



A NOVEL AND SIMPLE SYNTHESIS OF SCHIFF BASE MATERIAL AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

The ligand p-Methyl isonitrosophenylacetate has been used as chelating agent in the present course of study. This reagent is shown to be potential ligand for the extraction of transition metals. p-Methylisonitrosophenylacetate was synthesized by using p-cresol, acetic anhydride and n-amyl nitrite. The Fe(III), Cr(III) and Ir(III) complexes of schiff base derived from p-Methyl isonitrosophenylacetate have been synthesized and characterized on the basis of elemental analysis, conductivity, magnetic measurement, IR and NMR spectral studies. The conductivity data of the complexes suggests their non-electrolyte nature. The biological activity of schiff base and their metal complexes are studied against gram positive bacteria *B. Subtilis* and *Staphylococcus aureus* and gram negative organism *E.coli* and *Pseudomonas aeruginosa* bacterial strains by disc diffusion technique which showed that these novel compounds possess promising antimicrobial activities.

Keywords: p-cresol, p-Methyl isonitrosophenylacetate, metal complexes, spectroscopy, antimicrobial activity

1. Introduction

There are numerous literature reviews on the synthesis and characterization of schiffbase metal complexes by Vogel [1]. Generally transition metal ions are used to prepare coordination complexes, as there is possibility of synthesizing a variety of complexes with variable oxidation states, different coordination geometries and interesting physicochemical properties.

The inclusion of biologically active ligand into organometallic complexes offers much scope for the design of novel drug with enhanced targeted activity. Studies on such complexes indicate that new mechanism of action is possible when combining the bioactivity of the ligand with the properties inherent to the metal leading to the possibility of their application in many fields. Schiff's bases and their complexes continue to attract many researchers because of their wide applications in food industry, dye industry, catalysis, antimicrobial activity and pharmacological application like antitumor, antifungal, antibacterial, antimicrobial by Muller et. al. and Ross et. al. [2-3].

On reviewing the literature, it is proved that metal complex exhibits greater biological activity as the free organic compounds by Malhotra et. al. [4]. Increase of biological activity reported by the implementation of the transition metals of schiff bases by Fouad et. al. [5]. Since after successful administration of cisplatin as a choice of drug in the treatment of cancer in humans, many researchers have screened Pd complexes for anticancer by Ali et.al. [6] and antitumor by Malic et.al. and Saraf et.al.[7, 8] activities with more or less success. Versatility of schiff base ligands and biological, analytical and industrial applications of their complexes make further investigations in this area highly desirable. Isonitroso ketones are of great interest since it has the ability to chelate metal ion through nitrogen and or oxygen donor centers. The interaction of metal ion with ligand containing oxygen and nitrogen as donor atom were undertaken by many chemists by Saraf and Raut et.al. [9]. The ligand p-methyl isonitrosophenylacetate (L) has been synthesized and its metal complexes with Cr(III), Fe(III) and Ir (III) were synthesized. The structure of ligand

and metal complexes had been characterized by FTIR, H NMR spectroscopy. The biological activity also studied against gram positive and gram-negative bacteria for ligand and metal complexes.

2. Experimental

2.1 Materials and methods:

All chemical used were of analytical grade and of highest purity available and used without further purification. P-cresol and n-amyl alcohol were obtained from M/S Merck chemicals. Metal (II) chlorides and acetate salts were also obtained from Merck. Solvents used were distilled and purified before used.

2.2 Synthesis of p-methyl isonitrosophenylacetate :

p-cresol (100ml) and acetic anhydride(120ml) was taken in round bottom flask. Fused sodium acetate (4g) was added to it and reflux for 1hour. The reaction mixture was cooled and poured into ice cold water. Liquid layer was separated by separating funnel and distilled to obtain pure p-methyl phenyl acetate at 210°C-212°C

Dissolved 12g of sodium in 250ml of absolute alcohol and to this solution, added in small portion, and with cooling, first 60ml n-amyl nitrite and then 70ml of p-methyl phenyl acetate. This mixture was allowed to stand for 2 days in well stopper bottle in a refrigerator. At the end of this time, the brown sodium salt was filtered and dried in air. The dried sodium salt was dissolved in a minimum quantity of ice cold water and treated with a calculated quantity of glacial acetic acid. Precipitate p-methyl isonitrosophenylacetate was then filtered through suction, and dried in vacuum. The crude product was recrystallized from benzene

2.3 Preparation of Fe(P-MINPA)₃ :

Ferric nitrate solution was prepared by dissolving 0.403 g. in a minimum volume of alcohol and equal volume of water was added. Similarly p-MINPA solution was prepared by dissolving 0.537 g of it in a minimum volume of alcohol and equal volume of water was added. The iron solution was added to reagent solution drop by drop with constant stirring by maintaining the PH of the solution mixture at 6.5-6.8 by HCl/NH₄OH solution. The solution mixture was digested on boiling water bath for about 3 hrs. A colored complex was separated

out, it was filtered. Washed several times with hot water and aqueous alcoholic solution. It was dried and analysed for iron, carbon, hydrogen and nitrogen.

