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Template Synthesis, Spectroscopic Characterization and Biological Screening of Oxa-aza Macrocyclic Complexes of Mn (III)

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ABSTRACT

New $N_2O_3$ macrocyclic complexes of the type $[\text{MnL(CH}_3\text{COO})_2(CH}_3\text{COO}]$ (where $L=17$-membered trioxa-diazamacrocycle) have been synthesized by template (1+1) cyclocondensation of 4,7,10-trioxatridecane-1,13-diamine and aryl $\alpha$-diketones such as 1-phenyl-1,2-propanedione, benzil and 4,4'-dimethylbenzil in the presence of trivalent metal salt. The complexes have been characterized with the help of elemental analyses, molar conductance, magnetic measurement, electronic, IR, H$^1$NMR and mass spectral studies. On the basis of above studies, a six coordinate distorted octahedral geometry has been proposed for all these complexes. These macrocycles were also tested for their in vitro antimicrobial activities on some pathogenic bacteria strain Streptomyces griseus (gram-positive), Escherichia coli (gram-negative), Staphylococcus aureus (gram-positive), Pseudomonas aeruginosa (gram-negative) and antifungal activities against Trichoderma Reesei, Aspergillus niger, Penicillium funiculoseum and Fusarium oxysporium.

Key words: Trioxa-diazamacroycles, $H^1$NMR, IR, Mass Spectral Studies and Antimicrobial Activity.

INTRODUCTION

Coordination chemistry of macrocyclic complexes has been a fascinating area of research all over the world due to its importance in analytical, biomedical and environmental field (Firdaus et al. 2009; Ilhan et al. 2007; Chaudhary et al. 2003; Parker et al. 1996; Anupma et al. 2015; Guy et al. 1976). They are thermodynamically, kinetically more stable and more selective metal ion binders than their open chain analogues (Sharma et al. 2015; Tarafder et al. 2001; Chandra et al. 2001). Some macrocyclic derivatives are fundamental unit in
biological functions such as photosynthesis, transport of oxygen in mammalian (Agnus et al. 1984; Chandra et al. 2002; Luneau et al. 1988). They mimic important biological ligands in their structure and functions like metalloproteins, hemerythrin and enzymes (Rajni et al. 2013). Macrocyclic ligands containing oxygen-nitrogen donor atoms are important complexing agent for cations, anions and molecules (Khndar et al. 2007). Condensation reactions between carbonyl compounds and primary amines are the most common reactions in the synthesis of new macrocyclic complexes (Kumar et al. 2014; Ugras et al. 2006; Prasad et al. 2007). Macrocyclic compounds have been explored for their antibacterial (Deshmukh et al. 2010; Rosu et al. 2006), fungicidal (Tyagi et al. 2011), anticonvulsant (Bhasin et al. 2009) and catalytic activities. The available literature has also evidenced about their antioxidant and anti-HIV activities. A huge number of manganese macrocyclic complexes have been reported in literature. The interaction of Co(II), Ni(II), Zn(II) and Cd(II) with pyridine-derived ligand containing a $N_2O_2$-donor set, has been investigated (Valencia et al. 2000). Dioxatriaza-[22][DBF]$N_2O_3$ macrocyclic ligand containing a rigid dibenzofuran group (DBF), to metal cations and structural studies of their metal complexes have been carried out (Li et al. 2006). This 22-membered macrocyclic ligand synthesized by $[1+1]$ condensation of 4,6-dibenzo[d]furandicabaldehyde with 4,7,10-trioxadecane-1,13-diamine and with pyridine-derived ligand containing a $N_2O_2$-donor set, has been investigated (Valencia et al. 2000). Dioxatriaza-[22][DBF]$N_2O_3$ macrocyclic ligand containing a rigid dibenzofuran group (DBF), to metal cations and structural studies of their metal complexes have been carried out (Li et al. 2006). This 22-membered macrocyclic ligand synthesized by $[1+1]$ condensation of 4,6-dibenzo[d]furandicabaldehyde with 4,7,10-trioxadecane-1,13-diamine and aryl $\alpha$-diketones such as 1-phenyl-1,2-propanedione, benzil and 4,4'-dimethylbenzil in the presence of trivalent metal salts in butanolic medium. These complexes are characterized by molar conductance, magnetic measurement, electronic, $H^1$NMR, IR, mass spectral studies and in vitro antibacterial and antifungal activities.

