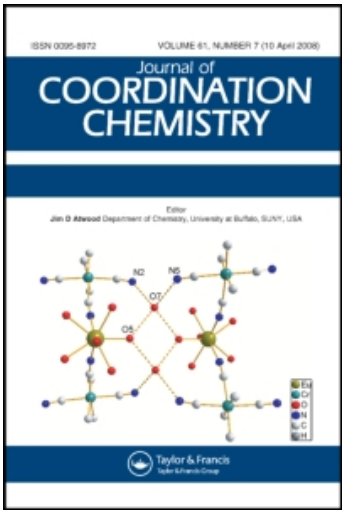

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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF METAL COMPLEXES OF CEPHALOTHIN

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The interactions of cephalothin (Hcephalo) with transition metal(II) ions and imidazole have been investigated. The complexes [M(cephalo)Cl], (where M=Mn(II), Co(II), Ni(II), Pd(II)) and [M(cephalo)(Im)Cl] (where M=Ni(II), Cu(II), Zn(II), Im=Imidazole) were obtained and characterized by physicochemical and spectroscopic methods. The IR and the ^{1}H-NMR spectra of the complexes suggest that the cephalothin behaves as a monoanionic tridentate ligand. They have been screened for antibacterial activity against several bacteria, and the results are compared with the activity of cephalothin.

Keywords: Antibacterial; Cephalothin; Imidazole; Nickel; Palladium; Manganese

INTRODUCTION

The antibiotic cephalothin belongs to the first generation cephalosporins and the resistance to it may be related to the inability of the antibiotic to reach its sites of action, to alterations in the penicillin-binding proteins that are targets of the cephalosporins, or to bacterial enzymes (β-lactamase) that can inactivate the cephalosporin. Many gram-positive microorganisms release relatively large amounts of β-lactamase into the surrounding medium and they can destroy the β-lactamic antibiotics by hydrolysis of the β-lactam ring, as the most prevalent mechanism of resistance [1–3]. The structure of cephalothin can be seen in Fig. 1.

Many metals possess toxicological and pharmacological properties in the form of metal complexes but the problem is that some of these lose their activity *in vivo* because proteins appear to have better affinities than the studied ligands for metal ions, which are inactivated once embedded in the proteins [4,5]. It seems that the presence of coordination sites from nitrogen heteroaromatic rings such as imidazoles or pyridines is important to have some biological activity that is not affected by biological chelators [4–7]. That is why we have undertaken investigation of both imidazole-containing complexes and 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 activities. As continuation of our work on metal interactions with

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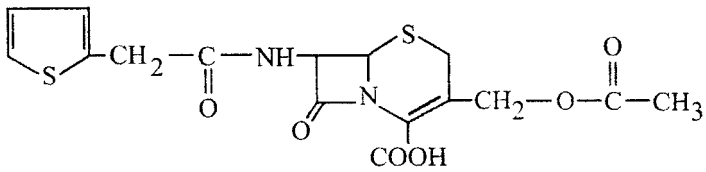

FIGURE 1 The structure of cephalothin.

β-lactamic derivatives [8–11], we report the synthesis and characterization of metal complexes of cephalothin.

EXPERIMENTAL

Physical Methods

IR spectra of the ligand and its metal complexes as KBr pellets were recorded in the 4000–400 cm^{-1} range with a Perkin-Elmer Series 2000 spectrophotometer. FTIR spectra as polyethylene pellets were registered between 450–120 cm^{-1} using a Brucker IFS 66 V spectrophotometer. UV–Vis spectra were recorded using a Perkin-Elmer recording spectrometer. C, H, N and S were analyzed by the microlabs in the Venezuelan Institute of Scientific Research (IVIC). Magnetic susceptibility was measured on a Johnson Matthey Susceptibility Balance at room temperature using $HgCo(NCS)_4$ as calibrant. EPR spectra were recorded on a Brucker ECS 106 spectrometer by the X-band method. 500 MHz ^{1}H-NMR spectroscopic measurements were performed on a Brucker AM-500 NMR spectrometer, using TMS as an internal reference and deuterated dimethylsulfoxide as solvent.

Antibacterial Activity Test

In vitro antibacterial activities of cephalothin 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, *Klebsiella pneumoniae* ATCC 556, *Salmonella enteriditis* 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 15 lb pressure before inoculation. The bacteria were cultured for 24 h at 36°C in an incubator. Mueller Hinton broth was used for preparing basal media for the bioassay of the organisms. Nutrient agar was poured onto a Petri plate and allowed to solidify. The test compounds in DMSO solutions were added dropwise on a 10 mm diameter filter paper disc placed in the center of the agar plates. The plates were then kept at 5°C for 1 h and transferred to an incubator maintained at 36°C. The width of the growth inhibition zone around the disc was measured after 24 h of incubation. Four replicates were taken for each treatment.

