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Antibacterial active tetraaza macrocyclic complexes of Chromium (III) with their spectroscopic approach

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Abstract : A novel macrocyclic Cr(III) compounds have been synthesized by treating tetraaza macrocycles with chromium chloride in methanol. These complexes were characterized with the help of elemental analyses, conductance measurements, magnetic susceptibility measurements, electronic, infrared, thermal and mass spectral studies. On the basis of these studies, a five coordinate square pyramidal geometry for all of these complexes have been proposed. The biological activities of these complexes have been tested *in vitro* to evaluate their activity against Gram +ve and Gram –ve bacteria and were found to be more active than streptomycin and ampicillin.

Keywords: Synthesis; Cr(III) complexes; N_4 macrocyclic Schiff bases; Antibacterial studies

1. Introduction

The chemistry of macrcocyles and its complexes have attracted the interest of both inorganic and bioinorganic chemists in recent years[1-5] due to their unique structural properties[6] and biological activities[7] .The field of macrocyclic chemistry of metals is developing very rapidly because of its importance in the area of coordination chemistry[8] . Macrocyclic compounds and their derivatives are interesting ligand-system because they are good hosts for metal anions, neutral molecules and organic cation guests[9] . The metal-ion and host guest chemistry of macrocyclic compounds are very useful in fundamental studies viz. in phase transfer catalysis and biological studies[10] . The family of complexes with aza-macrocyclic ligands has remained a focus of scientific attention for many decades[11] . Furthermore, transition metal macrocyclic complexes have received a great attention because of their biological activities, including antiviral, anticarcinogenic[12] , antifertile[13] , antibacteria[1,3] and antifungal[14] .Literature survey reveals that most of the existing orthophthalaldehyde (OPA) based macrocyclic metal compounds were synthesized by template method involving the direct treatment of OPA with diamine in the presence of metal salts[15-18] . However, to the best of our knowledge, there are no reports on the synthesis of macrocyclic Cr(III) compounds derived from o-phthalaldehyde. In view of these interesting aspects, we have synthesized and characterized the Cr(III) macrocyclic Schiff base complexes. The present paper deals with the striking structural features, synthesis, characterization and biological applications of these complexes.

2. Experimental

2.1. Materials and Methods

All chemicals used were of AR grade. Six macrocyclic ligands viz. 7,8,9,18,19,20-hexahydrodibenzo[*g*,*p*][1,2,4,5,10,11,13,14]-octaazacyclooctadecine-8,19-dione[HBOADO], 7,8,17,18-tetrahydrodibenzo[*f*,*n*][1,2,4,9,11,12]-hexaazacyclohexadecine-8,17-dione[TBACD], 3,4,5,6,7,8,9,10-octahydro-2,5,8,11-benzotetra azacyclotetradecine[OBACD], 7,8,9,18,19,20-hexahydrodibenzo[*g*,*p*][1,2,4,5,10,11,13,14]-octa azacyclooctadecine-8,19-dithione[HBOADT], 7,16-dihydrodibenzo[*e*,*l*][1,3,8,10]tetraazacyclo tetradecine-7,16-dithione[DBACDT] and 7,8,17,18-tetrahydrodibenzo[*f*,*n*][1,2,4,9,11,12]hexa azacyclohexadecine-8,17-dithione[TBAHD] were newly prepared and characterized. Chromium chloride was purchased from s.d.-fine. Organisms like Bacillus subtilis (MTCC–619), Staphylococcus aureus (MTCC–96), Escherichia coli (MTCC–722) and Klebsiella pneumonia (MTCC–109) from IMTECH, Chandigarh were used for antimicrobial studies.

2.2. Measurements

The melting points of all the macrocyclic ligands and macrocyclic metal compounds were obtained on a Buchi- 510 melting point apparatus. The percentages of

carbon, hydrogen, nitrogen in macrocyclic metal compounds were determined using a Perkin Elmer 2400 elemental analyzer. The IR spectra were recorded using KBr/CsBr pellet technique in a Perkin-Elmer 283 IR spectrophotometer. FAB mass spectrometer was used to obtain mass spectra. UV-Visible spectra were recorded with a Shimadzu UV-160A, a UV-Visible double beam spectrophotometer equipped with matched quartz cells of path length 1 cm. Conductance measurements were done on 10^{-3} M solution of compounds in DMSO at room temperature using Digisun Digital conductivity meter model DL-909. Gouy balance calibrated with $Hg[Co(NCS)_4]$ was used for the determination of magnetic susceptibilities of complexes in solid state at room temperature.

