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Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2010, 2(6):369-380

Kinetics, equilibria studies and antimicrobial activity of Iodomethyl (ligand) Cobaloximes and their role as DNA binding and Photocleavage agents

C. Shobha Devi[1], Penumaka Nagababu[2], Mynam Shilpa[1] and Satyanarayana S[1]*

[1]Department of Chemistry, Osmania University, Hyderabad, India
[2]Institute of Chemistry, Acadamia Sinica, Taipei, Taiwan

ABSTRACT

A series of Iodomethyl complexes with various N-donor ligands were synthesized & characterized by UV/VIS, IR, MS, ^{1}H & ^{13}C -NMR spectral methods. Pseudo first order reaction kinetics and binding studies of $[ICH_2Co(DH)_2OH_2]$ complex with aliphatic primary amines, aromatic and heteroaromatic amines such as Methylamine, Ethylamine, Propylamine, Aniline, Benzyl amine, Phenylalanine, Pyridine, and Amino pyridine were investigated. Comparison of equilibrium constants and rate constants indicates that the order is $K_{PA}>K_{EA}>K_{MA}$; $K_{APy}>K_{Py}$; $K_{BA}>K_{aniline}$; the rate of substitution of H_2O varies with the pKa of the incoming ligand establishing the existence of nucleophilic participation of the ligand in the transition state. The DNA binding properties of $[ICH_2Co(DH)_2OH_2]$ complex was investigated by absorption, emission & viscosity measurements. Photo activated cleavage of pBR-322 DNA by this complex was also studied. Further, all these cimplexes were screened for their antimicrobial activity.

Keywords: Cobaloxime; Co-C bond; Equilibrium constants; Molecular mechanics; Calf-thymus DNA; Photocleavage.

INTRODUCTION

Model complexes for Vitamin B_{12} such as alkyl(aquo)cobaloximes have played an important role in understanding the behavior of the ligand substitution reactions of Vitamin B_{12} and the role of Co-C [1,2] bond in the co-enzyme VitaminB_{12}. The study of coenzyme Vitamin B_{12} complexes unfolds the mechanism of B_{12} dependent enzymatic reactions especially at the initial stages of the B_{12} catalytic cycle. In order to understand better structure and reactivity of cobalamines, simple models have been proposed and investigated [3, 4, 5, 6 & 7]. Randaccio et al [8, 9 & 10] studied the structural and solution properties of rhodoximes, compared the cobaloximes and rhodoximes and discussed the basis of electronic and steric effects. These studies have furnished a foundation for understanding the mechanism of Co-C bond cleavage in the vit B_{12} coenzyme. Equilibria and

kinetics for axial ligation of bromomethyl(aquo)cobaloxime and binding of various Pyridines with methyl and ethyl(aquo)cobaloximes were reported by Bhoopal et al [11,12] and Navaneetha et al [13]. To provide further information concerning the ligand substitution reactions we have studied Equilibria and Kinetics of axial ligation of Iodomethyl(aquo)cobaloxime with N- donor ligands. These were chosen because

i) They form stable complexes. ii) Ligands with a wide range of pKa iii) Significant spectroscopic changes occur in their co-ordination so that reactions are readily followed spectroscopically and iv) Their co-ordination chemistry is expected to be simple.

EXPERIMENTAL SECTION

Aliphatic, aromatic, heteroaromatic amines and CT-DNA were obtained from Sigma and were used as received. KCl, HPLC grade MeOH, AcOH and HCl were obtained from Fluka. Dipotassium hydrogen phosphate, potassiumdihydrogen phosphate, potassium phosphate, tris, sodium acetate and potassium hydroxide were obtained from Acros. Double distilled, deionized water was used throughout. By using supercoiled (CsCl purified) pBR-322 DNA (Bangalore Genie, India), Photocleavage properties have been studied.

1.1 *Preparation of [$ICH_2Co(DH)_2L$]*

[$ICH_2Co(DH)_2OH_2$] complex was prepared by the procedure of Brown et al [14 ,15]. These alkyl(aquo)cobaloximes are photolabile due to Co-C bond, particularly in solution. All work with alkyl(aquo)cobaloximes was performed in dim light (in dark room) and solutions were covered with aluminium foil. [$ICH_2Co(DH)_2L$] complexes were isolated by mixing 1:3 ratio of Iodomethyl(aquo)cobaloxime and the desired base ligand (L) in methanol. This mixture was heated at 40-50^oC by constant stirring for 1-2 hrs. Then minimum amount of distilled water was added, the resulting precipitate of yellow powder was filtered washed with distilled water, 95% methanol, ether and dried in vacuo(yields were 70-80%).