Materials and Methods

All chemicals were analytical grade where possible and purchased from Aldrich and used without further purification. Solvents were distilled by standard techniques

before use. The [M(cephalo)Cl] complexes were prepared by mixing cephalothin (2 mmol) and metal(II) salts: $MnCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$ or $PdCl_2$ (1 mmol) in MeOH (40 mL), then pH of the solution was adjusted to 8.0 with 0.5 M NaOH solution and the reaction mixture was stirred at room temperature for about 8 h and then left to stand overnight. The volume was then reduced by rotary-evaporation. The precipitated complexes were filtered off, washed with water, MeOH and ether and dried under vacuum at room temperature. All syntheses were carried out under nitrogen. The procedure adopted for the synthesis of [M(cephalo)(Im)Cl] complexes was exactly analogous to that described above but inplace of the metal salt solutions, a mixture of metal salts $CuCl_2 \cdot 2H_2O$, $ZnCl_2$ or $NiCl_2 \cdot 6H_2O$ (1 mmol) and imidazole (2 mmol) in MeOH was added. Alternatively the same complexes have been obtained by mixing sodium cephalothinate and distilled water as solvent.

RESULTS AND DICSUSSION

The elemental analyses agree well with the proposed formulae of the complexes. The manganese(II) and cobalt(II) complexes are belge and dark red, respectively, while the nickel(II) and copper(II) complexes are green. The palladium(II) complex is maroon and the zinc(II) complex is white. The complexes are air-stable solids, soluble in DMSO and DMF, slightly soluble in acetonitrile and insoluble in methanol and water. The conductivity values measured in DMSO at room temperature fall in the range of nonelectrolytes [13] suggesting that the chloride ion is coordinated to the metal ion. Attempts to form complexes of a well-defined stoichiometry, in the above mentioned conditions, with silver(I), iron(II), mercury(II), magnesium(II) and cadmium(II) ions were unsuccessful. Analytical data of the compounds are given in Table I.

The IR spectra of cephalothin and its complexes are similar. The lactamic (C=O) band appears at 1730 cm^{-1} in the spectrum of cephalothin while the amide (C=O) band appears at 1650 cm^{-1}; the complexes show these bands at around 1685 and 1640–1660 cm^{-1}, respectively. All this suggests that coordination of the ligand occurs through the oxygen atom from the lactamic carbonyl group rather than the amide carbonyl group. The lactamic carbonyl bands were substantially shifted toward lower frequencies (40–60 cm^{-1}) relative to the value of the uncomplexed cephalothin while the amide carbonyl bands were not shifted significantly.

A carboxylate ligand can bind to the metal atom either as a monodentate or bidentate ligand, giving changes in the relative positions of the antisymmetric and symmetric

TABLE I Elemental analyses for the complexes

Compound	*Found (Calcd.) %*			
	C	*H*	*N*	*S*
[Mn(cephalo)Cl]	39.7 (39.5)	3.3 (3.1)	5.5 (5.8)	13.6 (13.2)
[Co(cephalo)Cl]	39.5 (39.2)	3.4 (3.1)	5.4 (5.7)	13.0 (13.1)
[Ni(cephalo)Cl]	39.7 (39.3)	3.5 (3.1)	5.8 (5.7)	13.4 (13.1)
[Pd(cephalo)Cl]	35.7 (35.8)	2.9 (2.8)	5.2 (5.2)	11.4 (11.9)
[Ni(cephalo)(Im)Cl]	40.9 (41.0)	3.6 (3.2)	10.5 (10.1)	11.1 (11.5)
[Cu(cephalo)(Im)Cl]	40.2 (40.6)	3.7 (3.2)	10.2 (10.0)	11.5 (11.4)
[Zn(cephalo)(Im)Cl]	40.1 (40.5)	3.4 (3.2)	10.6 (10.0)	11.6 (11.3)

TABLE II Main vibrational frequencies (cm^{-1})

Compound	*ν(CO)lact*	*ν(CO)amide*	*ν(COO)asym*	*ν(COO)sym*	$\Delta\nu$
[Na(cephalo)]	1730	1650	1620	1400	220
[Mn(cephalo)Cl]	1690	1660	1615	1380	235
[Co(cephalo)Cl]	1690	1660	1610	1390	220
[Ni(cephalo)Cl]	1680	1640	1610	1380	230
[Pd(cephalo)Cl]	1780	1650	1580	1380	200
[Ni(cephalo)(Im)Cl]	1670	1630	1610	1360	250
[Cu(cephalo)(Im)Cl]	1690	1640	1620	1380	240
[Zn(cephalo)(Im)Cl]	1680	1640	1580	1380	200

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–S stretching vibrations at 280–320 cm^{-1} for the metal complexes supports coordination by the ligand as a tridentate monoanionic chelating agent [15]. The coordination of the thiophene S atom to the metal ion is not the only explanation of these absorption bands; alternatively, the S atom of the dihydrothiazine ring can coordinate to the metal ions in solid complexes, however from steric constraints, coordination of this S atom along with the COO and lactamic CO groups is not possible. Therefore, coordination of thiophene is suggested, despite being poor ligand [16]. The main IR frequencies can be seen in Table II.

