Spectroscopic and Antimicrobial Aspects of Macrocyclic Complexes of Lead (II) and Palladium (II)

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ABSTRACT

Lead (II) and palladium (II) macrocyclic complexes have been prepared by the template process using macrocyclic ligands. This affords a method to synthesize these complexes with different ring sizes. The macrocyclic ligands ($MacL^n$) react with $PbCl_2$, $Pb(NO_3)_2$, $Pb(OAc)_2.3H_2O$ or $PdCl_2$ and different diamines in 1:1:1 molar ratio in refluxing methanol to give several solid complexes of the type $[Pb(MacL^n)(R_B)X_2]$ and $[Pd(MacL^n)R_B]Cl_2$ (where, X = Cl, NO_3, or OAc). Initially the ligands and their complexes were characterized by elemental analysis, molecular weight determinations and conductivity measurements. The mode of bonding was established on the basis of IR, ^{1}HNMR, ^{13}C NMR, ^{207}Pb NMR and mass spectral studies. The macrocyclic ligand coordinates through the four azomethine nitrogen atoms which are bridged by benzil moieties. IR spectra suggest that the pyridine nitrogen is not coordinating. The palladium complexes exhibit tetra coordinated square-planar geometry whereas, a hexacoordinated octahedral geometry is suggested for lead complexes, All the complexes are monomeric. The antifungal and antibacterial activities of the ligands and their metal complexes have also been recorded and discussed.

INTRODUCTION

The chemistry of macrocyclic complexes is of significant interest due to the use of such complexes as dyes and pigments as well as MRI contrast agents and models for naturally occurring macrocyclic systems. The design of host molecules as receptors for the recognition of substrate anion guest molecules in aqueous solution is a very important target from an environmental, industrial and health-related point of view with multiple potential applications /1,2/.

The coordinating chemistry of square-planar metal complexes involving nitrogen donor ligands has excited great interest among chemists in recent years due to the applications of these compounds in catalysis

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/3/ and their relevance to bioinorganic systems. For instance, the complexes of platinum(II) and palladium(II) exhibit potent antitumor activity /4/. It is known that activity is associated with the configuration of the Pt (II) and Pd(II) complexes /5/. Macrocyclic ligands are chemically interesting as they show metal exchange (transmetallation) reactions which are useful for the synthesis of new metal complexes. A current review /6/ on synthetic chlorophyll and haemoglobin reveals the importance of polyazamacrocycles as oxygen carriers. Transition metal complexes of nitrogen donor ligands have been studied in detail, on account of their stereochemistry and wide practical utility /7/.

Porphyrinic macrocycles have been widely employed in the construction of synthetic light harvesting arrays owing to their desirable optical and photochemical features, as well as the desire to mimic the properties of photosynthetic light-harvesting antennas /8/. The chemistry of macrocyclic complexes has received much attention and such compounds have been extensively studied in recent years /9/. Multidentate Schiff bases have been used extensively in the preparation of various complexes of transition metals and main group elements, since such polydentate ligands provide unusual stabilization of different oxidation states as well as a rigid coordination sphere for the central element. The chemistry of these ligands is specially developed for transition metals /10/ because of their catalytic properties.

Some transition metal (II) complexes of polyaza macrocyclic ligands containing coordinated secondary amino groups are chemically oxidized to metal (II) complexes containing a higher degree of unsaturation in the ligand /11/. Macrocyclic complexes are thermodynamically quite stable and are involved in a variety of biochemical processes /12/. Interestingly, few reports /13/ have appeared in the literature where azacyclans have been shown to act as effective and selective catalysts for the electro and photochemical reduction of carbon dioxide. Keeping these facts in mind, we have synthesized and characterized the macrocyclic complexes of palladium (II) and lead (II) by the template process using the macrocyclic ligand and various diamines. This article deals with the striking structural features, synthesis and appreciable biological applications of these complexes.

