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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF MIXED ASCORBIC ACID - NICOTINAMIDE METAL COMPLEXES

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ABSTRACT
The present study aimed at synthesizing copper(II) and zinc(II) complexes of mixed ascorbic acid and nicotinamide and physiochemically characterize by solubility test, melting point, conductivity test, infrared, electronic and proton nuclear magnetic resonance techniques. The result of the physiochemical studies indicated 1:1 stoichiometry and were supported by the spectroscopic data. The antimicrobial activities of the mixed complexes were carried out against Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Penicillum, spp Aspergillus flavus and Aspergillus niger. The result of the infrared data showed that ascorbic acid coordinates through the oxygen of the carbonyl group and that of enolic C-2 hydroxyl group, while nicotinamide coordinates through the nitrogen atom of the pyridine ring. The result of the antimicrobial studies showed that the mixed complexes have higher inhibitory activity than the original ligands against the tested bacteria and fungi species.
KEY WORDS: Ascorbic acid, Nicotinamide, Synthesis, Antimicrobial, Complexes.

INTRODUCTION

Antimicrobial resistance is fast becoming a global concern with rapid increase in multidrug- resistance bacteria. Some previously treated pathogens are now becoming untreatable (Saha *et al*, 2009). An extensive work have been reported on the investigation of some drug- metal complexes (Ajibade et *al*, 2007; Adediji *et al*, 2009; Obaleye *et al*, 1997; Hariprasath, et al, 2010; Lawal and Obaleye, 2007; Ajibade and Idemudia, 2010). In most cases, the metal complexes usually have higher inhibition or activity against the organism compared with the corresponding ligands.

In continuation of our studied on the mixed ligand metal complexes, we decided to report the synthesis, characterization and antimicrobial activity of mixed complexes of ascorbic acid and nicotinamide.

MATERIALS AND METHODS

The metal salts used for the synthesis were obtained and used as supplied from British Drug House Chemical Limited, England. Ascorbic acid and Nicotinamide were obtained from Swiss Chemical Limited Lagos, Nigeria. Cultures of the micro-organisms used were obtained from Department of Microbiology, University of Ilorin. Ilorin, Nigeria.

Synthesis of Mixed complexes of Ascorbic Acid and Nicotinamide

The procedure described by Abd El Wahab and El-Sarrag (2004) was used to synthesized the complexes, with slight modification. 5 mmole of metal salts ($ZnCl_2$ $NiCl_2.6H_2O$, $CuSO_4$, $ZnSO_4$, $CoCl_2.6H_2O$, $CuCl_2$, and $FeCl_3.6H_2O$) was dissolved in 10ml of methanol, followed by addition of 10 mmole (1.761g) of ascorbic acid and 10 mmole (1.731g) of nicotinamide. The mixtures were refluxed for 3 hours and kept for 2 weeks before formation of precipitate. No precipitates was formed from $NiCl_2.6H_2O$, $CuSO_4$, $ZnSO_4$, $FeCl_3.6H_2O$ and $CoCl_2.6H_2O$. The precipitates from $CuCl_2$ and $ZnCl_2$ were characterized to determine their structures.

Antimicrobial Studies

The antimicrobial activities of the ligands and the complexes were determined by methods described by Collins(1970). The antibacterial activity was determined on the seeded nutrient agar on which 0.5m diameter wells were punched. Different concentrations (50µg/ml and 100 µg/ml) of sterile filtered solutions of the ligands were made using DMSO as solvents, 0.1ml of each concentration was applied into the wells and incubated at 37^0C for one to three days. DMSO was used as control.

The antibacterial activity of the compounds was estimated on the basis of the size of the inhibition zone formed around the wells on the seeded nutrient agar. The bacterial species used include: *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. They were obtained from the Department of Microbiology, University of Ilorin.

For the determination of antifungal activity of the compounds, three fungal species were used including *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* species. The fungi were cultured on potato dextrose agar. The inoculation method used was as described by Abd El-Wahab and El-Sarrag (2004), Chohan *et al* (2006).

The fungal cultures were incubated at 30^0C for 48 hours. The antimicrobial activity was estimated on the basis of the size of inhibition zone formed around the well on the seeded agar plates and the inhibition growth in percentage was determined on the basis of the average diameter of bacteria or fungal colony on the growth medium compared to the respective control.

