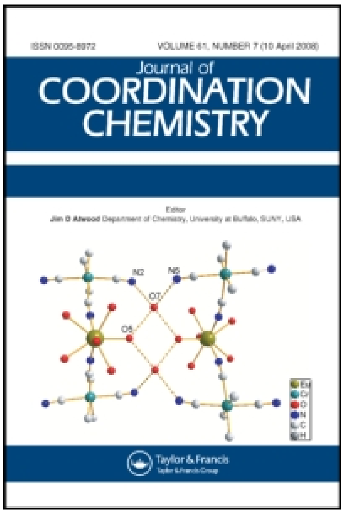

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Synthesis and antibacterial activity of cephradine metal complexes

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Cephradine (Hcephra) interacts with transition metal ions to give [Fe(cephra)Cl_2] and [M(cephra)Cl] complexes (M = Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)) which were characterized by physicochemical and spectroscopic methods; a tetrahedral geometry is suggested for their structures where cephradine behaves as monoanionic tridentate ligand. The complexes have been screened for antibacterial activity against several bacteria, and the results showed that all metal complexes tested had lower antibiotic activity than the free ligand.

Keywords: Cephradine; Transition; Metal complexes; Antibacterial

1. Introduction

The explosive growth of the cephalosporins during the past decades makes a system of classification desirable. Although cephalosporins may be classified by their chemical structure, clinical pharmacology, resistance to beta-lactamase or antimicrobial spectrum [1–3], the well accepted system of classification by generations is very useful. The antibiotic cephradine belongs to the second-generation cephalosporins. The second-generation cephalosporins have somewhat increased activity against gram negative microorganisms, but are much less active than the third generation agents. The structure of cephradine is shown in figure 1.

Many drugs possess modified toxicological and pharmacological properties when they are in the form of metal complexes. The most widely studied metal in this respect is copper(II) which has proved beneficial in diseases such as tuberculosis, gastric ulcers, rheumatoid arthritis and cancers [4–7]. These results encouraged us to investigate the coordination chemistry of antibiotics with transition and d^{10} metal ions in an attempt to examine the modes of binding in the solid state and to study biological activity. In continuation of our work [8–11] we report here the synthesis, characterization and bactericidal activity of cephradine metal complexes.

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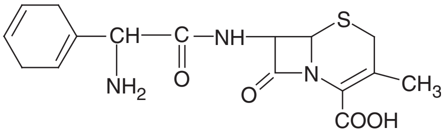

Figure 1. The structure of cephradine.

2. Experimental

2.1. *Physical methods*

The IR spectra of the ligand and its metal complexes were recorded as KBr pellets in the 4000–400 cm^{-1} range with a Perkin-Elmer Series 2000 spectrophotometer. FTIR spectra as polyethylene pellets were registered between 450 and 120 cm^{-1} using a Bruker IFS 66 V spectrophotometer. UV-vis spectra were recorded using a Perkin-Elmer recording spectrometer. C, H, N and S were analyzed on a LECO CHNS 932 model microanalytical instrument. Metal contents were estimated spectrophotometrically on an atomic absorption spectrometer. Thermogravimetric analyses were performed with a Cahn RG electromicrobalance in air at a heating rate of 4°C min^{-1} up to 400°C. Magnetic susceptibilities were measured on a Johnson Matthey Susceptibility Balance at room temperature using mercury(II) tetrathiocyanato-cobaltate(II) as calibrant. EPR spectra were recorded on a Bruker ECS 106 spectrometer by the X-band. 300 MHz ^{1}H NMR spectra were recorded on a Bruker NMR spectrometer, using TMS as an internal reference and deuterated dimethylsulfoxide as solvent.

2.2. *Antibacterial activity test*

In vitro antibacterial activities of cephradine and its complexes were tested using the paper disc diffusion method [12]. The chosen strains were G(+) *Staphylococcus aureus* ATCC 25923 and G(−) *Proteus mirabilis* ATCC 35659, *Shigella sonnei* ATCC 25931, *Salmonella enteritidis* ATCC 497, *Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 35939. The liquid medium containing the bacterial subcultures was autoclaved for 20 min at 121°C and at 15 lb pressure before inoculation. The bacteria were then cultured for 24 h at 36°C in an incubator. Muller Hinton broth was used for preparing basal media for bioassay of the organisms. Nutrient agar was poured onto a plate and allowed to solidify. The test compounds (DMSO solutions) were added dropwise to a 10 mm diameter filter paper disc placed at the centre of each agar plate. The plates were kept at 5°C for 1 h then transferred to an incubator maintained at 36°C. The width of the growth inhibition zone around the disc was measured after 24 h incubation. Four replicas were made for each treatment.

