Mn(II), Ni(II), and Cu(II) Complexes of Artmisinin Derivatives: Synthesis, Characterization and Antimicrobial Activities.

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ABSTRACT

A range of transition metals ligated by sesquiterpene lactone-artesunate (ATS) have been investigated. Syntheses of Cu(II), Ni(II), and Mn(II) complexes of decahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]1,2-benzodioxepin-10-ol hydrogen succinate were prepared by a direct aqueous substitution reaction in methanol. Structural elucidations of the complexes were determined using X-Ray Diffractrometry along with elemental analysis and FT-IR. The IR absorption revealed that ATS acts as monodentate specie through carbonyl group on coordination. It also acts as bidentate specie upon deprotonation of the carboxylic group.

The [Cu(ATS)ac.H_2O] was further characterized with X-ray powder diffraction studies which showed that it has a tetrahedral geometry. Corresponding complexes and their ligands were screened against bacteria and fungi: *Staphylococcus aureus, Pseudomonas aeruginosa*, *Proteus hauseri* ATCC 13315, *E. coli* ATCC35218, *Rhizopus stolonifer,* and *Aspergillus niger,* respectively, to investigate their antimicrobial activities. [$Mn(ATS)_2Cl_2$] showed the highest inhibitory level over Ni(II) and Cu(II) complex and the parent ligand drug(ATS).

(Keywords: transition metal complex, artesunate, antimicrobial activities)

INTRODUCTION

Among the challenges faced by bioinorganic chemists is how to overcome the resistance posed by pathogens to all classes of anti-malaria drugs [1, 2, 3]. Malaria is a major threat to health especially in Africa [4]. Current estimate indicate that at least one to three million children die of malaria related cases each year [5]. Therefore, recent research is on how to generate effective malaria vaccine. Artesunate (ATS) is a member of the artemisinin group of drugs that treat malaria with decreased efficacy or potency due to emergence of resistance which has been occurring to all classes of anti-malaria drugs and as a result brought a decline in anti-malarial drug efficacy. It is capable for killing the asexual forms of plasmodium at erythrocytic stage. It is effective against *Plasmodium falciparium* and *Plasmodium vivax* especially against malarial pararasites resistant to chloroquine[6,7,8]. Artesunate drug is a semi- synthetic derivative prepared from dihydroartemisinin (DHA) by reacting with succinic acid anhydride in basic medium [9]. Its molecular formula is $C_{19}H_{28}O_8$ with molecular mass of 384.421gmol^{-1} and melting point of 135.5^{o}C. It is a water soluble drug and may therefore be given by injection [10].

Interestingly, incorporation of metal ions into some drugs is receiving great attention in the field of chemotherapy. Some metal ions stabilize the drugs thereby allowing them to be deposited in the right location without any degradation or loss in composition [11]. Metal complexes as a metallo-pharmaceuticals have been reported to be capable of enhancing biological activity in the area of antimicrobial and antiviral properties and could be effective against diseases [12]. The cisplatin- Pt(II) as anti-cancer [14] and anti-parasitic [14] among others have been established. It is therefore necessary to know that metal ion such as manganese stabilizes the anti-malaria drugs [13]. Also, the administration of

Fe-artesunate to uninfected mice was discovered to bring about increase in the concentration of serum creatinine which suggests abnormal kidney function [15, 16, 17].

In furtherance to the earlier report of the complexation behavior of artemisinin [18], we present the synthesis, characterization and antimicrobial activities of Cu(II), Ni(II), and Mn(II) complexes artesunate with the view to exploit the therapeutic values of the complexes.

MATERIALS AND METHODS

All the chemicals (metal salts inclusive) used in the experiment were of analytical grade and used without further purification. They were purchased from Aldrich Chemical Company in Great Britain, and were used as received. Artesunate was pharmaceutical grade (Swiss Pharma). All solvents used were purified and dried by vacuum, distillation according to standard methods. The bacteria and fungi were obtained from Fidson Health Plc. (Lagos State, Nigeria) and Nigeria Institute of Medical Research, Lagos State, Nigeria and cultured at the department of microbiology, University of Agriculture, Abeokuta (UNAAB).

