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RESEARCH ARTICLE

Bioassay studies of cobalt (II) complexes of modified diamine

Saeed-UR-REHMAN (✉), Muhammad IKRAM, Sadia REHMAN and Shah NAWAZ

Coordination compounds of modified diamine, the basic unit of which are ethylenediamine, with that of Co (II) are prepared. The modified diamines are ethylenediaceticacid (EDDA) and N,N,N,N-tetaraethylene-1,2-diamine (TEEDA). These diamines are characterized through ^{1}H-NMR, ^{13}C-NMR, elemental analysis and IR techniques. Cobalt (II) complexes of these two ligands were prepared and characterized by physical measurements including elemental analysis, IR, UV-Visible, magnetic susceptibilities and conductance measurements. Antibacterial activities are also carried out in order to investigate the biological activity upon complexation. They were screened against four pathogenic bacteria like *Escherichia coli, Pseudomonas aeroginosa, Klesbella pneumonia* and *Staphylococcus aureus*. The results showed significant enhancement in activities.

Keywords EDDA, TEEDA, cobalt complexes, antibacterial studies

1 Introduction

Many studies stressed the role of metal ions in biological processes, whereas the inorganic pharmacology started to be an important field, and more than 25 inorganic compounds have been pursued and are being used in various therapies as antibacterial, antiviral and anticancer drugs [1–9].

In many such cases the chelating agent is a complex polyfunctional moiety which can virtually enclave the metal in an organic sphere. The geometries of the corresponding metal complexes also offer potency in biological applications. It has also been shown that chelation to the metal center tend to make biologically inactive compounds active by reorienting the active sites at the forefront for interaction with the biological systems [10–15].

Received February 11, 2011; accepted February 21, 2011
Institute of Chemical Sciences, University of Peshawar, Peshawar 25120, Pakistan
E-mail: srachem@yahoo.com

All these evidences highlighted the importance of metal coordination with chelating agents in drugs and therapeutics. For this purpose we selected complexation of the synthesized ligands with cobalt (II) metal ion, keeping in view the very importance of this metal.

Cobalt is the center of many metal proteins like Vitamin B12 [16], and the center of hydrogen transfer enzymes like glutamate mutase, methylmalonyl CoA isomerase, dioldehyrase, ethanolamine ammonia lyase, ribonucleotide reductase and many others [17–26], therefore we got interest in complexation of cobalt with modified diamines adding to our previous work carried out on diamines and their complexation [10,11,27]. These ligands by themselves are very potent in offering poly chelation sites [27].

In the context of our current interest in methodologies for constructing substituted diamines, we envisioned a new and versatile access to novel chiral diamines. This strategy involves the synthesis of the ligand by the removal of primary and secondary hydrogens from the corresponding amines with alkyl halide to bear diamines of the same class with different substituents (Figs. 1 and 2).

The prepared complexes were then screened against various pathogenic strains by using agar well diffusion method. This work is the continuation of our previous work in the field of complexation of various modified diamines.

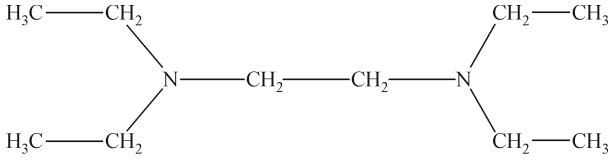

Figure 1 Structure of ligand of N,N,N,N-tetaraethylene-1,2-diamine (TEEDA)

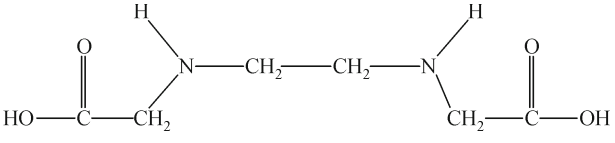

Figure 2 Structure of ligand of ethylenediaceticacid (EDDA)

