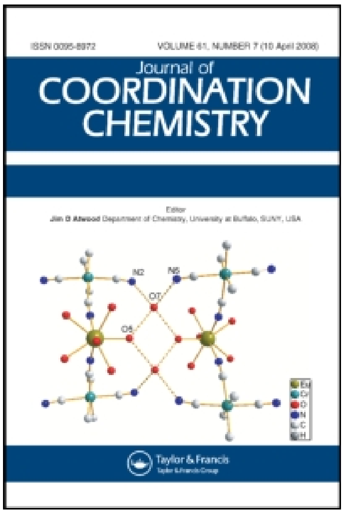

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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME METAL COMPLEXES CONTAINING CLAVULANIC OR NALIDIXIC ACID

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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME METAL COMPLEXES CONTAINING CLAVULANIC OR NALIDIXIC ACID

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The interaction of clavulanic acid (HClav) and nalidixic acid (HNal) with transition metals has been investigated. The complexes $[M(\mathrm{Clav})_2(\mathrm{H_2O})_2]$ (M = Cu(II) or Ni(II)) and $[\mathrm{Cu(Nal)_2(H_2O)_2}]$ and $[\mathrm{Fe(Nal)_2(H_2O)Cl}]$ were obtained by reaction of the ligands with metal ions in methanolic solution under a nitrogen atmosphere. They were characterized by physicochemical and spectroscopic methods. IR spectra of clavulanic complexes showed changes in the carbonyl group on the β-lactamic ring, indicating that this oxygen atom participates in coordination to the metal ions, while in the nalidixic complexes, the coordination to the metal ions is only through the carboxylate group. The ligands behave as monoanionic bidentate ligands and the structure and bonding of the chelates are discussed based on the spectroscopic data, and an octahedral geometry is suggested for their structures. *In vitro* antibacterial activity of the ligands and the complexes were tested using the filter paper diffusion method and the chosen strains include Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimorium, Staphylococcus aureus, Bacillus cereus, Proteus vulgaris and Klepsiella pneumoniae.

Keywords: Antibacterial; Clavulanic acids; Nalidixic acid; Cu(II); Ni(II); Fe(III)

INTRODUCTION

Clavulanic acid is a β-lactamic antibiotic while the nalidixic acid is a 4-quinolone derivative related to oxilinic acid (Fig. 1). These compounds are antibacterial agents employed as specific inhibitors of the bacterial

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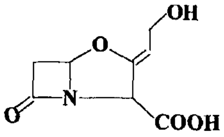
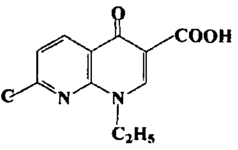


CLAVULANIC ACID



NALIDIXIC ACID

FIGURE 1 Structure of the ligands.

DNA-gyrase, provided with different binding sites towards metal ions for the formation of metal complexes [1–6]. The structure and the chemical constitution of an antimicrobial compound is clearly a valuable piece of information. Where the structure is known it is studied carefully to determine whether a structural analogy exists with the whole or part of a biologically important molecule. A structural analogy with a biological molecule may point directly to the site of biochemical action of an inhibitor. In the past few years we have been investigating the coordination chemistry of antibiotics and Schiff-base compounds with transition and d^{10} metal ions in an attempt to examine their mode of binding in the solid and to study their biological activity [7–10].

EXPERIMENTAL

Physical Methods

Fourier transform infrared (FTIR) spectra of the ligand and its metal complexes as KBr pellets were recorded in the spectral range 4000–400 cm^{-1} with a Perkin Elmer Series 2000 apparatus. FTIR spectra as polyethylene pellets were registered between 450–120 cm^{-1} by using a Brucker IFS 66 V spectrophotometer. Spectra were scanned with a resolution of 2 cm^{-1}. Two hundred scans were accumulated for both the mid and far FTIR measurements. EPR spectra were recorded on a Brucker ECS

106 spectrometer by the X-band method (9.76 GHz); α-α'-diphenyl-β-picrylhydrazide free radical was used as the g marker. UV-Visible spectra were recorded using a Perkin Elmer recording spectrometer. The metals of the complexes were determined by atomic absorption spectroscopy after destruction by a hot concentrated 1:1 mixture of HNO_3 and $HClO_4$. Carbon, nitrogen and hydrogen were analyzed by the microlabs in the Venezuelan Institute of Scientific Research (IVIC). Magnetic susceptibilities were measured on a Johnson Matthey Susceptibility Balance at room temperature using $HgCo(NCS)_4$ as calibrant.

