CEFOPERAZONE METAL COMPLEXES: SYNTHESIS AND CHARACTERIZATION

J.R. ANACONA[1], ALINA BRAVO[2] AND MARIA E. LOPEZ[1]*

[1]*Departamento de Química. Universidad de Oriente. Apartado Postal 208. Cumaná. Venezuela*
[2]*Departamento de Bioanálisis. Universidad de Oriente. Apartado Postal 208. Cumaná. Venezuela*

(Received: January 4, 2012 - Accepted: September 3, 2012)

ABSTRACT

Cefoperazone (Hcefopz) interacts with transition metal(II) ions to give [M(cefopz)Cl] complexes (M = Fe, Co, Ni, Cu and Cd) and [Fe(cefopz)Cl]Cl which were characterized by physicochemical and spectroscopic methods. The spectra indicated that the antibiotics act as monoanionic multidentate NO_3 chelating agent towards metal ions, via the amides, and carboxylate and N-azomoiety. The complexes are non-toxic, insoluble in water and common organic solvents and probably have polymeric structures.

Keywords cefoperazone sodium; antibiotic; metal complexes; antibacterial activity

INTRODUCTION

The cephalosporin antibiotics are comprised of several different classes of compounds with dissimilar spectrums of activity and pharmacokinetic profiles. All "true" cephalosporins are derived from cephalosporin C which is produced from *Cephalosporium acremonium*. Cephalosporins are usually bactericidal against susceptible bacteria and act by inhibiting mucopeptide synthesis in the cell wall resulting in a defective barrier and an osmotically unstable spheroplast. The exact mechanism for this effect has not been definitively determined, but beta-lactam antibiotics have been shown to bind to several enzymes (carboxypeptidases, transpeptidases, endopeptidases) within the bacterial cytoplasmic membrane that are involved with cell wall synthesis [1-4]. The different affinities that various beta-lactam antibiotics have for these enzymes (also known as penicillin-binding proteins; PBPs) help explain the differences in spectrums of activity of these drugs that are not explained by the influence of beta-lactamases. Like other beta-lactam antibiotics, cephalosporins are generally considered to be more effective against actively growing bacteria. The cephalosporin class of antibiotics is usually divided into three classifications or generations. The third generation cephalosporins retain the gram positive activity of the first and second generation agents, but in comparison, have much expanded gram negative activity [5,6]. Cefoperazone belongs to the third generation cephalosporins and in continuation of our work about metal based drugs [7-12] we report here the synthesis and characterization of cefoperazone metal complexes. The chemical structure of cefoperazone monosodium is shown in Figure 1.

EXPERIMENTAL

Physical methods

The 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 450–120 cm^{-1} using a Bruker IFS 66V 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. The halogen content was determined by combustion of the solid complex (30 mg) in an oxygen flask in the presence of a KOH-H_2O_2 mixture. The halide content was then determined by titration with a standard $Hg(NO_3)_2$ solution using diphenyl carbazone as an indicator. Thermograms were recorded on a simultaneous thermal analyzer, STA-6000 (Perkin Elmer) instrument at a heating rate of 4ºC min^{-1} up to 200°C. Magnetic susceptibilities were measured on a Johnson Matthey Susceptibility Balance at room temperature using Pascal's constants for the diamagnetic corrections and mercury(II) tetrathiocyanato-cobaltate(II) as calibrant. EPR spectra were recorded on a Bruker ECS 106 spectrometer by the X–band.

Materials and methods

All chemicals were commercially obtained in their purest form and were used without further purification. Solvents were redistilled by standard techniques before use. The complexes were prepared by mixing cefoperazone sodium salt (1 mmol) and metal salts: $FeCl_2.4H_2O$, $FeCl_3.6H_2O$, $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$ or $CdCl_2$ (1 mmol) in methanol (40 cm^3). The reaction mixture was then stirred at room temperature for *ca.* 5h, and a coloured precipitate formed. The precipitated complexes were filtered off, washed with water, methanol and ether and dried under reduced pressure at room temperature. All syntheses were carried out under a nitrogen atmosphere.

