STUDIES ON CEPHRADINE AND ITS COMPOUNDS WITH TIN(II), LEAD(II), MANGANESE(II) AND IRON(II)

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Abstract

New complexes of tin(II), lead(II), manganese(II) and iron(II) with antibacterial cephradine have been isolated and characterized by elemental analysis, IR, Electronic, Magnetic moment, ^{57}Fe, Mössbauer, ^{1}H NMR, ^{13}C and ^{119}Sn NMR spectral studies. The spectral data suggested hexacoordinated state for these complexes. Conductivity data suggested that they behave as non-electrolytes. The formulation of the complexes of the type $[M(L)_2Cl_2]$, {where, M = Sn(II), Pb(II), Mn(II) and Fe(II) and L = cephradine} showing octahedral geometry. In order to evaluate the effect of metal ions upon chelation, cephradine and its complexes have been screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Introduction

The development and design of new products with the potential for the use as biologically active compounds has recently become a burgeoning topic within the biological sciences[1-15] and chemistry in particular[16-19]. Evidences supporting the introduction of metallic elements in several biological process are rapidly accumulating[20-22]. Schubert[23] and Kirschner[24] have investigating the antibacterial, antiviral and anticancer activities of more than 25 inorganic compounds, which included the metal atom as potentially significant part of the molecule. They suggested[24,25] that the transfer of metal ions from the ligand to the cancer-associated viruses was a mechanism for releasing the anticancer drug in the locality of the tumor. Due to the significant nature of the metallic ions, their metal complexes are now, being included in the search for ideal anticancer drugs. Such significant examples of which are palladium and platinum of 6-mercaptopurine that destroy[26] adenoca, rcinomas and similarly, the complexes of dialkyldithiophosphate which reduce some tumors[27]. A wide range of activities of even the simplest complexes of metallic elements has been reviewed by Rosenberg[28] who noticed that the viral-induced cancers all respond to the treatment with even the simplest metal-amino halide complexes. It has also been demonstrated that chelation/complexation tend to make inactive substances/ligand active and active compounds/drugs become more active and less toxic[29,30]. All these evidences however, highlight the need to study and evaluate more the biological applications of metallic elements for therapeutic potentials.

Cephradine is a first generation cephalosporin class of antibiotic[31], used against Gram-positive cocci and Gram-negative bacilli. It has similar structural and antibacterial relationship to that of its closely related analogue cephalexin. It contains the (NH_2), (COOH), (NH) and (C=O) functional groups and its molecular model reveals that its structure is suitable for chelation/complexation. A detailed biological evaluation of the copper(II) and zinc(II) complexes of cephalexin has been reported[32]. In order to evaluate the biological comparison of both the analogues, we, therefore, report in this paper the preparation, characterization and

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Experimental

All chemicals and solvents used were of Analar grade. $SnCl_2$, $PbCl_2$, $MnCl_2.4H_2O$ and $FeCl_2.4H_2O$ were used as obtained from B.D.H.

Synthesis of the Complexes

A methanolic solution (25 mL) of tin chloride (0.90 g; 4.7 mmol) was added to a magnetically stirred sodium salt of cephradine (3.31; 9.4 mmol) in distilled water (15 mL). The reaction mixture was refluxed for 6 h and cooled to room temperature. On cooling white precipitate was formed, filtered and washed with methanol and ether, and dried *in vacuo*. The complex [tin(II) cephradine] was recrystallized in benzene.

All other heterocyclic metal complexes were formed following the same method.

Analytical Methods and Physical Measurements

The molecular weights were determined by the Rast Camphor Method. Conductivity measurements were made with a systronic model 305 conductivity bridge in dry dimethylformamide. The IR spectra of the solid samples were recorded as KBr discs on a Nicoletmagna FTIR 550 spectrophotometer. Electronic spectra recorded on UV-160A, Shimadzu spectrophotometer in the range 200-600 nm using methanol as the solvent. The ^1H NMR and ^{13}C NMR spectra were recorded on a FX 90 Q spectrometer in DMSO-d_6 using TMS as the internal standard. ^{119}Sn NMR spectra of the compounds were also recorded on the same spectrometer at 33.35 M Hz.

