Research Article

Studies and Characterization of Macrocycle Metal Chelates of Trivalent and Their Antimicrobial Activities

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ABSTRACT
The synthesis, physico-chemical investigation and biological studies of some chromium, managanese, iron complexes of tetraaza Schiff base macrocycles are described. The ligands were obtained by condensation of *ortho*phthalaldehyde with different diamines in methonal. The metal complexes of Mn (III), Cr(III), Fe(III), were synthesized and isolated as solid products and characterized by analytical means as well as by spectral techniques such as FT-IR, Mass and UV-Vis spectrometry. These complexes were also tested for their *in vitro* antimicrobial activities against some bacterial strains to assess their inhibiting potential and the activities shown by these complexes were compared with standard drugs.

Keywords: Mn(III), Cr(III), Fe(III), complexes, Macrocyclic Schiff bases, Antibacterial studies.

INTRODUCTION

Macrocyclic ligands are polydentate ligands with their donor atoms either incorporated into or attached to a cyclic backbone.[1,2] Macrocyclic ligands are considerably attractive in the quest for new chemistry,because they offer a wide variety of donor atoms, ionic charges,c oordination numbers,a nd geometry of the resultant complexes.[3,4] The understanding of the metal ion chemistry of macrocyclic ligands has important implications for a range of chemical and biochemical applications. Many metal complexes of naturally occurring porphyrins,co rrins,a nd phthalocyanines have been investigated because of their potential as dyestuffs or pigments.[5,6] The macrocyclic ligand complexes are involved in a number of fundamental biological and catalytic systems.[7,8]The importance of such complexes, for example, to the mechanism of photosynthesis,[9] to the transport of oxygen in mammalian and other respiratory systems,a nd to potency towards DNA binders with a high potential in anti-tumor therapy,ha s provided a motivation for investigation of the metal ion chemistry of these systems,as well as of cyclic ligand systems.[10,11]

METRIAL AND METHOD

EXPERIMENTAL

All chemicals and solvents used were of reagent grade 2,6-diacetylpyridine was purchased from Aldrich, U.S.A. and diacetyl from Redial, West Germany.

SYNTHESIS AND ISOLATION OF COMPLEXES

(1) *Chromium and Iron chelates*

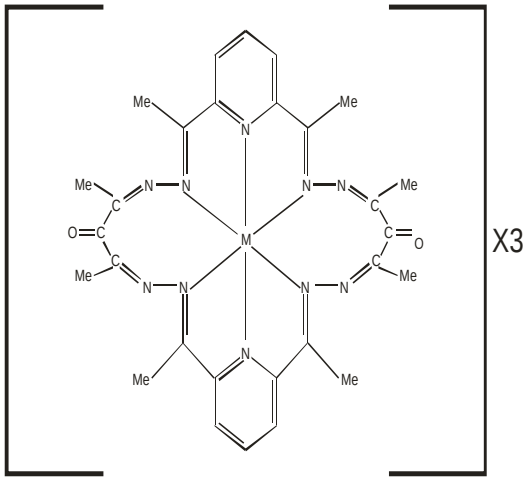
100ml methanolic solution of 3.0g (0.02 mole) 2,6 diacetylpyridine and 2.2g (0.02 mole) dicacetylidhydrazide were mixed and heat under reflux for an hour. To this refluxing mixture metal salt (0.01 mole) dissolved in minimum quantity of methanol was added followed by 2-3 drops of methanol KOH and refluxing continued for eight to ten hours. The resulted solution was concentrated and kept in desiccators for ten to twelve days. The dark greenish (chromium) and reddish (iron) crystals separated were filtered off, and washed and recrystallised from methanol and dried at 110^0.

(II) ***Manganese and Cobalt complexes***

Manganese (III) acetate was prepared by the standard method (42). Diacetylidhydrazide 2.2g (0.02 mole) in 25 ml. methanol was added 50 ml. methanol solution of 2,6-diacetylpyriding 3.0g (0.02 mole) and heat under reflux for one hour. To this mixture 2.3g (0.01mole) of Mn (III) acetate dissolved in 3oml. methanol was added, followed by 2&3 drops of glacial acetic acid and refluxing continued for eight to ten hours. The solution was concentrated to half of its volume and kept in desiccator for three days. The dark coloured crystals separated were recrystallised from methanol and dried. Yield ~ 35%.

Nitrato complexes were synthesised from metal nitrates, bromo and thiocyanato complexes were prepared by adding potassium bromide and ammonia thicyanate, respectively to methanolic solution of metal chloride and filtering off precipitate of KC1 and NH_4C1.