UV-Visible spectra were recorded on Elico BL-198 spectrophotometer with temperature control. Kinetics and binding studies were done using Elico SL-171 single beam spectrophotometer provided with a thermostat. IR Spectra were recorded on Perkin-Elmer 1600 FT-IR spectrophotometer using KBr pellets. ^1H & ^{13}C NMR spectra were recorded on varian Gemini 200 MHz NMR spectrometer. Samples were prepared by dissolving in DMSO –d_6. (spectral data are give in tables)

RESULTS AND DISCUSSION

Spectral analysis

We have prepared various Iodomethyl(L)combaloximes by replacing H_2O from [$ICH_2Co(DH)_2OH_2$] is shown in Fig 1. The IR spectral data for Iodomethyl complexes are reported in Table-1. The disappearance of peak from 2900 cm^{-1} to 3433 cm^{-1} & appearance of peaks near 420 cm^{-1} (Co-N) region [16] indicates the formation of Iodomethyl (ligand) cobaloximes by replacing H_2O. The characteristic absorption bands due to DMG ligands in these cobaloximes do not shift largely by the change in axial ligands. This suggests that the strength of the Co-N (equatorial) bonds are very strong due to Co→N=C π bond. The definite relationship between the frequency shifts and the consecutive order of axial ligands were observed (Table-1). As the donating power of the base ligand increases, the (O-H…H) at ~1770 cm^{-1} and (C=N) at ~1570 cm^{-1} shifts to a lower wave number region while, (N-O) at ~1230 cm^{-1} and 1085 cm^{-1}, (Co-N) at ~510 cm^{-1} shifts to higher one. These results can be interpreted as follows. The

coordination of more electron donating base to Co atom causes the increase in electron density in Co atom which facilitates the back donation from Co(III) to the nitrogen atoms of dimethyl glyoximato ligands resulting in the increase in electron densities in C=N and N-O bonds. The facilitated back donation from cobalt to nitrogen atoms of dimethylglyoxime lowers the C=N stretching frequency.

The electronic spectra of Iodomethyl(aquo)cobaloxime in MeOH shows spin allowed $^1A_1g \rightarrow {}^1T_1g$ transition [17] in the region ~22000 cm^{-1} due to I CH_2^- to Co(III) σ donation. This band disappears or the intensity drastically decreases in Iodomethyl(ligand) cobaloximes. The charge-transfer spectra of the trans $[ICH_2Co(DH)_2L]$ complexes show a band 33000 cm^{-1} due to intra ligand π-π* transition of the coordinated dimethylglyoxime[18]. A bond occurring around 27500 cm^{-1} are assigned to the L→Co(III) in these complexes. The σ(DH)→σ*Co(III) is masked by the intense short wavelength bands of Iodomethyl(ligand)cobaloximes[19]. LCMS spectra of $[ICH_2Co(DH)_2H_2O]$ gave molecular ion peak at 448m/z. In all these complexes ^{1}H-NMR shows a sharp singlet at around 2.10-2.2ppm (Table-2) corresponds to the equatorial methyl protons of dimethyl glyoxime and a singlet ~3.05ppm corresponding to CH_2I protons (Fig 2). The chemical shifts for the aromatic and heteroaromatic rings are in the aromatic region as expected and observed in the down field when compared to free ligands. All other complexes follow the same trend of downfiled shift and the data presented in Table-2.

In the ^{13}C NMR spectra, the peak around 12 ppm corresponds to four equatorial methyl groups of dimethyl glyoxime. The C=N group chemical shifts observed at around 150ppm (Table 3). The chemical shifts for the aromatic and heteroaromatic rings are in 125-140ppm as expected and observed in the down field when compared to free ligands. Fig 2 Shows ^{1}H & ^{13}C –NMR spectra of $[ICH_2Co(DH)_2OH_2]$ complex.

Kinetic and equilibria studies were done by the procedures of Sridhar et al [20, 21]. Fig. 3 shows Absorption spectra of Iodomethyl aquo complex and variation of the absorption spectrum of the complex in presence of different concentrations of ligand (MA). Depending on the pKa values of the ligands the binding studies were performed in the pH range above and below the pKa values. The K_{app} values for the ligation of $[ICH_2Co(DH)_2OH_2]$ with N-donor ligands were determined as a function of pH by spectrophotometry (Fig 4). The equilibrium constants for the axial ligation of $[ICH_2Co(DH)_2OH_2]$ by N-donor ligands is dependent upon the pKa values of the conjugated acid of the ligands. As the pH increases the apparent binding constant increases. Up to the pKa of the ligand, log K_{app} increases with pH, but above pKa log K_{app} is independent of pH. The K_{app} value below the pKa value is very low due to the protonation of the ligand, but as the pH increases the ligand is deprotonated and binds strongly to Co^{III}, thus K_{app} increases. The affinities of the ligands follow the order: PA>EA>MA(Table-4); among aromatic amines 4-aminopy>Py; Benzylamine> Aniline. The stability order can be explained by considering the HSAB principle, basicity of the ligands and their ability of π-bonding and σ donation. In all these cases formation constants increased with increase in basicity. The rate of ligand substitution is pH dependent. The rate of the reaction increases drastically near the pKa of the ligand. The slope of the plot of k_{obs} Vs concentration of the ligand gives the second order rate constant k_{on}, at a given pH(Table-4). The plot of k_{obs} Vs pH given in Fig 5 clearly indicates that as the pH increases, k_{obs} increases as the deprotonated form of the ligand is readily available at higher pH. The second-order rate constants increase as the nucleophilicity of the ligand increases. As the pH is increased the rate of formation of complex increases, at the same time at any given pH as the concentration of ligand increases (for a fixed concentration of $[ICH_2Co(DH)_2OH_2]$ complex) the k_{obs} increases. This is in accordance with the order of the Keq values (Table-4). The rate of formation of complex for various ligands increases in the order: PA>EA>MA; Benzylamine>Aniline.