2 Results and discussion

The ligands and solid complexes are characterized by elemental analyses, NMR (proton and ^{13}C). The composition contents of carbon, hydrogen and nitrogen are within the permissible limit to those calculated values. The ^{1}H-NMR of N,N,N,N- tetaraethylene-1,2-diamine (TEEDA) ligand shows a triplet at 1.02 ppm corresponding to the hydrogens of methyl attached terminally, a quartet at 2.55 ppm for methylene

protons attached to methyl groups at one end and to nitrogen atoms at the other and a triplet at 2.63 ppm for methylene protons attached to each other and to nitrogen atoms of amine. The intensity ratio is in agreement with their population. The ^{1}H-NMR for ethylenediaceticacid (EDDA) show a triplet at 2.63 ppm for the embedded methylene protons splitting each other, a triplet at about 3.05 ppm for terminal methylene groups attached to carboxylic groups at one end and to secondary nitrogens of amine at the other. Carboxylic singlet appears at about 7 ppm in the spectrum. The ^{13}C-NMR spectrum of TEEDA shows three single peaks at 11.5, 47 and 50 ppm, each corresponding to terminal methyl carbons, methylene carbons neighboring to the methyl groups and the embedded methylenes, respectively. The integration ratio and the *J*-value well fit the synthesized TEEDA compound.

Similarly, the ^{13}C-NMR spectrum of EDDA also shows three single peaks. The embedded methylene gives a peak at around 50 ppm. The methylene attached to carboxylic group splits up at 54 ppm, and 180 ppm is assigned to carboxylic carbon. Elemental analytical data of the ligands and its complexes are given, which show closeness to the theoretical values, and the percentage fits well the formula calculated. The ligand TEEDA behaves as a bidentate ligand and helps in determining the geometries of the corresponding complex, whereas the ligand EDDA behave as a tetradentate ligand. Table 1 lists the molar conductance, melting points and magnetic moment values. The molar conductance values indicate that the complexes are non-electrolytic.

In the FTIR spectrum of TEEDA, N-H peak around 3300 cm^{-1} is not observed showing the tertiary amine. The infrared spectra of the Co (II) complex of TEEDA show that there is shift and broadness of N-C frequency around 1235 cm^{-1}. Hence the bidentate nature of TEEDA ligand is shown. The far IR spectra show the M-X bonding for both TEEDA and EDDA. They also show the M-N stretch around 500 cm^{-1}. The infrared spectrum of EDDA shows an alteration of N-H and carboxyl frequency, which characterizes the tetradentate ligand. A broad peak observed at 3340 cm^{-1} in the IR band is assigned to the metal to water coordination [28,29]. The details are given in Table 2.

2.1 [Co(EDDA) 2H$_2$O] complex

The magnetic moment of cobalt EDDA complex shows three unpaired electrons. The solution spectrum of cobalt (II) EDDA complex is shown in Fig. 3 and $^4T_{1g}\rightarrow{}^4T_{2g}$, $^4T_{1g}\rightarrow{}^4A_{2g}$ and $^4T_{1g}\rightarrow{}^4T_{1g}$ are the three assigned transitions to the bands at 19 230, 15 500 and 14 800 cm^{-1}. And this shows the octahedral symmetry since the ligand is attached to the metal center through four sites including the two ammine sites of attachment and the two carboxylic sites. The intensities and bandwidths are also in accordance with O_h symmetry.

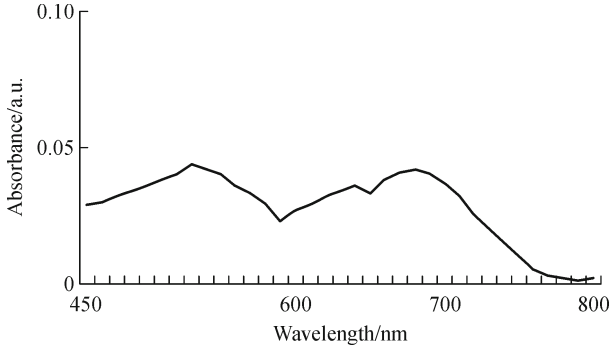

Figure 3 Visible spectrum of [Co(EDDA).2H$_2$O]

2.2 [Co(TEEDA)Cl$_2$] complex

The magnetic moment of cobalt complex is 4.73 B.M, indicating three unpaired electrons. The solution spectrum of

