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SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND PRELIMINARY BIOLOGICAL STUDIES OF Fe(III) AND Ni(II) COMPLEXES OF TWO NEW THIOLATES; 2,6-DIAMINO-3,5-DITHIODIPHENOYLPYRIDINE (DDPP) AND PHENOYLBENZYLMERCAPTAN(PBM).

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ABSTRACT
Phenoylbenzyl mercaptan (PBM) was prepared by condensing benzoyl chloride with benzyl mercaptan in the presence of pyridine. Also 2,6-diamino-3,5-dithiophenoylpyridine was obtained by the condensation of 2,6-diamino-3,5-dithiopyridine in the presence of pyridine. The Fe(III) and Ni(II) complexes of these ligands were synthesized using the chloride salts. All isolated compounds were characterized on the basis of their melting point, electronic, infrared, and NMR spectral data.
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INTRODUCTION

Co-ordination chemistry of iron(III) is of much interest not only to the chemist but also to the biologist. This is much more due to the physiological role played by iron in living tissues [1]. Iron(III) complexes of a plethora of ligands have been synthesized and characterized and it will be an impossible task trying to recapture all of them.

Inorganic pharmaceuticals derived from iron range from the phospholipid-encapsulated iron (III) sulphate [2] to the iron complexes of salicyclic acid [3], and bis(salicyl)glycine [4]. Also, iron(III) complexes of pentaazamacrocyclic ligands $[FeCl_2L]^+$ complexes have been known to protect cells from free radical damage [5]. In addition, carcinostatic properties had been reported for 4-ferrocenylbutanoic acid [6]. Of recent Rousin salts, $[Fe_2S_2(NO)_4]^{2-}$ and $[Fe_4S_3(NO)_7]^-$ as well as iron nitrosyl prophyrin complexes are being investigated for possible use in sensitizing tissues to radiotherapy and reduce toxic shocks [7].

Nickel (II) complexes of glycoside containing triamine ligands have shown high potency in inhibiting the growth of the pathogenic yeast, *Candida albicans* [8]. It is also noteworthy that several sulphur-containing compounds show marked biological activities [9].

Recently a new Ni(II) enzyme, acireductone dioxgenase (ARD) which catalyses oxidative breakdown of acireductone to 2-methylthioprppionate in *klebsiella pneumonia* was discovered [10]. In this dioxgenase, the sulphur atom is ligated to the Ni(II) centre. This has generated a lot of interest in ARD chemistry and its synthetic models are being prepared. Also current research in targeted towards ligands that coordinate through sulpur atom.

The present effort embodies the synthesis of Fe(III) and Ni(II) complexes of two new ligands DDPP and PBM. They have been characterized by means of their electronic, IR, NMR spectra as well as microanalysis and conductance measurements. Also ligands and complexes were screened for antibacterial and antifungal activities

EXPERIMENTAL

Reagents/Instruments

Nickel (II) chloride and iron (III) chloride were products of Fluka, Switzerland. Benzoylchloride, benzylmercaptan and 2,6-diamino-3,5-dithiopyridine were obtained from BDH and were used as supplied. All weighing were carried out using a P162N Mettler weighing balance. The melting points of the ligands and complexes were measured using a 4017 model of John-Fisher melting point apparatus. The electronic spectra of the ligands and complexes were obtained on CECIL CE 9050 spectrophotometer, FTIR data of the ligands and complexes were recorded as Nujol Mulls on Buck Scientific model 500 IR Spectrophotometer. Proton and carbon-13 NMR spectra were run with YH 200 MHz Varian Mercury NMR spectrometer using $CDCl_3$ and DMSO as solvents respectively. The proton NMR peaks were observed at 200MHz whereas the carbon-13 spectra were observed at about 50MHz. All the spectroscopic analysis was performed at the Central Analytical Laboratory of Obafemi Awolowo University, Ile-Ife, Nigeria. Elemental analysis was done using LECO-CHNS 932

microanalysis apparatus at Department of Pure and Applied Chemistry, University of Strathclyde, UK.