Antibacterial Activity Test

In vitro antibacterial activity of the ligand and the complexes were tested using the paper disc diffusion method [11]. The chosen strains include G(+) S. aureus, Proteus vulgaris, Salmonella typhimorium and B. cereus and G(−) P. aeruginosa, E. coli and K. Pneumoniae. The liquid medium containing the bacterial subcultures was autoclaved for 20 min at 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 in a plate and allowed to solidify. The test compounds in DMSO solutions were added dropwise on a 10 mm diameter filter paper disc placed in a centre 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 reactants and solvents were analytical grade where possible, purchased from Aldrich and used without further purification. All solvents were distilled by standard techniques before use. The clavulanic complexes were prepared by mixing potasium clavulanate (2 mmol) and metal(II) chloride salts: $CuCl_2 \cdot 2H_2O$ or $NiCl_2 \cdot 6H_2O$ (1 mmol) in MeOH (40 mL) and the reaction mixture was stirred at room temperature for 10 h, and then left to stand overnight. Solvent was then removed by rotary evaporation until a precipitate appeared. After cooling, the solid was filtered, washed with water, MeOH and ether and dried in *vacuo* at room temperature. The nalidixic complexes were prepared at pH 7 by mixing nalidixic acid (2 mmol), sodium hydroxide (2 mmol) and $CuCl_2 \cdot 2H_2O$ or $FeCl_3$ (1 mmol) in MeOH (40 mL) and the reaction mixture was stirred at room temperature for five days and then left to stand overnight. Evaporation of the solvent afforded precipitates which were washed and dried as mentioned before. All syntheses were carried out in the presence of nitrogen.

RESULTS AND DISCUSSION

Elemental analyses agree well with the stoichiometry of the complexes (Tab. I). The copper(II) and nickel(II) clavulanic complexes have green and dark green color respectively, while the copper(II) and iron(III) nalidixic complexes are blue and yellow, respectively. All appear to be air stable solids, soluble in DMSO, slightly soluble in CH_3CN and insoluble in MeOH and water with the exception of the iron complex which is water soluble. The experimental conductivity values measured on 10^{-3} M solutions in DMSO at room temperature, fall in the range of non electrolytes [12]. This strongly suggests that the iron(III) complex has the chlorine atom attached to the central ion. Thermal gravimetric analysis of the complexes shows a mass loss equivalent to two water molecules at 150°C, which means that these water molecules are coordinated to the metal. All the attempts to form complexes of a well defined stoichiometry, in the above mentioned conditions, with manganese(II), cobalt(II), zinc(II), and cadmium(II) and lanthanum(III) ions were unsuccessful.

The coordination site and the bonding properties of the ligands are discussed on the basis of spectroscopic analysis; IR spectroscopy is an important tool to determine the site of coordination. The IR spectra of the ligand and the respective complexes show similarities to one another and were recorded in the region between 4000 – 200 cm^{-1}. In the infrared spectrum of the clavulanic acid changes in frequency and intensity are

TABLE I Elemental analyses[a] for the metal complexes

Compound	*C*(%)	*H*(%)	*N*(%)
$[Cu(Clav)_2(H_2O)_2]$	38.9	4.3	5.3
	(38.7)	(4.0)	(5.6)
$[Ni(Clav)_2(H_2O)_2]$	38.8	3.9	5.9
	(39.1)	(4.1)	(5.7)
$[Cu(Nal)_2(H_2O)_2]$	51.5	4.2	10.2
	(51.3)	(4.6)	(10.0)
$[Fe(Nal)_2(H_2O)Cl]$	50.3	4.4	10.1
	(50.4)	(4.2)	(9.8)