EXPERIMENTAL

A clean and well dried glass apparatus fitted with quick fit interchangeable standard ground joints was used throughout the experimental work. All the solvents used were dried and purified by standard methods. Melting points were determined in sealed capillary tubes and are uncorrected. Fused calcium chloride towers were used to prevent the back diffusion of moisture from the vacuum pump. Palladium (II) chloride purchased from E. Merck was used as such. $PbCl_2$, $Pb(NO_3)_2$, $Pb(OAc)_2 .3H_2O$ (BDH) and the primary diamines, i.e. 1,3-diaminopropane, 1,2-phenylenediamine or 2,6-diaminopyridine were used as obtained from E. Merck.

Preparation of the Ligands : ($MacL^1$ and $MacL^2$)

The ligands ($MacL^1$ and $MacL^2$) were prepared by dissolving benzil in approximately 40mL of ethanol in a 100 mL round bottom flask. To this the calculated amount of diamines (2,6-diaminopyridine or 1,2-phenylenediamine) in ethanol was added in 2: 1 molar ratio. The reaction mixture was refluxed for 6-8 hours on a ratio head. It was then concentrated to half of the volume. The solution was cooled and the coloured crystalline compound that separated out was filtered. This was purified by recrystallization in the same solvent and dried *in vacuo*. The analysis and physical properties of these ligands are given in Table 1.

Synthesis of the Lead (II) Macrocyclic Complexes

A weighed amount of the methanolic solution of the ligands ($MacL^1$ and $MacL^2$) was taken into a dry 100 mL round bottom flask. The solution of ligands were treated with diamines, i.e. 2,6-diaminopyridine or 1,2-phenylene diamine, and metal salts, i.e., $PbCl_2$, $Pb(NO_3)_2$ or $Pb(OAc)_2\ 3H_2O$, in 1:1:1 molar ratio. The resulting mixture was refluxed for 7-10 hours. The contents were kept at room temperature overnight after reducing the volume of the solution to half, which led to the crystallization of the coloured complexes which were separated out. The crystals were washed with hot water, then with dry methanol so as to ensure their purity, and dried. The complexes were further purified by recrystallization from 1:1 solution of methanol and benzene and dried again *in vacuo*. The synthetic details and elemental analysis of the resulting lead complexes are listed in Table 1.

Synthesis of the Palladium (II) Macrocyclic Complexes

The macrocyclic complexes of palladium(II) were prepared by the template condensation reactions of ligands $MacL^1$ or $MacL^2$ and diamines, i.e. 1,3-diaminopropane, 2,6-diaminopyridine or 1,2-phenylenediamine, in the presence of $PdCl_2$ in 1:1:1 molar ratio in dry methanol. The contents were refluxed for about 7-10 hours on a ratio head. It was then concentrated to half of the volume by removing the solvent. The reaction mixture was transferred to an evaporating dish and kept overnight at room temperature. The coloured solid products separated out. They were collected, washed repeatedly with hot water then with dry and cold MeOH so as to ensure their purity and dried. The complexes were further purified by recrystallization from equimolar ratio of methanol and benzene and dried again under reduced pressure. The details of these reactions and analyses of the resulting products are recorded in Table 1.

Analytical Methods and Physical Measurements

The purity of the complexes was checked by TLC on silica Gel-G using anhydrous methanol and benzene (1:1) as solvent. Each of the compounds moves as a single spot indicating the presence of only one component and hence their purity.