2.3. *Materials and methods*

All chemicals were reagent grade, purchased from Sigma and used without further purification. Solvents were redistilled by standard techniques before use. The complexes were prepared by mixing cephradine (2 mmol) and metal salts: $MnCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$ or $ZnCl_2$ (1 mmol) in

Table 1. Elemental analyses for the complexes.

Compound	Found (Calcd)%				
	C	H	N	S	Metal
[Mn(cephra)Cl] · $2H_2O$	40.7 (40.5)	4.5 (4.7)	8.6 (8.8)	6.5 (6.8)	11.8 (11.6)
[Fe(cephra)Cl_2] · $3H_2O$	35.9 (36.3)	4.7 (4.6)	8.3 (7.9)	6.5 (6.1)	10.7 (10.6)
[Co(cephra)Cl] · $2H_2O$	39.7 (40.1)	4.8 (4.6)	9.1 (8.8)	7.2 (6.7)	12.1 (12.3)
[Ni(cephra)Cl]	43.8 (43.4)	4.5 (4.1)	9.7 (9.5)	7.6 (7.2)	13.5 (13.3)
[Cu(cephra)Cl] · $3H_2O$	38.4 (38.3)	4.8 (4.8)	8.3 (8.4)	6.2 (6.4)	12.9 (12.7)
[Zn(cephra)Cl]	42.4 (42.8)	3.8 (4.0)	9.5 (9.4)	7.3 (7.1)	14.9 (14.6)

MeOH (40 cm^3), then the pH of the solution was adjusted to 8.0 with 0.5 M NaOH. The reaction mixture was then stirred at room temperature for ca 6 h, and left to stand overnight. The volume was then reduced by rotary-evaporation and the precipitated complexes were filtered off, washed with water, MeOH and ether and dried under reduced pressure at room temperature. All syntheses were carried out under a nitrogen atmosphere. The precipitated complexes are colored, crystalline and obtained in 25–35% yield.

3. Results and discussion

The elemental analyses (table 1) agree well with a 1 : 1 metal to ligand stoichiometry for all complexes which are air-stable solids, soluble in DMSO and DMF and slightly soluble in MeCN. The conductivity values measured in DMSO at room temperature, fall within the usual range for non electrolytes [13] suggesting that the chloride ion is coordinated to the metal ion. Based on the results, the formulas [Fe(cephra)Cl_2] and [M(cephra)Cl] have been assigned to the complexes. Thermograms of the hydrated metal complexes indicate endothermic decompositions in the 85–110°C range due to loss of water of hydration and also revealed that the complexes are stable with no coordinated water and solvent molecules. Attempts to form complexes of a well-defined stoichiometry, in the above mentioned conditions, with silver(I), iron(II), cadmium(II), mercury(II) and chromium(III) ions were unsuccessful.

3.1. *IR spectra*

The IR spectra of cephradine and its complexes are similar and have been assigned mainly for those specific frequencies directly involved in complex formation. The main IR spectral data are presented in table 2. The lactam (C=O) band at 1750 cm^{-1} in the spectrum of cephradine is missing in the spectra of complexes. The amide (C=O) band of the ligand appears at 1650 cm^{-1} while the complexes show this band at around 1650–1660 cm^{-1} range. Thus coordination of the ligand occurs through the oxygen from the lactam carbonyl group rather than the amide carbonyl group where the shift was not significant. The exception was the copper(II) complex, the spectrum of which suggests that coordination of the ligand occurs through the oxygen atom from the amide carbonyl group. The absorption band of the amide carbonyl moiety of the copper(II) complex was shifted to higher frequency due to greater rigidity shown by this group when coordinated.

Table 2. Selected vibrational data (cm^{-1}).

Compound	ν(CO) lactam	ν(CO) amide	ν(COO) asymmetric	ν(COO) symmetric	$\Delta\nu$ (COO)	ν(M–N)
[(Hcephra)]	1750	1650	1600	1400	200	
[Mn(cephra)Cl] · 2H_2O		1660	1620	1380	240	460
[Fe(cephra)Cl_2] · 3H_2O		1650	1620	1380	240	450
[Co(cephra)Cl] · 2H_2O		1650	1630	1390	240	465
[Ni(cephra)Cl]		1660	1630	1380	250	460
[Cu(cephra)Cl] · 3H_2O	1750	1680	1620	1390	230	475
[Zn(cephra)Cl]		1650	1630	1390	240	465

The band at 1600 cm^{-1}, corresponding to the carboxylate asymmetrical stretching, is shifted to higher frequencies (1620–1630 cm^{-1}) after complexation with the metal(II) ions, indicating coordination through that group. The remaining carboxylate bands, namely ν_{sym}(COO), γ(COO), ω(COO) and ρ(COO), formerly at 1400, 780, 604 and 525 cm^{-1}, respectively, also change as a result of coordination. Furthermore, a carboxylate ligand can bind to the metal atom either as a monodentate or a bidentate ligand, giving changes in the relative positions of the antisymmetric and symmetric stretching vibrations [14]. The IR spectra of the complexes give a separation value of $>$200 cm^{-1} suggesting monodentate bonding for the carboxylate group.

