

STRUCTURAL, SPECTRAL AND BIOLOGICAL STUDIES OF NEWLY SYNTHESIZED 2-ACETYL-1-NAPHTHOLSEMICARBAZONE AND ITS COPPER(II) COMPLEX

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ABSTRACT

Organic synthesis utilizes Schiff base reactions to create carbon-nitrogen bonds, with Schiff bases serving as bi, tri, or tetra dentate ligands that can form highly stable complexes with transition metals. In various enzymatic reactions, Schiff bases derived from semicarbazones play a crucial role as intermediates, facilitating the interaction between an enzyme and either an amino or carbonyl group of the substrate. One of the key catalytic mechanisms in biochemical processes involves the condensation of a primary amine, typically found in a lysine residue of the enzyme, with a substrate's carbonyl group to produce an imine or Schiff base. Many of these compounds exhibit biological activities, including antibacterial, antifungal, and antitumor properties. This paper summarizes the synthesis and spectroscopic analysis of 2-acetyl-1-naphtholsemicarbazone (2-Ac-1-naphtholSc) Schiff base and its Cu(II) complex.

Keywords: Schiff Bases, Transition Metal Complexes, Spectroscopic Analysis and Biological Activity.

Introduction

Schiff bases are commonly used organic compounds that contain the azomethine (imine) group ($-RC=N-$). These compounds are typically created by combining a primary amine with an active carbonyl compound. The initial documentation of the condensation reactions between primary amines and carbonyl compounds was published by Hugo Schiff. Since then, Schiff bases have been widely utilized as intermediates and ligands for coordinating transition and inner transition metal ions, as well as for coordinating anions. Aldimines are formed when an aldehyde's carbonyl group is involved, while ketoinimes are formed when a ketone's carbonyl group is involved.

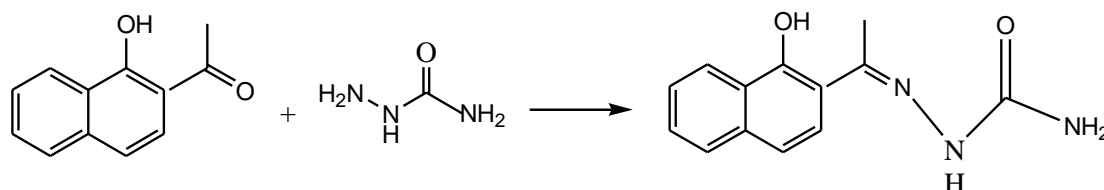
The Schiff base ligands have the potential to possess various substituents with either electron-donating or electron-withdrawing groups, resulting in unique chemical characteristics. These ligands have frequently been utilized as chelating agents in coordination chemistry. It is widely recognized that the active sites of numerous metallo biomolecules rely heavily on the coordination between oxygen and nitrogen atoms and metals.

Experimental

- **Synthesis of Semicarbazone Ligand:** 2-acetyl-1-naphtholsemicarbazone (2-Ac-1-naphtholSc) was prepared by condensing semicarbazide hydrochloride (0.05mol) with carbonyl compound 2-acetyl-1-naphthol (0.05mol) in methanol. The mixture was refluxed over water bath about 5 hours. After being left to cool overnight, the solution yielded needle-shaped crystals. The product's IR and NMR spectroscopy analysis provided insight into the arrangement of semicarbazone ligands.

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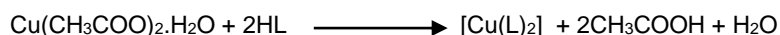


Scheme 1: Formation of 2-acetyl-1-naphtholsemicarbazone (2-Ac-1-naphtholSc)

Table 1: Physical properties and analytical data of synthesized semicarbazones

Molecular formula	% Yield	Colour	M.P (°C)	Analysis (%) found (cal.)				Mol. Wt. found (cal.)
				C	H	N	O	
C ₁₆ H ₁₇ N ₃ O ₂	74	Yellow	202	67.65 (67.84)	5.92 (6.00)	14.36 (14.84)	11.55 (11.30)	281 (283)