Preparation of Ligands

Synthesis of 2,6-diamino-3,5-dithiodiphenoyl pyridine (DDPP)

Preparation of DDPP was an adaptation of Vogel's method [12]. 5.8cm^3 (0.05 mole) benzoyl chloride, 2.925g (0.025 mole) 2,6-diamino-3,5-dithiopyridine and about 2-5cm^3 of pyridine were mixed in a 500cm^3 round-bottomed. The mixture was treated with stirring in a water bath until HCl fumes ceased. Then 2cm^3 of distilled water and about 2cm^3 pyridine were added to eliminate the excess of the reagent. Pale green solid was separated and was recrystallized with dilute ethanol. Yield was 56%.

Synthesis of Phenolylbenzylmercaptan (PBM)

2.9cm^3 (0.025 mole) benzoyl chloride, 2.33cm^3 (0.025mole) benzylmercaptan and about 2-5 cm^3 of pyridine was mixed in a 500cm^3 round-bottomed flask. The mixture was heated with stirring over a water bath until HCl fume ceased. Then about 2cm^3 of distilled water and about 2cm^3 of pyridine were added to eliminate excess reagent. The mixture was allowed to cool and white needle like crystals separated. The product was recrystallized with dilute ethanol. Yield was 87.9%.

Preparation of Complexes

Synthesis of Fe(III) Complex of DDPP

1.623g (0.01 mole) of $FeCl_3$ was dissolved in 10cm^3 absolute ethanol and mixed with 0.009g (0.02 mole) of DDPP previously dissolved in 10cm^3 of absolute ethanol. The mixture was refluxed for 30 minutes. A pale product separated which was washed with absolute ethanol and dried at room temperature. Also equimolar amounts of $NiCl_2.2H_2O$, and DDPP were mixed following above scheme to prepare the Ni(II) complex.

Synthesis of Ni(II) Complex of PBM.

1.297g (0.01 mole) of $NiCl_2.2H_20$ dissolved in absolute ethanol was added to 0.01g (0.02 mole) of PBM also dissolved in ethanol. The mixture was refluxed for 30 minutes. The solution was allowed to cool and dirty white needle-like crystals separated. The product was washed with absolute ethanol and dried at room temperature. The Fe(III) complex was not isolated.

Antibacterial activity of 2,6-diamino-3,5-dithiodiphenyl pyridine (DDPP) and phenylbenzylmercaptan (PBM) and their respective Fe(III) and Ni(II) complexes.

The antimicrobial properties of two ligands ; 2,6-diamino-3,5-dithiodiphenyl pyridine (DDPP) and phenylbenzylmercaptan (PBM) and their respective Fe(III) and Ni(II) complexes were determined against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 2091) using the agar-well diffusion method [10]. Briefly, each Mueller-Hinto agar (and Sabouraud dextrose agar for *Candida albicans*) plate was inoculated with 0.1 ml of 3 hours broth culture of each test organism. Wells (7 mm in diameter and 2.5 mm deep) were cut into the inoculated agar and labelled from one to seven. 50 µl of 20mg/ml of DDPP, PBM, Fe(III)DDPP, Fe(III)PBM, Ni(II)PBM were placed in wells labelled 1 to 6; and 20% v/v dimethylsulfoxide (DMSO) was delivered into the 7th well. The set up was left on the laboratory bench for one hour for the solutions and DMSO to diffuse into the media. The plates were then incubated at 37°C for 24 hours. Assessment of antibacterial activity was based on the measurement of the diameter of inhibition zone (IZD) around the wells. The experiment was replicated three times and the mean IZD was recorded to the nearest whole millimetre.