The presence of M–N stretching vibrations (from NH_2 group) at 450–475 cm^{-1} for the metal complexes (absent in the free ligand) suggests coordination by the ligand as a tridentate monoanionic chelating agent [15]. The coordination of the –NH_2 group to the metal ion is not the only explanation of these absorption bands, alternatively, the N atom of the CONH group could coordinate to the metal ions in solid complexes, however steric constraints prevent coordination of this N atom along with the COO and lactam CO groups. Finally, the bands in the 360–380 cm^{-1} region observed in the complexes, and absent in the cephradine, are tentatively assigned to ν(M–O) vibrations.

3.2. *^{1}H NMR studies*

In the ^{1}H NMR spectrum of cephradine three groups of doublets given by both CO–CH and N–CH on the beta-lactam ring and NH appeared at 4.95, 5.48 and 9.03 ppm, respectively. One group of four resonance signals consistent with an AB system attributed to S–CH_2 on the dihydrothiazine ring was observed in the 3.18–3.45 ppm region with a coupling constant 17.2 Hz for J_{AB}. Furthermore, coupling between NH_2 and the adjacent CH could not be distinguished and a broad single signal due to NH_2 protons was observed at 3.84 ppm. A multiplet in the range 5.60–5.67 ppm due to 1,4-dihydrobenzene protons was also present. Comparison of the ^{1}H NMR spectrum of cephradine with that of the diamagnetic complex, shows that there is a downfield shift in the frequency of amino protons (3.96 ppm), confirming coordination of this group to the metal ion.

3.3. *Electronic spectra*

The UV-vis spectra of cephradine and its complexes in DMSO have two absorption maxima at 260 and 300 nm assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions within the organic ligand. The manganese(II) and iron(III) complexes show two absorption bands at 370 and 400 nm probably due to spin-orbit forbidden transitions. The cobalt(II) complex has two bands centered at 380 and 490 nm which are assigned to the spin-forbidden and $^4A_2 \rightarrow {}^4T_1(P)$ transitions respectively, for cobalt(II) in tetrahedral complexes [16, 17], however, the presence of a six-coordinate structure cannot be ruled out. The nickel(II) complex also has one absorption band at 580 nm attributable to a d–d electronic transition. The copper(II) complex exhibits two absorption bands at 440 and 650 nm; the low energy band corresponds to the $^2B_{1g} \rightarrow {}^2A_{1g}$ transition in square planar or distorted tetrahedral geometries [18, 19]. The high energy band at 440 nm is assigned to a charge-transfer transition, metal → ligand or vice versa.

3.4. *Magnetic measurements*

From the molar magnetic susceptibility values, corrected magnetic moments were calculated using Pascal's constants. Both, the manganese(II) and the iron(III) complexes have magnetic moments of 5.6 and 5.9 B.M. respectively, consistent with high spin d^5 systems with five unpaired electrons. The rather low magnetic moments suggest diamagnetic impurities. The cobalt(II) complex has a magnetic moment of 4.1 B.M. while the expected value for tetrahedral complexes is 4.4–4.8 B.M. The rather low value of the magnetic moment of the complex would point to a not purely tetrahedral geometry of Co(II) and a tendency towards square-planar; the latter geometry is characterized by lower magnetic moments (2.1–2.8 B.M.) [20]. The nickel(II) complex has a magnetic moment of 3.4 B.M. as predicted for high spin d^8 systems with two unpaired electrons, and indicates a tetrahedral structure for the complex [21]. The copper(II) complex has a magnetic moment of 2.5 B.M. at room temperature and falls in the range associated with d^9 systems with one unpaired electron. The high value of the magnetic moment for the copper(II) complex could be explained, in part, by the fact that spin-orbital coupling in the ion can mix the ground state representing no orbital momentum with higher levels of identical multiplicity, resulting in a small orbital contribution [22], however, the presence of excess metal ion cannot be discarded. Although lowered moments can be accounted for by antiferromagnetic interactions between the ions, higher moments would require ferromagnetic interactions which are significantly rarer.