In the ^{1}H-NMR spectrum of cephalothin two single peaks attributed to methyl (3H) and COOH appeared at 1.99 and 10.2 ppm, respectively. Three groups of double peaks given by CH–S and N–CH on the β-lactamic ring and NH appeared at 4.95, 5.48 and 9.03 ppm, respectively. Three groups of four resonances consistent with an AB system attributed to S–CH_2 on the dihydrothiazine ring, CH_2CO and CH_2–O were observed in the 3.18–3.49, 3.60–3.90 and 4.74–5.03 ppm regions with coupling constants 17.4, 8.5 and 12.0 Hz for J_{AB}, respectively. One group of multiple peaks and two groups of double peaks due to the thiophene ring (3H) were observed at 6.92 and 7.35 ppm, respectively. With the exception of the zinc(II) complex the experimental results show that the complexes are paramagnetic and the peaks of protons of different groups in their ^{1}H-NMR spectra were very broad and could not be distinguished. The ^{1}H-NMR spectrum of the zinc(II) complex slightly changed as compared with that of the corresponding ligand and the signals appeared downfield, as expected, due to increased conjugation on coordination. Absence of the signal assigned to the COOH proton of cephalothin confirm deprotonation and suggest formation of a metal–COO bond.

The UV–Vis spectra of cephalothin and its complexes in DMSO present two absorption maxima at 260–270 and 280–320 nm assigned to $\pi \rightarrow \pi^*$ transitions within the organic ligand. The manganese(II) complex shows a very weak absorption at 390 nm probably due to a spin-orbit forbidden transition. The cobalt(II) complex presents a broad band centered at 350 nm while the nickel(II) and palladium(II) complexes showed two bands at 320–360 and 370–380 nm which may be assigned considering T_d symmetry around the metal, to $^3T_1(P) \rightarrow {}^3A_2(F)$ and $^3T_1(P) \rightarrow {}^3T_2(F)$ transitions, respectively. The copper(II) complex exhibits a broad band centered around 670 nm falling in the range of those usually reported for five-coordinate copper(II) complexes [17]. All these bands have extinction coefficients below 300, contrary to the charge transfer bands which can be quite intense.

From the molar magnetic susceptibility values, corrected magnetic moments were calculated using Pascal's constants. The manganese(II) complex has a magnetic moment of 5.5 BM as predicted for a high-spin d^5 system with five unpaired electrons, while the cobalt(II) complex has a magnetic moment of 3.9 BM typical of high-spin d^7 systems with three unpaired electrons. The nickel(II) and palladium(II) complexes have magnetic moments in the 2.8–3.0 BM range as predicted for high-spin d^8 systems with two unpaired electrons. The copper(II) complex has a magnetic moment of 2.1 BM at room temperature and falls in the range associated with d^9 systems with one unpaired electron. The rather high value of the magnetic moment for the freshly prepared copper(II) complex could be explained, in part, by the fact that spin-orbital coupling can mix the ion levels of identical multiplicity, resulting in a small orbital contribution [18], however, the presence of impurities cannot be discarded.

The room temperature EPR spectrum of the powder sample of the manganese(II) complex showed a single broad signal with a poor resolution of the hyperfine structure. The calculated g_{av} value of 1.9 agrees well with the results obtained previously for high-spin manganese(II) complexes. The EPR spectrum of the copper(II) complex also showed a single broad signal with poor resolution of the hyperfine structure on both sides of the main signal. The calculated g values, $g_{||} = 2.18$ and $g_{\perp} = 2.07$ indicate [19] that the unpaired electron most likely resides in the $d_{x^2-y^2}$ orbital having $^2B_{1g}$ as a ground state term. The axial symmetry parameter, $G = 2.6$, obtained by the relation $(g_{||} - 2)/(g_{\perp} - 2)$, supports [20] the contention that there is an exchange interaction between the copper centers ($G < 4$).

Structure of Complexes

The coordination chemistry of some β-lactamic antibiotics with transition and d^{10} metal ions has been reported [8–11,21]. In our case, the cephalothinate 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 of cephalothin occurs through the carboxylate and lactamic carbonyl oxygen atoms seems likely from molecular models. It is feasible that the metal ions in the [M(cephalo)Cl] complexes are four-coordinate with one molecule of cephalothin and the chloride anion at the vertices of a tetrahedron. On the other hand, the metal complexes containing imidazole are probably five-coordinate having tetragonal pyramidal or trigonal bipyramidal geometries. However, the presence of binuclear structures cannot be eliminated. Despite the crystalline nature of the products none proved suitable for X-ray structure determination.

