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Synthesis, Structural Characterization and Antimicrobial Potency of Anthranilic Acid Based Mn(II) Schiff Base Complex

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Abstract A Schiff base ligand derived from 4-(N,N-dimethylamino)benzaldehyde and 2-aminobenzoic acid with its Mn(II) complex was synthesized and characterized by solubility test, determination of water of hydration, melting point/decomposition temperature, infrared spectroscopy, elemental analysis, atomic absorption spectroscopy, molar conductivity and magnetic susceptibility measurements. The complex was coral red solid, air stable and soluble in most of the organic solvents used. The molar conductance value suggests its non-electrolytic nature. The melting point of the Schiff base was found to be 177 ^{o}C while the decomposition temperature of the complex was 202 ^{o}C suggesting its thermal stability. The atomic absorption spectroscopy and elemental analysis data revealed the formation of the complex in 1:2 Metal–ligand ratio. The determination of water of hydration showed that the complex is hydrated. The infrared spectral data indicate that the Schiff base ligand exhibits bidentate nature coordinated to the metal ion via the nitrogen atom of the azomethine (C=N) and deprotonated carboxylate oxygen (COO^{-}). On the basis of magnetic susceptibility, a four – coordinate tetrahedral geometry was proposed for the complex. The Schiff base and its metal(II) complex were screened for antibacterial activity against *Staphylococcus aureus*, *Echerichia coli* and *Salmonellatyphi*, and antifungal activity against *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus*. The complex showed enhanced activity against the tested microorganisms.

Keywords Tetrahedral, elemental analysis, antibacterial, antimicrobial, 2-aminobenzoic acid

Introduction

A Schiff base is a nitrogen analogue of aldehyde or ketone in which the C=O group is replaced by C=N with the nitrogen atom connected to an aryl or alkyl group, but not hydrogen [1]. It is a condensation product of primary amine with aldehyde or ketone and was first reported by Hugo Schiff in 1864. These compounds are also known as anils, imines or azomethines [2,3].

Metal complexes of Schiff bases exhibit a broad range of biological activities which include antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic activities. They also find applications in agriculture and industrial fields [4].

Ahmed and Kassem [5] prepared four new Schiff bases by condensation of 2-amino- pyridine-3-ol with 3,4-dihydroxybenzaldehyde (I), 2-hydroxybenzaldehyde (II), 5-bromo-2-hydroxybenzaldehyde (III) and 4-dimethylaminobenzaldehyde (IV). The structures of these compounds were characterized on the basis of elemental

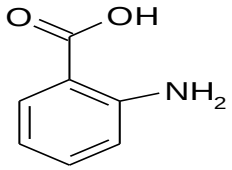
analysis, IR and ^{1}HNMR spectroscopy. Also, the electronic absorption spectra were recorded in organic solvents of different polarity. Biological activities against bacterial and fungal species representing different microbial categories were also studied.

A new ligand, 4-(dimethylamino)benzaldehyde(5-phenyl-1.3.4-oxadiazole-2-yl) hydrazone and its Cr(III), Fe(III), Co(II), Ni(II) and Cu(II) complexes were synthesized [6]. The structures of the complexes were established by elemental analysis, conductance and magnetic susceptibility measurements, IR, ^{1}HNMR and mass spectroscopy. The IR spectra suggested the bidentate nature of the ligand coordinating with metal ion via oxadiazole nitrogen and azomethine nitrogen. The magnetic studies suggested a tetrahedral geometry for Ni(II) and Cu(II) complexes while an octahedral geometry was suggested for Cr(III), Fe(III) and Co(II) complexes.

Anu *et al.* [7] reported the synthesis of Cu(II) Schiff base complex derived from para-dimethylaminobenzaldehyde and 2-aminophenol. The complex was characterized by molar conductance, IR, ^{1}HNMR, ^{13}CNMR, UV-Visible spectroscopy and elemental analysis. The elemental analysis revealed the formation of 1:2 M-L ratio with general formula $[CuL_2Cl_2]$. The molar conductance suggests that the complex is non-electrolyte. The UV-Visible spectra indicated an octahedral stereochemistry for the complex.

