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EFFECT OF COBALT (II), COPPER (II), NICKEL (II) AND ZINC (II) METAL IONS ON THE BIOLOGICAL ACTIVITY OF TRIAZINE DERIVED LIGAND

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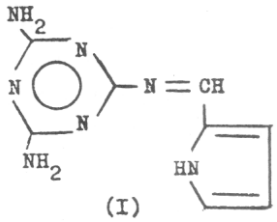
ABSTRACT

A new Schiff-base triazine-derived ligand and its cobalt (II), copper (II), nickel (II) and zino (II) metal complexes have been synthesised and characterised on the basis of elemental analysis, molar conductance, infrared, HNMR and electronic spectral, and magnetic susceptibility data. In order to asssess the effect of metal ions on the antibacterial activity, the ligand and its complexes have been screened against bacterial species Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. In all cases the activity/potency has substantially increased on complexation against one or more bacterial species.

Introduction

Many studies concerning the relationship between metal ions and the bioability of drugs have been a subject of great interest (Shields et al., 1960, Kirshner et al., 1966, Wacker & Vallee 1959, Albert & Rees 1953, Furst 1960, Dawyer & Mellor 1964, William 1971, Weinberg 1960). A number of known biochemical reactions which are catalysed by enzymes contain metal ions such as Zn, Co, Mo etc. Many metal chelates which have been proved (Cleare 1974, Cleare et al., 1973) to be more carcinostatic than the unchelated drugs and similarly, many antibacterial drugs when are cornplexed/chelated, their bioability is effectively altered (Chohan & Kausar 1992, Chohan & Pervez 1992, Chohan & Sheikh 1987).



(I)

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In order to understand the apparent role of metal ions and their possible mode of action, we have commenced a study programme which concerns the synthesis of such compounds which are known to possess antibacterial activity and likely to have biological functions, and their various metal complexes and evaluated for their antibacterial activity. In continuation to the same, in the present studies we wish to report a new biologically active compound (I) and its cobalt (II), copper (II), nickel (II) and zinc (II) complexes and have, also explored the relative changes that occur on their coordinating properties and antibacterial action upon complexation/chelation.

Material and Methods

Chemical and instrumentation

All chemicals and solvents used were analytical grade. Metals were used as their chloride salts. 4frared and absorption spectra were recorded on a A-10 Hitachi spectrophotometer and Hitachi double beam U-2000 spectrophotometer using glass cells of 1cm. thicken H-NMR spectra of the ligand was obtained in DMSO-d_6 using Rio spectrometer. Caductance was measured on a conductance meter YSI model-32 and magnetic susceptibility on a Gouys balance. Elemental analysis of CHN was carried out on a Colemar automatic analyser. All melting points were taken on a Gallenkamp melting point apparatus and are uncorrected.

The antibacterial activity of metal complexes in comparison to the ligand was studied on wild type pathogenic bacterial species collected from infected patients admitted in a local Bahawal Victoria Hospital, Bahawalpur. These studies were carried out with the help of Microbiology Laboratory, Department of Pathology, Quaid-e-Azam Medical College, Bahawalpur.

Preparation of Ligand

N-(2-Pyrrolylmethylene) 2, 4, 6-triamino-1, 3, 5-triazine

Pyrrol-2-carboxaldehyde (0.95g) (0.O1M in absolute ethanol (15m1) was added to a stirred hot ethanolic solution (20m1) of melamine (1.26g, 0.O1M). Then 2-3 drops of concentration sulphuric acid were added and mixture refluxed for 2h, during which a solid product was formed. The resultant mixture was then cooled, filtered with hot ethanol (2 x 5ml) and dried to give (I) (51% yield) mp 180°C. IR (nujol) 3220, 3160, 2930, 2020, 1695, 1630, 1615, 1605, 1545, 1110, 950, 785 cm^{-1}. H-NMR (DMSO-d_6) (ppm) 5.5 (4H, s, 2xNH_2), 6.8 (1H, dd, azomethine), 6.15 (2H, dd, H-1, 2), 7.75 (1H, d, 11-3), 7.9 (1H, s, NH). Found: C, 47.33; H, 4.42; N, 48.29% $C_8H_9N_7$ requires C, 47.31; H, 4.43; N, 48.25%.

Preparation of metal complexes

An ethanolic solution (15m1) of ligand (I) (0.02M) was added with stirring to an aqueous solution (20ml) of metal (II) chloride (0.OIM) respectively. The mixture was refluxed to the boiling point for lh. The precipitated solid product was formed which was cooled, filtered, washed with hot ethanol (2 x 5 ml) and dried. The crude product thus obtained was crystallised in aqueous ethanol to give the respective metal complexes 1(48%), 2(50%),1(45%) and 4(48%).