**MATERIAL AND METHODS**

**Material**

All the chemicals used were of AR grade. 4,7,10-trioxadecane-1,13-diamine (Merck), 1-Phenyl-1,2-propanedione (Sigma-Aldrich), Benzil (Merck), 4,4'-Dimethylbenzil (Fluka) were used as supplied and $n$-butanol (Merck) was distilled before use. Mn(CH$_3$COO)$_3$.2H$_2$O was prepared from Mn(CH$_3$COO)$_2$.4H$_2$O (Aldrich) (Heiba et al. 1969).

**Analytical methods and measurements**

Manganese was determined volumetrically by EDTA using eriochrome black T as indicator. C, H, and N were carried out using an elemental analyzer FLASH 2000. Molar conductances were measured at room temperature in DMF by a Systronic Direct Reading Conductivity Meter-304 using a glass cell having cell constant 1.0 cm$^{-1}$. Magnetic measurements were carried out on Gouy balance calibrated with Hg[Co(NCS)$_4$]. The IR spectra were recorded as KBr pellets in the region 4000-400 cm$^{-1}$ on Shimadzu-8400 S FTIR spectrophotometer. $^1$H NMR spectra were recorded in DMSO-d$_6$ on JEOL FX 90 QFT NMR spectrometer at 90 MHz using TMS as a reference. Electronic spectra (DMSO) were recorded in the region 200-800 nm on a Hitachi U-2000 spectrophotometer. The mass spectra were recorded on WATERS, Q-TOF MICROMASS (LC-MS).
Pharmacology

Test microorganisms

Eight microbial strains (four bacterial and four fungi) were selected on the basis of their clinical importance in causing disease in humans. Clinical laboratory bacterial isolates of *Streptomyces griseus* (gram +ve, MTCC-706), *Escherichia coli* (gram -ve, MTCC-1652), *Staphylococcus aureus* (gram +ve, MTCC-737) and *Pseudomonas aeruginosa* (gram -ve, MTCC-1688) and fungal isolates of *Trichoderma Reesei* (MTCC-164), *Aspergillus niger* (MTCC-282), *Penicillium funiculosum* (MTCC-1013) and *Fusarium oxysporium* (MTCC-2480) were screened for evaluation of antibacterial and antifungal activities of the synthesized macrocyclic complexes.

Medium

Mueller Hinton Agar (for bacteria) and Sabouraud’s dextrose agar, SDA (Merck) (for fungi) were used for the biological assay.

Determination of Antibacterial Assay

In vitro antibacterial activity of the samples gram positive and gram negative bacterial strains by the agar well diffusion method (Ahmad et al. 2001). The Mueller Hinton agar was melted and cooled to 48-50°C and a standardized inoculum (1.5×10^8 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 μl) was introduced in the well (6 mm). The plates were incubated overnight at 37°C. The antimicrobial spectrum of the chemical compounds was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, streptomycin. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean values are presented.

Determination of Antifungal Assay

Antifungal activity of the experimental sample was investigated by agar well diffusion method (Ahmad et al. 2001). The yeasts and saprophytic fungi were subcultured onto Sabouraud’s dextrose agar, SDA (Merck, Germany) and respectively incubated at 37°C for 24 h and 25°C for 2-5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106 cells/ml. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 ml of several dilutions of fresh chemical compounds was administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in mm). All experiments were made in triplicate and means were calculated.

Synthesis of macrocyclic complexes

All the macrocyclic complexes were synthesized by template method. Mn(CH₃COO)₃.2H₂O (3.2 mmol, 0.858 g) was dissolved in 20 ml of n-butanol.

To this, a solution of 1-phenyl-1,2-propanedione (3.2 mmol, 0.474 g) in ~15 ml n-butanol was added drop wise with constant stirring. It was followed by the addition of a butanolic solution of 4,7,10- trioxatridecane-1,13-diamine (3.2 mmol, 0.705 g) drop wise with constant stirring which was continued for ~ 6 h.
A brownish black solid appeared which was filtered, washed with petroleum ether and dried under reduced pressure.