RESULTS AND DISCUSSION

Cefoperazone has two ionisable groups (pKa = 2.55 and 9.55); it thus exists predominantly as a monoanionic at a physiological pH. The elemental analyses (Table 1) agree well with a 1:1 metal to ligand stoichiometry for all the complexes. They are air-stable solids. The mononuclear complexes are coloured, insoluble in water and other common organic solvents such as ethanol, benzene, acetone, acetonitrile and ether but soluble in DMF and DMSO. The general formulae [M(cefopz)Cl] and [Fe(cefopz)Cl]Cl have been assigned to the complexes. Thermograms of the hydrated metal complexes, provided as supplementary information, indicate endothermic decompositions in the 80–90 °C range due to the loss of molecules of water of hydration, and also reveal that the complexes are stable with no coordinated water and solvent molecules. Attempts to form complexes of a well-defined stoichiometry, under the above-mentioned conditions, with chromium(III), copper(I), zinc(II), mercury(II) and manganese(II) ions were unsuccessful. The conductivity values measured in DMSO at room temperature fall in the range of non-electrolytes [13] suggesting that the chloride ion is coordinated to the metal(II) ions. The exception was the iron(III) complex, [Fe(cefopz)Cl]Cl, which show to be 1:1 electrolyte.

IR spectra

The IR spectra of cefoperazone and its complexes are similar and have been assigned mainly to those specific wavenumbers directly involved in complex formation. The main IR wavenumbers are recorded in Table 2. Generally the ring carbonyl absorption frequency will be shifted to higher wave numbers as the ring becomes more and more strained. Thus, the lactam ν(C=O) and the 2,3 piperazinedione ν(C=O) bands appear at 1750 and 1690 cm^{-1} respectively in the spectra of cefoperazone and in all the metal complexes [14], the exception was the [Fe(cefopz)Cl] complex which presents the lactam ν(C=O) band at 1710 cm^{-1}. The (amide I) ν(C=O) band of the ligand appears at 1660 cm^{-1} while the complexes showed a negative shift, at around the 1645–1640 cm^{-1} range indicating coordination through oxygen [15]. A shift of the amide(II) band towards higher frequencies, indicates nonparticipation of the nitrogen atom in the coordination [16]. All this suggests that coordination of the ligand occurs through the oxygen atom from the amide carbonyl groups rather than the lactam and piperazinedione carbonyl moieties where the shifting was not significant, although the binding through b-lactamic carbonyl group cannot be ruled out in the [Fe(cefopz)Cl] complex. The band at 1610 cm^{-1}, corresponding to the carboxylate asymmetrical stretching, is shifted to higher wavenumbers (1620–1630 cm^{-1}) after complexation with the metal(II) ions, thus indicating coordination through that group. The remaining carboxylate bands, namely ν_{sym}(COO), γ(COO), ω(COO) and ρ(COO), formerly at 1400, 785, 610 and 530 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

e-mail: juananacona@hotmail.com

and symmetric stretching vibrations [14]. The IR spectra of the complexes give a separation value of $\Delta\nu > 200$ cm^{-1} suggesting monodentate bonding for the carboxylate group. The presence of (M-N) stretching vibrations in the 450–490 cm^{-1} range for the metal complexes (absent in the free ligand) provide evidence that the tetrazole moiety is bonded to the metal ion through the nitrogen atom. The coordination of the tetrazole 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 these N atoms along with the COO and lactam CO groups. Furthermore, the C–N–C stretching and the N–H stretching vibrations of the CONH residues observed in free cefoperazone at 1180 and 3240 cm^{-1} respectively, either do not shift or show a slight shift in all the metal complexes indicating that these N atoms were not involved in coordination. This result suggests coordination by the ligand as a tetradentate monoanionic NO_3 chelating agent. The bands in the 350–400 cm^{-1} region observed in the complexes, and absent in the free cefoperazone, are tentatively assigned to ν(M–O) vibrations.

Table 1.- Elemental analyses for the complexes.