Table 1 Conductance, melting points and magnetic moments data

Complex	Solvent	Melting point/°C	Molar conductance/(S·cm^{-1})	Cor × M × 10^{-6} /c.g.s	μ_{eff}/B.M
$C_{10}H_{24}N_2$	—	93	—	—	—
$C_{10}H_{24}Cl_2CoN_2$	DMSO	170	0.7	9320.35	4.73
$C_6H_{12}N_2O_4$	—	281	—	—	—
$[C_6H_{10}CoN_2O_4]$	DMSO	51	1.2	3997.82	5.0

Table 2 Assignment of absorption bands in IR spectra for ligands and their complexes/cm^{-1}

Complex	Stretching frequency of N-H	Stretching frequency of C-N	Other significant bands	M-X
$C_6H_{12}N_2O_4$	3330sh	1220m	2943, 3480, 1720m	—
$[Co(C_6H_{12}N_2O_4)2H_2O]$	3360s	1220m	2940, 3420b, 1720m	3340bd
$C_{10}H_{24}N_2$	Not observed	1213w, 1240w	2920m, 1300w	—
$[Co(C_{10}H_{24}N_2)Cl_2]$	Not observed	1233b, 1236w	2920m, 1300w	400s

sh = Sharp; s = Small; w = Weak; m = Medium; bd = Broad; X = Cl- or H_2O

cobalt (II) complex is given in Fig. 4. A set of three bands in the range of 15000 to 18000 cm^{-1} are assigned to $^4A_2(F) \rightarrow {}^4T_1(P)$ transition, ν_3, in T_d symmetry. The low energy transition $^4A_2(F) \rightarrow {}^4T_1(F)$, ν_2, is not observed. The intensities and bandwidths are in accordance with T_d symmetry and are identical with those of $[Co(daco)Cl_2]$ and $[Co(dach)Br_2]$ [30,31].

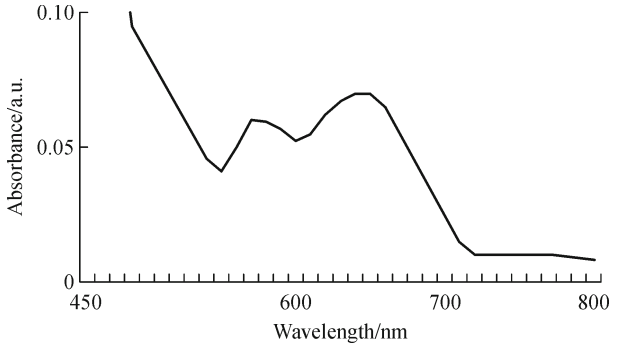

Figure 4 Visible spectrum of $[Co(TEEDA)Cl_2]$

2.3 Bio-assay investigations

The complexes of TEEDA and EDDA were investigated in terms of their bioactivity and the results are reported in Tables 3–6. This study shows that the metal complexes become more biologically active as compare to neat organic moiety. The complexes of ABH have been screened against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staph aureus*, and *Klesbiela pneumonae*. The results are reported in Tables 3–6. The comparison of the activities is also shown with comparative graphs in Figs. 5–8, revealing increase in inhibition properties upon complexation.

3 Experimental

3.1 Materials and methods

All the chemicals and solvents used were of analytical grade. Metal (II) salts of cobalt were used as chlorides and carbonates, obtained from Riedel-de-Haen, Germany and

Table 3 Antibacterial activity of EDDA against four pathogenic bacteria

Concentration/[μg/(20 μL)]	Zones of inhibition/mm			
	Escherichia coli	*Pseudomonas aeruginosa*	*Klesbiella pneumonae*	*Staphylococcus aureus*
20	0	0	1.0	0
40	0	0	1.0	0
60	0	0	1.0	0
80	0	0	2.0	0
100	0	0	4.0	1.0
120	0.6	0.4	4.0	1.0

Table 4 Antibacterial activity of $[Co(EDDA)2H_2O]$ against four pathogenic bacteria

Concentration/[μg/(20 μL)]	Zones of inhibition/mm			
	Escherichia coli	*Pseudomonas aeruginosa*	*Klesbiella pneumonae*	*Staphylococcus aureus*
20	0	0	2.0	0
40	0	0	2.0	0
60	0	2.0	4.0	0
80	4.0	2.0	4.0	0
100	4.0	5.0	4.0	0
120	4.0	6.0	5.0	0