El-Ajaily *et al* [8] prepared four new Ni(II), Cu(II), Rh(II) and Pt(IV) Schiff base complexes derived from 4-dimethylaminobenzaldehyde and 4-aminoantipyrine. These complexes were characterized by elemental analysis, molar conductivity, thermogravimetric analysis, magnetic moment measurement, infrared, electronic and mass spectra. The elemental analysis for C, H and N showed the formation of 1:1 [M:L] complexes. The magnetic moment data showed paramagnetic phenomena for Ni(II) and Cu(II) complexes and diamagnetic phenomena for Rh(II) and Pt(IV) complexes.

Anthranilic acid also called 2-aminobenzoic acid is an odourless white or yellow solid of chemical formula $C_7H_7NO_2$, molar mass of 137.14 gmol^{-1}, density of 1.412 gcm^{-3}, melting point of 146 -148 ^{o}C and a boiling point of 200 ^{o}C.

(a) 

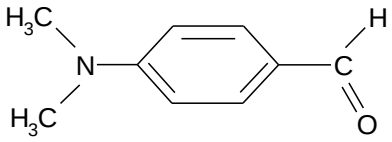
(b) 

Figure 1: Structure of anthranilic acid (a) and 4-N,N-Dimethylaminobenzaldehyde (b)

4-N,N-Dimethylaminobenzaldehyde (1, 11-3D structure) is a bifunctional aromatic skeleton possessing the aldehydic functional group (CHO) para to an activating substituent, dimethylamino group [-N(CH$_3$)$_2$]. It is a white crystalline powder with a melting point of 72-75^{o}C and boiling point of 176 – 177^{o}C. It has a molecular weight of 149.19 g/mol with a molecular formula of $C_9H_{11}NO$ and log P value of 1.8. It has two hydrogen bond acceptors with no hydrogen bond donor. It is stable at room temperature, although it is sensitive to light [9]. We report herein the results of the synthesis, characterization and antimicrobial activity of Mn(II) complex with Schiff base derived from 4-(N, N-dimethylamino)benzaldehyde and anthranilic acid.

Experimental

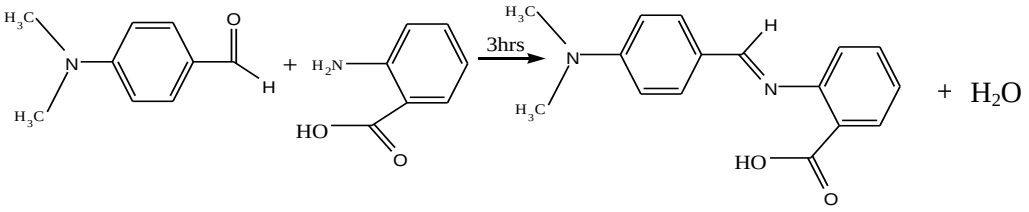
Materials and Methods

All chemicals used were of Analar grade and used as supplied. All weighing was carried out on college B154 Metler Toledo electric balance. Melting point and decomposition temperatures were determined on Stuart SMP 10 melting point apparatus. Determination of water of hydration was done on Gallenkamp Hotbox Oven Size Two [10]. Infrared spectra were recorded using FT-IR Nicolel IS10 Thermoscientific in the region 4000-400cm^{-1} at the Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria. The conductivity of 10^{-3} M solution of the complex was measured in Dimethylformamide (DMF) using Siemens WPA CM35 Conductivity meter, at the Department of Biochemistry, Bayero University, Kano, Nigeria. The room temperature Magnetic susceptibility measurements were carried out at the Department of Pure and Industrial Chemistry, Bayero University, Kano, Nigeria, using Sherwood MK1Magnetic susceptibility balance using Hg[Co(SCN)$_4$] as standard, and diamagnetic

corrections were applied using Pascal's constant [11]. The metal content was determined using Atomic Absorption spectrophotometer 210 VGP [12]. The elemental analysis of CHN was carried out at OEA labs., Callington, United Kingdom using a CE instruments (thermo) EA1110 Elemental Analyser using Xperience software. The system was single tube configured for CHN mode and was calibrated to acetanilide traceable to NIST. The antimicrobial Studies were carried out in dimethylsulphoxide (DMSO) using Disc diffusion method [13]. Three bacterial isolates viz: *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* and three fungal isolates viz: *Aspergillus flavus, Aspergillus fumigatus and Aspergillus niger* were obtained from Aminu Kano Teaching Hospital through the Department of Microbiology, Bayero University, Kano, Nigeria for *in vitro* antimicrobial screening. Ampicilin capsule and Nyastatin tablet were used as positive controls in the antibacterial and antifungal studies respectively.