Results and Discussion

All these complexes are coloured solids, which do not have sharp melting points. These are decomposed above 215°C. The conductivity values measured for 10^{-3} M solutions in anhydrous dimethylformamide are in the range 14-21 ohm^{-1} cm^2 and mol^{-1} showing them to be non-electyrolytes. Molecular weights of the complexes indicated the monomeric nature of the complexes. Elemental analyses agree well with the stoichiometry and chemical formula $[M(L)_2Cl_2]$, where, M = Sn(II), Pb(II), Mn(II) and Fc(II) and L = Cephradine. The physical properties and analytical data of the complexes are given in Table 1.

Table 1 - Physical Properties and Analytical Data of the Metal Complexes.

Empirical formula and M.P. (°C)	Analysis (%) Found (Calcd.)				Mol. Wt. Found. (Calcd.)
	C	H	N	Cl	
$C_{32}H_{36}N_6O_8S_2SnCl_2$	37.5	3.4	6.7	6.9	986
163	(37.7)	(3.5)	(8.2)	(7.0)	(1017)
$C_{32}H_{36}N_6O_8S_2PbCl_2$	34.6	2.7	6.7	5.7	1088
179	(34.7)	(2.9)	(7.6)	(6.4)	(1105)
$C_{32}H_{36}N_6O_8S_2MnCl_2$	39.8	3.6	7.4	6.8	933
156	(40.0)	(3.7)	(8.7)	(7.3)	(961)
$C_{32}H_{36}N_6O_8S_2FeCl_2$	39.9	3.6	7.72	6.6	937
181	(40.0)	(3.7)	(8.7)	(7.3)	(960)

The IR spectra of the complexes in comparison to the uncomplexed cephradine are listed in Table 2 with some tentative important characteristic assignments[33]. The IR spectrum of the cephradine shows some

characteristic bands at 3340, 3247, 1766 and 1745 cm^{-1} mainly due to the $\nu(NH_2)$, $\nu(NH)$, $\nu(COOH)$ and $\nu(C=O)$ vibrations, respectively. The metal complexes also contained other bands indicative of the coordination of the ligand with the metal ions. The band due to $\nu(COO)$ asym at 1766 cm^{-1} in the spectrum of the ligand shifted to lower frequency (10-15 cm^{-1}) in all the metal complexes indicative of the complexation[34]. New absorption bands assigned to $\nu(COO)$ and $\nu(M-O)$ appeared at 1566-1577 cm^{-1} and 419-429 cm^{-1} which were only observed in the spectra of the complexes. This in turn, indicated that the carboxyl group is coordinated to the metal ion. Also, the band due to $\nu(NH_2)$ at 3340 cm^{-1} was found shifted to lower wave number in the spectra of its metal complexes. A new band at 450-459 cm^{-1} assigned to $\nu(M-N)$ was evolved in the spectra of the complexes indicating[35] involvement of the $\nu(NH_2)$ group via nitrogen in the coordination. In the far infrared region a band at 351-367 cm^{-1} was found in the complexes assigned to $\nu(M-Cl)$ modes. It in turn, suggested that two chloride atoms are also coordinated to the metal atom. These evidences support the octahedral geometry for these complexes.

Table 2 - IR Spectra Data (cm^{-1}) of the Metal Complexes.

Compound	νNH_2	νNH	$\nu(COOH)$	$\nu C=O$	$\nu(COO)$	$\nu(M-N)$	$\nu(M-Cl)$	$\nu(M-O)$
Cephradine	3340	3247	1766	1745	-	-	-	-
$[Sn(L)_2Cl_2]$	3537	3246	-	1743	1754, 1571	459	367	425
$[Pb(L)_2Cl_2]$	3541	3244	-	1741	1759, 1566	454	351	419
$[Mn(L)_2Cl_2]$	3545	3242	-	17.40	1755, 1577	450	355	423
$[Fe(L)_2Cl_2]$	3529	3253	-	1737	1750, 1565	452	364	429

The electronic spectrum of the $[Mn(L)_2Cl_2]$ exhibited absorption bands at 587, 449 and 376 nm attributed to $^6A_{1g} \rightarrow {}^4T_{1g}$, $^6A_{1g} \rightarrow T_{2g}$ and $^6A_{1g} \rightarrow {}^4t_{1g}$, respectively[36]. The electronic spectrum of the $[Fe(L)_2Cl_2]$ display a weak intensity band at 868 nm[37]. These are found to be consistent with the octahedral geometry for these complexes.