The complexes are soluble in common organic solvents, insoluble in benzene and carbon tertrachloride. The complexes are stable upto 350^0.



RESULT AND DISCUSION

[A] PHYSCIAL MEASUREMENTS

(i) ***Magnetic measurements***

The magnetic measurements were made using ' Princeton Applied Research' vibrating sample Magnetometer, model 155 incorporation digital read out. The electromagnet of the magnetometer was fed from a polytronic constant current regulator, type CP-200. The current was so adjusted as to produce a magnetic field of 5000 gauss. The instrument was calibrated using a standard nickel pellet and cross checked against $H_gCo(CNS)_4$. These measurements were carried out at University Service and Instrumentation Centre, University of Roorkee, Roorkee.

(ii) ***Infrared spectral measurements.***

Infrared spectra of the complexes in the conventional region (4000-250 cm^{-1}) were recorded on a Beckman IR-12 spectrophotometer in potassium bromide pellets. Far infrared spectra (650-200 cm^{-1}) were recorded in nujol mull on a Beckman IR-12 spectrophotometer. The measurements were carried out in Department of Chemistry, University of Roorkee and Regional Sophisticated Instrumentation Centre, Indian Institute of Technology, Madras.

(iii) ***Electronic spectral studies.***

The electronic spectra of the complexes at room temperature were recorded in nujol mull or dimethyl form amide on Carl Zeiss Specord UV-vis spectrophotometer. These measurements were carried out in the Department of Chemistry, University of Roorkee, Roorkee.

(iv) ***Conductance measurements***

The conductance of complexes in conductivity water at room temperature was measured by Toshniwal type CL01/01 conductivity bridge using dip type cell. The conductance values for different electrolytes in various solvents are given in Table-6.

(v) ***P^H measurements***

The P^H of the solutions was measured at room temperature by Elico (LI-10) P^H meter, using combined electrodes.

(vi) *Chemical analysis and estimation of metal contents*

The analysis of C, H and N were carried out by Micro analytical techniques. Halidas were determined by Volhard's method.

The metal contents in all the complexes were estimated as follows:

A known quantity of the complex was heated with a very small amount of concentrated sulphuric acid and then repeatedly with concentrated nitric acid until all the organic mater was decomposed. The residue was dissolved in doubly distilled water or sometimes dilute hydrochloric acid was added to it and made upto known volume, from which the metals were estimated by standard literature methods (248, 249, 250). Mostly 0.1M EDTA was added to determine the metal contents volumetrically using Erichrome Black T. sulphosalicylic acid or murexide as indicators.

(vii) *Chemical and solvents*

Analytical grade samples of organic compounds, solvents and metal salts have been used through out the investigations.

[B] METHODS OF CALCULATION

(i) *Magnetic Susceptibility*

The value of effective magnetic moment was calculated using the relation.

$$\mu_{eff.} = 2.84 \sqrt{\frac{RMT}{}} \text{ BM}$$

Where,

R= Reading displacement on the magnetometer

M= Molecular weight of the complexes.

T= Absolute temperature at which measurement is carried out.

H= Magnetic field applied (in gauss)

W= Weight of the complex sample used for magnetic measurement (in grams) and where

X_M=corrected molar magnetic susceptibility at a given temperature T.

(ii) *Ligand field parameters*

The method and relevant references for the calculation of various ligand parameters, viz., Ds, Dt, Dq^{xy}, Dq^{Z} and parameters of Normalised Spherical Harmonic (NSH) Hamiltonian Theory viz, DS, DT, DQ, DQ^{L} and DQ^{Z} have been dealt with in the preceding chapters at appropriate places.