Table 1

Analysis and Physical Characteristics of the Ligands, Palladium and Lead Complexes

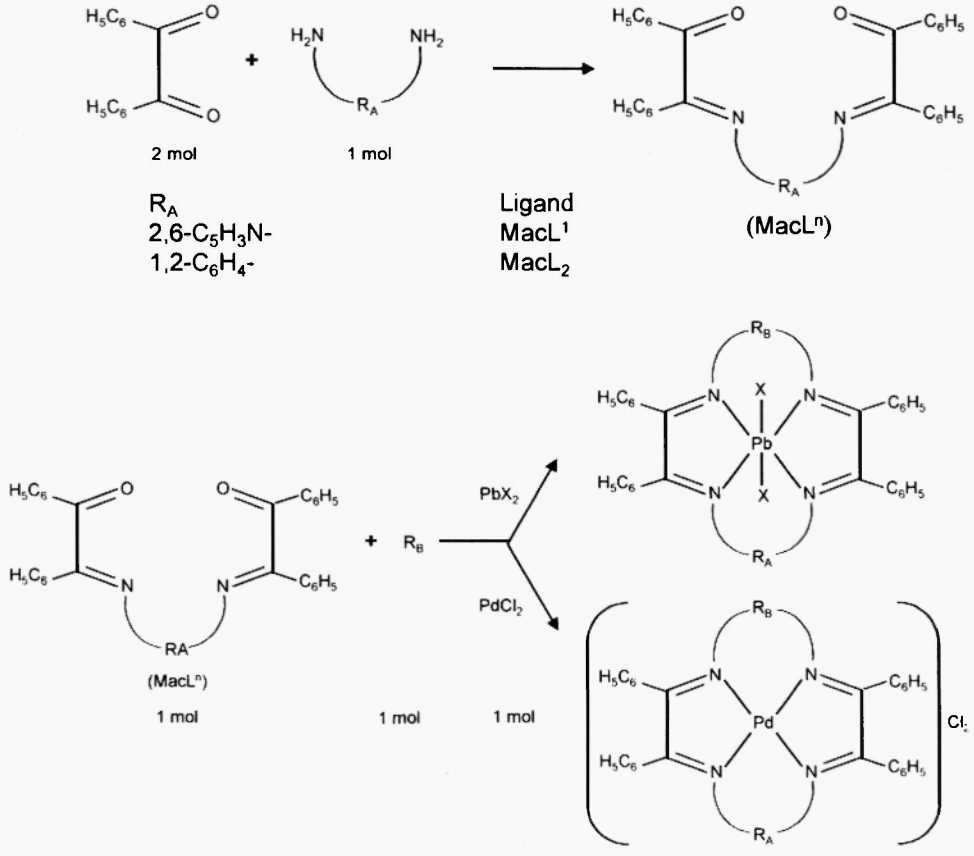
Reactants (g)			Compound Formed (Molecular Formula)	M.P. (°C) and Colour	% Analysis Found (Calcd.)			Mol. Wt. Found (Calcd.)
Metal Salt	Ligand	Diamines			N	C	M	
-	$C_{13}H_{23}N_3O_2$ (MacL^1)	-	-	99 Green	8.09 (8.51)	-	-	469 (493)
$PbCl_2$ (0.3975)	$C_{33}H_{23}N_3O_2$ (0.5172)	$C_5H_7N_3$ (0.1133)	$[Pb(C_{18}H_{26}N_6)Cl_2]$	109 Green	9.51 (9.95)	7.95 (8.39)	24.07 (24.52)	813 (844)
$Pb(OAc)_2.3H_2O$ (0.6239)	$C_{13}H_{23}N_3O_2$ (0.8118)	$C_5H_7N_3$ (0.1795)	$[Pb(C_{38}H_{21}N_6)(OAc)_2]$	126 Dark Green	8.97 (9.42)	-	22.76 (23.23)	866 (891)
$Pb(OAc)_2.3H_2O$ (0.3123)	$C_{13}H_{23}N_3O_2$ (0.4054)	$C_6H_8N_2$ (0.0890)	$[Pb(C_{19}H_{27}N_4)(OAc)_2]$	111 Yellowish Green	7.43 (7.86)	-	22.75 (23.25)	909 (890)
$PdCl_2$ (0.2401)	$C_{33}H_{23}N_3O_2$ (0.6682)	$C_3H_{10}N_2$ (0.1004)	$[Pd(C_{36}H_{29}N_5)Cl_2$	136 Light Brown	9.42 (9.88)	9.60 (10.00)	14.55 (15.01)	684 (708)
$PdCl_2$ (0.2235)	$C_{13}H_{23}N_3O_2$ (0.5221)	$C_5H_7N_3$ (0.1375)	$[Pd(C_{38}H_{21}N_6)]Cl_2$	107 Off White	10.86 (11.30)	9.11 (9.53)	13.83 (14.30)	767 (743)
-	$C_{14}H_{24}N_2O_2$ (MacL^2)	-	-	82-84 Light yellow	5.25 (5.68)	-	-	461 (492)
$Pb(OAc)_2.3H_2O$ (0.5742)	$C_{34}H_{24}N_2O_2$ (0.7456)	$C_6H_8N_2$ (0.1637)	$[Pb(C_{40}H_{28}N_4)(OAc)_2]$	168 Yellowish brown	6.68 (6.30)	-	22.78 (23.28)	858 (889)
$Pb(NO_3)_2$ (0.4221)	$C_{34}H_{24}N_2O_2$ (0.6278)	$C_6H_8N_2$ (0.1378)	$[Pb(C_{10}H_{28}N_4)(NO_3)_2]$	116-120 Chocolate brown	8.93 (9.38)	-	22.68 (23.13)	867 (895)
$PdCl_2$ (0.2657)	$C_{34}H_{24}N_2O_2$ (0.7381)	$C_3H_{10}N_2$ (0.1111)	$[Pd(C_{37}H_{30}N_4)]Cl_2$	99 Cream	7.51 (7.91)	9.56 (10.01)	14.90 (15.03)	671 (707)