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100 \quad 1$$

Where A =diameter of the bacterial or fungal growth in the controlB = diameter of the bacterial or fungal growth in the test plate.

RESULTS

Table 1 showed the results of the physical properties of the free ligands and the mixed complexes. The two mixed ligand complexes decomposed above 360 ^{0}C. The high melting point confirmed the formation of the complexes. The mixed complexes also have different colours. The blue colour of the Cu (II) complex suggests a tetrahedral geometry (Lawal, 2010). The results of molar conductance in DMSO solutions showed that the mixed ligand complexes are electrolytes.

Table 1: Result of some physical properties of mixed complexes of Ascorbic acid and Nicotinamide

Compounds	Colour	Mpt (^{0}C)	%Yield	RF	Conductivity (scm^{2}mol^{-1})	%Metal	
Ascorbic acid (Asc)	white	190- 192	-	0.61	64.5	-	
Nicotinamide (Nic)	white	130- 132	-	0.50	2.5×10^{-2}	-	
Zn (Asc) (Nic) Cl	white	□ 360	48.1	0.88	121.0	10.2 (14.0)	
Cu (Asc) (Nic) Cl	Blue	□ 360	40.0	0.85	96.0	12.4 (13.2)	

The results of the infrared spectra of the ligands and the mixed complexes are presented in Table 2.The infrared spectra of the free ligands were compared with those of the mixed complexes. The absorption bands at 3874 cm^{-1} and 3742 cm^{-1} in the free ascorbic acid due to

N-H stretching vibrations, have been shifted to 3840 cm^{-1} in Cu(Asc)(Nic)Cl and 3331 cm^{-1} in Zn(Asc)(Nic)Cl. The absorption band at 3519 cm^{-1} and 3188 cm^{-1} in the free ascorbic acid due to O-H stretching has been shifted to 3421 cm^{-1} in the Cu(II) complex (Anderson and Co, 2004). The strong bands at 1763 cm^{-1} in ascorbic acid and at 1685 cm^{-1} in the free nicotinamide , were assigned to C=O stretching vibrations, these bands have shifted in the mixed complexes to 1624 cm^{-1}. The bands at 546 cm^{-1} and 516 cm^{-1} were assigned to M-O vibrations and those at 656 cm^{-1} and 685cm^{1}were assigned to M-N vibrations respectively (David, 2000).

Table 2: Infrared spectra data for the ligands and drug-metal complexes

COMPOUND	**υ(N-H)**	**υ(O-H)**	**υ(C=O)**	**υ(C-O)**	**υ(M-O)**	**υ(M-N)**
Ascorbic acid (Asc)	3874w 3742w	3519s 3188s	1763s 1685s	1038s 1150s	- -	- -
Nicotinamide (Nic)	3351m		-	1381s	-	-
Cu(Asc)(Nic)Cl	3331m,b	-	1576w	-	685s 656s	546m,b
Zn(Asc)(Nic)Cl	-		1624w,b	1089s	-	516w,b

S= strong, w= weak, m= medium, b= broad

Table 3: UV- Visible spectra of the ligands and the mixed –metal complexes

COMPOUND	WAVELENGTH(nm)	ENERGIES(cm^{-1})	ASSIGNMENT
Ascorbic acid (Asc)	254	39937	π-π
Nicotinamide (Nic)	218	45872	π-π
	280	35714	n-π
Cu(Asc)(Nic)Cl	414	24155	MLCT
Zn(Asc)(Nic)Cl	402	24876	MLCT

Table 4: Antibacterial activities of the ligands and their mixed complexes showing the zone of inhibition against the organisms.

	E coli		***Staphylococcus***		***Bacillus subtilis***	
			Zone of inhibition (mm)			
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
Ascorbic acid (Asc)	NA	NA	NA	NA	NA	NA
Nicotinamide (Nic)	14±1.2	20±1. 1	15±1.6	17±1.3	19±1.2	21±1.1
Mixed complexes						
Zn(Asc)(Nic)Cl	26±1.2	30±1.5	21±1.4	24±1.8	28±1.8	35±2.5
Cu(Asc)(Nic)Cl	24±1.6	27±1.9	19±0.9	21±1.1	23±2.1	30±1.9

NA = No activity Values are mean of 3 replicates ± SD.