Preparation of the Schiff base ligand (DBAB)

The Schiff base was prepared by adding 75 cm^3 ethanolic solution of 2-aminobenzoic acid (4.11g, 0.03 mol) to the same volume of ethanolic solution of 4-(N,N-dimethylamino)benzaldehyde (4.47g, 0.03 mol). The mixture was refluxed with stirring for 3 hours. The resulting solution was evaporated to half its volume and the precipitated product was separated, washed twice with 15 cm^3 hot ethanol and dried over anhydrous $CaCl_2$ in a desiccator [14]. The equation for the reaction is shown in scheme 1.



Scheme 1: *Preparation of the Schiff base ligand*

The name of the Schiff base is 2-[4-(N,N-dimethylamino)benzylidene]benzoic acid (DBAB).

Synthesis of the Manganese(II) complex

The complex was synthesized by dissolving 0.015 mol (4.02g) of the Schiff base ligand (DBAB) in 75 cm^3 hot ethanol which was added with stirring to 75 cm^3 ethanolic solution of 0.0075 mol of the Mn(II) chloride and refluxed for 1 hour. On cooling to room temperature, the coloured complex precipitated out, was separated, washed with 15 cm^3 ethanol and dried over anhydrous $CaCl_2$ in a desiccator [14].

Determination of Water of Hydration

The water of hydration was determined by separately placing 0.2 g of each of the complexes in a weighed watch glass which was placed in an oven at 110 ^oC until constant weight was obtained. The net loss of weight was recorded after cooling as weight of water of hydration [10]. The percentage of water of hydration was calculated using the following relation:

$$\% \text{ of water of hydration} = \frac{\text{weight loss}}{\text{weight of sample taken}} \times 100$$

Results and Discussion

Results

Table 1: Physical and Analytical Data of the Schiff base and its Mn(II) Complex

Compound	M. wt. (g/mol)	Colour	Yield (%)	M.P (^oC)	D. Temp (^oC)	Λm ($\Omega^{-1}cm^2mol^{-1}$)	μ_{eff} (B.M)
DBAB	268.16	Orange red	54.69	177	-	-	-
$[Mn(DBAB)_2].10H_2O$	769.26	Coral red	57.55	-	202	7.60	5.92

Where DBAB is $C_{16}H_{16}N_2O_2$, M. Wt. = Molecular Weight, M.P=Melting point, D. Temp.=Decomposition Temperature

Table 2: Solubility Test of the Schiff base and its Mn(II) Complex

Compounds	Solvents								
	Acetone	**CCl_4**	**Chloroform**	**DMF**	**DMSO**	**Ethanol**	**Methanol**	**Nitro benzene**	**water**
DBAB	S	SS	S	S	S	S	S	SS	IS
$[Mn(DBAB)_2].10H_2O$	S	IS	SS	S	S	S	S	SS	IS

Where DBAB is $C_{16}H_{16}N_2O_2$, S= soluble, SS=Slightly soluble, IS=Insoluble

Table 3: Microanalytical Data of the Schiff base and its Mn(II) Complex

Compound	M. wt. (g/mol)	Microanalysis % Found (Calculated)			
		C	**H**	**N**	**M**
DBAB	268.16	71.35 (71.62)	5.91 (6.01)	10.11 (10.44)	-
$[Mn(DBAB)_2].10H_2O$	769.26	50.49 (49.96)	5.28 (6.49)	7.22 (7.28)	6.92 (7.14)

Where DBAB is $C_{16}H_{16}N_2O_2$, M. Wt. = Molecular Weight

Table 4: Relevant IR Spectral Frequencies (cm^{-1}) Of the Schiff base and its Mn(II) Complex

Compound	***v*(OH)**	***v*(C=O)**	***v*(C=N)**	***v*(COO^-)**	***v*(M-N)**	***v*(M-O)**
DBAB	-	1680.41	1611.17	1485.25	-	-
$[Mn(DBAB)_2].10H_2O$	3448.09	1636.54	1588.24	1457.62	559.42	473.02