2.3. Synthesis of macrocyclic Cr(III) complexes

A methanolic solution (20 mL) of macrocyclic ligand (L) was mixed to the chromium chloride solution (20 mL, in methanol) in equimolar ratio, with constant stirring and continued for about 3-4 hours. The resulting solution was concentrated under reduced pressure and a few ml of diethylether was added to initiate the crystallization. The precipitate formed was separated by suction filtration, washed with methanol, diethylether, vacuum dried to get a crystalline compound and was recrystallized using dichloromethane and diethylether solvent mixture (Yield 70-75%).

2.4. Antimicrobial testing by agar diffusion:

Antimicrobial testing was done by cup plate method[19] . Sterile Petri dishes were taken to which 27 ml of molten agar is added and allowed to solidify and set for 1hr. Then 50 ml of the 24 hrs culture of a test organism was taken on to the agar plate and spread evenly with the sterile cotton swab. Six mm wide bores were made on the agar using a borer. The solutions of the macrocyclic metal compounds were added in to each of the bores in appropriately using a sterile tip with micropipette and labeled as Petri dishes. A similar plate was prepared by replacing macrocycle by Streptomycin sulphate. This was taken as a standard against bacteria. These dishes were then incubated at 37°C for 24 hr. The inhibition zone formed by the compounds against the particular test bacterial strain determined the antibacterial activities of the Cr(III) compounds. The mean value obtained for three individual replicates was used to calculate the zone of growth inhibition of each sample. The activities of compounds were interpreted either active or inactive. The minimum inhibitory concentration required was also found when a series of dilutions were tested.

2.5. Determination of minimum inhibitory concentration (MIC):

The minimum inhibitory concentration was determined by liquid dilution method[20] . Stock solutions of Cr(III) complexes with 2.5 µg/ml, 5 µg/ml, 10µg/ml, 20 µg/ml, 50µg/ml and 100 µg/ml concentrations were prepared with aqueous methanol solvent. The solutions of standard drugs like Streptomycin, Ampicillin and Rifampicin were also prepared in the same concentrations. Inoculum of the overnight culture was prepared. In a series of tubes 1 ml each of macrocyclic Cr(III) complex solution with different concentrations were taken and 0.2 ml of the inoculum was added to each tube. Further 3.8 ml of the sterile water was added to each of the test tubes. These test tubes were incubated for 24 hrs and observed for the presence of turbidity. The absorbance of the suspension of the inoculum was observed with spectrophotometer at 555 nm. This method was repeated by replacing Cr(III) complexes with drugs like Streptomycin, Ampicillin and Rifampicin for comparison.

3. Results and Discussion

In the present investigations, six new macrocyclic Cr(III) complexes were synthesized by treating chromium chloride with six N_4 donor macrocyclic ligands separately. These complexes are crystalline solids, non-hygroscopic, freely soluble in DMF or DMSO. The percentages of carbon, hydrogen and nitrogen were determined experimentally using CHN analyzer. The percentage of chromium in macrocyclic Cr(III) complexes was determined by using gravimetric procedure[21] . The tests for anions are positive before decomposing and after decomposing the chelates with concentrated HNO3 showing their presence outside as well as inside of coordination sphere. Several attempts failed to obtain a single crystal suitable for X-ray crystallography. However, the analytical, spectroscopic and magnetic data enable us to predict the possible structure of the synthesized complexes. The physical and analytical data (Table-1) for the newly synthesized macrocyclic Cr(III) complexes is in good agreement with the proposed molecular formulae viz. $[Cr(L)Cl]Cl_2$ (Where L= tetraaza macrocyclic ligand).

3.1. Infrared spectral data

T In the IR spectra of macrocyclic Cr(III) complexes, a medium intensity band due to $\upsilon_{C=N}$ was shifted towards lower side about 20-30 cm^{-1} compared to ligand spectra and appeared in the range of 1625-1600 cm^{-1}. This supports the fact that the ligands coordinate to the metal ions through the nitrogen of C=N group in all the complexes[22]. This fact is further supported by the appearance of a band in the region of 525-505 cm^{-1} assignable to υ_{M-N} vibration[15] . However, in the complex-**3** υ_{NH} band was observed at 3314 cm^{-1}. This band was shifted towards lower side about 30 cm^{-1} compared to the ligand spectrum indicates the coordination to the metal through nitrogen of NH group [23]. The bands present in the range 300-320 cm-1 may be assigned due to ν(M─Cl) vibration[2-5] .The characteristic bands due to the $\upsilon_{C=O}/\upsilon_{C=S}$ in the spectra of respective macrocyclic Cr(III) compounds were remain almost unshifted[24]. All the characteristic bands due to the aromatic rings were also

present in the expected regions[2] in all the macrocyclic Cr(III) compounds (Table-2).

