Fertility Inhibitors Macrocyclic Complexes of Bivalent Manganese : Synthetic, Spectroscopic and Medicinal Approach

R.V. Singh[1*], Ashu Chaudhary[1] and Anita Phor[2]

[1]*Department of Chemistry University of Rajasthan, Jaipur-302004, India*
[2]*Hindu College Sonipat - 131001, India*

ABSTRACT

The modern physico-chemical, spectroscopic and biochemical methods have proved an important tool to elucidate the constitution of transition metal complexes. This paper presents a brief account of the synthesis, spectroscopic and medicinal aspects of tetraazamacrocyclic compounds of manganese(II). Sixteen to eighteen membered tetraamide macrocyclic ligands $DTTD^1$ and $DTTD^2$ have been synthesized by the condensation of 1,2-diaminoethane and 1,3-diaminopropane with phthalic acid in the presence of condensing reagents dicyclohexylcarbodiimide and 4-dimethylaminopyridine. On reduction these macrocyclic ligands give new tetraazamacrocycles TTD^1 and TTD^2 which form complexes with manganese (II) nitrate and manganese (II) acetate. Based on chemical analyses, molecular weight determinations, conductance measurements, magnetic moment, IR spectra, ^1H NMR, ^{13}C NMR spectra, electronic spectra, mass spectra and X-ray spectral analysis, an octahedral geometry has been assigned to the newly synthesized products. The formulation of the complexes of the type $[Mn(TTD^n)X_2]$ [where, n = 1 or 2, X= (NO_3) or (CH_3COO)] has been established on the basis of chemical composition. The possibilities of potential uses of these complexes as fungicides and bactericides, studied *in vitro*, are also discussed. The testicular sperm density, sperm morphology, sperm mortality, density of cauda epididymis, spermatozoa and fertility in mating trials and biochemical parameters of reproductive organs of rat have been examined and discussed.

INTRODUCTION

The chemistry of macrocyclic complexes has received much attention and such compounds have been extensively studied in recent years /1/. Dramatic progress has been achieved in the field of macrocyclic chemistry on account of its various applications /2/ in bioinorganic chemistry. Catalysis, extraction of metal ions from solution and the activation of small molecules gave impetus to this endeavor. In view of the

*Author for Correspondence : E-mail : kudiwal@datainfosys.net; Fax : +91(141) 2708621

presence of two possible potential donor atoms, viz nitrogen and oxygen, the coordination chemistry of amide macrocyclic deserves special attention. It has been shown that amide macrocyclic compounds bear the dual structural features of macrocyclic tetraamines and oligopeptides and can stabilize higher oxidation states in some of the metal ions /3/. There are many examples of macrocyclic synthesis, mixtures of two or more donor sites have also been employed to tune of the selectivity and stability /4/. The current interest is often inspired by some other applications /5/ and importance in the development of industrial area /6/.

Transition metals have aroused great interest in researchers, due to their biopotency, unusual stereo-and magneto-chemistry and ability to form multiple complexes. The eagerness to reveal the characteristics of transition metals has led to innovations in the area of model compounds /7-9/, in enzymatic mechanism /10-11/, noble stoichiometric /12/ and catalytic oxygen transfer systems. The complexes of manganese play an important role in photochemical reactions /13,14/. Several manganese complexes are known to exhibit antifungal /15/ and antileukemic activities. In view of these interesting aspects we have synthesized and characterized the tetraazamacrocyclic complexes of manganese(II) in the presence of dicyclohexylcarbodiimide and 4-dimethylaminopyridine as condensing reagents for the condensation of 1,2-diaminoethane, 1,3-diaminopropane and phthalic acid. The present paper deals with the striking structural features, synthesis and appreciable biological applications of these complexes.

EXPERIMENTAL

The chemicals including dicyclohexylcarbodiimide and 4-dimethylaminopyridine and phthalic acid (Fluka) were used as such. 1,2-Diaminoethane, 1,3-diaminopropane and $LiAlH_4$ were used as obtained from E.Merck. $Mn(NO_3)_2.4H_2O$ and $Mn(CH_3COO)_2.4H_2O$ were used without further purification.

Synthesis of the Ligands:

3,4 : 11, 12-Dibenzo-2,5,10,13-tetraoxo-1,6,9,14-tetraazacyclohexadecane (DTTD^1) :

The reaction is carried out in 2 : 2 molar ratios of 1,2-diaminoethane and phthalic acid. A catalytic amount of 4-dimethylaminophyridine and an appropriate amount of dicyclohexylcarbodiimide (1.3274g;6.43mmol) in a minimum amount of dichloromethane at 0°C were kept magnetically stirred in a two-necked round bottom 100 mL flask. The reaction was followed by the addition of 1,2-diaminoethane (0.3866 g; 6.43 mmol) and phthalic acid (1.0681g; 6.43 mmol) in dichloromethane. The resulting reaction mixture was stirred for about 10-12 hours at 0°C. The solid product was obtained by filtration and washing with dichloromethane. The white product thus obtained was recrystallised from benzene and dried *in vacuo*.

3,4:12,13-Dibenzo-2,5,11,14-tetraoxo-1,6,10,15-tetrazacyclooctadecane (DTTD^2) :

Similarly DTTD^2 was prepared by reacting (1.0750g; 5.21 mmol), dicyclohexylcarbodiimide, (0.3868g; 5.21mmol), 1,3-diaminopropane and (0.8662 g; 5.21 mmol) phthalic acid.

1,6,9,14-Tetrazacyclohexadecane (TTD^1) :

The reaction is carried out in 1 : 2 molar ratios of ligand DTTD^1 and $LiAlH_4$. The ligand DTTD^1 (0.8765 g; 2.30 mmol) was dissolved in tetrahydrofuran and cooled at 0°C. $LiAlH_4$ (corresponding to DTTD^1) in tetrahydrofuran was stirred for about 10 hours in an ice bath. The reaction mixture was then stirred under reflex for 75 hours. After cooling it, 10 mL of water and 10 mL of 15% aqueous sodium hydroxide were added to the reaction mixture at 0°C. The solid material was filtered and the residue washed with tetrahydrofuran The filtrate and tetrahydrofuran washing were concentrated under reduced pressure to give TTD^1 as liquid.