The valucs $\mu\beta$ for the complex $[Mn(L)_2Cl_2]$ were observed at 5.88 B.M. and are within the range required for an octahedral grometry[38].

The mössbauer speatrum of the complex $[Fe(L)_2Cl_2]$ has been recorded. The values of the isomer shift (0.27 mm δ^{-1}) and quadrupali splitting (0.65 mm δ^{-1}) at room temperature are characteristic of six coordinated low spin iron complex[39].

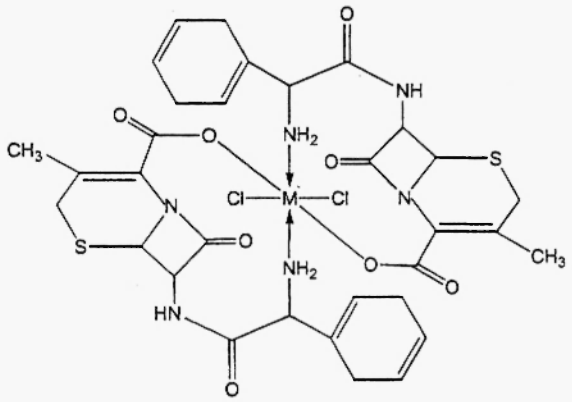
^1H and ^{13}C NMR spectra of the uncomplexed cepharadine and its $[Sn(L)_2Cl_2]$ and $[Pb(L)_2Cl_2]$ complexes have been recorded in DMSO-d_6 with TMS as internal reference and are summarized in Table 3. The spectra of cephradine exhibited peaks that are expected[40] for its structure. Signals for the NH and COOH protons in the spectra of uncomplexed cephradine at δ 10.8 ppm disappeared in the complexes. This indicated that cephradine is coordinated to the metal atom by deprotonation. In the metal complexes the aromatic proton signals appeared downfield due to increased conjugation during coordination[41] are characteristic of six-coordinated low spin iron complex[39].

Table 3 - ^{1}H NMR and ^{13}C NMR (δ, ppm) Spectral Data of Metal Complexes.

Compounds	^{1}H NMR (ppm)	^{13}C NMR (ppm)
Cephradine	2.1 (s, 6H, CH_3), 46 (d, 2H, NCH), 7.1 (m, 4H, Ph), 7.1 (m, 4H, Ph), 7.4 (m, 2H, Ph), 8.1 (d, 2H, β-lactam) 8.3 (m, 4H, hetero-Ph), 8.4 (m, 2H, hetero-Ph).	13.3 (CH_3), 62.8 (CHN), 117.6 (β-lactam), 119.6, 122.2, 124.6 (hetero-Ph), 148 2, 125.4, 127.0, 129.1, 172.2 (C=O), 177.2 (COO), 211.6 (β-lactam)
$[Sn(L)_2Cl_2)]$	2.1 (s, 6H, CH_3), 46 (d, 2H, NCH), 7.1 (m, 4H, Ph), 7.1 (m, 4H, Ph), 7.4 (m, 2H, Ph), 8.1 (d, 2H, β-lactam) 8.3 (m, 4H, hetero-Ph), 8.4 (m, 2H, hetero-Ph).	13.3 (CH_3), 62.8 (CHN), 117.6 (β-lactam), 119.6, 122.2, 124.6 (hetero-Ph), 148 2, 125.4, 127.0, 129.1, 172.2 (C=O), 177.2 (COO), 211.6 (β-lactam)
$[Pb(L)_2Cl_2)]$	2.1 (s, 6H, CH_3), 46 (d, 2H, NCH), 7.1 (m, 4H, Ph), 7.1 (m, 4H, Ph), 7.4 (m, 2H, Ph), 8.1 (d, 2H, β-lactam) 8.3 (m, 4H, hetero-Ph), 8.4 (m, 2H, hetero-Ph).	13.3 (CH_3), 62.8 (CHN), 117.6 (β-lactam), 119.6, 122.2, 124.6 (hetero-Ph), 148.2, 125.4, 127.0, 129.1, 172.2 (C=O), 177.2 (COO), 211.6 (β-lactam)

The ^{119}Sn NMR spectrum of the $[Sn(L)_2Cl_2]$ complex gives signal at δ-589 ppm indicating coordination number six in the complex around tin atom[42].