Conductivity measurements in dry DMF were performed with a Systronics Conductivity Bridge type 305 and molecular weights were determined by the Rast Camphor method /14/. IR spectra of the solid samples were recorded as KBr discs on a model Nicolet Magna FTIR-550 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL FX-90Q spectrometer with Me_4Si as an internal standard. The chemical shift values were determined in ppm relative to an external reference Me_4Si. Nitrogen and chlorine were estimated by Kjeldahl's and Volhard's methods, respectively /15/. Lead was estimated as lead sulphate and palladium was estimated gravimetrically /16/. ^{207}Pb NMR spectra were recorded on a JEOL FX 200 spectrometer.

RESULTS AND DISCUSSION

The elemental analysis and spectral data suggested the formation of the ligands ($MacL^1$ and $MacL^2$) along with the macrocyclic complexes $[Pb(MacL^n)(R_B)X_2]$ and $[Pd(MacL^n)(R_B)]Cl_2$. The resulting macrocyclic complexes are coloured solids, stable at room temperature and non-hygroscopic. They are soluble in organic solvents, DMF and DMSO. Molecular weights of the complexes indicated the monomeric nature of the complexes. The conductivity values of 10^{-3} M solutions of the lead compounds in the anhydrous DMF are in the range 10-28 $ohm^{-1}cm^{2}$ mol^{-1} showing them to be non-electrolytes, while those of the palladium complexes appear in the range (205-220 $ohm^{-1}cm^{2}$ mol^{-1}) expected for 1:2 electrolytes /17/.

A proposed scheme of the synthetic route for ligands and their complexes is given below :



Where, R_B = 1,3-diaminopropane, 2,6-diaminopyridine or 1,2-phenylenediamine, and
X = Cl, NO_3 or OAc