1,6,10,15-Tetraazacyclooctadecane (TTD^2) :

The ligand TTD^2 was synthesized analogous to the above ligand. The ligand DTTD^2 (0.8216 g; 2.01 mmol) and $LiAlH_4$ (0.1528 g; 4.02 mmol) were used for this synthesis.

Synthesis of the Complexes :

Dinitrato/acetato(1,6,9,14-tetraazcyclohexadecane)manganese(II)./Mn(TTD^1)X_2/ :

The reaction is carried out in a 1 : 1 molar ratio of ligand TTD^1 and metal salt. To a methanolic solution of metal salt (0.9467 g; 3.77 mmol) was added a methanolic solution of TTD^1 (corresponding to metal salt). The reaction mixture was stirred for about 15-18 hours. The resultant solid product obtained was filtered off and washed with methanol and dried in vacuo.

Dinitrato/acetato (1,6,1015-tetraazacyclooctadecane) manganese (II) /Mn(TTD^2)X_2/ :

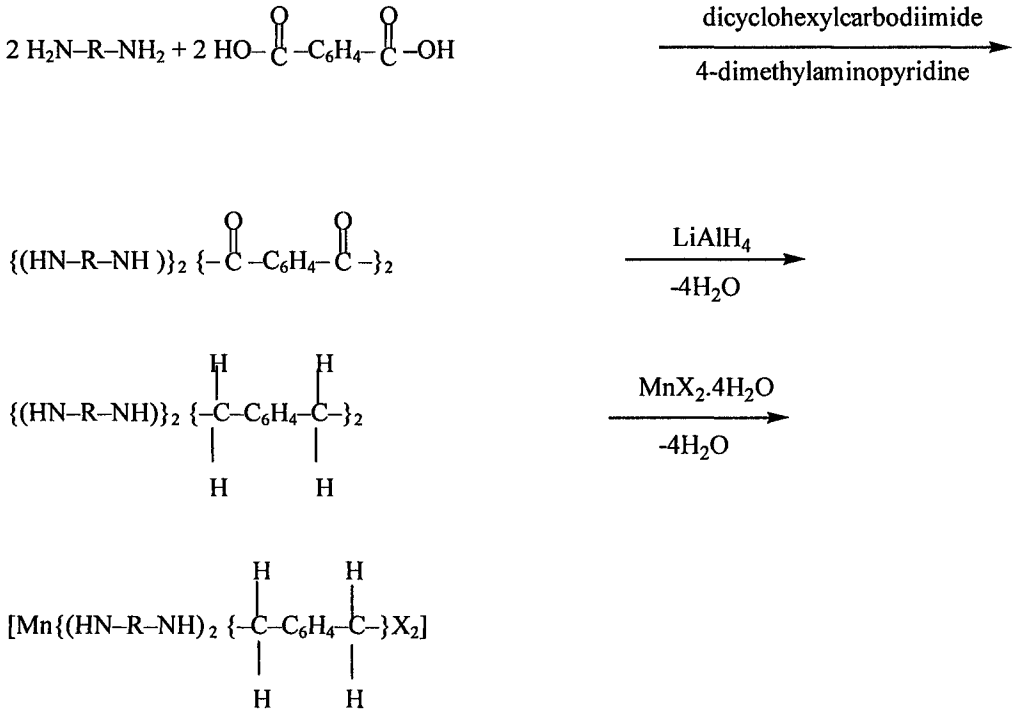
These complexes were prepared using the above mentioned method for /Mn(TTD^1)X_2/. Ligand TTD^2 was used in this synthesis in place of the ligand TTD^1.

Physical Measurements and Analytical Methods :

The molecular weights were determined by the Rast Camphor Method. Conductivity measurements in dry dimethylformamide were performed with a conductivity bridge type 305. Infrared spectra were recorded on a Nicolet Magna FT–IR 550 spectrophotometer in KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 using TMS as standard on a JEOL FX-90Q spectrometer. Electronic spectra in dimethylsulphoxide were recorded on a UV–160A Shimadzu spectrophotometer. X-ray powder diffraction spectra of the compounds were obtained on the Philips model P.W. 1840 automatic diffractometer using Fe(Kα) target with Mg filter. The wavelength used was 1.9373 Å and the reflections from 5 – 65Å were recorded. The mass spectra of the compounds were recorded on a JEOL FX 102/ DA – 6000 mass spectrometer/data system using Argon/Xenon (6 KV, 10 mA) as the FAB gas. m-Nitrobenzyl alcohol was used as the matrix. Manganese was estimated gravimetrically. Nitrogen was estimated by Kjeldahl's method. Carbon and hydrogen analyses were performed at Central Drug Research Institute, Lucknow.

RESULTS AND DISCUSSION

A series of sixteen- to eighteen-membered tetraazamacrocyclic ligands and their manganese (II) complexes were derived by the condensation of phthalic acid with 1,2-diaminoethane or 1,3-diaminopropane in the presence of condensing reagents dicyclohexylcarbodiimide and 4-dimethylaminopyridine. The reactions proceeded as given in equation 1.



where:

R= 1,2- C_2H_4 or 1,3- C_3H_6

X= (NO_3) or (CH_3COO) (Eqn. 1)

All the complexes are coloured solids. They are soluble in most of the organic solvents. The experimental conductivity values measured for 10^{-3}M solutions in anhydrous dimethylformamide are in the range 16-32 ohm^{-1} cm^2 mol^{-1} showing that they are non-electrolytes. This indicated that the anions are coordinated to the manganese metal in these complexes. The physical properties and analytical data of the complexes are given in Table 1.