RESULTS AND DISCUSSION

The synthesis of these ligands was based on the well-known condensation reaction of acyl chlorides with thiols. The reaction of benzoyl chloride with 2,6-diamino-3,5-dithiopyridine afforded 2,6-diamino-3,5-dithiodiphenoylpridine herein after denoted DDPP according to Scheme (1). Also the reaction of benzylmercaptan with benzoyl chloride yielded phenoylbenzoyl mercpatan (PBM) as seen in scheme (2). Reaction of $FeCl_3$ and $NiCl_2.2H_2O$ with either DDPP or PBM afforded the complexes.

Physical Properties

The physical properties of these ligands and their complexes are presented in Table 1.

Table 1: Physical properties of the ligands and complexes

Compound	Colour	Melting Point /°C	Yield (%)
DDPP	Pale Green	178 - 180	52
PBM	White	115 - 117	61
Fe(III)DDPP	Dirty Green	220 - 223	70
Ni(II)DDPP	Dirty Green	195 - 197	65
Ni(II)PBM	Dirty White	124 - 126	60

Analytical Data

The analytical data of these ligands are presented in Table 2.

Table 2: Analytical data of the ligands.

Compound	Molecular Mass		%C		%H		%N		%S	
	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	found
DDPP	366	360	62.30	62.46	4.10	4.38	11.48	10.89	17.49	19.12
PBM	228	231	73.68	71.50	5.26	6.10	-	-	14.03	15.01

There is a good agreement between what is expected and what was observed as regards the analytical data. This also infers that the complexes did not crystallize as polymers. The crystalline solids were found to be stable and on storage for about 24 months their nature has not changed.

Electronic Spectra

Table 3 describes the electronic spectral results of these ligands and its complexes.

Table 3: Electronic Spectral Data (nm) of Compounds

Compound	ν_1	ν_2
DDPP	246.8 (1.696)	-
PBM	257.0 (2.673)	-
Fe(III)DDPP	234 (2.581)	344.0 (1.453)
Ni(II)DDPP	258 (3.092)	345.2 (2.046)
Ni(II)PBM	246.6 (2.854)	348.0 (1.328)

DDPP and PBM showed sharp intense bands at 246.8nm and 257.0nm respectively. These peaks have been assigned specifically to $\pi \rightarrow \pi^*$ transitions of aromatic rings. The electronic data of Fe(III) DDPP shows two bands in the regions 234nm and 344.0nm. The band at 234 nm is mainly due to ligand transition whereas the metal d← d transition likely results in the broad band at 344.0 nm with low extinction. However, for most octahedral Fe(III) complexes, bands in this area are due to charge transfer phenomenon which masks the d ←d transition [10]. The Ni(II) complexes have absorption bands similar to that of Fe(III). The peaks between 246 nm to 258nm results from $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transfer in the ligands but shifts to slightly higher wavelength are due to complexation which distortes the $n \rightarrow \pi^*$ transitions. The bands at 345-348nm (28985.50 – 28735.63cm^{-1}) have been ascribed to $^3A_2g \rightarrow {}^3T_1g(P)$ in an octahedral field. The same observation has been made for hydrazone complexes of Ni(II) [11].

Infrared Spectra

Tables 4 and 5 show the peaks and assignments of the IR absorptions of the ligands and complexes.

Table 4: Infrared absorption frequencies (cm^{-1}) in Nujol Mulls of DDPP and its Fe(III) and Ni(II) complexes

DDPP	Fe(II)DDPP	Ni(II)DDPP	Assignment
3455 (br)	3430.9 (br)	3354.66 (br)	ν(N – H)
	3142 (sh)	3121.14 (sh)	
2853.19(s)	2890 (s)	2891	ν(C – H)
		2851	
2800 (s)	2805 (s)		
	2349 (w)	2327.54	
1694.97 (s)	1628.8(br)	1602 (br)	ν (C = 0)
1461.62 (s)	1462.33 (s)	1462(s)	ν(C – C) of ring
1377.30(s)	1377.35 (s)	1377.66(s)	
1289.10(w)	1287.56 (w)		ν(C – N)
1255 (w)			
1125.80 (w)		1157 (w)	
	1028.59 (w)	1034.69 (s)	
935.09 (w)			ω(C – H)
760(w)	721.73 (w)	721.98 (w)	ν(C – S)
710.40(s)			
690.09 (m)			Ring breathing
547.05 (w)	512.50 (w)	543.30 (w)	
	451.20 (w)	415.35 (w)	ν(M – O), ν(M – N), or ν(M – S)
	409.52 (w)	395.16 (w)	

s=strong, m= medium, br=broad, sh=shoulder, w=weak

Table 5: Infrared absorption frequencies (cm^{-1}) in Nujol Mulls of PBM and its Ni(II) complex