Where DBAB is $C_{16}H_{16}N_2O_2$

Table 5: Antibacterial Activity of the Schiff base and its Mn(II) Complex

Compound	Concentration (µg/disc)	Bacterial Zone of Inhibition (mm)		
		Staphylococcus aureus	***Echerichia coli***	***Salmonella typhi***
DBAB	60	12	10	11
	30	10	08	08
	15	09	-	07
$[Mn(DBAB)_2].10H_2O$	60	17	15	13
	30	14	13	07
	15	11	10	-
Amoxicillin (control)	60	28	31	27
	30	22	28	21
	15	15	19	17

Where DBAB is $C_{16}H_{16}N_2O_2$

Table 6: Antifungal Activity Test of the Schiff base and its Mn(II) Complex

Compound	Concentration (µg/disc)	Fungal zone of inhibition (mm)		
		Aspergillus flavus	***Aspergillus Niger***	***Aspergillus Fumigatus***
DBAB	60	10	-	12
	30	09	-	10
	15	-	-	07
$[Mn(DBAB)_2].10H_2O$	60	16	09	17
	30	13	07	14
	15	09	-	11
Nystatin (Control)	60	27	20	28
	30	18	16	23
	15	14	10	17

Where DBAB is $C_{16}H_{16}N_2O_2$

Discussion

The Schiff base, DBAB was prepared in good yield by condensation of 2-aminobenzoic acid and 4-(N,N-dimethylamino)benzaldehyde. It was orange red, non – hygroscopic crystalline solid. The synthesized Mn(II) Schiff base complex was coral red in colour due to d-d electronic transition [11]. The melting point of the ligand was found to be 177^{o}C while the decomposition temperature of the complex was 202^{o}C suggesting its thermal stability [15]. This could be as a result of the effective bond that exists between the metal ion and the bidentate ligand forming chelate [16]. The analytical data are presented in table 1.

The solubility of the Schiff base and the complex was determined in some common organic solvents. The results are presented in table 2. The Schiff base and the complex were soluble in most of the organic solvents used but insoluble in water and tetrachloromethane, slightly soluble in acetone, chloroform and nitrobenzene.

Molar Conductance Measurements

The molar conductance of the 10^{-3} M solution of the metal complex was determined and found to be 7.60 ohm^{-1}cm^{2}mol^{-1}. This low value indicates that the complex is non-ionic in nature and is therefore non- electrolyte [8,17,18]. The result is presented in Table 1.

Microanalysis

The elemental analysis of the Schiff base and its complex has been determined. The calculated and experimental values are in good agreement (Table 3). The elemental analysis of the Schiff base ligand showed that the formula can be represented by $C_{16}H_{16}N_2O_2$while the complex is analyzed as $[Mn(DBAB)_2].10H_2O$.

IR Spectroscopic Analysis

The infrared spectra of the Schiff base and its Mn(II) complex have been determined and the results are presented in Table 4.

The IR spectrum of the Schiff base showed the absence of bands at 1660 and 3240 cm^{-1} due to carbonyl ν(C=O) and amino $\nu(NH_2)$ stretching vibrations of the starting materials and, instead, a strong new band at 1611 cm^{-1} assigned to the azomethine, ν(C=N) linkage appeared [19]. This suggested that amino and aldehyde moieties of the starting materials are absent and have been converted into Schiff base [20]. The comparison of the IR spectra of the Schiff base and its Mn(II) complex indicates that the absorption bands appearing at 1611 and 1680 cm^{-1} due to ν(C=N) and ν(C=O) respectively shifted to lower frequencies of 1588 and 1636 cm^{-1} respectively, indicating the involvement of the azomethine nitrogen and carboxyl oxygen in coordination [8, 21]. This was further supported by weak intensity non-ligand bands at 559 and 473 cm^{-1} which are ascribed to ν(Mn-N) and ν(Mn-O) stretching vibrations, respectively [20,22]. The IR spectrum of the complex exhibits broad band at 3346 cm^{-1} attributable to ν(OH) stretching vibration which confirmed the presence of water of hydration [23,24].

Magnetic Susceptibility Measurement

The magnetic susceptibility measurement of the Schiff base complex was determined at room temperature. The magnetic moment of the solid Mn(II) complex was calculated as 5.92 B.M, indicative of five unpaired electrons in a tetrahedral environment [14,25].