On the basis of the above observations, it is tentatively suggested that all the complexes show an octahedral geometry (Fig.1) in which two cephradine molecules act as bidentate and at axial positions two chlorides are coordinated to the metal atom.



M = Sn(II), Pb(II), Mn(II) and Fe(II)

(Fig. 1)

Biological Properties

Bioefficacies of the synthesized compounds were checked *in vitro* and *in vivo*. The *in vitro* antifungal, activities of the ligands and their complexes have been evaluated against several fungi by the Radial Growth Method[43]. The compounds were directly mixed with the medium in different concentrations. Controls were also run and three replicates were used in each case. The linear growth of the fungus was obtained by measuring the diameter of the fungal colony after four days (Table 4). The amount of growth inhibition in each of the replicates was calculated by :

$$\% \text{ inhibition} = \frac{(\delta c - \delta t) \times 100}{\delta c}$$

Where δc is the diameter of the colony on the control plate and δt is the diameter of the fungal colony on the test plate.

The synthesized metal complexes, in comparison to the uncomplexed cephradine were also screened for their antibacterial activity against pathogenic bacterial species, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The paper disc diffusion method was adopted for the determination of antibacterial activity. The compounds were tested at a concentration of 30 μg/0.01 mL in DMF solution using paper disc diffusion method[43]. The susceptibility zones were measured in diameter (mm) and the results are reported in Table 5. Cephradine and its metal complexes individually were found to be biologically active showing various degrees of inhibitory effect on the growth of the tested bacterial species. The bacterial results evidently show that the complexation improved the antibacterial activity.

Table 4 - Fungicidal Screening Data of Metal Complexes.

Treatment	Concentration (ppm)	*Alternaria alternata*			*Fusarium oxysporum*		
		R_1 (angular value)	R_2 (angular value)	R_3 (angular value)	R_1 (angular value)	R_2 (angular value)	R_3 (angular value)
$[Sn(L)_2Cl_2)]$	25	45 (42.13)	43 (40.98)	38 (38.06)	46 (42.71)	39 (28.65)	40 (39.23)
	50	54 (47.29)	53 (46.72)	51 (45.57)	52 (46.15)	55 (47.87)	52 (46.15)
$[Pb(L)_2Cl_2)]$	25	35.0 (36.27)	39 (28.65)	40 (39.23)	34 (35.67)	35 (26.27)	39 (38.65)
	50	48 (43.85)	52 (46.15)	50 (45.00)	46 (42.71)	50 (45.00)	51 (45.57)
$[Mn(L)_2Cl_2)]$	25	46 (42.71)	48 (43.85)	50 (45.00)	39 (28.65)	42 (40.40)	43 (40.98)
	50	55 (47.85)	56 (49.02)	54 (47.29)	54 (47.29)	55 (47.87)	54 (47.29)
$[Fe(L)_2Cl_2)]$	25	41 (39.82)	39 (38.65)	40 (39.27)	37 (37.46)	38 (38.06)	35 (36.27)
	50	52 (46.15)	53 (46.72)	54 (47.29)	52 (46.15)	45 (42.13)	50 (45.00)

Table 5 - Antibacterial Screening Data of the Cephradine and its Metal Complexes.

	Escherichia coli	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
$[Sn(L)_2Cl_2)]$	++++	+++	+++
$[Pb(L)_2Cl_2)]$	+++	+++	+++
$[Mn(L)_2Cl_2)]$	++	++	++
$[Fe(L)_2Cl_2)]$	+++	++	+++
Cephradine	++	+	++

Inhibition zone diameter mm (% inhibition) :

+ = 6-10 (27-45%), ++ = 10-14 (45-64%), +++ = 14-18 (64-84%), ++++ = 18-22 (82-100%)

Percentage inhibition values are relative to inhibition zone (22 m) with 100% inhibition.

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