Basic Theory

Physical Measurements: The elemental analysis (C, H, & N) were performed by using Flash–EA1112 micro-analyzer and a HPMOD1106 microan at the Institute of Chemistry, Chinese Academy of Science, (ICCAS), China. Vibrations spectral were recorded in KBr/Nujol using NaCl and CsCl cells on Unicam 360 FT-IR, Perkin Elmer UV Winlab 6.0.3.0730/ Lambda 25 1.27 spectrophotometer and d sp dffractometer. All the other physical measurements and analytical procedures were used according to literatures [19].

Properties of the Artesunate Used: Artesunate (ART) has the following properties:

M F = $C_{19}H_{28}O_8$ M.wt: 384gmol^{-1} ; M.pt: 135.5^{0}C
IR(KBr,cm^{-1}) 3437, 2924, 1735, 1457, 1370, 1319
UV-Vis (methanol): λ_{max}, 226 nm
NMR (CD_3OD): (CH_2C=O) 170ppm, Cq 104ppm, 6CH_2 (20-40ppm), CH_3 (10-20ppm),O-CH(90ppm)
Conductivity: 8.730 x 10^{-3} Ω^{-1}cm^{-1}

Synthesis of the Complexes

Synthesis of Cu-ATS Complex: A mixture of Artesunate (0.845 g, 2.2 mmol) and $Cu(ac)_2.H_2O$ (0.399 g ,2.0 mmol) in freshly distilled 20mL methanol was refuxed for 3h. The greenish solution produced a blue precipitate after 2h. The reaction mixture was cooled in ice bath, concentrated and filtered. The residue was washed with diethylether and dried *in vacuo*.

Yield: (0.85 g, 1.5 mmol) 66%, M.wt: 582, M.pt. (decomp. at temp >198^{o}C) anal. calcd. for $C_{19}H_{28}O_8Cu2acH_2O$ C,47.4;H,6.19; Cu,8.6 Found: C,47.52;H,6.20
IR(KBr,Cm^{-1}): 3373,2918,1136,1721
UV-Vis (methanol) λ, nm: 619
Conductivity: 1.687 x 10^{-4} Ω^{-1}cm^{-1}

Synthesis of Ni-ATS Complex: A mixture of Artesunate (0.768 g, 2.0 mmol) and $NiCl_2.6H_2O$ (0.54 g, 2.0 mmol) in freshly distilled 20 mL methanol was refluxed for 12h. The light brown color of the solution changed to dark-brown with no precipitate formation after 24h constant stirring. The reaction mixture was filtered. The mixture was placed in an ice bath overnight followed by slow evaporation for 2 weeks. The solid product obtained was washed with diethyl ether and dried *in vacuo*.

Yield: (0.77 g, 1.5 mmol) 73%, M.wt: 530, M.pt (decp.at temp)>250^{o}C, Anal.Calcd. for $C_{19}H_{28}O_8Ni2Cl$,: C,43.02;H,5.66; Ni,6.26; Found:C,43.05;H,5.50
IR(KBr,cm^{-1}) : 3437, 2924,1735,1457,1370,1319
UV-Vis (methanol) λ, nm: 259, 352;
Conductivity: 1.60 x 10^{-4} Ω^{-1}cm^{-1}

Synthesis of Mn- ATS Complex: A mixture of Artesunate (0.423 g, 1.1 mmol) and $MnSO_4$ (0.168 g,1.0 mmol) in freshly distilled 20ml methanol was refuxed for 3h. A milky solution produced a white precipitate after 2h. The reaction mixture was cooled in ice bath, concentrated and filtered. The residue was washed with diethylether and dried white powdery product was stored *in vacuo*.

Yield: (0.85 g, 1.5 mmol) 66%, M.wt: 582,
M.pt(decomp. at temp >230^{o}C) anal. calcd. for $C_{19}H_{28}O_8Mn.SO_4$ C,47.4;H,6.19; Cu,8.6 Found: C,47.52;H,6.20
IR(KBr,Cm^{-1}): 3373,2918,1139,1749