Molecular- mechanistic Studies

Molecular mechanics is a tool of increasing importance for structural investigations of coordination and organometallic chemistry [22, 23, 24 & 25]. By means of MM2 parameterization, the optimized structures resulting from axial ligation of $[ICH_2Co(DH)_2OH_2]$ by different ligands were calculated with Bio Med CA Cache 5.02 software. Bond length and bond strain values are then evaluated. Table-5 illustrates the values that are obtained by MM2 optimization. As the basicity of trans ligand or steric crowding of trans ligand increases Co-C bond becomes weak. This is evidenced by the increase in Co-C bond length when H_2O is replaced by basic ligand.

Antimicrobial Activity

Antitumor, anticancer and antimicrobial activity [13] has been reported for some Co(III) complexes substituted with imidazoles and Pyridines. Thus antimicrobial activity was attempted for the complexes we have synthesized. The antimicrobial activities of $[ICH_2Co(DH)_2OH_2]$ & $[ICH_2Co(DH)_2L]$ compounds were determined in vitro using different microorganisms by the standard disc method [26]. The following bacteria were used: Bacillus, Staphylococcus, E.coli and Fusarium(Fungus) . Bacterial cultures were sub-cultured in nutrient broth medium and incubated at 37^oC for 18h and the logarithmic or exponential phase was achieved. Filter paper discs of 4mm size were prepared by using Whatmann filter paper no.1, and on to each of these discs a 5 µl of a solution of the complex in DMSO was added. At the end of the incubation period the zones of inhibition were measured (Table-6). From the zone of inhibition test (Table-6) it has been found that when agar plates were supplemented with antibiotics, e.g. Bacillus, the inhibition area was 6-14. When the same agar plates were supplemented with Fungal species it has been observed that the zone of inhibition was less or insensitive. From the above results it is seen that these complexes posses antibacterial activity (Fig 7). The anti microbial activity increased on the concentration of the compounds increased. It is known that in a complex the positive charge of the metal is partially shared with donor atoms present in the ligand and there may be π electron delocalization over the whole chelating ring. This increases the lipophilic character of the complex and favors its permeation through the lipid layer of the bacterial membrane.

DNA binding Studies

Absorption Studies

The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques [27, 28]. The absorption spectra of the complex $[ICH_2Co(DH)_2OH_2]$ in the absence and presence of calf thymus DNA in Tris buffer are illustrated in Fig 8. In the UV region, the intense absorption bands observed for Co^{III} complexes are attributed to intraligand π-π* transition of the coordinated groups. Addition of increasing amounts of CT DNA results in hypochromism and a moderate bathochromic shift of the UV spectrum of the complex $[ICH_2Co(DH)_2OH_2]$. These spectral data may suggest a mode of binding that involves a stacking interaction between the complex and the base pairs of DNA. By varying the DNA concentration (10-100µM) & maintaining the complex concentration constant. Absorbance values were recorded after each successive addition of DNA solution. The data were fitted to the following equation[29] to obtain the intrinsic binding constant K_b.

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

Where [DNA] is the concentration of DNA in base pairs. ε_a ,ε_f,& ε_b are the apparent, free and fully bound metal complex extinction coefficients respectively. In the plots $[DNA]/(\varepsilon_b-\varepsilon_f)$ vs [DNA], K_b is given by the ratio of slope to intercept. The binding constant K_b of $4.3x10^4$ M^{-1} was obtained from the decay of the absorbance. The K_b values are in the same order of

Bromomethyl(aquo)cobaloxime [30] & Aquabis(ethane-1,2-diamine)ethyl cobalt III complex [31].

Fluorescence Spectroscopic Studies

The complex $[ICH_2Co(DH)_2OH_2]$ exhibits luminescence in Tris buffer (pH7.0) at room temperature with a maximum at 520nm. Binding of the complex to DNA was found to increase the fluorescence intensity. The emission spectra of the complex in the absence and presence of CT DNA are shown Fig 9. Upon addition of CT DNA the emission intensity increases steadily. This observation is further supported by the emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher. The ion $[Fe(CN)_6]^{4-}$ distinguish between differentially bound Co(III) species and positively charged free complex ions readily as the ions are quenched by $[Fe(CN)_6]^{4-}$. The complex bound to DNA can be protected from the quencher, because highly negatively charged $[Fe(CN)_6]^{4-}$ would be repelled by the negative DNA phosphate backbone, hindering the quenching of the emission of the bound complex. The method essentially consists of titrating a given amount of DNA-metal complex by increasing the concentration of $[Fe(CN)_6]^{4-}$ and measuring the change in fluorescence intensity. The ferrocyanide quenching curves for this complex in the presence and absence of CT DNA are shown in Fig 10. The absorption and fluorescence spectroscopy studies determine the binding of the complex with DNA.