Antibacterial studies

Preparation of disc

A ligand/metal complex (30mg) in dimethylformamide (DMP) (0.0lml) was applied to a disc prepared from blotting paper (3mm size) with the help of a micropipett. The discs were left in an incubator for 48 h at 37°C and then applied to a bacteria grown agar plates.

Preparation of agar plates

For this purpose, minimal agar was used for the growth of specific bacterial species. For Staphylococcus species, a blood agar base with low pH was used. The blood agar base (40g) was first suspended in cold distilled water (1L) and heated to boiling. It was then sterilised at 120°C for 15 minutes and later, allowed to cool at 50°C. Then 5% sterile defibrinated sheep blood was added to it and mixture was poured into previously washed and sterilised petri dishes which were stored at 4°C for inoculation. For the preparation of agar plates for Escherichia coli, Mac Conkey agar (50g) obtained from Merck Chemical Company, was suspended in freshly distilled water (1L). It was allowed to soak for 15 minutes and then boiled with constant shaking on a water both until the agar was completely dissolved. The mixture was autoclaved for 15 minutes at 120°C and then poured into previously washed and sterilised petri dishes and stored at 4°C for inoculation.

Procedure of inoculation

It was done by means of a platinum wire loop which was first made red hot on a flame, allowed to cool in air and then used for the application of previously described bacterial strains. The preculture was first prepared in 2ml of nutrient broth by selecting a suitable bacterial colony and later on transferred to a nutrient broth which was incubated for 2h at 37°C. Then 500ml of the culture was spread on the agar plate and incubated for 2 h at 37°C.

Application of discs

A sterilised forecep was used for the application of paper discs to the already incubated agar plate. When the discs were applied, they were then incubated at 37°C for 24 h.

Results and Discussion

The newly synthesized ligand was prepared by following the same procedure as reported earlier by us (Chohan & Kausar 1992, Chohan & Farooq 1993). It is a solid compound which is stable at room temperature. It is soluble in water, dimethylformamide (DMF) and dimethylsulphoxide (DMSO) and insoluble in other solvents. The structural determination of the ligand was done with the help of their IR, H-NMR and microanalytical data.

The IR assignment of the ligand show same characteristic absorption bands at 3220, 3160, 1630, 1545 and 1615$cm^{-1.}$ These bands are assigned to -NH of pyrrol, - NH2, azomethine linkage (>CN-), C = C and C = N of heterocyclic ring stretches respectively. The disappearance of a band at 1760cm^{-1} and appearance of a new and at 1630cm^{-1} due to azomethine linkage confirm the formation of ligand (I). H-NMR spectra also displays signals assignable to the expected number of protons. Also the microanalytical data of CHN was found to be in a complete agreement with the molecular structure of the title ligand.

All the metal complexes $_{(1\text{-}4)}$ of this ligand were prepared by the stoichiometric reaction of the respective metal (II) chloride and ligand in a molar ratio M:L =1:2. All the complexes are air and moisture stable solid compounds. The conductivity measurements of these complexes in aqueous solution (12-15 ohm^{-1} cm^2 mol^{-1}) suggest their non-electrolytic nature (Glasstone 1956).

Magnetic susceptibility

The magnetic susceptibility measurements (Table 1) for all the solid complexes are indicative of three unpaired electrons for Co(II) ion having Meg 4.18 BM, one unpaired electrons for Cu(II) ion (matt 1.45 BM) and two unpaired electrons for each Ni(II) ion having mefr 3.44 BM which suggest (Balhausen 1962) octahedral geometry for Co(II) and Ni(II) complexes and distorted octahedral geometry for Cu(II) complex. Zn(II) complex was found to be diamagnetic.

Infrared spectra

The binding of the ligand to the metal ions was investigated by comparing spectra of free ligand with metal complexes (Table 1) and the following con are drawn:

i) The IR spectra of ligand and their metal complexes exhibit a band at 3 attributed to -NH_2 vibrations indicating that the ligand is certainly not to the metal atom through this group.
ii) The IR spectra of complexes show that (C= N) heterocyclic) band at 1615cm^{-1} in the spectra of ligand shift towards lower frequency side by 10cm^{-1} indicative (Nakamoto 1978) of participation of heterocyclic nitrogen in the coordination.
iii) The IR spectra of complexes indicate that C=N band in the spectra of ligand at 1630cm^{-1} due to azomethine linkage is shifted towards frequency by 5cm^{-1} respectively, indicating the ligand to be coordinated to the metal ions through azomethine linkage.
iv) The new band appearing in the spectra of complexes and not observed in the spectra of ligand at 480cm^{-1} due to M-N mode respectively indicate that ligand is also coordinated to the metal ion by nitrogen of pyrrol ring.

