposed structure for $[Co(ampicillin)_2(OH_2)_2]^{2+}$ ion.

The solution spectrum of the complex in visible and near infrared regions consists of four distinct bands situated at ν_1 = 8130 cm^{-1} (ε 40.8), ν_1=9.615 cm^{-1} (ε 41.6), ν_2 = 14.286 cm^{-1}(ε

51.6) and ν_3 = 20,000 cm^{-1} (ε 66.0). These extinction values are within the range commonly observed for octahedral complexes of organic ligands. Therefore one can easily assume an approximate octahedral geometry around the metal ion in this complex. The observed bands may be assigned to electronic transitions within d orbitals (as shown in Table 1). Further transition energy ration (ν_2 cm^{-1}/ ν_1 cm^{-1} = 1.75) is quite close to the theoretical value of 2.1 calculated for octahedral complexes of Co(II) [10]. The experimental ratio is usually lower than the theoretical one. The observation that absorption spectrum of this complex consists of four bands, indicates a lower symmetry species in solution. A high spin Co(II) ion (a d^7 system) in six coordinated complexes is expected to have a distor-

Table-1: The solution Spectrum, Molar Extinction and Assigned Electronic Transitions of Cobalt(II) - Ampicillin Complex

	λ_{max} (cm^{-1})	ε cm^{-1} $mole^{-1}$ 1	Electronic Transitions
ν_1	8,130 9,615	40.8 41.6	$^4T_{2g} \leftarrow {}^4T_{1g}$
ν_2	14,286	51.6	$^4A_{2g} \leftarrow {}^4T_{1g}$
ν_3	20,000	66.0	$^4T_{1g}(P) \leftarrow {}^4T_{1g}$

ted octahedral environment. In complexes where only one kind of ligands are bonded to Co(II) ion, small distortions are expected which appear as broadening of the bands. On the other hand, if different ligands are attached to the metal ion, the resulting complex is highly distorted and some of the absorption bands actually split up to show increased number of bands. The lowest energy band ν_1 in six coordinated complexes of Co(II) ion is usually found in the range 8,000 - 10,000 cm^{-1}. A number of complexes such as $[Co(NH_3)_6]^{2+}$, $[Co(PyO)_6]^{2+}$ and $[Co(N(C_2H_5)_3)_6]^{2+}$ exhibit single, broad bands in this region whereas $[Co(2\text{-}MePy)_2(NO_3)_2]$ has two bands (at 7,486 (sh) and 8,764 cm^{-1}) in the same region of spectrum, showing a highly distorted octahedral environment around the metal ion [11,12]. The absorption spectrum of $[Co(ampicillin)_2(OH_2)_2]^{2+}$ ion consists of four bands, two of which are situated in near infrared region at 8,130 and 9,615 cm^{-1}. These two bands seem to arise from the splitting of ν_1 showing a highly distorted octahedral environment around the metal ion in the complex. Further a trans arrangement of water molecules around the metal ion is assumed because the various atoms of bulky ampicillin molecules will be at larger distance from each other in such as position and lead to a more stable complex.

Antibacterial Properties of the Complex

The cobalt (II)-ampicillin complex was tested for its antibacterial activity in comparison with pure ampicillin against a number of bacteria such as Salmonela arizona, Staphylococcus pyogenus, Proteus vulgaris, Enterobector agrogenes and Klebsiella pneumoniae. The results of these studies are reproduced in figures 2 and 3 and Table 2. It is found that the complex is active against all of these bacteria except Enterobector agrogenus. The complex is found to

Table-2: Effect of Ampicillin and its Cobalt(II)-Complex on Various Microorganism

Micro-organism	Bacterial Growth in Presence of Ampicillin			Bacterial Growth in Presence Cobalt(II)-ampicillin Complex		
	50 µg	100 µg	200 µg	50 µg	100 µg	200 µg
1. Staphlococcus pyogenus	+++	++	++	++	+	-
2. Salmonela arizonae	+++	++	++	++	+	-
3. Proteus vulgaris	++	++	++	+++	+	-
4. Klebsiella pneumoniae	++	++	++	++	+	-

be more active than pure ampicillin against all of the above noted bacteria.

If same concentrations of ampicillin and its complex are used, the later inhibits the growth of Staphylococcus pyogenus more than the former as shown in Fig.2, a & b. Further, even 200 µ g of ampicillin is not sufficient for killing the bacteria completely whereas relatively low concentration (140 µ g) of the complex completely inhibits the growth of Staphylococcus pyogenus.

The antibacterial activity of the complex against Salmonela arizonae is also more than ampicillin itself, as is evident from Fig.2 c & d. Only 40 g of the complex has as much ability to inhibit the growth of this bacteria as at least 100µg of ampicillin. If the dose of the complex is increased beyond this limit, a bacteriocidal effect (i.e. complete inhibition of bacterial growth) starts which, in its turn, is complete at about 170 µ g concentration of the sample. However bacteriocidal effect is not observed for ampicillin even at concentrations higher than 170 µg.