PBM	Ni(II)PBM	Assignment
2918.84(s)	2918.85(s)	ν (C – H)
2858.64 (w)	2857.45(s)	
	2675.93	
1700 (w)	1694.68	ν(C = 0)
1669.26 (m)	1619.51	
1650(w)		ν(C - C)
1453.03	1454.52	
1400 (m)	1377.60	γ (ring)
1325 (m)	1287.24	$\omega(CH_2)$
1215 (m)		
1127.08 (m)	1176.54	γ (CH_2) ring vibration
1069.69 (m)	1028.11	
999.31 (m)		ω(C - H)
914.09 (s)	915.37 (s)	
804.45 (s)	804.36 (s)	ν(C - S)
706.83 (s)	750 (w)	
	710 (s)	
537.50 (s)		Ring vibration, γ (C - C)
	412.12 (w)	ν(M - 0)
	339.50 (w)	ν(M - S)

s=strong, m= medium, br=broad, sh=shoulder, w=weak

In the ligands and their complexes, the most significant bands are those of $\nu(NH_2)$ which appears at 3455cm^{-1} in DDPP, 3430.9cm^{-1} in Fe(III)DDPP and at 3354.66cm^{-1} in Ni(II)DDPP. The shifts in the peaks, suggest the participation of NH in bonding to the metals. As expected, the spectrum of PBM does not show any peak for NH. ν(C=0) is seen at 1694.97cm^{-1} in DDPP and at 1669.26 and 1700cm^{-1} in PBM. However Fe(III)DDPP and Ni(II)DDPP have the ν(C=0) centered at 1628.8 and 1602cm^{-1} respectively suggesting likely ligation through the oxygen atom of the carbonyl group. The peaks at 760 cm^{-1} and 710.40 cm^{-1} for DDPP and 708.83cm^{-1} for PBM is likely due to ν(C–S) stretch. In Fe(III)DDPP this peak shifted to 721.73cm^{-1} and in Ni(II)DDPP, it shifted to 721.98cm^{-1}. In Ni(II)PBM, this peak was not observed. These results also implicate the lone pair electrons on the S atom to have participated in bonding to the metals. Absence of any peaks between 2500 – 2700cm^{-1} indicate the non-existence of ν(S-H), implying that the thiol group was used in the formation of the ligands and not the $-NH_2$ group.

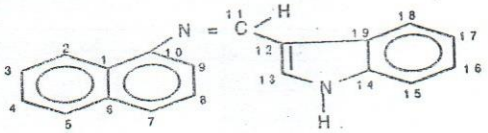
NMR Spectra

The Proton and Carbon -13 NMR spectra of the ligands are shown in Figures 6 and 7 respectively.

Table 6: ^{1}H NMR Data of the Ligands

DDPP	
^{1}H(δ)	Assignments
7.95 (10H, m)	aromatic protons on the side rings
7.52 (1H, s)	proton in the middle pyridine ring
7.6 (4H, s)	protons on the two amino groups of the pyridine ring
PBM	
7.8 (5H, m)	aromatic protons on A side rings
7.5 (5H, m)	aromatic protons on B ring
2.5 (2H, s)	$(CH_2–S)$ protons