IR Spectra

The preliminary identification of the macrocyclic ligands and their complexes has been obtained from their infra-red spectra. The IR spectra of the ligands have bands due to ν(C=O) at 1670-1681 cm^{-1}. In the metal complexes, the infra-red spectra show the appearance of the ν(C=N) absorption /18/ at around 1620-1600 cm^{-1}. The appearance of this band, coupled with the absence of the ν(C=O) band, provide conclusive evidence of the condensation of the diamine with the benzil. This contention is supported by the presence of bands at 1470 - 1490 and 1365 - 1385 cm^{-1} characteristic of the benzil moiety and may be assigned to ν_{asym} C_6H_5 and ν_{sym} C_6H_5, respectively. Aromatic ring stretching (C.....C) /19/ bands are present at 1600, 1584 and 1492 cm^{-1}. The spectra do not show any changes in the pyridine ring vibrations and it appears that in these complexes the nitrogen atom of pyridine does not participate in the coordination. Strong and sharp bands for C-H stretching and bending vibrations appear at 2835 and 1415 cm^{-1}, respectively /20/. The presence of aromatic C-N band in the complexes appeared at 842 cm^{-1}. It is apparent from the spectra that both amino groups of the diamines react with the oxygen atom of benzil, forming a two-carbon atom bridge between the two amino groups, similar to those reported in coordinated amines /21/. The frequencies of the ν(C=N) vibrations indicated coordination through this site. Thus, in the presence of metal salts a quadridentate macrocyclic complex is formed, which coordinates through azomethine nitrogens, while pyridine nitrogen does not take part in the coordination.

The appearance of new bands in the spectra of the metal complexes in the far IR region 415-450 cm^{-1} due to ν(M-N) vibrations /22/ unequivocally supports the coordination of the imine nitrogens to the metal ions. In the spectrum of the nitro complex, bands around 1254, 1012 and 865 cm^{-1} are in agreement with the monodentate nature of the nitrito group /23/.

^{1}H NMR Spectra

The proton magnetic resonance spectra of the ligands, as well as their respective metal derivatives, have been recorded in DMSO-d_6 using TMS as an internal standard. The spectra of the complexes do not show any signal corresponding to the primary amino protons. This suggests that the proposed macrocyclic skeleton has been formed. The ^{1}H NMR spectra of Pd(II) complexes display a quintet at δ2.15 - 2.25 ppm due to β-protons and a triplet at δ3.60 - 3.70 ppm assigned to the α-protons of the propylene bridge, in the case of 1,3-diaminopropane. The complex pattern of the aromatic protons were observed at δ7.27 - 8.33 ppm in the spectra of the ligands. A downfield shifting in the position of this complex pattern strongly supports the coordination of the azomethine nitrogens to the central metal atom.

Mass Spectra

The FAB mass spectrometry is receiving increased attention for studying the structures of coordination complexes. This technique provides gentle ionization of the volatile samples, resulting in no or minimal fragmentation of the parent ions. The FAB mass spectra of palladium complexes [{$Pd(C_{38}H_{26}N_6)$}Cl_2] and

$[\{Pd(C_{37}H_{30}N_4)\}Cl_2]$ were recorded on a JEOL SX 102/DA-6000 mass spectrometer /data system using Argon/Xenon as the FAB gas. The accelerating voltage was (10KV) and the spectrum was recorded at room temperature. m-Nitrobenzyl alcohol (NBA) was used as matrix. The mass spectrum of $[\{Pd(C_{38}H_{26}N_6)\}Cl_2]$ shows the molecular ion peak at m/z 743, which confirms the monomeric nature of the complex. The prominent fragments are as follows: 666 $[\{Pd(C_{32}H_{21}N_6)\}Cl_2]^+$; 589 $[\{Pd(C_{26}H_{16}N_4)\}Cl_2]^+$; 565 $[\{Pd(C_{24}H_{16}N_6)\}Cl_2]^+$; 537 $[\{Pd(C_{24}H_{16}N_4)\}Cl_2]^+$; 533 $[\{Pd(C_{28}H_{20})\}Cl_2]^+$ and 460 $[\{Pd(C_{19}H_{13}N_3)\}Cl_2]^+$. Another peak, appearing at m/z 672, is due to the loss of two chlorine atoms from the parent ions.