Similarly, Mn(III) complexes of other oxaazamacrocycles derived from 4,7,10-trioxatridecane-1,13-diamine and benzil or 4,4'-dimethylbenzil were synthesized.

**RESULT AND DISCUSSION**

The reaction of Mn(CH₃COO)₃.2H₂O with 4,7,10-trioxatridecane-1,13-diamine and different α-diketones such as 1-phenyl-1,2-propanedione, benzil and 4,4'-dimethylbenzil in 1:1:1 molar ratios have resulted in the formation of Mn(III) complexes of 17-membered N₂O₃ macrocycles (Scheme 1).

The resulting macrocyclic complexes are brownish balck solids. These complexes are quite stable at room temperature but decompose at high temperature. All the complexes are insoluble in water, carbon tetrachloride, metanol, chloroform and ethanol but solublle in dimethylsulphoxide and dimethylformamide. The characteristics and analyses of the complexes are given in Table 1.

![Scheme 1. Synthesis of Mn(III) trioxa-diazamacroyclic complexes](image)

**Molar conductances**

The molar conductance values for all the macrocycles of Mn(III) (10⁻³ M) were determined in DMSO. These values were found in the range of 142-150 ohm cm² mol⁻¹ indicating 1:2 electrolytic nature (Ravinder et al. 2009; Kumar et al. 2008).

**Infra-red spectra**

In free α-diketones 1-Phenyl-1,2-propanedione, Benzil, 4,4'-Dimethylbenzil, IR bands at 1680-1720 cm⁻¹ have been assigned due to ν(C=O). A band observed at 3200-3400 cm⁻¹ region in the spectra of 4, 7, 10-trioxatridecane-1, 13-diamine, assigned due to ν(NH₂).
Table 1. Analysis and characterization Mn(III) macrocyclic complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Color and Decomp. Temp. (°C)</th>
<th>Yield (%)</th>
<th>Found (Cal. %)</th>
<th>Λm (Ω cm² mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Mn(C₁₉H₂₈O₃N₂)(OAc)]_₂</td>
<td>Black (248)</td>
<td>58</td>
<td>9.60 (9.73)</td>
<td>5.00 (4.96)</td>
</tr>
<tr>
<td>(OAc)₂</td>
<td></td>
<td></td>
<td>53.00 (53.19)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.50 (6.61)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.00 (4.96)</td>
<td></td>
</tr>
<tr>
<td>[Mn(C₂₄H₃₀O₃N₂)(OAc)]_₂</td>
<td>Black (250)</td>
<td>47</td>
<td>8.60 (8.77)</td>
<td>4.50 (4.47)</td>
</tr>
<tr>
<td>(OAc)₂</td>
<td></td>
<td></td>
<td>57.30 (57.51)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.25 (6.27)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.50 (4.47)</td>
<td></td>
</tr>
<tr>
<td>[Mn(C₂₆H₃₄O₃N₂)(OAc)]_₂</td>
<td>Blakish brown (255)</td>
<td>48</td>
<td>8.50 (8.39)</td>
<td>4.40 (4.28)</td>
</tr>
<tr>
<td>(OAc)₂</td>
<td></td>
<td></td>
<td>88.50 (58.72)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.50 (6.62)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.40 (4.28)</td>
<td></td>
</tr>
</tbody>
</table>

In the infra-red spectrum of manganese (III) complexes, these bands {υ(C=O) and υ(NH₂)} are disappear and a new medium intensity band in 1580-1620 cm⁻¹ confirm the condensation of > C=O group of β-diketones and -NH₂ group of oxa-azadiamine and formation of macrocyclic schiff base. These bands may be assigned due to υ(C=N) stretching vibrations (Raman et al. 2003). A weak to medium intensity absorption bands in the region 420-500 cm⁻¹, which are absent in free ligands, may be assigned due to υ(Mn-O) vibrations (Shelke et al. 2011). A band in the region 2850-2930 cm⁻¹ can be assigned due to aliphatic υ(C-H) stretching vibrations. A medium intensity absorption band in the region 1400-1500 cm⁻¹ may be assigned for phenyl ring absorption (Prasad et al. 2007).