Table 7: ^{13}C NMR Data of the Ligands

Position	^{13}C(δ)	Structure Showing Carbon Numberings for DDPP
C1, C15	129.271	
C2, C16	129.931	
C3, C17	129.472	
C4, C18	129.962	
C5, C19	129.986	
C6, C14	129.992	
C9	131.411	
C8, C10	133.581	
C11,C12	133.621	
C7, C13	168.017	
Position	**^{13}C(δ)**	**Structure Showing Carbon Numberings for PBM**
C8	40.0	
C2, C11	129.188	
C1, C10	129.224	
C3, C12	129.977	
C4, C13	130.011	
C5, C14	131.449	
C9	133.328	
C6	133.482	
C7	168.063	

For DDPP, the peak at 7.6ppm and integrated for 4 protons is due to two $-NH_2$ protons. The peak centered at 7.52 ppm has been assigned to the protons of the pyridine ring whereas the peak centered at 7.95ppm is due to the 10 aromatic protons in the side.

For PBM, the peak at 2.5ppm is due to the S-CH_2 group. The peaks centered at 7.5ppm and 7.90 ppm are assigned to the five aromatic proton on the B ring bearing the carbonyl group and the A ring bearing the $-CH_2-$ group respectively. The absence of any bands between 3.5 – 3.8 ppm suggests the absence of S-H protons. The results are in conformity with the structures suggested for the ligands.

The ^{13}C NMR spectra of DDPP and PBM are shown in Table 7. For DDPP, the aromatic carbons on the two symmetrical side rings appear between 129.271 to 129.992 ppm. The C = O carbonyl appear at 168.017 ppm. The peaks at 39 - 40.998 ppm are due to the solvent. Similar observations were made for PBM. The aromatic carbons appear between 129.188 to 133.482 ppm. The C=O carbon shows at 168.063ppm. The ^{13}C spectral results are in agreement with structures given for the ligands.

Antimicrobial properties

Antimicrobial properties of DDPP, PBM and their Ni(II) and Fe(III) complexes are presented in Table 8.

Table 8: Antimicobial properties of DDPP, PBM and their metal complexes.

Microorganism	Mean inhibition zone diameter (± sem)*					
	DDPP	PBM	Fe(III)DDPP	Ni(II)DDPP	Fe(III)PBM	Ni(II)PBM
Staphylococcus aureus (ATCC 25923)	16±0.02	20±0.2	0	16±0.01	0	15±0.07
Bacillus subtilis (ATCC 6633)	13±0.02	0	11±0.05	14±0.02	0	0
Pseudomonas aeruginosa (ATCC 27853)	10±0.01	11±0.00	0	11±0.00	0	11±0.01
Escherichia coli (ATCC 25922)	0	0	0	0	0	0
Candida albicans (ATCC 2091)	12±0.04	12±0.05	0	12±0.03	0	12±0.08

* mean of three replicates (measured in mm)

The results indicate that the ligands and their metal complexes did not inhibit the growth of E. coli strain used in this study. At a concentration of 20mg/ml DDPP was activity against S.aureus, B.subtilis, P. aeruginosa and C. albicans while PBM showed the same pattern of activity but not on B. subtilis. Overall PBM with inhibition zone diameter of 20 against 16 for DDPP is a better agent for combating S. aureus than DDPP. Ni(II)-DDPP shows the same range of activity as the ligand. For Ni(II)PBM there is mild activity against B. subtilis, S. aureus and C. albicans. Also the Ni(II)PBM is a better antimicrobial agent than the ligand PBM in suppressing the growth of B. subtilis. Apart from this, complexation did not import better antimicrobial properties for the other cases.

Conclusion

Two new thiolates and their Fe(III) and Ni(II) complexes have been successfully prepared and partially characterized. The ligands most probably coordinated to the metals from the sulphur atom and the carbonyl oxygen and also for the DDPP ligand the amino groups are also involved in bonding giving $Fe_2(DDPP)_2$ where the iron has an octahedral geometry. The same octahedral geometry has been given to the Ni(II) complex where the ligand is hexadentate overall. PBM is bidentate and for $Ni(PBM)_2$ giving a probable square-planar geometry. The ligands have shown moderate activity against some multi-drug resistant microbes.

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