Compound	Found (Calcd.) %					
	C	N	H	S	Cl	M
[Fe(cefopz)Cl]Cl.3H_2O	36.7	15.8	3.6	7.5	8.4	6.3
[Fe($C_{25}H_{32}N_9O_{11}S_2Cl_2$)]	(36.4)	(15.3)	(3.9)	(7.8)	(8.6)	(6.8)
[Fe(cefopz)Cl]	40.5	16.9	3.3	8.8	4.5	7.7
[Fe($C_{25}H_{26}N_9O_8S_2Cl$)]	(40.8)	(17.1	(3.6)	(8.7)	(4.8)	(7.6)
[Co(cefopz)Cl].H_2O	39.8	16.3	3.6	8.1	4.9	8.2
[Co($C_{25}H_{28}N_9O_9S_2Cl$]	(39.7)	(16.7)	(3.7)	(8.5)	(4.7)	(7.8)
[Ni(cefopz)Cl]	40.4	17.5	3.8	8.3	4.6	8.4
[Ni($C_{25}H_{26}N_9O_8S_2Cl$)]	(40.6)	(17.1)	(3.6)	(8.7)	(4.8)	(8.0)
[Cu(cefopz)Cl].H_2O	39.6	16.3	3.4	7.9	4.4	8.3
[Cu($C_{25}H_{28}N_9O_9S_2Cl$)]	(39.4)	(16.6)	(3.7)	(8.4)	(4.7)	(8.4)
[Cd(cefopz)Cl]	37.8	15.6	2.8	8.2	4.3	
[Cd($C_{25}H_{26}N_9O_8S_2Cl$)]	(37.9)	(15.9)	(3.3)	(8.1)	(4.5)	

Electronic spectra

The UV-Vis spectra of cefoperazone and its complexes in DMSO present absorption maxima at 255–270 nm assigned to a $\pi\rightarrow\pi^*$ transition due to molecular orbital energy levels originating in the NC–S moiety [17,18]. An intraligand band at 290-320 nm is related to the $\pi\rightarrow\pi^*$ transitions within the tetrazole moiety. The band in the 360-380 nm region is ascribed to an intraligand transition of the $n\rightarrow\pi^*$ type in accordance with the literature data for transitions due to sulphur atoms [17, 19]. The fact that the bands due to sulphur atoms are not shifted suggests that these atoms are not involved in coordination to metal ions. The local symmetry around metal(II) ions may belongs to the point group C_{3v} assuming trigonal bypiramidal geometry, therefore, an accurate band assignment is not possible due to the multicomponent nature of the bands. The iron(III) complex showed very weak absorption bands probably due to spin-orbit forbidden transitions. The iron(II) complex showed two weak bands at 420 and 600 nm. The cobalt(II) complex in DMSO solution presents one absorption maxima at 480 nm presumably due to intraligand excitation. Because of the insaturation of the cefoperazone, the intense uv absorption has a tail in the visible region and this hampers assignment of the relatively week d-d transitions of the cobalt(II) and iron(II) ions. The nickel(II) complex showed a broad absorption band at 580-650 nm range attributable to a d-d electronic transition. The copper(II) complex exhibits a d-d transition as a broad band centered at 680 nm falling in the range of those usually reported for five-coordinate copper(II) environments [20].

Magnetic measurements

From the molar magnetic susceptibility values, corrected magnetic moments were calculated using Pascal's constants. The magnitudes of the magnetic moments fall within the ranges associated with high spin ions in octahedral fields and normally they are unlikely to be of value in discriminating between the metal ions in six or five coordinate geometries. The iron(III) complex have a magnetic moment of 5.72 B.M. which is consistent with high spin d^5 systems with five unpaired electrons and an $S = 5/2$ ground state. The iron(II) complex has a magnetic moment of 4.65 B.M. which corresponds to a high spin d^6 systems with four unpaired electrons and an $S = 2$ ground state. Since the experimental value obtained for the magnetic moment of cobalt(II) in the cobalt(II) complex is 4.12 BM. while the calculated value for a d^7 high spin electronic distribution is 3.87 BM we conclude that cobalt(II) in [Co(cefopz)Cl] is in a five coordinate or octahedral geometry with a high spin configuration. The experimental value obtained for the magnetic moment for nickel(II) in [Ni(cefopz)Cl] complex is 3.17 B.M. which is close to the expected value for a five coordinate geometry (3.20–3.40 B.M.) [21]. For [Cu(cefopz)Cl] the experimental magnetic moment measured is equal to 2.22 B.M. while the calculated one for a d^9 configuration is equal a 1.73 B.M. suggesting the presence of excess metal ions in the complex. Although lowered moments can be accounted for by antiferromagnetic interactions between the ions, higher moments would require ferromagnetic interactions which are significantly rarer.

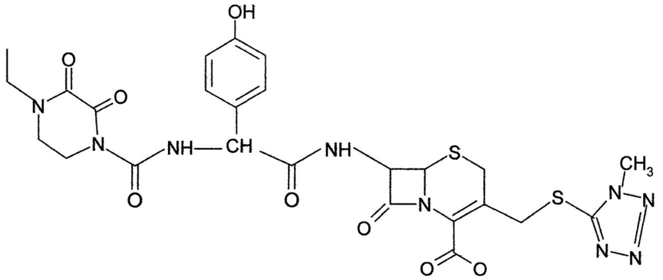

Figure 1. The structure of cefoperazone anion.