[a] Calculated values are given in parenthesis.

observed, when complexed, on the band corresponding to the stretching vibration of the carbonyl group which occurs at 1782 cm^{-1}. The spectra of the clavulanic complexes show this band around 1630 cm^{-1} suggesting that coordination of ligand occurs through the oxygen atoms from the carbonyl groups. On the other hand, in the IR spectrum of the nalidixic acid the band corresponding to the stretching vibration of the carbonyl group on the benzene ring occurs at 1715 cm^{-1} while the spectra of the nalidixic complexes show this band around 1720 cm^{-1} suggesting that the carbonyl oxygen atom does not coordinate to the metal ions. In all the compounds the metal is coordinated by the carboxylate group either as a unidentate or as a bidentate ligand assigned from the relative positions of the antisymmetric and symmetric stretching vibrations [13]. The presence of M–O stretching vibrations at 460 and 270 cm^{-1} for the metal complexes support coordination of the ligand as bidentate chelating agent [13]. A broad diffuse band of medium intensity in the 3500–3400 cm^{-1} region may be assigned to OH stretching vibration for the lattice water.

Corrected magnetic moments have been calculated from the molar magnetic susceptibilities using Pascal's constants [14] and the values are within the range 1.8–2.1 BM for the copper(II) complexes indicating that these complexes have one unpaired electron. The nickel(II) complex has a magnetic moment value of 2.8 BM which is characteristic of systems with two unpaired electrons and greater than the spin only value, presumably due to the orbital contribution resulting from the transfer of an electron from the $d_{x^2-y^2}$ orbital to the d_{xy} orbital. The iron(III) complex has a magnetic moment value of 5.5 BM as predicted for high spin systems with five unpaired electrons. We assign O_h symmetry to these complexes.

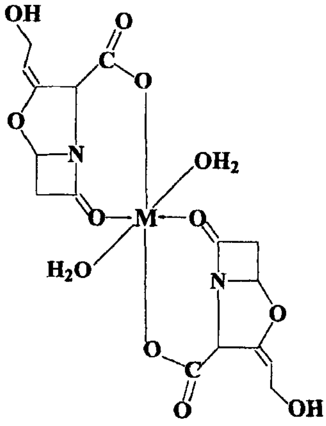
The room temperature EPR spectra of the powder samples of the $[Cu(Clav)_2(H_2O)_2]$ and $[Cu(Nal)_2(H_2O)_2]$ complexes showed a single broad signal with poor resolution of the hyperfine structure on both sides of the main signal. The calculated $g_\parallel$ and $g_\perp$ values appear in the 2.14–2.26 and 2.03–2.07 regions, respectively. The existence of $g_\perp$ values lower than those of their respective $g_\parallel$ values ($g_\parallel > g_\perp > 2.0023$) indicates [15] that the unpaired electron most likely resides in the $d_{x^2-y^2}$ orbital having $^2B_{1g}$ as a ground state. The axial symmetry parameter, $G = 4.7$, obtained by the relation $(g_\parallel - 2)/(g_\perp - 2)$, for the clavulanic complex, supports [16] the contention that there is no exchange interaction between the copper centers ($G > 4$), while for the nalidixic complex, $G = 3.7$, supports an exchange interaction.

The UV-visible spectra of the free ligands and the complexes in DMSO solutions present two major absorption maxima in two distinct regions:

38000–39000 and 35000–36000 cm^{-1} for the clavulanic compounds while at 38000–39000 and 27000–29000 cm^{-1} for the nalidixic complexes assigned to the $\pi \rightarrow \pi^*$ transitions within the organic molecule. In the electronic spectrum of the iron(III) complex, only two strong bands at 27000 and 24200 cm^{-1} are observed, hence no structural deductions can be made. The nickel complex showed two bands at 11700 and 17900 cm^{-1} which may unambigously be assigned [17] as $^3A_{2g} \rightarrow ^3T_{2g}$ and $^3A_{2g} \rightarrow ^3T_{1g}$ (F) transitions respectively, arising from the octahedral geometry of nickel(II). The copper complexes exhibit two discernable bands centered in the 15000–15600 cm^{-1} and 16000–16800 cm^{-1} regions assignable to $^2B_{1g} \rightarrow ^2B_{2g}$ and $^2B_{1g} \rightarrow ^2E_{2g}$ transitions, respectively, characteristic of an octahedral geometry [17].