Viscosity Studies

Further clarification of the interaction between Iodomethyl(aquo)cobaloxime with DNA was carried by Viscosity measurements [32, 33]. Optical photophysical probes are necessary, but not sufficient clues to support a binding model. Hydrodynamic measurements that are sensitive to length change (i.e. Viscosity and Sedimentation) are regarded as the most critical tests of binding in solution. Effects of the complexes on the viscosity of rod like DNA are shown in Fig 11. For the Iodomethyl(aquo)cobaloxime the viscosity of DNA increases with increase of the concentration of complex which is similar to that of proven DNA intercalator EtBr (Ethydium bromide). But the increase is less than that of EtBr. So this complex binds to DNA through groove binding.

Photoactivated Cleavage of pBR322 DNA by $[ICH_2Co(DH)_2OH_2]$ Complex

There has been considerable interest in DNA endonucleolytic cleavage reactions that are activated by metal ions [34, 35]. The delivery of high concentrations of metal ion to the helix, in locally generating oxygen or hydroxide radicals, leads to an efficient DNA cleavage reaction. DNA cleavage was monitored by reaction of supercoiled circular pBR-322 (form I) into nicked circular (II) forms. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the supercoiled form (form I). If scission occurs on one strand (nicking), the supercoils will relax to generate a slower-moving open circular form (form II). If both strands are cleaved, a linear form (form III) will be generated that migrates between forms I & II. Fig. 12 shows the gel-electrophoresis separations of plamid pBR-322 DNA after incubation with cobalt complex and irradiation at 360nm. Fig 12 reveals the conversion of form I and II after 60min irradiation in the presence of varying concentrations of complex. It was observed that, by increasing the concentration of complex form I gradually decreases and form II increases. This is the result of single-stranded cleavage of pBR-322 DNA. That neither irradiation of DNA at 360nm without cobalt complex nor incubation with cobalt complex but without light yields not a significant strand scission, it is most likely of cobalt complex is the important step leading to DNA Cleavage.

Table-1:IR Spectral data* for Iodomethyl cobaloximes [$ICH_2Co(DH)_2L$]

Where L	Co-N(N of L)	Co-N(N of DH)	C=N--O	CH_3(of DH)	C=N	H-O..H	CH_2	NH_2
Water	0	511.5	739.9	1229&1265	1565	2900-3433	-	-
Methylamine	432	516	742	1371&1455	1563	1765	2940	3275
Ethylamine	420	514	741.9	1356&1438	1560	1774	2926	3236
Propylamine	491	514.4	739.8	1371&1443	1563	1781	2930	3234&3438
Benzylamine	491	515.8	745	1364&1453	1560	1787	2929	3264&3432
Aniline	492	515	758	1366&1493	1567	1623	2918	-
Pyridine	491	515	764	1368&1444	1558	1654	2926	-
4AminoPyridi	490	515	736	1333&1435	1507	1647	2935	3435
Phenylalanine	469	524	745	1306&1455	1573	1631	2964	3062

**Recorded as KBr discs & values in cm^{-1}*

Table-2: ^{1}H-NMR Spectral data* for Iodomethyl cobaloximes [$ICH_2Co(DH)_2L$]

			Axial Ligand Protons				
Where L	eq CH3	I-CH2	CH3	CH2	CH2-NH2	NH2	Aromatic Protons
Water	2.1(s)	2.7	-	-	-	-	-
Methylamine	2.3(s)	3.06	1.84	-	-	2.06	-
Ethylamine	2.3(s)	3.05	1.08	-	2.2	1.8	-
Propylamine	2.3(s)	3.05	0.82	1.4	2.17	1.78	
Benzylamine	2.3(s)	3.09	-	3.3	-	2.15	7.1 to 7.3
Aniline	2.1(s)	3.19	-	-	-	2.04	7.5 to 7.9
Pyridine	2.2(s)	2.8	-	-	-	-	7.2 to 8.5
4AminoPyridine	1.9(s)	2.75	-	-	-	4.6	6.2 to 7.8
Phenylalanine	2.2(s)	2.7	-	2.5	3.3	3.5	7.2 to 8.0

**Dissolved in DMSO-d_6 & values in ppm relative to TMS*

Table-3: ^{13}C-NMR Spectral data* Iodomethyl cobaloximes [$ICH_2Co(DH)_2L$]