Table 1

Physical Properties and Analytical Data of the Macrocyclic Ligands and their Manganese(II) Complexes.

Compound	Empirical formula	M.P. (°C) and Colour	Yield (%)	Analysis (%) Found (Calcd.)				Mol. Wt. Found (Calcd.)
				C	N	H	Mn	
DTTD[1]	$C_{20}H_{20}O_4N_4$	186 White	53	63.10 (63.21)	5.10 (5.30)	13.42 (14.74)	-	367 (380)
DTTD[2]	$C_{22}H_{24}O_4N_4$	162 White	59	64.61 (64.76)	5.71 (5.92)	13.20 (13.70)	-	391 (408)
TTD[1]	$C_{20}H_{28}N_4$	178 Yellowish brown	63	74.02 (74.14)	8.47 (8.71)	16.90 (17.29)	-	301 (324)
TTD[2]	$C_{22}H_{32}N_4$	103 Yellowish brown	50	74.69 (74.85)	8.92 (9.13)	15.51 (15.87)	-	320 (353)
$[Mn(TTD^1)(NO_3)_2]$	$C_{20}H_{28}N_6O_6Mn$	203 Off white	48	47.57 (47.75)	5.38 (5.61)	15.60 (16.70)	10.40 (10.92)	479 (503)
$[Mn(TTD^2)(NO_3)_2]$	$C_{22}H_{32}N_6O_6Mn$	210 Off white	46	49.45 (49.66)	5.87 (6.06)	14.65 (15.79)	9.78 (10.32)	501 (532)
$[Mn(TTD^1)(CH_3COO)_2]$	$C_{24}H_{34}N_4O_4Mn$	198 Cream	42	57.88 (58.00)	6.63 (6.89)	10.32 (11.27)	10.56 (11.05)	471 (497)
$[Mn(TTD^2)(CH_3COO)_2]$	$C_{26}H_{38}N_4O_4Mn$	188 Cream	40	59.21 (59.36)	7.16 (7.28)	9.69 (10.65)	4.56 (11.56)	499 (526)

Infrared Spectra :

A comparative study of the IR spectra of the tetraamides, tetraazamacrocyclic ligands and their corresponding manganese (II) complexes confirmed the formation of the macrocyclic complexes with the proposed coordination pattern. The ligands $DTTD^1$ and $DTTD^2$ show the absence of bands corresponding to primary amino and hydroxy groups, which confirms their involvement in the formation of tetraamide macrocycles. The presence of four bands in the regions 1640-1648, 1485-1489, 1230-1239 and 620-632 cm^{-1} is due to amide I, amide II, amide III, and amide IV bands respectively /16/. This provides strong evidence for the presence of a closed cyclic product. A single sharp band observed for amide ligands $DTTD^1$ and $DTTD^2$ in the region 3275-3289 cm^{-1} is due to ν (NH) of amide group. Strong and sharp absorption bands that appear in the regions 2800-2925 and 1400-1450 cm^{-1} in all the complexes may be ascribed to the C-H stretching and bending vibrations, respectively /17/. In the IR spectra of tetraazamacrocyclic ligands TTD^1 and TTD^2, it is observed that the amide bands have been found absent on comparing with $DTTD^1$ and $DTTD^2$.

In the spectra of macrocylic complexes of manganese (II) as compared to their analogous metal free tetraaza ligands a slight negative shift in the ν (N-H) band that appeared in the region 3210-3235 cm^{-1} was observed. It is ascribed to the coordinated N-H stretching vibration. The shift to the lower frequency of ν (N-H) mode, along with the appearance of a new band in the region 303-329 cm^{-1} assignable to the ν(Mn-N) vibrations, suggested that the amide nitrogen is coordinated to the manganese /18,19/. The infrared spectral data of the ligands and their manganese(II) complexes are reported in Table 2. The nitro complexes exhibit a band in 243-258 cm^{-1} region assignable to ν (Mn-O) of ONO_2 group /20,21/.

^1H and ^{13}C NMR Spectra

The bonding pattern in the ligands has also been sustained by the proton magnetic resonance spectra. The ligands $DTTD^1$ and $DTTD^2$ do not show any signal corresponding to primary amino and alcoholic protons suggesting that the proposed macrocyclic skeleton has been formed through condensation reaction. Broad signals exhibited by the ligands $DTTD^1$ and $DTTD^2$ at δ 8.44 and 8.47 ppm, respectively, are due to the amide proton (CO-NH). The signals due to the methylene protons δ 3.39 – 3.68 ppm were also assigned for the ligands. A multiplet in the region δ 1.98 – 2.04 ppm may be attributed to the central methylene protons, –C–CH_2–C–. A multiplet of aromatic protons was observed at δ 7.28 – 8.05 ppm in the spectra of all the ligands.

The conclusions drawn from the IR and ^1H NMR spectra are parallel with the carbon-13 spectral data regarding the authenticity of the proposed skeleton. ^1H NMR and ^{13}C NMR spectral data of the ligands are listed in Table 3.

Electronic Spectra :

The electronic spectra of the complexes display weak absorption bands in the regions 17245-16930, 23827-23204 and 27020-26035 cm^{-1} for $^6A_{1g} \rightarrow {}^4T_{1g}$, $^6A_{1g} \rightarrow {}^4T_{2g}$ and $^6A_{1g} \rightarrow {}^4T_{1g}$, respectively. These data are in fair agreement with the octahedral geometry for the Mn(II) complexes /22/.

Table 2

Infrared Spectral Data (cm^{-1}) of Macrocyclic Ligands and their Manganese(II) Complexes.