The FAB mass spectrum of the another complex $[\{Pd(C_{37}H_{30}N_4)\}Cl_2]$ shows the molecular ion peak at m/z = 707, which confirms the monomeric nature of the compound. Other fragments were observed as follows: 665 $[\{Pd(C_{34}H_{24}N_4)\}Cl_2]^+$; 637 $[\{Pd(C_{34}H_{24}N_2)\}Cl_2]^+$; 631 $[\{Pd(C_{31}H_{26}N_4)\}Cl_2]^+$; 630 $[\{Pd(C_{31}H_{25}N_4)\}Cl_2]^+$; and 601 $[\{Pd(C_{37}H_{30}N_4)\}Cl_2]^+$. A peak, appearing at m/z 636, is due to the loss of two chlorine atoms from the parent ion.

^{13}C NMR Spectra

The ^{13}C NMR spectra of the macrocyclic complexes are comparable to those of their ligands and the assigned peaks positions are listed in Table 2. The shifting in the positions of carbon resonances attached to the azomethine nitrogen indicate the involvement of these atoms in coordination and support the formation of macrocyclic frame work, as reported earlier also /24,25/.

^{207}Pb NMR Spectra

The ^{207}Pb NMR spectra of the $[Pb(C_{38}H_{26}N_6)Cl_2]$ and $[Pb(C_{40}H_{28}N_4)(NO_3)_2]$ complexes give signals at δ 2205 and δ2240 ppm, respectively, indicating coordination number six in the complexes around the lead atom. These results are found to be in accordance with the results obtained by West *et al.* /26/ and Godwin *et al.*/27/.

On the basis of the above studies, we can say that the ligands are behaving as tetradentate chelating agents having four coordinating sites. Secondly, the X^- ions remain bonded to the metal atom. Therefore, a hexa-coordinated octahedral geometry around the lead atom and tetracoordinated square planar geometry around palladium atom seems to be reasonable.

Table 2

^{13}C NMR Spectral Data (δ, ppm) of the Ligands and their Corresponding Metal Complexes.

Compound	>C=O	>C=N	>C-N	R		Aromatic Carbons			
				C_3	C_2	C_1	C_2	C_3	C_4
$C_{33}H_{21}N_3O_2$(MacL^1)	174.48	156.72	142.87	131.21	135.30	127.16	126.60	126.24	125.32
[Pb(C$_{18}$H$_{26}$N$_6$)Cl$_2$]	-	150.79	139.45	134.19	138.82	129.31	128.16	127.99	127.46
[Pb(C$_{18}$H$_{26}$N$_5$)(OAc)$_2$]	-	150.11	140.07	133.53	138.44	129.48	128.69	128.25	127.58
[Pb(C$_{19}$H$_{27}$N$_5$)(OAc)$_2$]	-	152.98	139.88	132.94	137.51	129.56	129.22	128.30	127.85
[Pd(C$_{16}$H$_{29}$N$_5$)]Cl$_2$	-	154.31	140.37	134.83	140.96	128.15	127.72	127.55	126.46
[Pd(C$_{18}$H$_{26}$N$_6$)]Cl$_2$	-	153.37	142.16	134.23	139.52	128.33	126.88	126.21	125.40
$C_{34}H_{21}N_3O_2$(MacL^2)	175.52	157.22	143.67	134.50	136.68	127.67	127.28	126.51	125.79
[Pb(C$_{10}$H$_{28}$N$_4$)(OAc)$_2$]	-	153.92	141.84	130.52	139.52	129.60	129.52	128.70	127.30
[Pb(C$_{10}$H$_{28}$N$_4$)(NO$_3$)$_2$]	-	152.64	140.71	131.30	138.62	128.97	127.54	127.10	126.90
[Pd(C$_{17}$H$_{10}$N$_4$)]Cl$_2$	-	154.11	140.17	134.22	140.30	127.96	127.64	127.10	126.32