Where L	eq CH3	CH3	CH2	CH2-NH2	C=N	Aromatic Protons
Water	12.2	-	-	-	150.2	-
Methylamine	15.8	28	-	-	149.9	-
Ethylamine	12.3	17.3	-	36.8	149.8	-
Propylamine	12.6	25.6	32.5	44	150.1	-
Benzylamine	16.5	-	-	46	150	127,128&139
Aniline	12.4	-	-	-	151	114&128
Pyridine	12.28	-	-	-	149	125&138
4AminoPyridine	12.19	-	-	-	155	110&156
Phenylalanine	12.2	-	38.3	51.2	150	120,123&127

**Dissolved in DMSO-d_6 & values in ppm relative to TMS*

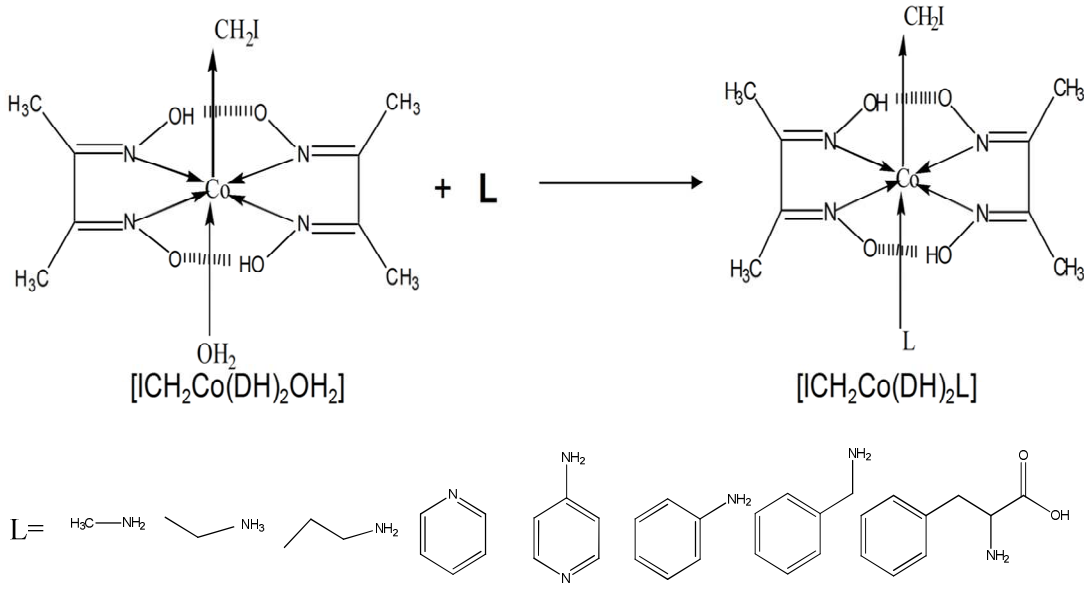
Table-4: Dependence of the Rate Constant k_{obs} for the axial Ligation of [$ICH_2Co(DH)_2OH_2$] complex on the concentration of [L] at 25^{o}C

	M/L	MA	EA	PA
$k_{obs}[s^{-1}]$	1:100	$3.0*10^{-4}$	$7*10^{-4}$	$1.3*10^{-3}$
	1:125	$6.5*10^{-4}$	$1.1*10^{-3}$	$1.6*10^{-3}$
	1:150	$1.4*10^{-3}$	$1.6*10^{-3}$	$2.4*10^{-3}$
	1:175	$2.1*10^{-3}$	$2.7*10^{-3}$	$3.2*10^{-3}$

	1:200	$2.6*10^{-3}$	$3.1*10^{-3}$	$4.3*10^{-3}$
k_{on}'		0.0242	0.0256	0.0304
pH		9.5	9.5	9.5
α		0.08351	0.072033	0.06332
k_{on}		0.2895	0.35539	0.482
Keq		1220.5	1836	2589

Table-5: Bond lengths [A^o] obtained from molecular mechanics studies with [$ICH_2Co(DH)_2OH_2$] and different N-donor ligands

Ligand	Co-N(1)	Co-N(1)	Co-N(1)	Co-N(1)	Co-C	Co-N(L)	Co-O
H_2O	1.755	2.11	1.616	1.467	3.122	-	3.39
MA	1.876	2.249	1.728	1.569	3.337	3.391	-
EA	1.913	2.292	1.761	1.599	3.402	3.456	-
PA	1.88	2.253	1.73	1.571	3.343	3.542	-
Aniline	2.055	2.462	1.892	1.718	3.655	3.713	-
BenAmine	2.121	2.542	1.953	1.773	3.773	3.833	-
Py	2.046	2.451	1.884	1.71	3.638	3.356	-
AminoPy	2.074	2.486	1.91	1.734	3.689	3.403	-
Phe-ala	2.309	2.766	2.125	1.93	4.106	3.798	-



[$ICH_2Co(DH)_2OH_2$] [$ICH_2Co(DH)_2L$]

Fig 1 The lilgand substitution reaction of [$ICH_2Co(DH)_2OH_2$] with various N-donor ligands