Compound	ν(NH)	Amide				C–H		ν(Mn–N)	ν(Mn–O)
		I	II	III	IV	Stretching	Bending		
$DTTD^1$	3289	1640	1485	1230	620	2922	1400	303	250
$DTTD^2$	3275	1648	1489	1239	632	2833	1422	320	245
TTD^1	3280	-	-	-	-	2800	1438	311	253
TTD^2	3271	-	-	-	-	2827	1408	319	248
$[Mn(TTD^1)(NO_3)_2]$	3215	-	-	-	-	2810	1414	325	245
$[Mn(TTD^2)(NO_3)_2]$	3210	-	-	-	-	2925	1429	329	256
$[Mn(TTD^1)(CH_3COO)_2]$	3227	-	-	-	-	2911	1450	322	258
$[Mn(TTD^2)(CH_3COO)_2]$	3235	-	-	-	-	2920	1446	317	243

Table 3

^{1}H and ^{13}C NMR Spectral Data (δ, ppm) of Macrocyclic Ligands.

	^{1}H					
Ligand	**CO-NH**		**-N-CH_2**	**C-CH_2-C**	**Aromatic**	
				$H_{2,5}$	$H_{3,4}$	
DTTD[1]	8.44	3.62	-	7.28	7.88	
DTTD[2]	8.47	3.68	1.98	7.45	8.05	
TTD[1]	-	3.48	-	7.40	7.94	
TTD[2]	-	3.39	2.04	7.33	7.83	

	^{13}C NMR					
	>C=O	**>N-CH_2**	**>N-CH_2-*CH_2***	**Aromatic**		
				$C_{1,6}$	$C_{2,5}$	$C_{3,4}$
DTTD[1]	178.26	40.78	-	134.27	129.41	124.49
DTTD[2]	175.20	44.42	36.70	129.86	127.55	125.10
TTD[1]	-	40.74	-	127.02	126.49	125.37
TTD[2]	-	45.20	37.19	128.06	127.22	125.04

Magnetic Moment :

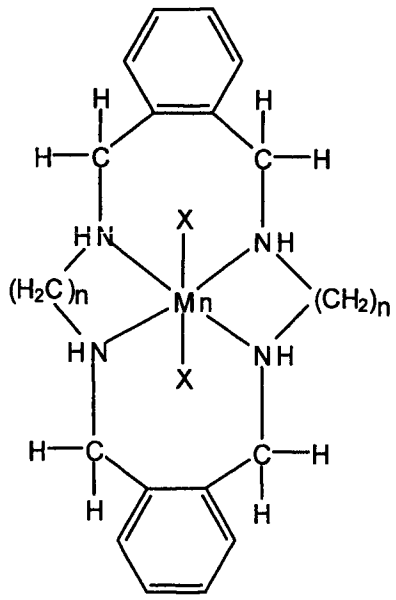
The μ_B values for the complexes observed at 5.82-5.89 BM are within the range required for an octahedral geometry /23/.

Mass Spectrum :

The mass spectrum of the compound $[Mn(TTD^2)(CH_3COO)_2]$ shows the molecular ion peak at m/z =526. The prominent fragments are at 454 for $[Mn(C_{23}\ H_{30}N_2O_4)]^+$,422 for $[Mn(C_{18}H_{30}N_4O_4)]^+$ and 382 for $[Mn(C_{20}H_{22}O_4)]^+$ due to the loss of $C_3H_8N_2$, C_8H_8 and $C_6H_{16}N_4$, respectively. Another peak appeared at m/z 408 due to the loss of two acetate groups from the parent ion.

X-ray Diffraction Studies :

The X-ray diffraction analysis of the compound $[Mn(TTD^1)(NO_3)_2]$ confirms the orthorhombic crystal system for this derivative having unit cell dimensions, a=13,.0202, b = 22.4347 and c=17.0650 and $\alpha=\beta=\gamma=90^\circ$. Miller indices *h, k* and *l* are given in Table 4. The structure shown in Figure 1 may be assigned to the complexes based on the preceeding spectral studies.



Where, X = NO_3 or CH_3COO
n = 2 or 3

Fig. 1

Table 4

X-Ray Powder Diffraction Data of the Compound /$Mn(TTD^2)(NO_3)_2$/

Peak No.	20 (deg.) (obs.)	d spacing (obs.)	h	k	l
1.	12.4	8.9693	1	3	1
2.	18.6	5.9942	0	3	2
3.	21.0	5.9942	0	5	5
4.	22.0	5.3155	2	1	3
5.	23.2	5.0767	1	1	4
6.	24.7	4.8174	2	1	4
7.	25.4	4.5290	1	5	3
8.	26.2	4.2739	0	2	5
9.	27.2	4.1195	0	5	4
10.	28.0	4.0041	2	1	5
11.	29.2	3.8429	4	4	1
12.	31.6	3.5577	0	6	4
13.	32.8	3.4309	1	5	5
14.	33.0	3.4107	5	3	0
15.	33.74	3.3380	3	8	0
16.	34.8	3.8393	1	2	7
17.	36.86	3.0688	5	5	2
18.	37.4	3.0213	3	9	1
19.	38.6	2.9308	0	9	4

Refined values $a = 13.0202$ $\alpha = \beta = \gamma = 90°$

$b = 22.4347$

$c = 17.0650$

(Orthorhombic system)

Biological Screening :

To assess the biological potential of the ligands and their macrocyclic complexes, laboratory and field experiments have been conducted. The following techniques have been used for the antimicrobial activities of these compounds.

Antifungal Activities

1. *Poisoned Food Technique*: The antifungal activity was evaluated by the food poisoned technique /24/.

2. *Spore Germination Method /25/ :*

The spore germination test includes depositions of chemicals on a slide, evaporation to dryness and addition of a drop of water containing spore of the test fungus.