Table 2. Magnetic Moments and electronic spectral data’s of macrocyclic complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>µB</th>
<th>3B₁₈→5A₁₈ (cm⁻¹)</th>
<th>3B₁₈→5B₂₈ (cm⁻¹)</th>
<th>3B₁₈→5E₈ (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Mn(C₁₉H₂₈O₃N₂)(OAc)]_₂</td>
<td>4.96</td>
<td>14230</td>
<td>16955</td>
<td>20340</td>
</tr>
<tr>
<td>(OAc)₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Mn(C₂₄H₃₀O₃N₂)(OAc)]_₂</td>
<td>4.89</td>
<td>14570</td>
<td>17125</td>
<td>20155</td>
</tr>
<tr>
<td>(OAc)₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn(C₂₆H₃₄O₃N₂)(OAc)]_₂</td>
<td>4.92</td>
<td>14175</td>
<td>17900</td>
<td>20125</td>
</tr>
<tr>
<td>(OAc)₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Magnetic Moments and electronic spectra

The magnetic moments (µm) for the manganese (III) macrocyclic complexes observed in the range 4.89 to 4.96 µB indicating their paramagnetic behavior (Table 2). This corresponds to four unpaired electrons (Hubin et al. 2001; Mandlik et al. 2002). Manganese (III) has a d⁴ (t²g⁴ e¹g) high spin configuration. The Mn^3+ cation subjected to John-Teller distortion. The distortion decreases the symmetry of the coordination site. The electronic spectra of Mn(III) complexes of oxa-azamacrocycles exhibits bands in the region 13950-14570 cm⁻¹, 16250-17900 cm⁻¹, 18500-20340 cm⁻¹ assigned due to 3B₁₈→5A₁₈, 3B₁₈→5B₂₈ and 3B₁₈→5E₈ transitions, respectively (Sharma et al. 2006). The high energy bands in the regions 34200-35550 cm⁻¹ may be assigned due to the charge transfer. These datas indicating a distorted octahedral geometry with D₄h symmetry around Mn(III) ion.
**1H-NMR Spectrum**

A peak in the region δ 2.48-2.59 ppm is observed in the macrocyclic complexes due to the residual methyl protons of the solvent DMSO-d6. The spectral data of free diamine are as follows: -NH2 (4H) singlet at δ 1.48 ppm, -NH2-CH2- (4H) triplet at δ 2.55 ppm, -CH2-CH2-O- (4H) multiplet at δ 1.67 ppm, -CH2-O (4H) triplet at δ 3.15 ppm and -O-CH2-CH2-O- at δ 3.45-3.48 ppm. A peak in free diamine due to the -NH2 at δ 1.48 ppm disappears in the spectra of complexes confirm the condensation of -NH2 group of diamine with >C=O group of ketone to give macrocycles having >C=N linkages (Sharma et al. 2013). In the metal complexes N-CH2 (δ 2.67 ppm) and -CH2-O (δ 3.29 ppm) peaks appeared at high field support the coordination of donor atoms to the metal ion. The aromatic protons (C6H5) exhibit a multiplet at δ 7.15-7.18 ppm. In the complexes aromatic C6H5 protons give raise a multiplet at δ 7.42-7.49 ppm (Prasad et al. 2004) (Table 3). This may be due to the non-equivalence of the aromatic protons as a result of restricted rotation.

**Table 3. 1H-NMR Spectral data (δ, ppm) of diamine and macrocyclic complexes.**

<table>
<thead>
<tr>
<th>Complex</th>
<th>Chemical Shifts (δ, ppm)</th>
<th>Ketone residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-NCH2a</td>
<td>-CH2b-</td>
</tr>
<tr>
<td>4,7,10-trioxadiaza-1,13-diamine</td>
<td>2.55 t</td>
<td>1.67 m</td>
</tr>
<tr>
<td><a href="OAc">Mn(C19H28O3N2)(OAc)</a>2</td>
<td>2.67 t</td>
<td>1.75 m</td>
</tr>
<tr>
<td><a href="OAc">Mn(C24H30O3N2)(OAc)</a>2</td>
<td>2.69 t</td>
<td>1.76 m</td>
</tr>
<tr>
<td>Mn(C26H34O3N2)(OAc)2</td>
<td>2.65 t</td>
<td>1.79 m</td>
</tr>
</tbody>
</table>