Table-6:Antimicrobial activity of the Co Complexes at 20 µg/ml

Complex	Bacterial Species		Fungal Species	
	Bacillus	Staphylococcus	E.Coli	Fusarium
[$ICH_2Co(DH)_2OH_2$]	11	8	6	7
[$ICHCo(DH)_2MA$]	12	10	6	0
[$ICH_2Co(DH)_2EA$]	12	9	7	0
[$ICH_2Co(DH)_2PA$]	7	4	14	3
[$ICH_2Co(DH)_2$Aniline]	8	8	8	0
[$ICH_2Co(DH)_2$BenA]	6	3	7	0

$[ICH_2Co(DH)_2Py]$	11	10	5	0
$[ICH_2Co(DH)_2APy]$	8	9	7	8
$[ICH_2Co(DH)Phe\text{-}ala]$	6	8	5	7
Tetracyclin		10-14		
Bacitracin		6-8		

Values of zone of inhibition (mm)

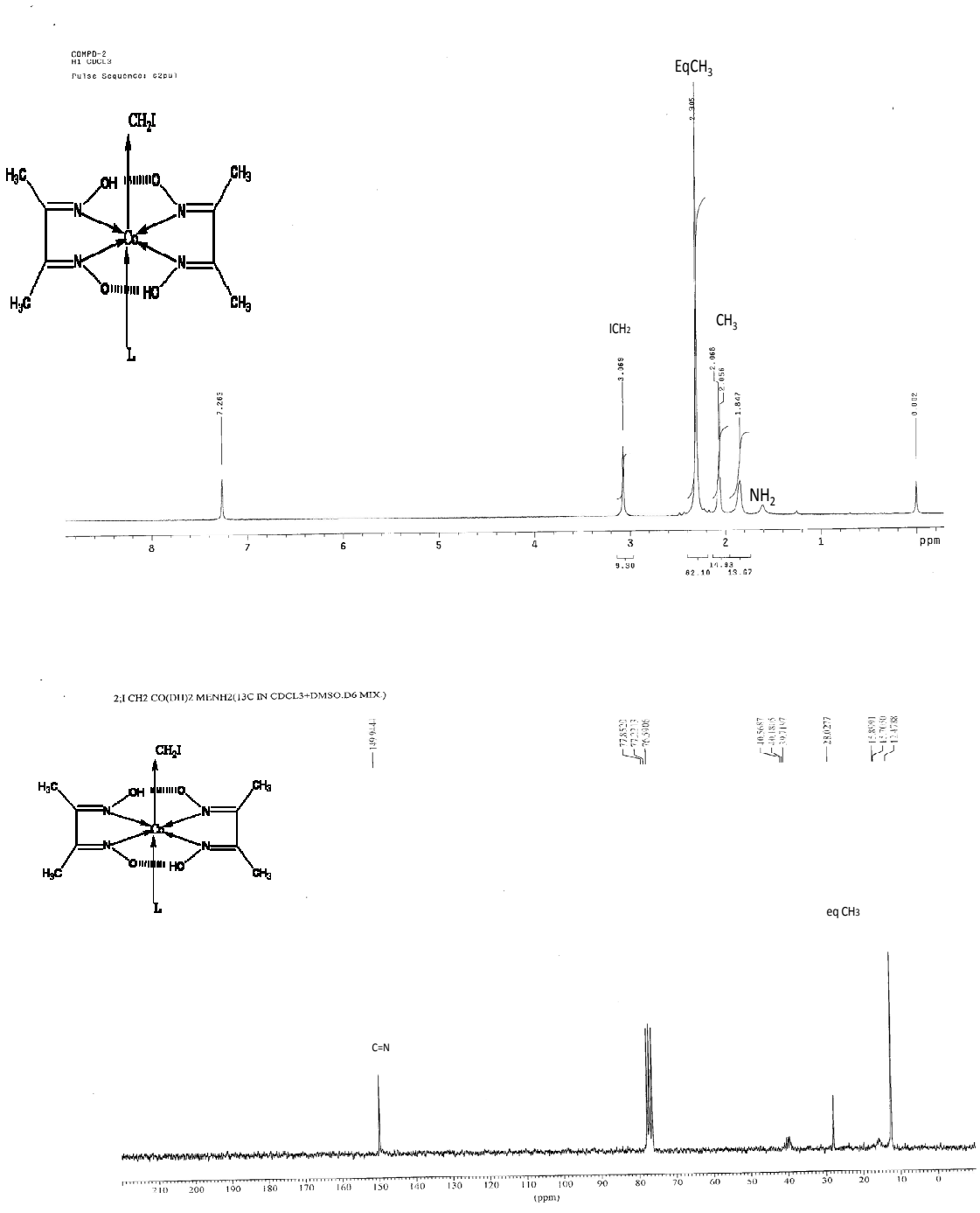


Fig 2 ^{1}H and ^{13}C-NMR spectra of $[ICH_2Co(DH)_2MA]$

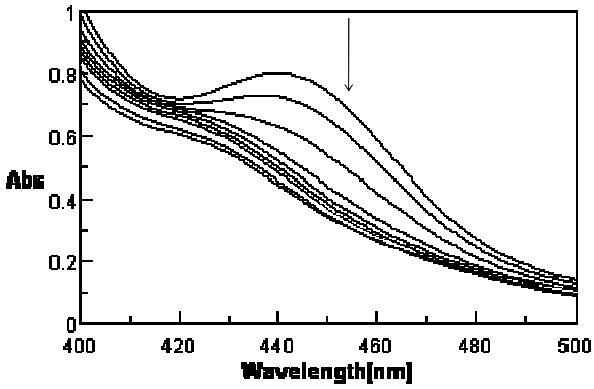