A drop of spore suspension was placed on a clear slide. The number of spores in the drop under low power magnification was determined and the suspension was adjusted to obtain nearly ten spores per microscopic field. These slides were incubated for 12 hours at 25°C in petri dishes working as moisture chambers. Slides were put in such a position that spore drops keep hanging. After incubations of 12 hours, spore germinations were counted. Each treatment was replicated thrice. In this technique total number of spores, number of germinated spores and number of ungerminated spores were counted. LD_{50} values were calculated to compare the activity of the ligands along with their chelates by plotting graphs between the concentration of ligand *vs* complex with the number of germinated spores. The organisms selected for this method are; *Alternaria alternata* and *Helminthosporium gramineum.*

Antibacterial Activity

Inhibition Zone Technique /26/:

In this technique sterilized hot nutrient agar medium and 5 mm diameter paper discs of Whatman No. 1 were used. The agar medium was poured into the petri plates. After solidifications, the petri plates were stored in inverted position so that there was condensation of water in the upper lid. Solutions of test compounds in methanol in 500 and 1000 ppm concentrations were prepared in which discs were dipped in solution of the test sample placed on seeded plates. The petri plates having these discs on the seeded agar should first be placed at low temperature for two or four hours to allow for the diffusion of chemicals before being incubated at suitable optimum temperature 28 ± 2°C for 24-30 hours. After the expiry of their incubation period, the zone of inhibition associated with the treated disc was measured in mm. The compounds were tested against *Pseudomonas cepacicola* (-), *Xanthomonas compestris* (-), *Escherichia coli* (-) and *Staphylococcus aureus* (+).

Antifertility Activity :

The antifertility activity of the ligands and their complexes was studied on male albino rats.

Healthy, adult male albino rats of sprague dawley strain were used in the present investigations. The rats were divided into seven groups of eight animals each. The group A served as vehicle (olive oil) treated control. In the group B, ligand DTTD[1] 25 mg kg^{-1} body weight suspended in 0.2 mL olive oil was given

orally for a period of forty five days. The animals of groups D and G received the same dose of compounds, respectively, for a similar period. The animals were screened for fertility test and autopsied for detailed pathological and biochemical studied. Reproductive organs were excised, blotted free of blood, weighed and fixed in Bouin's fluid for histological studies.

The sperm motility and density of *Cauda epididymal* spermatozoa was assessed. The protein, sialic acid /27/ and fructose were determined /27/. The activity of acid phosphate was estimated. The vaginal smear or vaginal plug confirmed the positive mating. The females were checked for implantation after 16 days of pregnancy. The results were analysed with the help of student 't' test.

RESULTS AND DISCUSSION

Antifungal and Antibacterial Screening :

Antifungal and antibacterial activities of representative ligands and their manganese complexes have been screened in Tables 5, 6 and 7. The results showed that there is considerable increase in the toxicity of the complexes as compared to the ligands.

On giving a closer look at these results a common feature which appears is that the bioactivity enhances undergoing chelation. This can be well ascribed to Tweedy's chelation theory /28/. According to this, the chelation reduces the polarity of the central atom mainly because of partial sharing of its positive charge with the donor groups and possible π-electron delocalisation over the whole chelation ring. This increases the lipophilic nature of the central manganese atom, which subsequently favours its permeation through the lipid layer of the cell membrane.

Solubility and concentration of the compounds play a vital role in ascertaining the extent of inhibition. The biological potency of these compounds may be attributed to their ability to inactivate various cellular enzymes which play a vital role in different metabolic pathways of these microorganisms. It has been proposed that the ultimate action of these compounds is the denaturation of one or more proteins of the cell as a result of which normal cellular processes are impaired. It may also be postulated that these complexes might act as uncoupling agents of oxidative phophorylation of ADP to ATP /29/. These agents are less effective for bacteria.

Representative ligands and their manganese complexes have been screened *in vivo* to study their potency on crop plants guar (guar blight by *Alternaria cymopsidal*) / mung (*Cercospora leaf spot*)/, using Percent Disease Incidence (PDI) technique.

The Percent Disease Incidence (PDI) is the area covered on foliage by specific disease and was calculated by using the following formula:

$$\text{Percent Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{Percent Disease Control} = \frac{\text{Number of treated plants} - \text{PDI in untreated plant}}{\text{PDI in untreated plants}} \times 100$$

Table 5

Antifungal Screening Data of Ligands and their Corresponding Macrocyclic Complexes of Manganese.

Compound	Percent inhibition after 4 days at 25 ± 2°C (Conc. in ppm)								
	Fusarium oxysporum			*Macrophomina phaseolina*			*Rhizoctonia bataticola*		
	50	**100**	**200**	**50**	**100**	**200**	**50**	**100**	**200**
$DTTD^1$	27	42	48	20.4	51	66	23.4	50	63
$DTTD^2$	26	48	57	30.2	44	48	32.0	53	59
TTD^1	35	42	62	28	46	62.4	34	43	65
TTD^2	34	45	52	32	54	58	-	-	57
$[Mn(TTD^1)(NO_3)_2]$	87	93	96	89	95	99	87	92	91
$[Mn(TTD^2)(NO_3)_2]$	87	90	94	85	91	93	92	96	93
$[Mn(TTD^1)(CH_3COO)_2]$	62	76	85	69	78	89	-	-	-
$[Mn(TTD^2)(CH_3COO)_2]$	62	68	70	59	77	81.7	74	80	86
Bavistin (Standard)	82	100	100	82	100	100	85	100	100

Table 6

Antifungal Screening Data of the Ligands and their Macrocyclic Complexes of Manganese(II).

Compound	Conc. ppm	*Alternaria alternata*			*Helminthosporium gramineum*		
		Total no. of spores	No. of germinated spores	No. of ungerminated spores	Total no. of spores	No. of germinated spores	No. of ungerminated spores
DTTD[1]	50	10	9	1	12	8	4
	100	10	7	3	12	4	8
	200	10	5	5	12	3	9
	400	10	2	8	12	2	10
DTTD[2]	50	10	7	3	12	7	5
	100	10	5	5	12	5	7
	200	10	2	8	12	4	8
	400	10	1	9	12	2	10
TTD[1]	50	10	7	3	12	7	5
	100	10	6	4	12	5	7
	200	10	3	7	12	4	8
	400	10	1	9	12	2	10
TTD[2]	50	10	6	4	12	2	10
	100	10	5	5	12	5	7
	200	10	3	7	12	7	5
	400	10	2	8	12	9	3
$[Mn(TTD^1)(NO_3)_2]$	50	10	8	2	12	7	5
	100	10	6	4	12	4	8
	200	10	4	6	12	3	9
	400	10	3	7	12	1	11
$[Mn(TTD^2)(NO_3)_2]$	50	10	6	4	12	7	5
	100	10	4	6	12	4	8
	200	10	3	7	12	3	9
	400	10	2	8	12	1	11

Comparison of LD_{50} Values (ppm) of Ligands and their Macrocyclic Complexes of Mangnese(II).