From the above observations it is concluded that ligand is possibly coordinated to the metal atom through C N, N=C and nitrogen of pyrrol ring by covalent bonding.

Electronic spectra

The electronic spectra of metal (11) complexes listed in Table-1 show different values of Amax with changed metal ions which are due to vibrations in perturbing influence of metal ions.

Co(II) complex exhibits absorption bands at 19425, 16550 and 8225 cm 1 assigned to the transition $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ (V_3), $^4T_{lg}$ (F) $\rightarrow {}^4A_{2g}$ (F) (V_2) and $^4T_{1g}$ (F) $\rightarrow {}^4T_{2g}$ (F) (V_1) in cotahedral geometry (Carlin 1968). The spectra of Cu(II) complex exhibits three broad bands in the region 30575, 22220 and 17555 cm^{-1}. The lower energy band may be assigned to the transition $^2Eg \rightarrow {}^2T_{2g}$ for distorted octahedral configuration (Lever 1968). The bands in the region 22220 and 30575 cm-1 can be attributed to ligand → Metal charge transfer.

The electronic spectra of Ni(II) complex showed d-d transitions in the region 26550, 14820 and 10210 cm4. These are assigned to the transitions $^3A_{2g}$ (F) $\rightarrow {}^3T_{2g}$ (P) (V_3), $^3A_{2g}$ (F) $\rightarrow {}^3T_{1g}$ (F) (V_2) and $^3A_{2g}$ (F) $\rightarrow {}^3T_{2g}$ (F) (V_1) respectively consistent with an octahedral environment (Figgis 1968). The absorption spectra of Zn (II) complex, similarly showed a charge transfer band at 27250cm^{-1} and a band at 13250cm^{-1} due to transitions $^2E_g \rightarrow {}^2T_{2g}$ in distorted octahedral environment (Figgis & Lewis 1968).

Based on the knowledge gathered from the above observations, it is thus proposed that all the complexes possess an octahedral geometry in which the ligand behaving as tridentate arrange themselves around the metal atom in such a way that a stable five membered chelate right is formed and thus, a stable configuration of the molecule is attained.

Table 1
Physical, analytical and spectral data of metal complexes

Complex No./ Mol. formula	MP (°C) (decomp)	M. moment (BM)	IR (cm^{-1})	λ_{max} (cm^{-1})	Elemental analysis % cal (found) C	H	N
1 $Co(L_I)_2Cl_2$	252-254	4.18	3192, 3160, 2950, 2675 1945, 1610 1625, 1595 1130, 1095, 872, 480	19425, 16550, 8225	35.83 (35.88)	3.35 (3.15)	36.57 (36.29)
2 $Cu(L_I)_2 Cl_2$	248-250	1.45	3195, 3160, 2950, 2672, 1945, 1610, 1625, 1590, 1130, 1095, 870, 480.	30575, 22220, 17555.	35.52 (35.81)	3.33 (3.12)	36.26 (36.28)
3 $Ni(L_I)_2 Cl_2$	242-244	3.44	3190, 3160, 2950, 2670, 1947, 1610, 1625, 1595, 1135, 1095, 870, 480.	26550, 14820, 10210.	35.84 (35.69)	3.36 (3.39)	36.59 (36.62)
4 $Zn (L_I)_2 Cl_2$	222-224	Dia	3190, 3160, 2955, 2670, 1945, 1610, 1625, 1595, 1130, 1090, 870, 480.	27250, 13250.	35.40 (35.31)	3.31 (3.58)	36.14 (36.08)

Antibacterial studies

The uncomplexed ligand and its metal complexes were tested for their antibacterial activity against bacterial species Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. The antibacterial activity was tested at concentration 30mg'O/Olml in DMF using paper disc diffusion method as reported earlier by us. The results of these studies reproduced in Table 2 show that ligand and all the metal complexes are biologically active against one or more bacterial species. In comparison to uncomplexed ligand the metal complexes have been shown to be more antibacterial.

Table 2
Antibacterial activity data of ligand and complexes

Ligand[a]/Complex No.[b]	Microbial species		
	a	b	c
I	+ +	+	+ +
1	+ + +	+ +	+ + +
2	+ + +	+ +	+ + + +
3	+ + +	+ + +	+ + +
4	+ + + +	+ +	+ + +

a = Uncomplexes ligand (L_I),
b = Same numbering as in Table-1.
Microbial species :
a = Escherichia coli,
b = Pseudomonas aeruginosa,
c = Staphylococcus aureus.
Inhibition zone measured in diameter,
+, 6-10mm; + +, 10-14mm; + + +, 14-18mm; + + + +, > 20mm.

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