Fig 3 UV/VIS Scan: Binding of the $[ICH_2Co(DH)_2OH_2]$ complex with varying concentrations of Methylamine at pH 9.5 and 25°C.

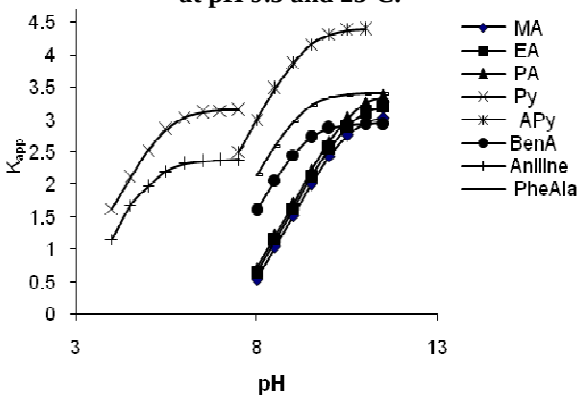


Fig 4 Dependence of the formation constants, log K_{app} on the pH for the axial ligation of the $CH_2Co(DH)_2OH_2]$ complex by different ligands at 25°C.

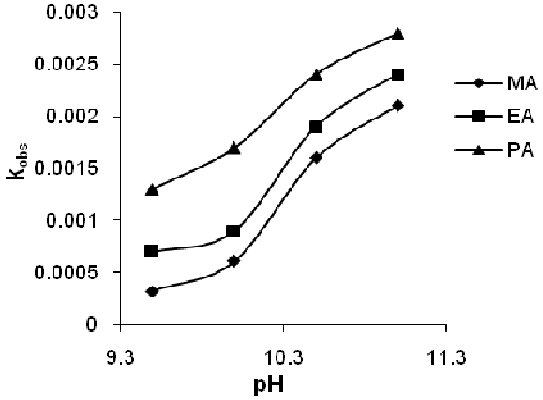


Fig 5 Dependence of the rate constants k_{obs} on the pH for the axial ligation of $[ICH_2Co(DH)_2OH_2]$ complex by MA, ET and PA

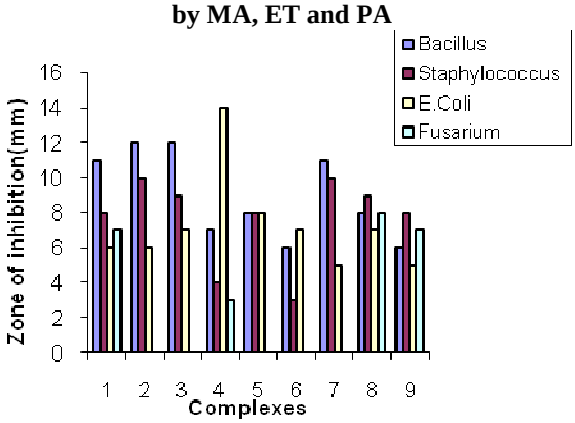


Fig 6 Bar graph showing the relative activity of the complexes

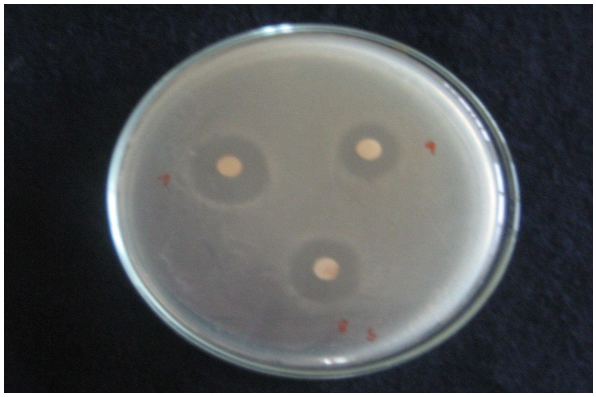


Fig 7 Zone of inhibition of Co complexes with Staphylococcus aureus.