Compound	*Alternaria alternata*	*Helminthosporium gramineum*
DTTD[1]	200	75
DTTD[2]	100	70
TTD[1]	130	67
TTD[2]	100	125
$[Mn(TTD^1)(NO_3)_2]$	120	60
$[Mn(TTD^2)(NO_3)_2]$	70	65

Table 7

Antibacterial Screening Data of Macrocyclic Ligands and their Manganese(II) Complexes.

Compound	Inhibition (mm) after 24 h (Conc. in ppm)							
	Pseudomonas cepacicola (-)		*Xanthomonas compestris (-)*		*Escherichia coli (-)*		*Staphylococcus aureus (+)*	
	500	1000	500	1000	500	1000	500	1000
$DTTD^1$	6	8	5	8	4	6	3	5
$DTTD^2$	4	6	4	7	5	7	-	-
TTD^1	7	11	-	10	4	7	4	7
TTD^2	5	8	6	8	5	6	-	-
$[Mn(TTD^1)(NO_3)_2]$	10	13	9	12	8	10	6	9
$[Mn(TTD^2)(NO_3)_2]$	7	9	5	8	7	9	5	8
$[Mn(TTD^1)(CH_3COO)_2]$	8	12	7	13	9	16	9	15
$[Mn(TTD^2)(CH_3COO)_2]$	9	13	8	-	6	13	-	14
Streptomycin (Standard)	3	5	3	5	15	18	15	17

Field experiments were laid out in randomized block design with three replications. The crops were raised in each plot. Compounds with a standard fungicides, bavistin [2-methoxycarbamyl) benzimidazole] were tried in addition to check (water spray).

After sowing of 25 days, the plants were inoculated artificially by spraying the conidial suspension. The conidial suspension was prepared by crushing the infected leaves in water. The inoculation was done late in the evening.

The first spray of the respective fungicide was given when lesions were first seen and spraying was repeated after 10 days. Disease intensity was recorded 10 days after the second spray. The data was analysed statistically and Percent Disease Control was worked out. It was observed that, although all the fungicides were significantly at par with each other and different from check, yet the performance of the complexes are higher than their respective ligands.

Antifertility Activity:

The testicular morphology, testicular sperm density, sperm motility, density of cauda epididymal spermatozoa and fertility in mating trials and biochemical parameters of reproductive organs with tetraamides and their manganese complexes *in vivo* were examined and discussed.

The results show that the ligands alone were able to inhibit fertility but due to the added synergistic effects of the manganese complexes, their activity becomes enhanced. The results are grouped under the following headings:

Body and Organ Weights :

Body weights of rat were not affected after their manganese complexes were administrated. However, the weights of testes, epididymis, seminal vesicle and ventral prostate were significantly decreased [Table 8].

Fertility Test

The sluggish motile spermatozoa was unable to fertilize normal cyclic females. The test was 68 to 92% negative in rats treated with the compounds.

Sperm Motility

The sperm motility declined significantly after treatment with compounds.

Sperm Density

The sperm density in testes and cauda epididymis declined significantly after treatment [Table 9].

Biochemical Changes

Total protein and sialic acid contents of testes, epididymis, ventral prostate and seminal vesicle were depleted significantly after treatment with ligands and their complexes. The acid phosphate levels of testes, epididymis and ventral prostate were also reduced significantly. A significant decrease in seminal vascular

Table 8

Effects of Macrocyclic Ligands and their Manganese(II) Complexes on Body Weight and Organ Weight of Male Rats.

Group	Treatment	Body weight		Testes	Epididymis	Seminal vesicle	Ventral prostate
		Initial	Final	mg/100 g body weight			
A	Control	180.0 ± 20.0	210 ± 150	1200 ± 90.5	415.0 ± 25.0	330.0 ± 20.70	250.0 ± 32.0
B	DTTD[1]	185.0 ± 15.0	220 ± 15.0[c]	900 ± 25.0[b]	330.0 ± 20.0[b]	250.0 ± 10.5[b]	235.0 ± 30.50[c]
C	TTD[1]	170.0 ± 14.0	180.0 ± 10.0[c]	870 ± 30.0[a]	295.0 ± 10.0[a]	210.0 ± 8.5	160.0 ± 7.5[a]
D	$[Mn(TTD^1)(NO_3)_2]$	190.0 ± 12.0	230 ± 18.0[c]	810 ± 15.0[a]	275.0 ± 12.0[a]	215.0 ± 18.0[a]	175 ± 5.5[a]
E	DTTD[2]	186.0 ± 10.0	215.0 ± 15.0[c]	630.0 ± 15.0[b]	215.0 ± 10.0[b]	180.0 ± 8.5[b]	145 ± 6.3[b]
F	TTD[2]	188.0 ± 150	222 ± 20.0[c]	600.0 ±13.0[b]	205.0 ± 18.0[b]	180.0 ± 10.0	130.0 ± 8.0[b]
G	$[Mn(TTD^2)(NO_3)_2]$	195.0 ± 10	230 ± 20.0[c]	730 ± 18.0[a]	250 ± 150[a]	210.0 ± 9.0[a]	168.0 ± 9.8[a]

Values means ± SE of seven determinations

a = $P \leq 0.05$

b = $P \leq 0.001$

c = $P \leq$ NS

Table 9

Sperm Dynamics and Fertility Test after Treatment with Macrocyclic Ligands and their Manganese(II) Complexes