2.4 Preparation of Cr(P-MINPA)₃ :

Chromium chloride solution was prepared by dissolving 0.403 g. in a minimum volume of alcohol and equal volume of water was added. Similarly p-MINPA solution was prepared by dissolving 0.537 g of it in a minimum volume of alcohol and equal volume of water was added. The chromium salt solution was added to p-MINPA solution drop wise with constant stirring and the pH of the mixture was maintained at 6-6.5 by HCl/NH₄OH. The solution was refluxed on water bath at 80-100°C for 4-6 hrs & then kept in vacuum desiccator for overnight a complex was separate out wash with hot water and analysed for chromium, carbon, hydrogen, and nitrogen.

2.5 Preparation of Ir(p-MINPA)₃ complex :

Aqueous solution of iridium Chloride and p-MINPA and solution (which was dissolve in minimum quantity of alcohol and equal volume of water) were mixed in the molar ratio of 1:2 and digested for 6 hours on 50°C, a yellow colour complex was formed and analysed for iridium, carbon, hydrogen, and nitrogen.

3. Results and discussion:

Synthesized compounds and ligands were screened against bacteria Escherichia coli by disc diffusion technique by Dubey et.al and Malic Suman et.al. [10, 11] using nutrient agar as medium. .

On the basis of physicochemical characteristics it has been found that the complexes are non-hygroscopic, stable at room temperature, insoluble in water but soluble in organic solvents. The magnetic moment data indicates that the Fe(III), Cr(III) and Ir(III) complexes are paramagnetic in nature. The molar conductance values for all the complexes in 1x10⁻⁴ m nitrobenzene are in the range of 9.5-14 ohm⁻¹ cm² mol⁻¹ suggesting their non-electrolytic nature by Kumar et.al.[12] and that no anion are present outside the coordination sphere. Elemental analysis data and molar conductance value for ligand and metal complexes given in Table 1.

Table 1: Elemental analysis data and magnetic moment value for ligand and metal complexes

Ligand/Complexes	Colour	% yield	% of C	% of H	% of N	% of M	μ_{eff}
p-MINPA	Colourless	98	60.24 (60.34)	5.33 (5.03)	7.66 (7.82)
Fe(p-MINPA) ₃	Brown	94	54.65 (54.68)	4.55 (4.58)	7.08 (7.11)	9.42 (9.46)	5.68
Cr(p-MINPA) ₃	Green	92	55.01 (55.10)	4.58 (4.57)	7.13 (7.11)	8.83 (9.46)	3.44
Ir(p-MINPA) ₃	Yellow	92	44.43 (44.42)	3.70 (3.52)	5.76 (5.78)	26.36 (26.38)	4.68

3.1 Infrared spectroscopy:

The IR spectra of the complexes indicate that the ligand behaves as bidentate and coordinates with metals via azomethine nitrogen and C=O group. The IR spectra of schiff base ligand p-MINPA shows sharp band observed for ligand at 1601 cm^{-1} , is due to azomethine C=N linkage which is shifted to lower frequency (1596 cm^{-1}) on going from ligand to its metal complexes due to coordination of azomethine nitrogen with metal ion by Sece et. al. [13] It is expected that coordination of nitrogen to the metal atom would reduce the electron density in the azomethine link and thus lower -HC=N absorption.

In the spectra of p-MINPA show pick at 1720 cm^{-1} which may be attributed to the ν C=O, The disappearance of this band in all the metal complexes indicating the involvement of

this group in complex formation by Saraf et.al. [14]. The presence of sharp band in the region $608\text{-}633\text{ cm}^{-1}$ in all the complexes due to the $>\text{M-N}$ coordination of azomethine nitrogen Ghosh et.al. and Saraf et.al. [15-16]. The appearance of $\nu\text{M-N}$ and $\nu\text{M-O}$ vibration support the involvement of N and O atoms in complexation with metal ions under investigation by Thomas et. al. [17]. The IR spectra of ligand and its complexes, the band at $1013\text{-}1047\text{ cm}^{-1}$ can be attributed to C-O bond by Ibrahim Demir et.al. [18]. The N O stretching vibration due to N-O of =NOH which found near 1267 cm^{-1} by Palm et.al. [19]. The absorption near 3319 cm^{-1} in p-MINPA is assigned to the hydrogen bonded OH stretching in the spectrum of the p-MINPA. The infrared spectral data of schiff base ligand and its metal complexes are listed in Table 2