UV-Vis (methanol) λ, nm: 600
Conductivity: 1.687 x 10^{-4} $\Omega^{-1}cm^{-1}$

Antimicrobial Analysis

The antimicrobial activities of the synthesized complexes and their parent ligands were tested against some pathogens, using the agar well diffusion method [20, 21]. Each complex and the parent ligand of 0.1g were dissolved in 3ml sterilized water. Holes were drilled in nutrient agar inoculated with the test organisms in Petri dishes and 14mg/ml, 7mg/ml, and 3.5mg/ml of the samples were dispensed in the holes. The Petri dishes were incubated at 37°C for 24hrs 10^8-10^9 cells/ml except those inoculated with fungi. Sterile paper discs (Whatman: 1.6 mm) with absorbed spice extract (30 µl/disc) were placed on the agar at certain intervals by pressing gently. After the plates were incubated at 35±0.1°C for 48 h, zones of inhibition were measured in mm and taken as a measure of the inhibitory power of the samples against the particular test organism. The experiments were repeated in duplicate for all of the test strains.

XRD data of Cu(II) ATS

Crystal of Cu(II) artesunate complex suitable for powdery XRD diffractometry studies was used. The instrument was first calibrated with the standard, corundum (Al_2O_3) so as to acquire spectra for the unknown sample and the Braggs principle was then applied. The detector used 95° reflection to deduce that the complex has the following data and hence possess a distorted tetrahedral geometry.

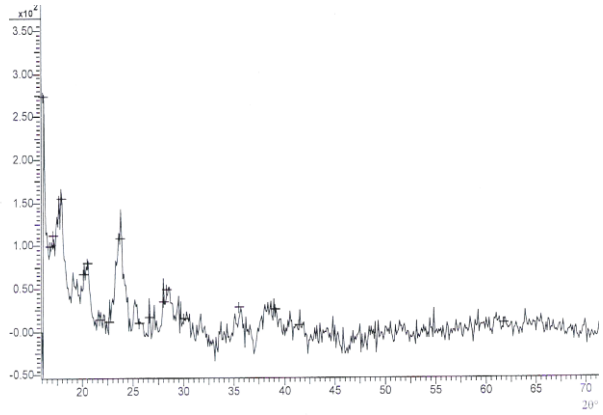


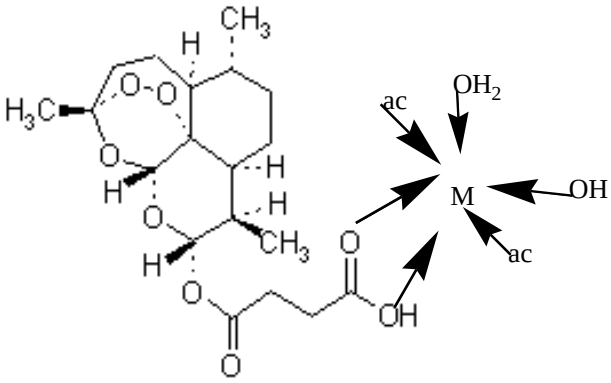
Figure 1: XRD Data of Cu(II) Artesunate Complex.

RESULTS AND DISCUSSION

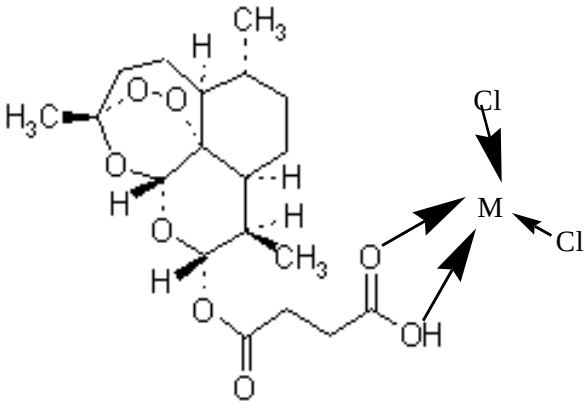
All the metal complexes were synthesized according to previously reported methods [14]. The interaction of each of the ligand with the metal salts resulted in the formation of the complexes in 1:1 stoichiometric ratio. The complexes are stable and non-hygroscopic solid compounds. All the prepared complexes were characterized on the basis of solubility, metal content, infrared and electronic spectra. All the complexes decomposed at higher melting points compared with the ligand attributed to strong (ionic) in the complexes. The metal complexes are not soluble in the solvent of the reaction but soluble in DMSO. The molar conductance values measured in methanol and acetone for the complexes are in (10^{-4}) which were lower than the ligand found in (10^{-3}) suggesting that the complexes are weak electrolytes. The ligand artesunate shows single absorption band in the region 226nm which is assigned to n→π* transition. A light band shift in the UV spectra relative to that of ligand with visible absorption *bands in the metal-artesunate complexes* have been attributed to metal complexation. The possibility of d^{10} electronic configuration of Copper ion leading to ^1S (no splitting) ground state spectroscopic symbol suggests the appearance of only band in the Cu-ATS complex while manganese also behave in the same pattern with ^6S (no splitting) symbol. The d-d spectra of the Ni(II) artesunate complex showed only two bands at 259nm and *352nm* even though three bands were expected : $^3T_{1g} \rightarrow {}^3T_{2g}(F)$, $^3T_{1g} \rightarrow {}^3A_{2g}(F)$ and $^3T_{1g} \rightarrow {}^3T_{1g}(P)$.