Group	Treatment	Sperm motility Cauda epididymis	Sperm density (million/ml)		Fertility test (%)
			Testes	Epididymis	
A	Control	80.0 ± 5.1	1.95 ± 0.10	55.5 ± 0.10	99% + ve
B	DTTD[1]	50 ± 3.0[b]	0.85 ± 0.15[b]	0.85 ± 0.15[b]	68% - ve
C	TTD[1]	35 ± 3.9[b]	0.65 ± 0.10[b]	0.65 ± 0.10[b]	80% - ve
D	$[Mn(TTD^1)(NO_3)_2]$	45.0 ± 2.5[a]	0.60 ± 0.12[b]	0.60 ± 0.12[b]	85% - ve
E	DTTD[2]	30.0 ± 2.9[b]	0.50 ± 0.10[b]	0.50 ± 0.10[b]	87% - ve
F	TTD[2]	24.0 ± 2.0[b]	0.62 ± 0.12[b]	0.62 ± 0.12[b]	88% - ve
G	$[Mn(TTD^2)(NO_3)_2]$	44.0 ± 3.0[a]	0.69 ± 0.15[b]	0.69 ± 0.15[b]	92% - ve

Values means ± SE of seven determinations

a = $P \leq 0.05$

b = $P \leq 0.001$

c = $P \leq$ NS

fructose contents was also noticed whereas the testicular cholesterol contents were increased significantly after the treatment with various compounds [Tables 10 and 11].

Discussion :

The present study revealed that administration of ligands and their manganese complexes caused a significant reduction in the weights of testes and other sex accessory glands. The prostate and seminal vesicle are well documented androgen dependent processes /31/. Sperm motility is considered as an important parameter in evaluating the fertility potential /32/. The tetraamide ligands and their manganese complexes significantly reduce the fertility of male rats. Since a number of androgen sensitive parameters (protein, sialic acid, fructose, acid phosphatase and total cholesterol) in target organs were found to be altered by these complexes, it is probable that the structure and function of epididymis and other sex accessory organs are changed.

Manganese chloride causes testicular germ cells in rats and rabbits /33/ and decreased libido and impotency in a man occupationally exposed to manganese /34/. Our findings indicate that the ligand TTD^2 and its manganese complex have a more pronounced effect of fertility on various biochemical parameters of reproductive organs as compared to other ligands and complexes discussed in this paper.

ACKNOWLEDGEMENT

One of the authors (AC) is thankful to CSIR New Delhi for financial assistance in the form of SRF vide Grant No. 9/149(288)/2K2, EMR1.

REFERENCES

1. H. Sigel, *Metal Ion in Biological Systems,* Vol. 2; Mixed-Ligand Complexes, Marcel Dekker, Inc., New York, 1993.
2. T.A. Khan, Syed S. Hasan, A.K. Mohamed, K.S. Islam and M. Shakir, *Synth. React. Inorg. Met-Org. Chem.*, **30(5)**, 815 (2000).
3. A.C. Hiremath, K.M. Reddy and M.B. Halli, *Asian, J. Chem.*, **5**, 35 (1993).
4. F.A. Catton and G.Willkinson, *Advanced Inorganic Chemistry,* John Wiley and Sons, New York, 1988; p. 730.
5. M.W. Hosseini, J.M. Lehn, S.R. Duff. K. Gu and M.P. Mertes, *J. Org. Chem.*, **52**, 1662 (1987).
6. R.M. Iyan, J.S. Brandshaw, S.A. Neilsen, J.D. Lamb, J.J. Christensen and D. Sen, *Chem. Rev.*, **85**, 271 (1985).
7. X.M. Chem, Z. T. Xu and X.C. Huang, *J. Chem. Soc., Dalton Trans*, **1994**, 2331.

Table 10

Effects of Ligands and their Manganes(II) Complexes on Biochemical Parameters (Total Protein and Sialic Acid) of Reproductive Organs of Male Rats.

Group	Treatment	Total protein (mg/g)				Sialic acid (mg/g)			
		Testes	Epididymis	Ventral prostate	Seminal vesicle	Testes	Epididymis	Seminal vesicle	Ventral prostate
A	Control	225.0 ± 15.0	200.0 ± 10.0	245.0 ± 10.0	260.0 ± 10.0	7.8 ± 0.9	6.90 ± 0.5	6.75 0 ± 0.6	7.10 ± 0.5
B	DTTD[1]	160.0 ± 20.0	165.0 ± 15.0	188.0 ± 10.0	200.0 ± 15.9[b]	5.0 ± 0.6[b]	5.1 ± 0.6	5.5 ± 0.3[b]	5.2 ± 0.5
C	TTD[1]	135 ± 10.0[a]	140.0 ± 10.0	150.0 ± 6.0[a]	160.0 ± 9.5[a]	4.0 ± 6.8[a]	5.1 ± 0.8	4.5 ± 0.4[a]	5.2 ± 0.4[b]
D	$[Mn(TTD^1)(NO_3)_2]$	110.0 ± 150[a]	125.0 ± 10.0	145.0 ± 6.5[a]	175 ± 10.0[b]	3.8 ± 0.3[a]	4.1 ± 0.8	4.9 ± 0.3[a]	4.6 ± 0.3[a]
E	DTTD[2]	99.0 ± 10.0[a]	100.0 ± 8.5[a]	120.0 ± 7.0[b]	105.0 ± 4.8[b]	3.9 ± 0.2	3.6 ± 0.4[a]	3.2 ± 0.2[b]	4.0 ± 0.5a
F	TTD[2]	105.0 ± 10.2[a]	110 ± 8.5[a]	122.0 ± 8.2[b]	108.0 ± 9.0[b]	4.1 ± 0.9	2.9 ± 0.4[b]	3.6 ± 0.2	3.2 ± 0.2b
G	$[Mn(TTD^2)(NO_3)_2]$	122.7 ± 12.0[a]	135 ± 10.4[a]	140.0 ± 8.0	158.0 ± 8.0	4.2 ± 0.6	3.0 ± 0.6	4.2 ± 0.5	4.5 ± 0.29