Table 2: IR spectra of Schiff base ligand and its metal complexes in cm^{-1}

Compound	Ar-H	CH ₃	C=N	C=O	C-O	M-N	N→O	OH of N-OH
p-MINPA	3037	2924	1601	1720	1047	-	-	3319
Fe(p-MINPA) ₃	3027	2924	1610	-	1047	604	1267	-
Cr(p-MINPA) ₃	3026	2940	1617	-	1028	589	1285	-
Ir(p-MINPA) ₃	3018	2926	1596	-	1043	599	1277	-

3.2 H NMR spectra:

H NMR Spectra of schiff base (p-MINPA) and their complexes were recorded in DMSO (Dimethyl sulfoxide) solution and TMS (Tetramethylsilane) used as internal standard.

The azomethine proton (-CH=N-) appears at 2.78δ , in metal complexes which confirm coordination of ligand with metal by azomethine nitrogen by Vashi et.al. [20]. NMR spectrum of (p-MINPA) show a peak around

8.606 δ due to the =NOH group. Two groups of band corresponding to $-\text{CH}_3$ and the aromatic proton in (p-MINPA) appears at 2.38 δ and 7.38 δ respectively. It may be mentioned that ethyl- α -isonitrosoacetate(HEINA), Isonitrosoacetylacetone by Thakkar et.al.[21](HINAA), Isonitrosoacetophenone (HINAP) and p-chloroisonitrosoacetophenone by Raut et.al.[22](HP-CIINAP), show =NOH proton resonance at 9.27 δ , 8.65 δ , 8.6 δ and 8.64 δ respectively.

NMR spectra of $\text{Fe}(\text{p-MINPA})_3$, $\text{Cr}(\text{p-MINPA})_3$ and $\text{Ir}(\text{p-MINPA})_3$ in DMSO

solution exhibit peak due to methyl, azomethine proton ($-\text{CH}=\text{N}-$) and aromatic ring proton and do not show any proton signal due to the =NOH group. This suggests that these complexes have been formed by the replacement of the proton exhibit at lower value compared to that of methyl proton in the reagent (p-MINPA). Further peak due to aromatic ring proton in these complexes occur at higher field side with respect to that of aromatic ring proton signal in (p-MINPA).

Table-3: NMR Spectra of p-MINPA and complexes
All value in δ scale

Compound	=NOH	CH=N	CH ₃	Aromatic ring	
				C ₁	C ₂
p-MINPA	8.60	2.78	2.38	7.38	6.84
$\text{Fe}(\text{p-MINPA})_3$	-	2.83	2.40	7.48	6.80
$\text{Cr}(\text{p-MINPA})_3$	-	2.70	2.48	7.48	6.83
$\text{Ir}(\text{p-MINPA})_3$	-	2.71	2.49	7.46	6.70

3.3 Antimicrobial activity:

The antibacterial activity data is presented in Table 4. The antibacterial activity of ligand and their metal complexes were screened on gram positive bacteria *Staphylococcus aureus* and *B.subtilis* and gram negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* by disc diffusion technique. The diameter of susceptibility zones measured in mm by Rehman et.al. [23]. Cups of 6 mm diameter

were then made with the help of sterile stainless steel bore and 0.1 ml. added to each cup. The antibacterial activity of ligand incubation period of 24 hours at 37°C and their complexes were tested by measuring inhibition zone observed around material. Ligand showed significant range of activity on growth of all selected bacterial stain. The results suggest that complexes increase the antibacterial activity.

Table 4: Antibacterial activity of ligand and its complexes

Ligand/Complexes	Gram + ve		Gram -ve	
	S.aureus	B. subtilis	E. coli	P.aeruginosa
p-MINPA	12	14	10	11
$\text{Fe}(\text{p-MINPA})_3$	20	20	18	17
$\text{Cr}(\text{p-MINPA})_3$	13	13	14	16
$\text{Ir}(\text{p-MINPA})_3$	14	22	13	14
Gentamycin	20	18	17	18

4. Conclusion :

In the present work a novel schiff base p- methyl isonitrosophenylacetate has been used as chelating ligand. The ligand coordinated to metal ion using azomethine N atom. The Fe(III), Cr(III) and Ir(III) complexes of schiff base derived from p-Methyl isonitrosophenylacetate have been synthesized

and characterized on the basis of elemental analysis, conductivity, magnetic measurement, IR and NMR spectral studies. The conductivity data of the complexes suggests their non-electrolyte nature. The biological activity of schiff base and their metal complexes are studied against gram positive bacteria and gram negative bacterial strains, which showed that

these novel compounds possess promising antimicrobial activities

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