In case of Proteus vulgaris, maximum antibacterial activity is observed at

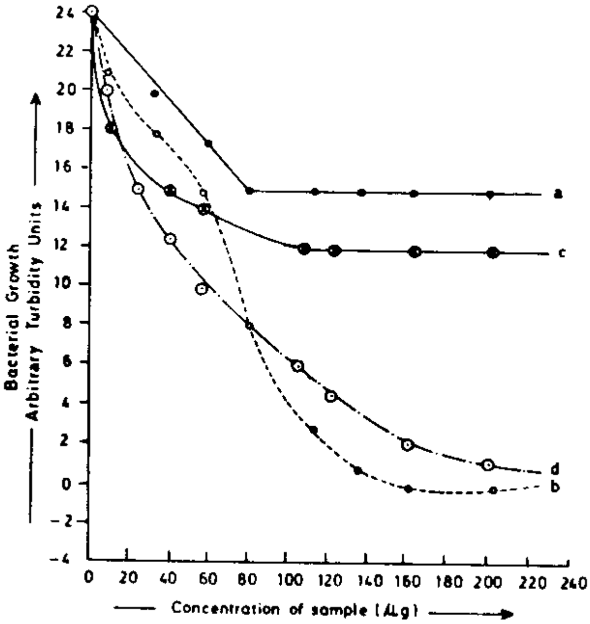


Fig.2 (a,b) Growth of Stephlo-coccus pyogenues vs concentration of ampicillin (-------0 ------0------0) and concentration of Cobalt (II)-ampicillin complex (-------0------0-----0). (c & d) Growth of Salmonela Arizonae vs concentration of ampicillin (-----0----0----) and concentration of Cobalt (II)-ampicillin Complex (----0----0----)

70 µ g concentration of the complex which is more than that of ampicillin. Ampicillin exerts only a bacteriostatic

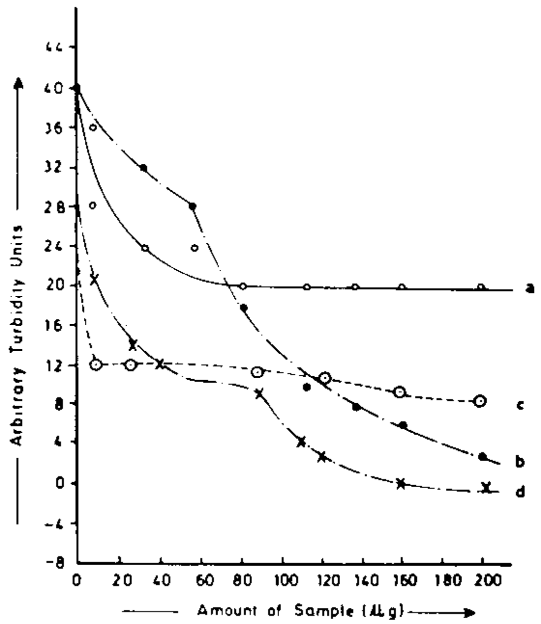


Fig.3: (a & b) Growth of Proteus Vulgaris vs concentration of ampicillin (----0------0----) and concentration of Cobalt (II)-ampicillin complex (------0-----0-----) (c & d) Growth of Klebsiella Pneumoniae vs concentration of ampicillin (-----0-----0-----) and concentration of Cobalt (II)-ampicillin complex (------x-----x-----).

(i.e. partial inhibition of bacterial growth) effect even above 200 μg level whereas the complex starts showing a bacteriocidal action at relatively lower concentration. The antibacterial properties of the complex and ligand against Proteus vulgaris are compared in Fig. 3, a & b.

The cobtalt (II) ampicillin complex shows a bacteriocidal effect at relatively lower concentration of 160 μg for Klebsiella pneumoniae whereas ampicillin remains bacteriostatic even at 200 μg concentration. Although amplicillin is also effective against Enterobector argrogenus, its complex is found ineffective against this bacterium.

It is clear from these studies that $[Co(ampicillin)_2(OH_2)_2]Cl.3H_2O$ amplicillin acts as an antibiotic. In most of the cases, the complexes act as a more powerful antibiotic killing most of the bacteria as compared to ampicillin which partially inhibits the growth of bacteria. At this stage, it is, however, not clear why the complex is more effective against certain type of bacteria than the ligand (drug) itself. It seems that the metal content of the complex is playing some role in its bacteriocidal activity. Work is in progress on pharmacological screening and determining lethal dose of the complex in biological systems.

Acknowledgement

The authors are grateful to the Pakistan Science Foundation for financial support (grant No. PSF/Res/C-IU/CHEM(54) and M/S Beecham Company, Karachi for supplying a generous sample of ampicillin. We wish to thank Mr. Zahir Khan, of National Institute of Health, Islamabad for his valuable help in antibacterial activity studies.

References

1. K.B.Swedo and J.H.Enemark, *J.Chem.Ed.*, **56**, *70 (1979)*
2. W.N.Lipscomb, *Chem.Soc.Rev.*, **1**, *319 (1972)*
3. B.Rosenberg, *Mot.Rev.*, **15**, *42 (1971)*
4. D.R.Williams, *Inorg.Chim.Acta.Rev.*, **8**, *123 (1972); Chem.Revs.*, **72**, *203 (1972)*
5. T.A.Connors, *Recent Results Cancer Res.*, **48**, *113 (1974)*
6. S.Kirschner, Y.K.Wei, D.Frances and J.Bergman, *J.Med.Chem.*, **9**, *369 (1966)*
7. F.P.Doyle, *Nature*, **191**, *1091,(1961)*
8. A.I.Vogel, "A Textbook of Quantitative Inorganic Analysis", 3rd Ed., (Longman, London 1961), P. 531.
9. B.N.Figgis and R.S.Nyholm,

J.Chem.Soc., *4190 (1958)*
10. A.B.P.Lever, Inorganic Electronic Spectroscopy", (Elsevier Publishing Company, Amsterdam, 1968), P. 320
11. (a) C.K.Jorgensen, *Advances Chem.Phys.*, **5**, *33 (1963)*
(b) R.W.Kiser and T.W.Lapp, *Inorg.Chem.*, **1**, *401 (1962)*
12. A.B.P.Lever and D.Ogden, *J.Chem.Soc.*, *2041 (1967)*