Values means ± SE of seven determinations

a = $P \leq 0.05$

b = $P \leq 0.001$

c = $P \leq$ NS

Table 11

Effect of Macrocyclic Ligands and their Manganese(II) Complexes on Biochemical Parameters (Acid Phosphate, Fructose and Cholesterol) of Reproductive Organs of Male Rats

Group	Treatment	Acid Phosphate			Fructose (mg/g)	Cholesterol
		Testes	Epididymis	Ventral prostate		
A	Control	3.9 ± 0.30	5.45 ± 0.06	3.05 ± 0.07	445.0 ± 30.0	7.60 ± 0.50
B	$DTTD^1$	2.7 ± .10[b]	3.41 ± 0.04	2.01 ± 0.01[a]	375.0 ± 20.0[a]	8.40 ± 0.50[a]
C	TTD^1	2.1 ± 0.01[a]	2.10 ± 0.001	1.65 ± 10.00	315.0 ± 10[a]	9.0 ± 0.2[a]
D	$[Mn(TTD^1)(NO_3)_2]$	2.0 ± 2.0	2.35 ± 0.05	1.68 ± 0.001[a]	330.0 ± 15.0[b]	9.6 ± 0.3[a]
E	$DTTD^2$	1.8 ± 0.02[a]	2.00 ±	2.25 ± 0.01	270.0 ± 17.0	9.9 ± 0.4[a]
F	TTD^2	2.0 ± 0.01[a]	1.9 ± 0.3[a]	1.4 ± 0.001[a]	290.0 ± 12.0[b]	9.78 ± 0.2a
G	$[Mn(TTD^2)(NO_3)_2]$	2.4 ± 0.03	2.20 ±	159.0 ±	300.0 ± 15.0[b]	9.7 ± 0.39

Values means ± SE of seven determinations

a = $P \leq 0.05$

b = $P \leq 0.001$

c = $P \leq$ NS

8. S. Ozawa, Y. Watanabe, S. Nakashima, T. Kistogawa and I. Morishima, *J. Am. Chem. Soc.*, **116,** 634 (1994).
9. P.I. Pessiki, S.V. Khangulov, D.M. Ho and G.C. Dismukes, *J. Am. Chem. Soc.*, **16,** 891 (1994).
10. K. Weigharadt, *Angew Chem. Int. Ed. Engl.*, **28,** 1153 (1990).
11. A. Willing, H. Folman and G. Aulig, *Eur, II, Biochem. Soc.*, **170,** 603 (1988).
12. A. S. Goldstein, R.H., R.H. Beer, R.S. Drago, *J. Am. Chem. Soc.*, **116** 2424 (1994).
13. J.W. Gobdes and W.H. Armstrong, *Inorg. Chem.*, **31,** 368 (1992).
14. M.L. Ludwig, K.A. Pattridge and W.C. Stallings, *Metabolism and Enzyme Function,* Academic Press, New York, 405, 1986.
15. R.D. Guieles, V.K. Yachanadra, A.E. Mc. Dermott, J.L. Cole, S.L. Dexheimer, R.D., Briti, K. Sauer and M.P. Klein, *Biochemistry*, **29**, 486 (1990).
16. A. Chaudhary, A. Phor and R.V. Singh, *Indian J. Chem.*, **41A**, 2536 (2002).
17. A. Chaudhary, S. Dave, R. Swaroop and R.V. Singh, *Indian J. Chem.*, **40A,** 757 (2001).
18. M.B.H. Howladar, M.S. Islam and M.R. Karim, *Indian J. Chem.*, **39A**, 407 (2000).
19. D.M. Adam and J.B. Carrool, *J. Chem. Soc., A*, **1968**, 1299.
20. R.H. Balundi and A. Chakravorty, *Inorg. Chem.*, **12**, 1981 (1975).
21. A.R. Katrityky, A.R. Hands and R.A. Jones, *J. Chem. Soc.*, 3165 (1958).
22. P.S. Mane, S.G. Shrokdar, B.R. Arbad and T.K. Chondheker, *Indian J. Chem.*, **40A**, 648 (2001).
23. R.A. Lal, A. Kumar and J. Chakravorty, *Indian J. Chem.*, **40A**, 422 (2001).
24. A. Chaudhary, S. Dave, R.K. Saini and R.V. Singh, *Main Group Met. Chem.*, **24,** 217 (2001).
25. S. Belwal, S. C. Joshi and R.V. Singh, *Main Group Metal Chem.*, **20,** 313 (1997).
26. A. Chaudhary and R.V. Singh, *Metal Based Drugs*, **8**, 315 (2002).
27. K. Sharma, S.C. Joshi and R.V. Singh, *Metal Based Drugs*, 7, 237 (2000).
28. A. Bansal, R.D. Singh and R.V. Singh, *Metal Based Drugs*, 7, 211 (2000).
29. S. Belwal, R.K. Saini and R.V. Singh, *Indian J. Chem.*, **37A**, 245 (1998).
30. S.C. Vyas *Fungicides,* Vol. 1, 2, 3, Oxford Indian Book House, New Delhi, 1995.
31. T. Mann and C. Mann Lutwak, in: *Male Reproduction Function and Semen,* Springer Verlag, Berlin, New York, 1981; p. 139.
32. G. Gupta, A.K. Srivastava and B.S. Shetty, *Indian J. Exptl. Biol.*, **33**, 281 (1995).
33. R.H. Dixon, *Reproductive Toxicity*, Raven Press, New York, 309 (1985).
34. P. Schulfer, H. Oyanguren, V. Maturana, A. Valenzuela, E. Cruz, V. Plaza, E. Schmid and R. Haddad, *Industrial Medicine and Surgery*, **26**, 167 (1957).