Table 5 Antibacterial activity of TEEDA against four pathogenic bacteria

Concentration/[μg/(20 μL)]	Zones of inhibition/mm			
	Escherichia coli	*Pseudomonas aeruginosa*	*Klesbiella pneumonae*	*Staphylococcus aureus*
20	0	0	1.0	1.0
40	0	0	1.0	2.0
60	0	0	1.0	4.0
80	3.0	1.0	2.0	4.0
100	4.0	2.0	2.0	5.0
120	6.0	4.0	2.0	6.0

Table 6 Antibacterial activity of [Co(TEEDA)Cl_2] against four pathogenic bacteria

Concentration/[μg/(20 μL)]	Zones of inhibition/mm			
	Escherichia coli	*Pseudomonas aeruginosa*	*Klesbiella pneumonae*	*Staphylococcus aureus*
20	0	0	1.0	1.0
40	4.0	0	1.0	3.0
60	4.0	2.0	6.0	5.0
80	4.0	4.0	6.0	6.0
100	4.0	6.0	6.0	8.0
120	4.0	6.0	6.0	9.0

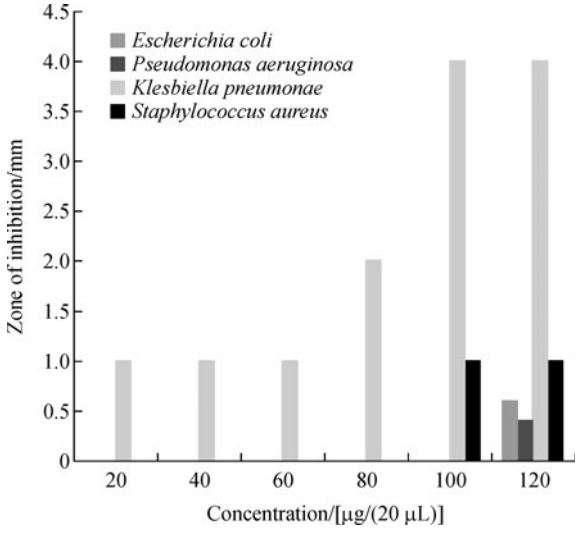

Figure 5 A comparative graph for the activity of EDDA against different pathogens at different concentrations

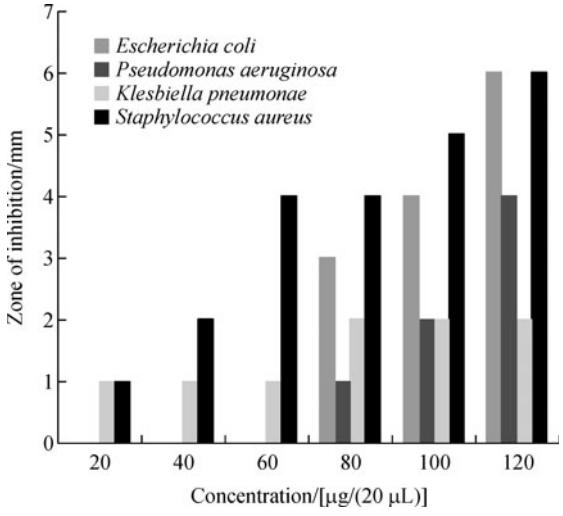

Figure 7 A comparative graph for the activity of TEEDA against different pathogens at different concentrations

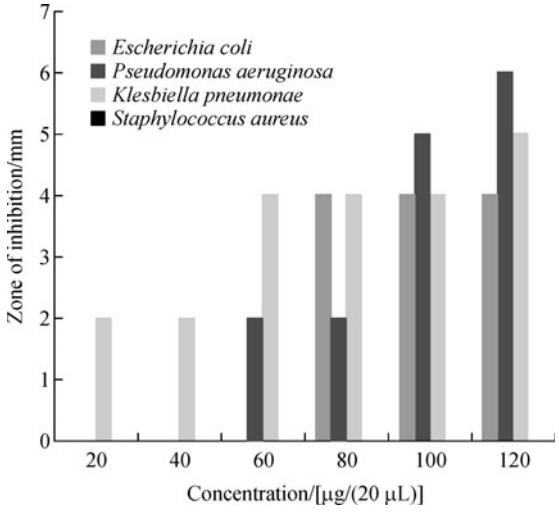

Figure 6 A comparative graph for the activity of [Co(EDDA)] against different pathogens at different concentrations