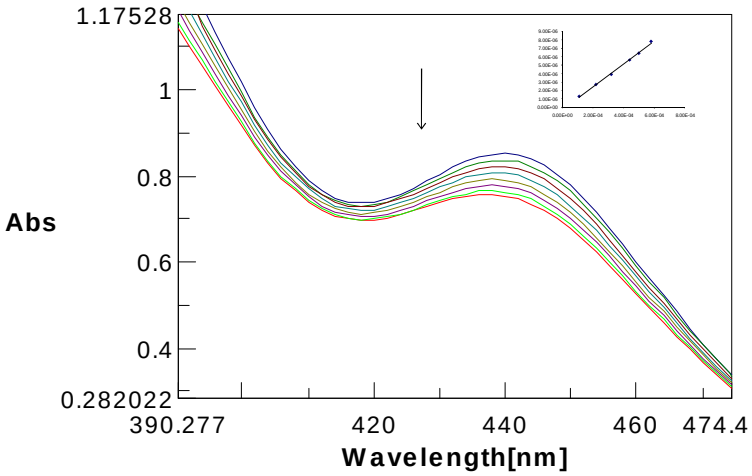


Fig 8 Absorption spectra of [$ICH_2Co(DH)_2OH_2$] complex in Tris-HCl buffer at 25^oC in the presenceof increasing amount of CT-DNA. Arrow indicate the change in absorbance upon increasing the DNA concentration.

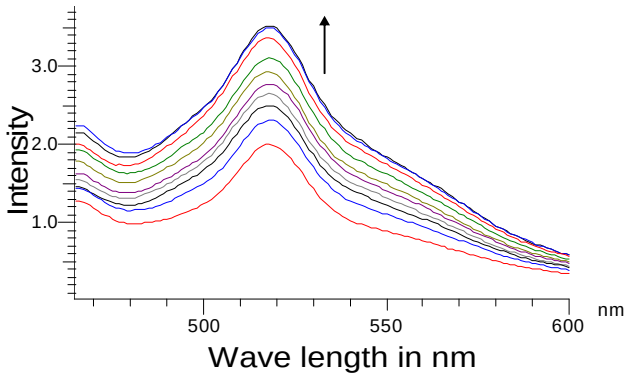


Fig 9 Emission spectra of of [$ICH_2Co(DH)_2OH_2$] in BPE buffer (pH7) in the absence and presence of CT DNA. The arrow shows the intensity change upon increasing DNA concentrations.

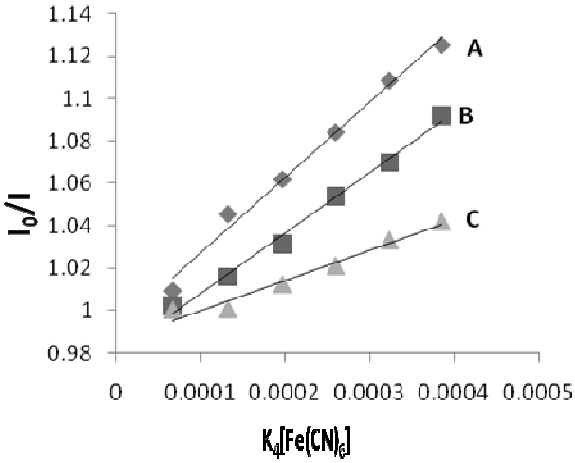


Fig 10 Quenching of [ICH_2 $Co(DH)_2$] With Potassium Ferrocyanide A. Without DNA B. With DNA. C. With Excess of DNA.

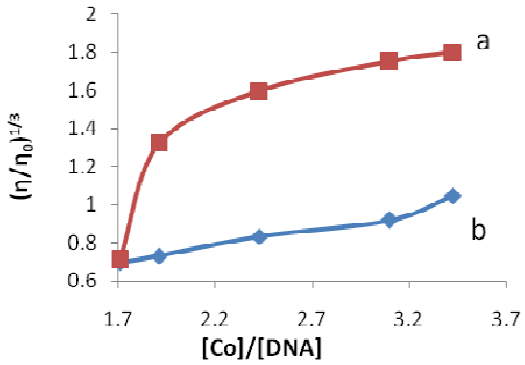


Fig 11 Effect of increasing amount of Ethidium bromide (a) and Iodomethyl aquo cobaloxime (b) on the relative viscosities of CT DNA at 25± 0.1°C.

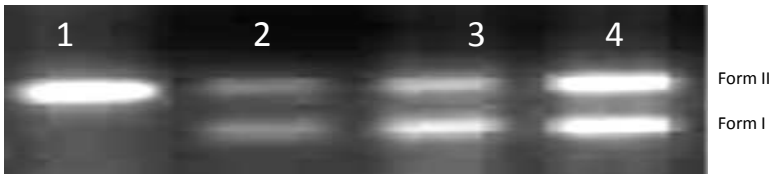


Fig 12 Photoactivated cleavage of pBR 322 DNA. Lane I Control plasmid DNA(100µM) (untreated pBR-322), Lanes 2-4 , addition of complex in amounts 5,10,15 µl. of 0.01M)

CONCLUSION

Iodomethyl(aquo)cobaloxime reacts with various N-donor ligands and forms stable complexes whose stability has been explained on the basis of basicity of the ligand. Py and 4-Amino Py bind to Co(III) through the N of the ring and other Primary amines through N of the NH_2 group.. The reported complexes possess a broad range antibiotic activity and show promise for the development of new antibiotics.

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