X-band EPR spectrum of the manganese(II) complex (^{55}Mn, $I = 5/2$) showed slight hyperfine splitting at 77 K. However, the splitting pattern was not observed for the same complex at room temperature and a single broad band was obtained centred at 3180 G. The EPR spectrum at 77 K of the powder sample of the copper(II) complex showed four lines (^{63}Cu, $I = 3/2$) and is anisotropic at higher magnetic field. The three peaks of low intensity in the weaker field region are considered to originate from the $g_\parallel$ component. The calculated g values, $g_\parallel = 2.21$ and $g_\perp = 2.06$ and $A_\parallel = 128 \times 10^{-4}\,\mathrm{cm}^{-1}$ indicate [23] that the unpaired electron most likely resides in the $d_{x^2-y^2}$ orbital having $^2B_{1g}$ as a ground state term.

3.5. *Structure of complexes*

The coordination chemistry of some beta-lactam antibiotics with transition and d^{10} metal ions has been reported [9–11, 24]. In our case, the cephradine ion has several potential donor atoms but, due to steric constraints, the ligand can provide a maximum of three donor atoms to any one metal center. The assumption that the coordination

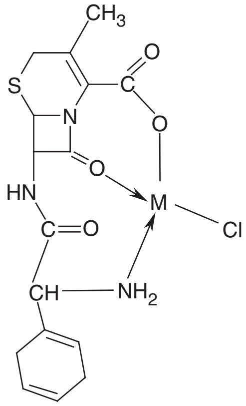

Figure 2. Tentative structure of the cephradine metal complexes.

of cephradine occurs through the carboxylate, lactam carbonyl and NH_2 groups seems likely from molecular models. It is feasible that the metal ions in the [M(cephra)Cl] complexes (where M = Mn(II), Co(II), Ni(II) and Zn(II)) are tetra coordinate with one molecule of cephradine and the chloride anion at the vertices of a tetrahedron. For copper(II) complex, the cephradine coordination through carboxylate, amide carbonyl and NH_2 groups is suggested. On the other hand, the iron(III) complex containing two chloride anions inside the coordination sphere is pentacoordinate having a tetragonal pyramidal or trigonal bipyramidal geometry; the presence of binuclear structures cannot be discarded. Despite the crystalline nature of the products none proved suitable for X-ray structure determination. The suggested structure is shown in figure 2.

3.6. *Microbiological screening*

The susceptibility of certain strains of bacterium towards cephradine and its metal complexes was judged by measuring the size of inhibition diameter. As assessed by colour, the complexes remain intact during biological testing. The antibiotic and its complexes presented bactericide diameters larger than 10 mm showing that they are sensitive [25, 26]. The average results are shown in table 3.

The results showed that the metal complexes are poorer bactericides as compared with the cephradine ligand against the same bacteria and under identical experimental conditions. The exception was [Cu(cephra)Cl] against *E. coli*. Only the copper(II) complex, was found to yield clear inhibition zones around the discs showing that it has a good activity as bactericide. The highest antibacterial activity was shown by the copper(II) complex against *S. aureus*. No compound showed activity against *P. aeruginosa* and the cobalt(II) and nickel(II) complexes show no activity at all against the bacteria.

The bactericidal action of cephradine results from inhibition of cell wall synthesis, so the bacteria die. In contrast with present results, it has been observed that for some cephalosporins [9–11, 24], their metal complexes showed higher bacterial toxicity than the free antibiotics. The relationship between chelation and bacterial toxicity is

Table 3. Antibacterial activity of the cephradine metal complexes.[a]

	Zone of inhibition (mm)					
Compound	S.A.	P.A.	E.C.	S.S.	P.M.	S.E.
[(Hcephra)]	38	0	0	24	26	30
[Mn(cephra)Cl] · $2H_2O$	13	0	0	0	14	0
[Fe(cephra)Cl_2] · $3H_2O$	10	0	0	0	10	0
[Co(cephra)Cl] · $2H_2O$	0	0	0	0	0	0
[Ni(cephra)Cl]	0	0	0	0	0	0
[Cu(cephra)Cl] · $3H_2O$	21	0	10	12	12	10
[Zn(cephra)Cl]	13	0	0	10	12	0

[a] S.A. *Staphylococcus aureus* ATCC 25923, P.A. *Pseudomonas aeruginosa* ATCC 10145, E.C. *Escherichia coli* ATCC 35939, S.S. *Shigella sonnei* ATCC 25931, P.M. *Proteus mirabilis* ATCC 35659. S.E. *Salmonella enteriditis* ATCC 497. All doses were 800 $\mu g\,disc^{-1}$. Estimated error ±1 mm.

very complex and is expected to be a function of steric, electronic and pharmakinetic factors, along with mechanistic pathways.

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