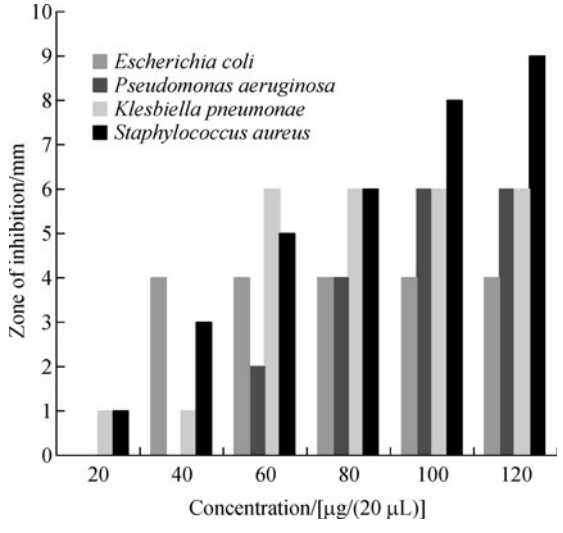

Figure 8 A comparative graph for the activity of [Co(TEEDA)Cl_2] against different pathogens at different concentrations

were used as such without further purification. Solvents were distilled at least twice before use. Elemental analyses were taken by HEJ Research Laboratories, Karachi. Melting points were recorded on Gallenkamp apparatus and reported as such.

Biologic activities were carried out by the stated procedure at Pakistan Medical Research Center, Khyber Medical College, Peshawar (PMRC).

3.2 Instrumentation

Molar conductances of the solution of the metal complexes were determined with a type HI 8333 conductivity meter. All measurements were carried out at room temperature with freshly prepared solution.

Magnetic susceptibilities were measured by Gouy method at room temperature using $Hg[Co(SCN)_4]$ as a standard [32], and the magnetic moments were thus calculated. The cations and anions were estimated by using analytical procedure [33].

Infrared spectra were taken in the range of 4000 – 600 cm^{-1} on PYE UNICAM Infrared Spectrophotometer in KBr disc. The far IR spectra were examined in CsI discs in the region of 400 – 200 cm^{-1}(T- IR SHIMADZU).

The absorption spectra of solution of complexes in the range of 400–800 nm using different solvents were obtained on Jasco DEC-1 Spectrophotometer with 1 cm matched quartz –cells.

Bioactivities were investigated using agar-well diffusion method [34]. 2–8 hours old bacterial strains inoculums containing approximately 104–106 colony forming units (CFU)/mL were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centers of at least 24 mm. Recommended concentration (100 μL) of the test sample 1 mg/mL in DMSO was introduced in the respective wells. Other wells were supplemented with DMSO and reference antibacterial drug, and maxipime served as negative and positive controls, respectively. The plates were incubated immediately at 37°C for 20 h. Activity was determined by measuring the diameter of the zones that showed complete inhibition (mm). Growth inhibition was compared with the standard drug maxipime for the selected bacterial strains. To clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions of DMSO alone and they showed no activity against any bacterial strains. All these complexes were found to be potentially active against these bacterial strains.

3.3 Synthesis of TEEDA

0.019 mol of ethylenediamine and 10 mL of dry ethanol were stirred in a flask at room temperature for 10–15 min. Then 0.0096 mol of dibromoethane was added and stiring was continued for another 30 min. 0.53 g solid KOH was added to the reaction mixture and refluxed for one hour. White precipitate of KBr was filtered off and the filtrate containing TEEDA was evaporated through rotary evaporator to get solid phase. Solid TEEDA was purified by dissolving in dry methanol. The purity was checked by TLC technique. The yield for TEEDA obtained was 55%.

$C_{10}H_{24}N_2$ calc. C(69.70%) H(14.04%) N(16.26%)., found C(69.43%), H(13.86%), N(16.04%).