EPR spectra of a powdered sample of the iron(III) complex at room temperature (RT, 300 K) and liquid nitrogen temperature (LNT, 77 K) were obtained, but in each case there was simply a strong broad band with no evidence of fine structures due to ^{57}Fe (2,25% natural abundance, I = ½). The g_{iso} = 2.01 at RT and 2.02 at LNT probably indicate an octahedral environment around iron(III) [22]. The EPR spectrum at LNT of the powder sample of the copper(II) complex (Figure 2) 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, $g_{\parallel}$ = 2.15 and $g_{\wedge}$ = 2.05 and $A_{\parallel}$= 125 x 10^{-4} cm^{-1} indicate that the unpaired electron most likely resides in the d_{x2-y2} orbital having $^2B_{1g}$ as a ground state term [23].

Table 2.- IR spectral data of the drug and the complexes (cm^{-1}).

Compound	νC=O lactam	νC=O νC=O dione amide I,II	νCOO asymm	νCOO symm	Δν
[Na(cefopz)]	1750	1690 1660, 1530			
[Fe(cefopz)CL]Cl.3H_2O	1750	1690 1645, 1570	1600	1380	220
[Fe(cefopz)Cl]	1710	1690 1640, 1570	1600	1380	220
[Co(cefopz)Cl].H_2O	1750	1690 1645, 1570	1600	1380	220
[Ni(cefopz)Cl]	1750	1690 1645, 1570	1600	1380	220
[Cu(cefopz)Cl].H_2O	1750	1690 1645, 1570	1600	1380	220
[Cd(cefopz)Cl]	1750	1690 1640, 1570	1600	1380	220

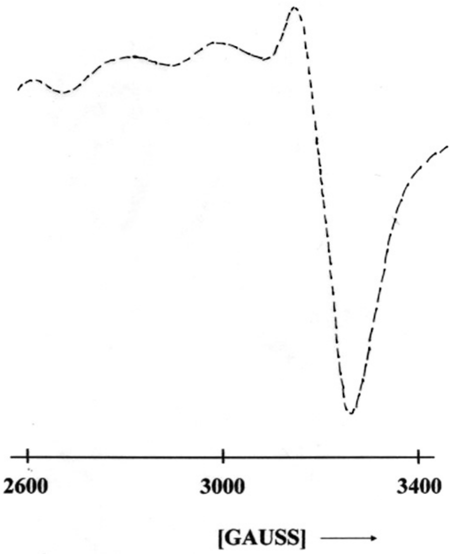

Figure 2. EPR spectrum of cefoperazone copper complex.

Structure of complexes

Despite the crystalline nature of the products, neither proved suitable for X-ray structure determination. The coordination chemistry of some beta-lactam antibiotics with transition and d^{10} metal ions has been reported [7-12, 24]. In our case, the cefoperazone anion has several potential donor atoms but, due to steric constraints, the ligand can provide a maximum of four donor atoms to any one metal center. The assumption that the coordination of cefoperazone occurs through the carboxylate, amide carbonyl moieties and N-tetrazole atom seems likely from molecular models. It is feasible that the metal ions in the [M(cefopz)Cl] complexes (where M = Fe(II), Co(II), Ni(II), Cu(II), and Cd(II)) containing one coordinated chloride anion are pentacoordinate and would probably have a tetragonal pyramidal or trigonal bipyramidal geometries. The poor solubilities of the complexes in all but strongly coordinating solvents suggests a polymeric structure, in which the cefoperazone ligand bridge between metal centres. We assume that each cefoperazone is bound to two metal ions (tetrazole ring and carboxylate group is on one M and the two amide moieties are on another metal ion). Although, it would not be appropriate to predict precisely the structure of these complexes, the structure (Figure 3) may tentatively be proposed.