Table 5:Antifungal activities test of the ligands and their mixed complexes showing the zone of inhibitionagainst the organisms.

Ligands/ Complexes	***Aspergillus flavus***		***Aspergillus niger***		***Penicillum sp***	
			Zone of inhibition (mm)			
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
Ascorbic acid (Asc)	17±1.1	20±1.9	22±2.5	28±3.1	12±1.4	20±0.9
Nicotinamide (Nic)	15±1.1	19±1.2	10±0.8	17±1.4	12±1.2	20±1.6
Mixed complexes						
Zn(Asc)(Nic)Cl	28±2.0	36±2.7	21±1.8	31±1.9	29±1.4	40±1.6
Cu(Asc)(Nic)Cl	30±2.1	33±2.6	27±1.9	35±2.0	32±1.4	38±1.8

Values are mean of 3 replicates ± SD.

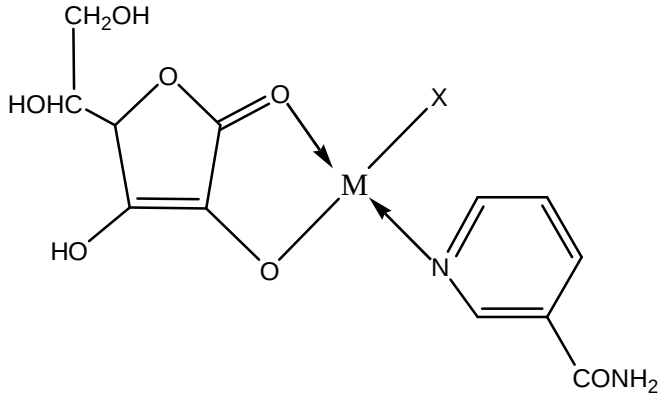
DISCUSSION

The results of the ultraviolet/ visible spectra are presented in Table 3. The visible spectra of ascorbic acid- nicotinamide showed only one absorption band at 414 nm for Cu (II) and at 402 nm for the Zn (II) complexes, which were assigned to metal ligand charge transfer (Lawal and Obaleye, 2013*)*.

The results of the antibacterial activities of the ligands and their mixed complexes are presented in Table 4. The antibacterial activities test of the ligands and their complexes were tested against three bacteria species; which include *Bacillus subtilis*, *Staphylococcusaureus and Escherichia coli*. The antibacterial activity of the compounds was estimated on the basis of the average size of inhibition zone formed around the well on the seeded agar plate.

The results of the antifungal activities test of the ligands and the mixed complexes are presented in Table 5. The antifungal activities test of the ligands and the mixed complexes are tested against three fungi species: *Aspergillus flavus, Aspergillus niger* and *Penicillum* species. In the mixed ligand complexes, Zn(II) complex of mixed ascorbate-nicotinamide complex, Zn(Asc)(Nic)Cl show the higher antifungal activities against *Aspergillus flavus*, when compared with Cu(II) complex of mixed ascorbate-nicotinamide complex Cu(Asc)(Nic)Clwhich showed the lower antifungal activity against *Aspergillus flavus*. Hence, the result of these studies showed that all the mixed vitamin-metal complexes are more effective antibacterial and antifungal against the tested species. It was observed that metal chelation has affected significantly the antimicrobial or bioactive behaviour of the ligands (Chester *et al,* 1987).

The mixed complexes were insoluble in deuterated chloroform, hence the ^{13}C-NMR signals obtained contain mainly the solvent peaks. On the basis of the physical and spectroscopic data obtained, the proposed structure for the mixed ascorbic acid –nicotinamide metal complexes is as shown in fig 1 below:



M = Zn(II), Cu(II)

X = Cl

Figure1: Proposed Structure for mixed Ascorbic acid- Nicotinamide metal Complexes

CONCLUSION

Copper(II) and Zinc(II) complexes of mixed ascorbic acid and nicotinamide have been formed and characterized using physical and spectroscopic methods. The results of these studies confirmed that the ligands are coordinating. The result of the antimicrobial studies showed that the mixed vitamin-metal complexes are more effective antibacterial and antifungal agents against the tested species.

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