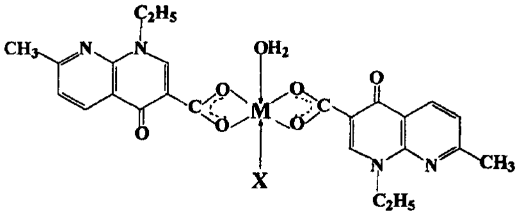
Structure of Complexes

Clavulanic and nalidixic acids have several potential donor atoms but, due to steric constraints, the ligands can provide a maximum of two donor atoms at any one time to coordinate a metal center. On the assumption that the coordination of the clavulanic acid occurs through the $O_{carboxylate}$ and $O_{carbonyl}$ atoms to the metal, as seems likely from molecular models, it follows that clavulanic acid behaves as a bidentate ligand. It is quite feasible that the copper(II) and nickel(II) ions are six coordinate with two molecules of clavulanic acid and water molecules at the vertices of an octahedron (Fig. 2). On the other hand, the nalidixic complexes of copper(II) and



M = Cu(II), Ni(II)

FIGURE 2 The tentative structure of the clavulanic complexes.



M = Cu(II), X = H_2O ; Fe(III), X = Cl

FIGURE 3 The tentative structure of the nalidixic complexes.

iron(III) are six coordinate with two molecules of the ligand and molecules of water or chloride ion, in any case, probably occupying *trans* axial positions, as can be seen in Figure 3. From models it appears that both a planar or approximately planar configuration of the ligand are possible but without a full structural analysis one cannot make a distinction between these possibilities. Despite the crystalline nature of the products none proved suitable for X-ray structure determination.

Biological Activity Test

The susceptibility of certain strains of bacterium towards the ligand and metal complexes was judged by measuring the size of inhibition diameter. The results are shown in Table II. The antibacterial activity of the complexes were compared with commercial samples of amoxiciline/clavulanic acid and nalidixic acid. In general, when the complexes were tested against the E. Coli and P. Aeruginosa strains they were found to possess higher activity than the acids themselves at concentrations of 200 μg/disc. In all cases

TABLE II Antibacterial activity of the metal complexes

		Zone of inhibition (mm)						
Compound	*(μg/disc)*	*E.C.*	*S.A.*	*B.C.*	*S.T*	*P.A*	*P.V*	*K.P.*
[Amox./Clav.Acid]	200	45	57	58	22	10	45	17
[$Cu(Clav)_2(H_2O)_2$]	200	55	56	55	18	10	39	11
[$Ni(Clav)_2(H_2O)_2$]	200	52	53	50	15	15	40	13
[Nalidixic Acid]	200	40	33	36	44	20	35	35
[$Cu(Nal)_2(H_2O)_2$]	200	50	43	42	53	30	48	41
[$Fe(Nal)_2(H_2O)Cl$]	200	45	37	40	48	25	45	39

E.C.: Escherichia Coli 10536, S.A.: Staphylococcus Aureus 6538, B.C.: Bacillus Cereus 9634.
S.T.: Salmonella Typhimurium 14028, P.A.: Pseudomonas Aeruginosa 9027.
P.V.: Proteus Vulgaris 9920, KY.: Klebsiella Pneumoniae 10031.

the nalidixic complexes were found to possess higher activity than that of nalidixic acid showing that they have a very good activity as bactericides.

Nalidixic acid is zwitterionic having a favorable solubility in acidic or basic solvents, while its solubility in water, methanol, ethanol, and chloroform is poor. However, the iron(III) complex of nalidixic acid is water soluble. This increase of hydrotropy can enhance the ability of drug molecules in crossing the membrane of a cell, and hence raise the biological utilization ratio and activity of the drug.

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