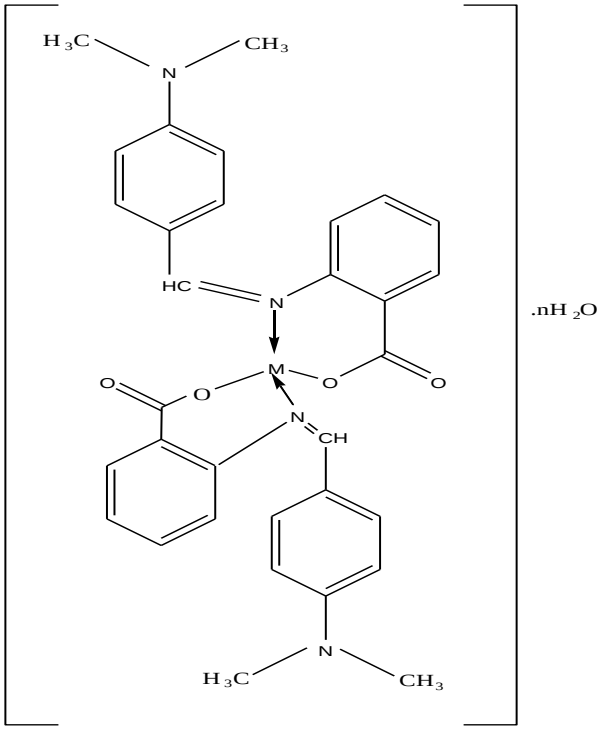
Antimicrobial Studies

The *in vitro* antibacterial activity of the Schiff base ligand and its Mn(II) complex have been carried out against one gram positive bacterium, *Staphylococcus aureus* and two gram negative bacteria viz: *Escherichia coli* and *Salmonella typhi* using disc diffusion method in dimethylsulphoxide (results are presented in Table 5). The Schiff base showed good activity against all the tested bacteria at all concentrations except *E. coli* which showed no inhibitory action at concentration of 15µg/disc. A comparative study of the growth inhibition zones of the Schiff

base and its Mn(II) complex indicates that the metal chelate exhibits higher antibacterial activities than the free ligand. The increase in the biological activity of the metal chelate was found due to the effect of manganese ion on the metal chelate which could be explained on the basis of overtone`s concept and Tweedy`s chelation theory [22]. According to this theory, on chelation, the polarity of the metal ion reduces to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups [25]. Furthermore, it increases the delocalization of the π electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity enhances the penetration of the complex into lipid membrane and thus blocks the metal binding sites on enzymes of microorganisms [15,25]. The metal complex also disturbs the respiration process of the cell and thus blocks the synthesis of proteins, which restricts further growth of the organism [15].

The antifungal activity of the Schiff base ligand showed that it is active against *Aspergillus flavus* and *Aspergillus fumigatus* at all concentrations for the latter and at concentrations of 60µg and 30µg/disc for the former, but no activity was recorded against *Aspergillus niger* (Table 6). The metal(II) complex showed enhanced activities against all the fungal strains at all concentrations except *Aspergillus niger* which showed no zone of inhibition at the concentration of 15µg/disc. The inactivity of the metal(II) complex at low concentration may be attributed to their probable lipophobic nature as a result of an outer protective layer called lipopolysaccharide. The outer layer provides additional fortification to the cell membrane, limiting the concentration of test compound streaming through the microbial cell wall [27, 28].

From the analytical results and available information from literature, the proposed molecular structure for the complex is shown in Fig. 2.



Where M=Mn(II) and n=10

Figure 2: Proposed Structure of the complex

Conclusion

The Schiff base ligand, DBAB and its metal(II) complex have been synthesized and characterized. The analytical data showed the formation of the complex in 1:2 metal – ligand stoichiometry. The complex was found to be non-electrolytes in DMF. The Schiff base ligand behaves as monoanionic bidentate ligand coordinated to the metal ion

through the azomethine nitrogen and carboxylate oxygen. On the basis of magnetic susceptibility, a four – coordinate tetrahedral geometry was suggested for the complex. The Schiff base and its complex showed promising activity against the bacterial and fungal isolates used.

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