3.2. Mass spectral analysis

The FAB mass spectra of Cr(III) macrocyclic complexes have been recorded. All the spectra exhibit parent peaks due to molecular ions (M+). The proposed molecular formula of theses complexes were confirmed by comparing their molecular formula weights with m/z values. This data is in good agreement with the respective molecular formulae. The isotope pattern calculations that represent in web-based and windows-based application to view the mass spectrum. The existing systems face a number of problems, such as slow performance of viewing the output, incomplete graph interface which not user friendly and low capability in output the values of large molecules such as inorganic complexes. Thus, to overcome this problem isotope pattern calculations are developed. This helps to calculate the exact molecular formula and molecular weights of the complexes. The isotope patterns of all the macrocyclic Cr(III) complexes are given Table 3.

3.3. Electronic spectral analysis

The electronic spectra of chromium(III) complexes show bands at ~9020-9350 cm^{-1}, 13040-13350 cm^{-1}, 17450-18340 cm^{-1}, 27440-27820 cm^{-1} and 34830 cm^{-1}. The spectral bands are consistent with that of five coordinated Cr(III) complexes, whose structure have been confirmed with the help of X-ray measurements[25] . On the basis of the analytical data, spectral studies and electrolytic nature of these complexes, a five coordinated square pyramidal geometry may be assigned for these complexes. Thus, assuming the symmetry C_4V for these complexes[26-27] the various spectral bands may be assigned as: $^4B_1 \rightarrow {}^4E^a$, $^4B_1 \rightarrow {}^4B_2$, $^4B_1 \rightarrow {}^4A_2$ and $^4B_1 \rightarrow {}^4E^b$.

3.4. Molar conductance, magnetic susceptibility and thermal studies

The molar conductance values for all the macrocyclic Cr(III) compounds (10^{-3} M) were determined in DMSO. These values were found between 150 and 175 $ohm^{-1}cm^2mol^{-1}$ indicating 1:2 electrolytic nature. The electrolytic nature of these compounds is due to the presence of two chloride ions outside the coordination sphere. The presence of chloride ions in these compounds was detected by the addition of silver nitrate reagent leading to the formation of white precipitate. Magnetic moments of chromium (III) complexes were found in the range of 4.15-4.52 B.M. at room temperature which is close to the predicted values for three unpaired electrons in the metal ion[28] . The thermal analysis data of Cr(III) complexes indicates that they are stable up to 240°C and hence exist in anhydrous state. The DTA curves show no endothermic peaks up to 240°C confirming the absence of lattice or coordinated water molecules in the complexes[29-31]. The sharp decomposition corresponding to the loss of organic moiety in complexes can be seen in the DTA curves which contained one sharp exothermic peak falling in the range of 240-285°C. The final product of decomposition of all the complexes above 630°C corresponds to metal oxide. Taking the loss of organic moiety as the decomposition temperature, the thermal stability of the Cr(III) complexes can be represented with respect to ligands as HBOADT > HBOADO > TBAHD > DBACDT > TBACD > OBACD. Though, it is difficult every time to explain the order of thermal stability in terms of the structure and the nature of the ligand, this order can be explained to some extent, on the basis of the steric factors such as bulkiness of the groups attached to the ligating groups, labile nature of ligand bonds and the number of chelate rings formed by each ligand [32 -34].

On the basis of analytical and spectral data, a five coordinated square pyramidal geometry (Scheme 1) have been tentatively proposed for all of these complexes.