The assignments of important IR bands of the complexes were based on literature values obtained for similar structural compounds of the ligands[9]. The ATS ligand shows absorption at 3437cm^{-1} for O-H carboxylic acid with moderate and broad intensity; while 1457cm^{-1} and 1370 cm^{-1} for out of plane bending vibration O-H. 2845 cm^{-1} band is attributed to C-H str. alkane bond. 1757 cm^{-1} (C=O str. for ester), 1646 cm^{-1} (C=C str. aromatic), 1319 cm^{-1} (C-O str.) these vibrations are also found in the complex except C=O frequency which has undergone bathochromic effect for Cu(II) and Mn(II) complexes while there was a total disappearance of the carbonyl group in Ni(II) artesunate complex attributed to coordination. The results of elemental analysis of the complexes are in agreement with the proposed formula. Degree of

crystalinity was confirmed with some sharp peaks values obtained in the XRD data.



Where M = Cu



Where M= Mn & Ni

The results of antimicrobial test of the compounds are shown in Table 1. From the analyses, the parent drug Mn-ART test drug exhibit the highest inhibitory value among all complexes except on *Staphylococcus*. The antimicrobial activity of the Mn-ART may be suggested to be due to its ions components capable to enhance the destruction of the microbial pathway [22].

Table 1: Result of the Sensitivity of the Artesunate and Complexes against the Microorganisms in mm.

Micro-organism/ pathogens	ART	Cu – ATS	Ni – ATS	Mn-ATS
Staphylococcus	Nil	4 ± 1.0	2 ± 1.1	3 ± 1.0
Pseudomonas	Nil	3	2	4
Aspergillus niger	6± 2.2	Nil	1± 0.5	12±2.1
Proteus hauseri	4±1.4	5±1.2	3±1.0	6±1.1
Rhizopus stolonifer	2±1.0	5±1.1	2±1.1	7±1.5

Values are mean of 3 replicates ± S.D

Artesunate the parent drug was detected to exhibit zero inhibition when screened against *P. aeruginosa* and *S. aureus*.

Table 2: The Antibacterial Activities of ART and its Complexes Evaluated by MIC(µg/ml).

Micro-organisms	Artesunate	Cu-ATS	Ni-ATS	Mn-ATS
Pseudomonas aeruginosa	-	>50,000	50,0000	>50,000
Straphylococcus aureus	-	>50,000	50,000	>50,000
Aspergilluss niger	>50,000	-	>50,000	>50,000
Proteus hauseri	>50,000	50,000	50,000	>50,000
Rhizopus stolonifer	-	>50,000	50,000	>50,000

The results show that the MIC of artesunate on *Aspergilluss niger* is greater than 50,000 µg/mL while that of Cu-ATS on *Straphylococcus aureus* and *Pseudomonas aeruginosa* also greater than 50,000 µg/mLwhile the MIC of Ni-ATS on *Aspergilluss niger* was higher than 50,000 µg/mL.

CONCLUSION

Three new complexes of artesunate were synthesized with copper, nickel and manganese metal salts. Artesunate acting as bidentate ligand through carbonyl (C=O) and hydroxyl(OH) group of carboxylic group evident from spectroscopic data resulting into tetra/octahedral coordinated compounds depending on the solvent effects during the synthesis.

The results of the antimicrobial effect posed by Manganese complex of the artesunate among other complexes and their parent ligand were better and could be a good potential therapeutic drug for antibacterial activity.

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