**Table 4. Antibacterial screening data of macrocyclic complexes (conc. in µg/ml).**

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. griseus (+ve)</td>
</tr>
<tr>
<td></td>
<td>MTCC-706</td>
</tr>
<tr>
<td><a href="OAc">Mn(C19H28O3N2)(OAc)</a>2</td>
<td>8</td>
</tr>
<tr>
<td><a href="OAc">Mn(C24H30O3N2)(OAc)</a>2</td>
<td>6</td>
</tr>
<tr>
<td><a href="OAc">Mn(C26H34O3N2)(OAc)</a>2</td>
<td>5</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>15</td>
</tr>
</tbody>
</table>
Mass spectra
The LC-MS mass spectra of Mn(III) macrocyclic complexes have been recorded. All the macrocyclic complexes show peaks corresponding to molecular ion \([M]^{+}\) and free macrocycle \([L]^{+}\) which confirm the formation of macrocyclic complexes. In addition to the peaks due to the molecular ions and the macrocycles, the spectra exhibit peaks assignable to various fragments arising from the thermal cleavage of the macrocycles and their complexes. The proposed molecular formulae of these complexes were confirmed by comparing their molecular formula weight with m/z values.

The molecular ion peak obtained for various complexes are as follows: (1) m/z = 564.7, (2) m/z = 626.1, (3) m/z = 654.2. \([\text{Mn(C}_{24}\text{H}_{30}\text{O}_{3}\text{N}_{2})(\text{OAc})]^{2}\) macrocyclic complex shows a low abundance molecular ion peak \((M^{+}\)) at m/z 626.1. Many other peaks are observed at m/z = 627.6, 508.6, 449.4, 394.5, 395.5, 317.4, 240.8, 372.2, 294.7 due to different fragments of complex. These data suggest the 1+1 cyclocondensation of \(\alpha\)-diketone and 4,7,10-trioxadecane-1,13-diamine.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T. reesei MTCC-164</td>
</tr>
<tr>
<td></td>
<td>40</td>
</tr>
<tr>
<td>([\text{Mn(C}<em>{24}\text{H}</em>{30}\text{O}<em>{3}\text{N}</em>{2})(\text{OAc})]^{2})</td>
<td>5</td>
</tr>
<tr>
<td>([\text{Mn(C}<em>{24}\text{H}</em>{30}\text{O}<em>{3}\text{N}</em>{2})(\text{OAc})]^{2})</td>
<td>4</td>
</tr>
<tr>
<td>([\text{Mn(C}<em>{26}\text{H}</em>{34}\text{O}<em>{3}\text{N}</em>{2})(\text{OAc})]^{2})</td>
<td>6</td>
</tr>
<tr>
<td>Ketakenazole</td>
<td>12</td>
</tr>
</tbody>
</table>

Antibacterial and antifungal activity
All synthesized macrocyclic complexes of Mn(III) were screened against pathogenic fungal and bacterial strains. Inhibitory activity of synthesized complexes compared with standard known antibiotics such as Streptomycin (antibacterial) and Ketakenazole (antifungal). All these synthesized macrocyclic complexes showed good antimicrobial activity. The data in Table 4 showed that the macrocyclic complexes show very low antibacterial activity against \(P. \text{ aeruginosa}\). On increasing the concentration inhibitory activity of complexes also increased. All the complexes showed higher activities against \(S. \text{ griseus}\) and \(E. \text{ coli}\). The activity of metal complexes was found to be similar to standard antibiotics which indicate that it has better antibacterial activity. Presence of aromatic group increases its inhibitory effect on one or more type of bacteria and fungi as compare to alkyl group in the same position. The antimicrobial activity can be explained on the basis of chelation theory. Chelation may enhance the biochemical potency of bioactive species. It enhances the penetration of complex in to lipid membrane and blocking of the metal binding sites in the enzymes of microorganisms.
CONCLUSION

Elemental analyses, molar conductances, magnetic measurement, H$^1$NMR, IR and mass spectral studies indicate that all the complexes are of the type \([\text{MnL} \quad \text{(CH}_3\text{COO)}_2] \quad \text{(CH}_3\text{COO)}_2\) with a hexa- coordinated octahedral environment around manganese ion. All the macrocyclic complexes are paramagnetic and have electrolytic nature. All the complexes have significant antimicrobial activity against bacterial strain as well as fungal strain.

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