3.4 Synthesis of EDDA

2 g (0.033 mol) of ethylenediamine in 10 mL of dry ethanol was taken and stirred at room temperature for about 10 min. 6.237 g (0.066 mol) of chloroacetic acid was added to this mixture with continued stirring for one hour. Solid KOH 1.84 g (0.033 mol) was added to this reaction mixture and refluxed for one hour. White precipitate of KBr was filtered off and the filtrate containing EDDA was evaporated through rotary evaporator to get solid phase. Solid EDDA was purified by dissolving in dry methanol. The purity was checked by TLC technique. The yield for EDDA obtained was 38%.

$C_6H_{12}N_2O_4$ calcl. C(40.91%) H(6.87%) N(15.90%)., found C(40.11%) H(6.70%) N(16.30%).

3.5 Synthesis of $[Co(TEEDA)Cl_2]$

0.8 g (0.003 mol) of $CoCl_2.6H_2O$ was dissolved in minimum amount of dry ethanol and 2 mL of 2,2-dimethoxy propane was added to it. The reaction mixture was stirred for two hours in order to dehydrate the metal salt. 0.57 g (0.0035 mol) of ligand TEEDA dissolved in minimum amount of dry ethanol was added to this reaction mixture and the resulting solution was heated and stirred for two hours. The mixture was left overnight and then concentrated by rotary evaporator. On cooling blue colored product was precipitated out. The product was filtered through sintered glass crucible and dried under vacuum at 50°C. The yield for the product was calculated to be 55%.

$C_{10}H_{24}Cl_2CoN_2$ Calcl. C(39.75%) H(8.01%) Cl(23.47%) Co(19.50%) N(9.27%)., found. C(39.61%) H(7.84%) Cl (23.30%) Co(19.26%) N(9.15%).

3.6 Synthesis of $[Co(EDDA)2H_2O]$

0.3 g (0.0028 mol) of $CoCO_3$ was dissolved in minimum amount of distilled water. The reaction mixture was got with stirring. 0.4 g (0.0025 mol) of ligand EDDA dissolved in minimum amount of distilled water was added to this reaction mixture and the resulting solution was heated up to 60°C in inert atmosphere until the CO_2 emission was completed. It took almost 10 min. The mixture was left overnight and then concentrated by rotary evaporator. On cooling pink colored product was precipitated out. The product was filtered through sintered glass crucible and washed by 5 mL mixture of ice-cold

water, ethanol and acetone. The dried product appeared to be stable in air. The yield for the product was calculated to be 40%.

C_6 H_{14} Co N_2 O_6 calcl. C(26.78%) H(5.24%) Co(21.90%) N(10.41%)., found C(25.67%) H(4.12%) Co(22.23%) N (11.41%).

4 Conclusion

The synthesized Co complexes of TEEDA and EDDA ligands show octahedral and tetrahedral geometries, respectively as shown in Figs. 9 and 10.

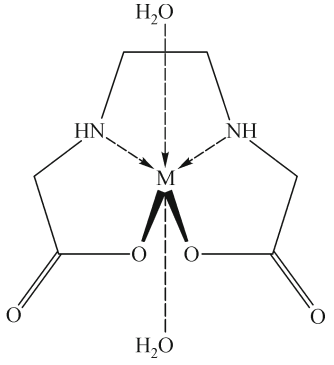

Figure 9 Proposed octahedral geometry for [Co(EDDA) $2H_2O$]

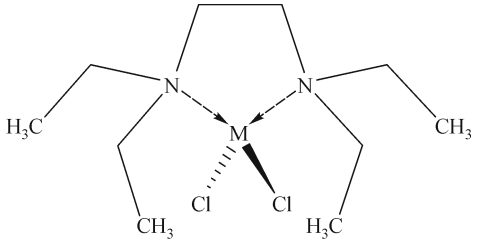

Figure 10 Proposed tetrahedral geometry for [Co(TEEDA)Cl_2]

Magnetic moment studies prove the assigned geometries. The synthesized ligands showed antibacterial properties. In comparison, the cobalt (II) metal complexes of these compounds showed more activity against one or more bacterial strains, thus introducing a novel class of metal-based bactericidal agents.

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