Table 1: Analytical data of divalent

Complexes	Colour	Found % Calcd. (%)						
		M	C	H	N	X	Δ_M Mhos Cm^{-2} Mol^{-1}	Mol. Wt.
1	2	3	4	5	6	7	8	9
[Mn ($C_{26}H_{30}N_{10}$)] Cl_2	Brown	9.47 (9.04)	51.63 (51.31)	4.44 (4.93)	23.18 (23.02)	11.57 (11.67)	133	(608)
[Mn ($C_{26}H_{30}N_{10}$)] Br_2	Light Brown	7.98 (7.89)	44.51 (44.76)	4.42 (4.30)	- (20.08)	22.67 (22.95)	139	(697)
[Mn ($C_{26}H_{30}N_{10}$)] $(NO_3)_2$	Dark Brown	8.02 (8.32)	47.71 (44.76)	4.38 (4.53)	25.59 (25.43)	18.17 (18.75)	130	(661)
[Mn($C_{26}H_{30}N_{10}$)] $(NCS)_2$	Brown	8.13 (8.42)	51.87 (51.45)	4.43 (4.59)	25.88 (25.72)	- (17.76)	144	(653)
[Fe ($C_{26}H_{30}N_{10}$)] Cl_2	Brownish Red	9.81 (9.19)	51.56 (51.23)	4.52 (4.92)	22.66 (22.98)	11.76 (11.65)	141	(609)
[Fe ($C_{26}H_{30}N_{10}$)] Br_2	Orange Red	8.23 (8.02)	44.74 (44.69)	4.53 (4.29)	20.56 (20.05)	22.84 (22.92)	154	(698)
[Fe ($C_{26}H_{30}N_{10}$)] $(NO_3)_2$	Brown	8.52 (8.45)	47.61 (47.12)	4.49 (4.53)	- (25.37)	18.81 (18.37)	164	(662)
[Fe($C_{26}H_{30}N_{10}$)] $(NCS)_2$	Brownish Red	8.15 (8.56)	51.41 (51.37)	4.33 (4.58)	25.89 (25.68)	- (17.73)	161	(654)
[Co ($C_{26}H_{30}N_{10}$)] Cl_2	Reddish Pink	9.18 (9.64)	50.67 (50.98)	4.53 (4.90)	22.64 (22.87)	11.87 (11.60)	151	(612)
[Co ($C_{26}H_{30}N_{10}$)] Br_2	Brownish Yellow	8.00 (8.41)	44.99 (44.50)	4.74 (4.27)	- (19.97)	22.66 (22.82)	159	(701)
[Co ($C_{26}H_{30}N_{10}$)] $(NO_3)_2$	Brown	8.63 (8.87)	46.61 (46.91)	4.81 (54.1)	25.52 (25.26)	18.82 (18.64))	155	(665)
[Co($C_{26}H_{30}N_{10}$)] $(NCS)_2$	Brownish Red	8.80 (8.98)	51.00 (51.14)	4.81 (4.56)	25.92 (25.57)	- (17.65))	162	(657)

[Ni ($C_{26}H_{30}N_{10}$)] Cl_2	Dark Green	9.53 (9.64)	- (50.98)	- (4.90)	22.26 (22.87)	11.42 (11.60)	141	(612)
[Ni ($C_{26}H_{30}N_{10}$)] Br_2	Green	8.69 (8.41)	44.44 (44.50)	4.58 (4.27)	19.87 (19.97)	22.79 (22.82)	148	(701)
[Ni ($C_{26}H_{30}N_{10}$)] $(NO_3)_2$	Blackish Green	8.15 (8.87)	46.19 (46.91)	4.59 (4.51)	- (25.26)	18.04 (18.64)	151	(665)
[Fi($C_{26}H_{30}N_{10}$)] $(NCS)_2$	Dark Green	8.51 (8.98)	51.55 (51.14)	4.13 (4.56)	25.47 (25.57)	- (17.65)	149	(657)
[Cu ($C_{26}H_{30}N_{10}$)] Cl_2	Blackish Brown	10.10 (10.30)	50.42 (50.60)	4.13 (4.86)	- (22.70)	11.87 (11.57)	136	(616.5)
[Cu ($C_{26}H_{30}N_{10}$)] Br_2	Dark Brown	9.27 (9.00)	44.63 (44.22)	4.13 (4.25)	19.62 (19.84)	22.70 (22.67)	142	(705.5)
[Cu ($C_{26}H_{30}N_{10}$)] $(NO_3)_2$	Dark Brown	9.59 (9.48)	46.09 (46.60)	4.80 (4.48)	- (25.09)	- (18.52))	139	(669.5)
[Cu($C_{26}H_{30}N_{10}$)] $(NCS)_2$	Blackish Brown	9.20 (9.59)	50.67 (50.79)	4.33 (4.53)	25.58 (25.39))	- (17.53)	143	(661.5)

PHARMACOLOGY

In Vitro Antibacterial Activity

Some of the synthesized macrocyclic complexes were tested for their in vitro antibacterial activity against some bacterial strains using spot-on-lawn on Muller Hinton Agar by following the reported method [4]. Four test pathogenic bacterial strains viz *Bacillus cereus* (MTCC 1272), *Salmonella typhi* (MTCC 733), *Escherichia coli* (MTCC 739), and *Staphylococcus aureus* (MTCC 1144) were considered for determination of Minimum Inhibitory Concentration (MIC) of selected complexes.