Biocidal Activity

The study of synthetic macrocyclic compounds is an important area of chemistry in view of their presence in many biologically significant naturally occurring metal complexes, such as metallo-proteins and chlorophyll. In these macrocyclic complexes, both the metal ion and the size of the ring play an important role /28/. Antimicrobial agents are chemical compounds biosynthetically or synthetically produced, which either destroy or usefully suppress the growth of metabolism of a variety of microscopic or submicroscopic forms of life. On the basis of their primary activity, they are more specifically called antibacterial, antifungal, antiprotozoal, antiparasitic or antiviral agents. Of the thousands of antimicrobial agents, only a small number are safe as chemotherapeutic agents, which are effective in controlling infectious diseases in plants, animals and humans. A much larger number are used in almost every phase of human activity, in agriculture, food preservation, water, skin and air disinfection /29/.

Activities of fungi and bacteria on several compounds give more important information about complexes. This prompted us to screen all the macrocyclic complexes and ligands to find out which part of the molecule is actually responsible for its physiological activity. Fungicidal and bactericidal activities of the ligands and their metal complexes against pathogenic fungi and bacteria are recorded in Tables 3 and 4.

Table 3

Antifungal Screening Data of the Ligands and their Corresponding Metal Complexes, Percent Growth Inhibition After 4 Days at 25 ± 2 °C (Conc. 25, 50, 100 and 200 ppm).

Compound	*Alternaria alternata*				*Alternaria brassicae*			
	25	50	100	200	25	50	100	200
$C_{33}H_{23}N_3O_2$(MacL^1)	43	55	66	77	40	51	61	69
$[Pb(C_{38}H_{26}N_6)Cl_2]$	50	66	76	90	46	63	80	88
$[Pb(C_{38}H_{26}N_6)(OAc)_2]$	53	69	82	98	50	67	78	92
$[Pb(C_{39}H_{27}N_5)(OAc)_2]$	52	67	79	94	48	61	74	90
$[Pd(C_{36}H_{29}N_5)]Cl_2$	45	57	69	78	44	52	63	70
$[Pd(C_{38}H_{26}N_6)]Cl_2$	47	56	67	80	46	54	62	73
$C_{34}H_{24}N_2O_2$(MacL^2)	40	51	62	70	36	47	55	62
$[Pb(C_{40}H_{28}N_4)(OAc)_2]$	51	66	78	89	48	60	72	81
$[Pb(C_{40}H_{28}N_4)(NO_3)_2]$	53	68	81	88	47	62	70	80
$[Pd(C_{37}H_{30}N_4)]Cl_2$	45	52	64	73	40	50	56	68
Bavistin	84	87	100	100	82	91	100	100

Table 4

Antibacterial Screening Data of the Ligands and their Corresponding Metal Complexes, Inhibition (mm) after 24 hours at 28 ± 2 °C (Conc. 500 and 1000 ppm)

Compound	*Xanthomonas compestris*		*Pseudomonas syringae*	
	500	1000	500	1000
$C_{33}H_{23}N_3O_2$ (MacL^1)	4	6	6	8
$[Pb(C_{38}H_{26}N_6)Cl_2]$	7	9	9	11
$[Pb(C_{38}H_{26}N_6)(OAc)_2]$	8	9	10	13
$[Pb(C_{39}H_{27}N_5)(OAc)_2]$	8	10	10	12
$[Pd(C_{36}H_{29}N_5)]Cl_2$	5	8	8	11
$[Pd(C_{38}H_{26}N_6)]Cl_2$	6	8	8	10
$C_{34}H_{24}N_2O_2$(MacL^2)	5	8	5	8
$[Pb(C_{40}H_{28}N_4)(OAc)_2]$	10	13	10	14
$[Pb(C_{40}H_{28}N_4)(NO_3)_2]$	9	12	9	12
$[Pd(C_{37}H_{30}N_4)]Cl_2$	7	10	6	10
Streptomycin	3	5	2	3