3.5. Antibacterial activity

Antibacterial activities of macrocyclic Cr(III) complexes were studied along with metal free ligands and three existing antibacterial drugs *viz.* streptomycin, ampicillin and rifampicin. Preliminary screening for all the complexes was performed at fixed concentrations of 1000 µg/ml. Based on the obtained values of relative zone inhibition[35] only three ligands **HBOACTD, DBACDT, TBAHD** and their complexes viz. **4, 5** and **6** were found to be very effective. The macrocyclic Cr(II) complexes showed more increased activity than the corresponding ligands. In addition, the above three complexes were found to be effective at different dilutions based on the activity. The minimum inhibitory concentration [36] of these three complexes was also verified by the liquid dilution method in which the effectiveness was observed at lower concentrations. The activity of these three complexes against gram +ve (*Bacillus subtilis, Staphylococcus aureus*) and gram–ve (*Escherichia coli, Klebsiella pneumonia*) bacteria were compared with the activity of existing antibacterial drugs like streptomycin, ampicillin and rifampicin and these complexes were found be very active than streptomycin, ampicillin (Table-4). The antimicrobial activity of macrocyclic ligands and their Cr(III) complexes is due to the presence of C=N group (Schiff base), which were obtained by the condensation of o-phthalaldehyde with diamines. In addition, it has been suggested that chelation reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with donor group within the whole chelate ring system[37,38]. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favours its permeation through the lipoid layer of the membrane thus causing the metal complex to cross the bacterial membrane more effectively thus increasing the activity of the complexes. Besides from this many other factors such as solubility, dipole moment, conductivity influenced by metal ion may be possible reasons for remarkable antibacterial activities of these complexes[39] . Especially, three ligands

HBOACTD, DBACDT, TBAHD and their complexes viz. **4, 5** and **6** complexes are more active may be due to the presence of thio group in corresponding macrocyclic ligands and their complexes (Figure 1).

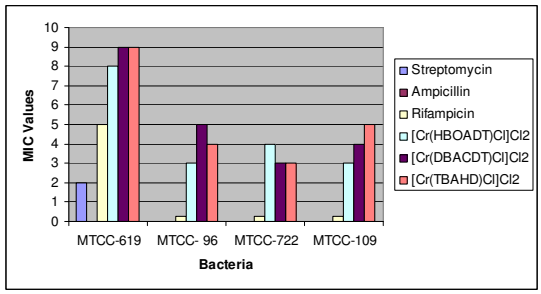
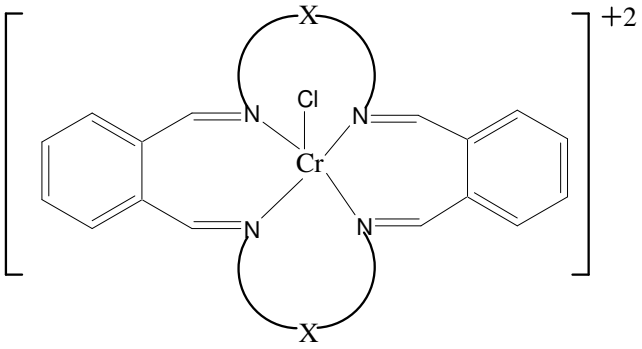


Figure 1. Comparison of MIC values (in µg/ml) of macrocyclic chromium complexes and standard drugs against different bacteria.

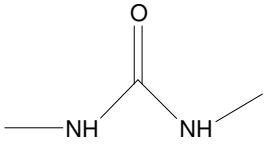
4. Conclusions

Six macrocyclic Cr(III) compounds were synthesized by treating chromium chloride with six tetraaza macrocyclic ligands separately. By the elemental, spectral analysis, five coordinated square pyramidal geometry has been assigned to these complexes. Since, two chloride ions are present outside the coordination sphere, these compounds have electrolytic nature. The macrocyclic Cr(III) compounds were found to have significant antibacterial activity. Out of six, three complexes viz. [Cr(HBOADT)Cl]Cl2, [Cr(DBACDT)Cl]Cl2 and [Cr(TBAHD)Cl]Cl2 were found to be very active than the streptomycin and ampicillin due to the presence of thio group in corresponding macrocyclic ligands.

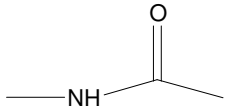


Where

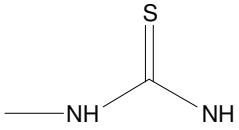
for HBOADO X=



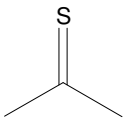
for TBACD X=



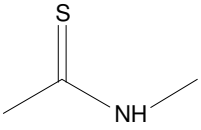
for HBOADT X=



for DBACDT X=



for TBAHD X=



Scheme 1: Representative structures of macrocyclic Cr(III) complexes

Table 1 : Physical, analytical and electronic spectral data of macrocyclic Cr(III) complexes.