Culture Conditions

The test pathogens were subcultured aerobically using Brain Heart Infusion Agar (HiMedia, Mumbai, India) at 37°C/24 hours. Working cultures were stored at 4°C in Brain Heart Infusion (BHI) broth (HiMedia, Mumbai, India), while stock cultures were maintained at −70°C in BHI broth containing 15% (v/v) glycerol (Qualigens, Mumbai, India). Organisms were grown overnight in 10 mL BHI broth, centrifuged at 5000 g for 10 minutes, and the pellet was suspended in 10 mL of phosphate buffer saline (PBS, pH 7.2). Optical density at 545 nm (OD-545) was adjusted to obtain 10^8 cfu/mL followed by plating serial dilution onto plate count agar (HiMedia, Mumbai, India).

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation. Antimicrobial activity of the compounds was evaluated using spot-on-lawn on Muller Hinton Agar (MHA, HiMedia, Mumbai, India). Soft agar was prepared by adding 0.75% agar in Muller Hinton Broth (HiMedia, Mumbai, India). Soft agar was inoculated with 1% of 10^8 Cfu/mL of the test pathogen and 10 mL was overlaid on MHA. From 1000X solution of compound (1 mg/mL of DMSO) 1, 2, 4, 8, 16, 32, 64, and 128X solutions were prepared. Dilutions of standard antibiotics (Linezolid and Cefaclor) were also prepared in the same manner. 5 µL of the appropriate dilution was spotted on the soft agar and incubated at 37°C for 24 hours. Zone of inhibition of compounds was considered after subtraction of inhibition zone of DMSO. Negative control (with no compound) was also observed.

BIOLOGICAL ASSAY

The minimum inhibitory concentration (MIC) shown by the complexes against these bacterial strains was compared with MIC shown by standard antibiotics *Linezolid* and *Cefaclor* (Table 2). Complex 1 showed an MIC of 8 µg/mL against bacterial strain *Escherichia coli* (MTCC 739), which is equal to MIC shown by standard antibiotic *Cefaclor* against the same bacterial strain. Complex 3 registered an MIC of 8 µg/mL, against bacterial strain *Bacillus cereus* (MTCC 1272), which is equal to MIC shown by standard antibiotic *Cefaclor* against the

same bacterial strain. Further complexes 3 and 7 showed a minimum inhibitory concentration of 32 µg/mL against bacterial strain *Salmonella typhi* (MTCC 733), which is equal to MIC shown by standard antibiotic *Linezolid* against the same bacterial strain. The MIC of complex 4 against *Escherichia coli* (MTCC 739) was found to be 16 µg/ml, which is equal to the MIC shown by standard antibiotic *Linezolid* against the same bacterial strain. Complex 6 registered an MIC of 4 µg/mL against bacterial strain *Staphylococcus aureus* (MTCC 1144) which is equal to MIC shown by standard antibiotic *Linezolid* against the same bacterial strain. Among the series under test for determination of MIC, complexes 1 and 3 were found most potent as compared to other complexes. However, complexes 2 and 5 showed poor antibacterial activity or no activity against all bacterial strains among the whole series. (Table 2).

Table 2: Minimum Inhibitory Concentration (MIC) shown by complexes against test bacteria by using agar dilution assay. (-) No activity, a: *Bacillus cereus* (MTCC 1272); b: *Staphylococcus aureus* (MTCC 1144); c: *Escherichia coli* (MTCC 739); d: *Salmonella typhi* (MTCC 733); Cefaclor and Linezolid are standard antibiotics.

Serial no.	Complexes	MIC (µg/mL)			
		a	b	c	D
(1)	$[Cr(C_6H_8N_4O_2)Cl]Cl_2$	32	32	8	64
(2)	$[Cr(C_6H_8N_4O_2)(NO_3)](NO_3)_2$	—	—	64	>128
(3)	$[Cr(C_6H_8N_4O_2)(OAc)](OAc)_2$	8	64	64	32
(4)	$[Mn(C_6H_8N_4O_2)(OAc)](OAc)_2$	64	>128	16	64
(5)	$[Fe(C_6H_8N_4O_2)Cl]Cl_2$	>128	64	64	>128
(6)	$[Fe(C_6H_8N_4O_2)(NO_3)](NO_3)_2$	32	4	64	>128
(7)	$[Fe(C_6H_8N_4O_2)(OAc)](OAc)_2$	>128	64	>128	32
	Cefaclor	8	2	8	16
	Linezolid	4	4	16	32

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