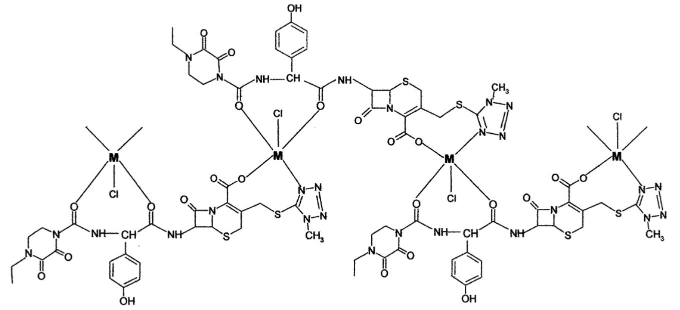

Figure 3. Tentative structure of the cefoperazone metal complexes [M(cefopz)Cl], [M = Fe, Co, Ni, Cu and Cd].

CONCLUSION

Cefoperazone complexes with different metal ions of 1:1 metal to antibiotic stoichiometry have been prepared. The coordination to metal occurs through the tetrazole, carboxylate and amide carbonyl groups although the binding through b-lactamic carbonyl group cannot be discarded in the [Fe(cefopz)Cl] complex. The solubility of the cefoperazone complexes in water and common organic solvents is reduced on complexation.

Supplementary information

Thermograms recorded on a simultaneous thermal analyzer, STA-6000 (Perkin Elmer) instrument at a heating rate of 4ºC min^{-1} up to 200°C are available free of charge as PDF file from the authors and the journal.

ACKNOWLEDGEMENTS

Our sincere thanks to the Comision de Investigacion from the Universidad de Oriente for providing financial support as well as Lic. Erasto Bastardo for elemental analyses.

REFERENCES

1. Bergan, T. (1987) *Drugs*, **34**, 89-104
2. Cunha, B. (1982) *Clin. Ther.*, **14**, 616-652
3. Neu, H. (1987) *Drugs*, **34**, 135-153
4. Williams, D.R. (1971) *The Metals of Life*, Van Nostrand Reinhold, London
5. Sorenson, J.R.J. (1976) *J. Med. Chem.*, **19**, 135-141

6. Brown, D.H., W.E. Smith & J.W.Teape (1980) *J. Med. Chem*. **23**, 729-733
7. Anacona J.R. & I. Rodriguez (2004) *J. Coord. Chem*. **57**, 1263-1269
8. Anacona J.R. & C.C. Gil (2005) *Transition Met. Chem*. **30**, 605-609
9. Anacona J.R. & A. Rodriguez (2005) *Transition Met. Chem.* **30**, 897-901
10. Anacona J.R. & F. Acosta (2006) *J. Coord. Chem*. **59**, 621-627.
11. Anacona J.R. & J. Estacio (2006) *Transition Met. Chem*. **31**, 227-231
12. Anacona J.R. & G. Da Silva (2005) J. Chil. Chem. Soc. **50**, 447-450
13. Geary, W. (1971) *Coord. Chem. Rev*. **7**, 81-122
14. Socrates, G. (1980) Infrared Characteristic Group Frequencies, John Wiley & Sons, Ltd. Great Britain
15. Barnes**,** D.J., R.L. Chapman, F.S. Stephens & R.S. Vagg (1981) *Inorg. Chim. Acta*, **51**, 155-162
16. Garg**,** B.S.**,** N. Bhojak, P. Dwivedi & V. Kumar (1999) *Transition Met. Chem.*,**24**, 463-466
17. Franchini, G.C., A. Giusti, C. Preti, L. Tosi & P. Zannini (1985) *Polyhedron*, **9**, 1553-1558
18. Hadjikostas, C.C., G.A. Katsoulos and S.K. Shakhatreh (1987) *Inorg. Chim. Acta*, **133**, 129-132
19. Castillo, M., J.J. Criado, B. Macias & M.V. Vaquero (1986) *Inorg. Chim. Acta*, **124**, 127-132
20. Hathaway, B.J. (1987) In: G. Wilkinson, R.D. Gillard and J.A. Cleverty (EDs.), Comprehensive Coordination Chemistry. Pergamon Press, New York
21. Salib, K.A.R., A.A.Saleh, S.A. El-Wafa & H.F.O. El-Shafiy, (2003) *J. Coord. Chem.,* **56**, 283-289
22. Goodwin B.A. & J.B. Raynor (1970) *Adv. Inorg. Chem. Radiochem*. **13**,135-632
23. Jain, M.C., A.K. Srivastava & P.C. Jain (1977) Jain, *Inorg. Chim. Acta*, **23**,199-203
24. Anacona, J,R, (2001) *J. Coord. Chem*. **54**, 355-365 .