Comp. No.	Cr(III) compound/ Molecular formula	Λ_M	Analyses (%) Found (Calculated)			
			C	H	N	Cr
1.	[Cr(HBOADO)Cl]Cl2$C_{18}H_{16}Cl_3N_8O_2Cr$	175	40.50 (40.43)	3.03 (3.02)	20.89 (20.96)	9.70 (9.72)
2.	[Cr(TBACD)Cl]Cl2 $C_{18}H_{14}Cl_3N_6O_2Cr$	164	42.73 (42.84)	2.79 (2.80)	16.63 (16.65)	10.36 (10.30)
3.	[Cr(OBACD)Cl]Cl2 $C_{14}H_{20}Cl_3N_4Cr$	150	41.79 (41.76)	5.00 (5.01)	13.89 (13.91)	12.90 (12.91)
4.	[Cr(HBOADT)Cl]Cl2$C_{18}H_{16}Cl_3N_8S_2Cr$	170	38.12 (38.14)	2.85 (2.84)	19.80 (19.77)	9.14 (9.17)
5.	[Cr(DBACDT)Cl]Cl2$C_{18}H_{12}Cl_3N_4S_2Cr$	165	42.68 (42.66)	2.38 (2.39)	11.04 (11.06)	10.28 (10.26)
6.	[Cr(TBAHD)Cl]Cl2 $C_{18}H_{14}Cl_3N_6S_2Cr$	158	40.22 (40.27)	2.60 (2.63)	15.62 (15.65)	9.75 (9.69)

Table 2 : Infrared spectral data of macrocyclic Cr(III) complexes.

Comp. No.	Cr(III) compound	Selected IR bands (cm^{-1})			
		$\upsilon_{C=N}$	υ_{NH}	Y_{Cr-N}	Anion peaks
1.	[Cr(HBOADO)Cl]Cl2	1580	3330	515	320
2.	[Cr(TBACD)Cl]Cl2	1590	3320	525	305
3.	[Cr(OBACD)Cl]Cl2	1595	3310	520	315
4.	[Cr(HBOADT)Cl]Cl2	1600	3340	505	300
5.	[Cr(DBACDT)Cl]Cl2	1586	-	510	302
6.	[Cr(TBAHD)Cl]Cl2	1582	3370	520	305

Table 3: Isotope pattern of macrocyclic Cr(III) compounds

Comp. No.	Molecular formula	Isotope pattern macrocyclic Ru(II) complexes
1.	[Cr(HBOADO)Cl]	533(99.0%), 534(33.5%), 535(100%,M^+), 536(32.8%),
	$Cl2C_{18}H_{16}Cl_3N_8O_2Cr$	537(37.0%), 538(11.5%).
2.	[Cr(TBACD)Cl]Cl2	503(99.2%), 504(32.0%), 505(100%,M^+), 506(31.0%),
	$C_{18}H_{14}Cl_3N_6O_2Cr$	507(36.0%), 508(10.8%)
3.	[Cr(OBACD)Cl]Cl2	401(100 %,M^+), 402(27.6%), 403(98%,), 404(25.9%),
	$C_{14}H_{20}Cl_3N_4Cr$	405(35.0%), 406(8.8%)
4.	[Cr(HBOADT)Cl]Cl2	565(91.0%), 566(31.5%), 567(100%,M^+), 568(34.0%),
	$C_{18}H_{16}Cl_3N_8S_2Cr$	569(42.0%), 570(13.5%), 571(8.5%).
5.	[Cr(DBACDT)Cl]Cl2	505(92.0%), 506(32.5%), 507(100%,M^+), 508(32.5%),
	$C_{18}H_{12}Cl_3N_4S_2Cr$	509(42.0%), 510(12.5%), 511(8.0%).
6.	[Cr(TBAHD)Cl]Cl2	535(91.6%), 536(31.3%), 537(100%,M^+), 538(33.5%),
	$C_{18}H_{14}Cl_3N_6S_2Cr$	539(41.5%), 540(13.2%), 541(8.3%).

Table 4: Minimum inhibitory concentrations of the Cr(III) complexes and existing antibiotics.

S. No.	Cr(III) compound	Range of concentration (0.25-10 µg/ml)			
		MTCC-619	MTCC- 96	MTCC-722	MTCC-109
1.	Streptomycin	02	-	-	-
2.	Ampicillin	-	-	-	-
3.	Rifampicin	05	0.25	0.25	0.25
4.	[Cr(HBOADT)Cl]Cl2	08	03	04	03
5.	[Cr(DBACDT)Cl]Cl2	09	05	03	04
6.	[Cr(TBAHD)Cl]Cl2	09	04	03	05

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