When the energy was minimized using MM2 calculations for the [M(cephalo)Cl] complexes, reasonable bond lengths and bond angles nearly tetrahedrals around the metal were obtained, suggesting that coordination of COO, lactamic CO and thiophene moieties to the metal are possible. The suggested structures can be seen in Fig. 2.

Microbiological Screening

The susceptibility of certain strains of bacterium towards cephalothin and its metal complexes was judged by measuring the size of inhibition diameter. As assessed by color the complexes remain intact during biological testing. The antibiotic and the

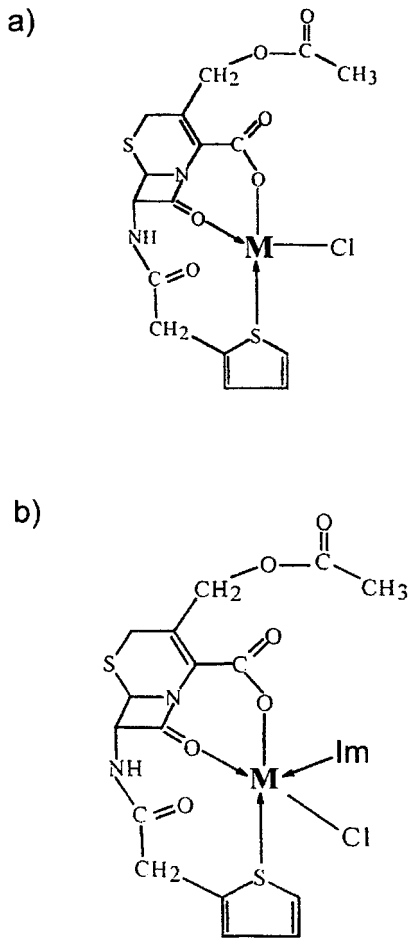

FIGURE 2 Tentative structure of the cephalothin metal complexes: (a) [M(cephalo)Cl]; (b) [M(cephalo)(Im)Cl].

complexes presented bactericide diameters larger than 20 mm being highly sensitive [22,23]. The average results are shown in Table III.

In general, the complexes [Mn(cephalo)Cl], [Ni(cephalo)Cl] and [Cu(cephalo)(Im)Cl] were found to have higher activity than that of cephalothin against the bacteria strains studied under the test conditions, showing that they have good activity as bactericides. However, all the compounds showed no activity against *P. aeruginosa*. The highest antibacterial activities were demonstrated by the manganese(II) and copper(II) complexes being about 38% higher than cephalothin activity. The cobalt(II) and palladium(II) complexes showed no activity againt *E. coli*, *K. pneumoniae* and *S. enteriditis*. Unfortunately there is only one pair of complexes with and without imidazole, [Ni(cephalo)(Im)Cl] and [Ni(cephalo)Cl], so it is hard to draw conclusions about the importance of the structural difference. Nevertheless, the comparison of their biological activities (Table III) does not support the idea that the presence of heterocyclic ligands, like imidazole, may enhance antimicrobial activity of the metal chelates.

TABLE III Antibacterial activity of the cephalothin metal complexes

Compound	*Zone of inhibition* (mm)				
	S.A	*E.C*	*K.P*	*S.E*	*P.M*
[Na(cephalo)]	35	26	30	24	34
[Mn(cephalo)Cl]	47	37	0	26	42
[Co(cephalo)Cl]	23	0	0	0	12
[Ni(cephalo)Cl]	42	35	39	36	8
[Pd(cephalo)Cl]	36	0	0	0	10
[Ni(cephalo)(Im)Cl]	24	14	44	0	14
[Cu(cephalo)(Im)Cl]	36	39	0	37	47
[Zn(cephalo)(Im)Cl]	44	27	28	0	0

S.A: *Staphylococcus aureus* ATCC 25923; E.C: *Escherichia coli* 35939; K.P: *Klebsiella pneumoniae* 556; S.E: *Salmonella enteriditis* ATCC 497; P.M: *Proteus mirabilis* ATCC 35659. All doses were 400 μg/disc. Estimated error ± 1 mm:

The metal-catalyzed solvolytic degradation of cephalosporins via β-lactam ring opening is well documented. Considering that metal coordination involves the β-lactum CO a significant increase in the lability of the β-lactam ring could be anticipated, however, as normally happens the antimicrobial activity is maintained or increased. The results in Table III can be understood considering that the enzyme probably serves primarily to hold catalytic groups or the substrate in the proper positions. Metal complexes of β-lactam antibiotics may change the stereochemistry required in solvolytic reactions on an enzyme surface.

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