EXPERIMENTAL

Radial Growth Method for Antifungal Activity

The antifungal activities were evaluated by Radial Growth Method /30/ using Czapek's agar medium, having the composition: glucose – 20g, starch – 20g, agar-agar –20g and distilled water 1000 mL, which was prepared in a flask and sterilized. To this medium was added the requisite amount of the compound, after being dissolved in methanol so as to get a certain final concentration (25, 50, 100 and 200 ppm). The medium was then poured into the Petri plates and a small disc (0.7 cm) of the fungus culture was cut with a sterile cork borer and transferred aseptically to the centre of a Petri dish containing the medium with a certain amount of the compound. These Petri plates were wrapped in polythene bags containing a few drops of alcohol and were placed in an incubator at 25 ± 2°C. The controls were also run and three replicates were used in each case. The colony diameter, after 96 hours, compared with control was taken as a measure of fungitoxicity. The amount of growth inhibition was calculated by the equation

$$\% \text{ inhibition} = \frac{(d_c - dt) \times 10}{d_c}$$

where, dc = Diameter of the fungal colony in control or check plate.
dt = Diameter of the fungal conlony in the test plate.

The organism used in these investigations included *Alternaria alternata* and *Alternaria brassicae*.

Paper Disc Plate Method for Antibacterial Activity

The activity against bacteria was evaluated by the inhibition zone technique /31/. Flat bottomed 90mm Pyrex Petri dishes were used. 15mL nutrient agar medium, having the composition peptone – 5g, beef extract – 5g, NaCl – 5g, agar-agar –20g and distilled water – 1000mL, was pipetted into the Petri dish. After the agar solidified, 5mL of warm seeded agar was applied. The seeded agar was prepared by cooling the molten agar to 40°C and then adding the amount of bacterial suspension. The compounds were dissolved in methanol in 500 and 1000 ppm concentrations.

Paper discs of Whatman No.1 filter paper with a diameter of 5mm were soaked in these solutions of varied concentrations. The discs were dried and placed on the medium previously seeded with the organism in Petri plates at suitable distances. The Petri plates were stored in an incubator at 28 $\pm$ 2°C for 24 hours. The zone of inhibition, thus formed around each disc containing the test compounds, was measured accurately in mm. The organisms used in the present investigations included *Xanthomonas compestris* and *Pseudomonas syringae*.

The experimental results show that there is an increase in the toxicity of the complexes as compared to the ligands, and the inhibition of the growth of the microorganisms was found to be dependent on the solubility, concentration, fineness of particles and the size of the metal ion.

The factors determining the potency of antimicrobials are as follows :

(a) Concentration of the substance
(b) Time of action
(c) pH of the medium
(d) Temperature and
(e) Nature of the organisms.

The enhanced antimicrobial activity of the metal chelates over their corresponding chelating agents may be explained by the chelation theory. Chelation reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups and possible π-electron delocalization over the whole chelate ring. This increases the lipophilic character of the metal complexes, which subsequently favour its permeation through the semi-permeable defences of cell membrane of the microorganism, thereby impairing the normal cell process /33/.

Mode of Action

Antimicrobials can attack various targets in microorganisms, as a consequence of which organisms either are destroyed or their growth is inhibited. Since the complexes inhibited the growth of microorganisms, it is assumed that the production of the enzymes is being affected and hence the microorganisms are unable to utilize the food themselves or the intake of ions decreases and, consequently, the growth ceases. At lower concentrations, when the enzyme is leached out, the growth of the microorganisms is slowed; though very little enzyme is being produced, its amount is sufficient for the need of the microorganism to grow; but a higher concentration proves fatal to the microorganisms. The higher concentration destroys the enzyme

mechanisms by blocking any of the metabolic pathways (viz., lipid, carbohydrates and amino acids) and hence due to lack of availability of proper food, the organism dies. Further, the results of biocidal activity have been compared with the conventional fungicide *Bavistin* and conventional bactericide *Streptomycin* used as standards.

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