- Synthesis of Copper Complex of Semicarbazone Ligand:** Copper is a vital natural element necessary for all living organisms and is the third most abundant trace element present in the human body, following iron and zinc. It is not only found in its metallic state but also in various forms of copper compounds, with Cu(I) and Cu(II) being the most common. Cu(I) compounds are typically unstable in water and are easily converted into Cu(II) compounds through oxidation. However, there are a few highly insoluble Cu(I) compounds such as CuCl and CuCN that remain stable in water. Additionally, Cu(I) can form complexes with chelating ligands, usually adopting tetrahedral or trigonal-pyramidal geometries. While there are also Cu(I) complexes with three or two coordinated ligands, those with five coordinated ligands are less common and often have at least one Cu-ligand bond that is significantly elongated. The literature contains a significant number of Cu(II) compounds, many of which are soluble in water. The study of Cu(II) complexes has been a focus of recent research. The reactions of Cu(CH₃COO)₂.H₂O with synthesised ligands in methanol are represented in the following equation



The powder of the complex has an amorphous structure and cannot be dissolved in water or ether. It can only be partially dissolved in solvents like CHCl₃, CH₂Cl₂, and MeCN, but can be completely dissolved in MeOH, DMF, and DMSO. The ligand acts as a bidentate or tridentate ligand after being deprotonated. The table provides information on the color, elemental analysis, and stoichiometry of the complex. The results of the elemental analysis for both the ligands and their metal complexes support the proposed composition, showing a 2:1 mole ratio of ligand to Cu(II) metal.

Table 2: Physical Properties and Analytical Data of Synthesized Copper Complexes

Molecular Formula	Yield (%)	Colour	M.P (°C)	Analysis (%), found (calcd.)						Mol. Wt. found (calcd.)
				C	H	N	O	S	Ni	
[Cu(2-Hy-1-naphtholSc) ₂] C ₃₂ H ₃₂ N ₆ O ₁ S ₂ Cu	65	Brown	176-178	59.30 (60.54)	5.55 (5.9)	12.97 (13.07)	12.35 (13.64)	-	9.8 (9.3)	697.5 (699)

Results and Discussion

- Infrared Spectral Studies:** The infrared spectrum of the ligand displayed distinct absorption peaks at 1595 cm⁻¹ for the -C=N group and at 3425 cm⁻¹ for the -NH group. Peaks at 3075 cm⁻¹ could be attributed to the stretching vibration of aliphatic C-H bonds, while the peak at 1725 cm⁻¹ was assigned to the C=O group of the semicarbazone compound. A strong peak at 1450 cm⁻¹ indicated the presence of aromatic C=C bonds. Table 3 presents the various bands observed in the IR spectra, which can be attributed to the different functional groups present in the molecules.

The $\nu(\text{C}=\text{O})$ peak of the semicarbazone ligand at $1625\text{-}1690\text{ cm}^{-1}$ disappeared in the complex due to the formation of M-O bonding between the oxygen and the central metal atom. The ligands displayed a sharp and strong peak at $1590\text{-}1618\text{ cm}^{-1}$, which is associated with $\nu(>\text{C}=\text{N}-)$ and was observed to shift to a lower frequency in the complex spectra. This could be due to a decrease in the bond order of $>\text{C}=\text{N}-$ as a result of the formation of $\text{N}\rightarrow\text{M}$ (Cu) bonds. The peak corresponding to the -OH group present in the ligands at $3420\text{-}3550\text{ cm}^{-1}$ was absent in the complex, indicating the coordination of the metal through the oxygen atom, leading to the deprotonation of the -OH group. The ligand displayed a strong peak at $1625\text{-}1700\text{ cm}^{-1}$, which could be attributed to the stretching vibration of phenolic C-O bonds. In the copper complex, this peak was observed at a higher frequency of $1720\text{-}1750\text{ cm}^{-1}$ due to the bonding of the ligand through the phenolic oxygen atom. Based on these observations, the following structures can be assigned to the copper complex.

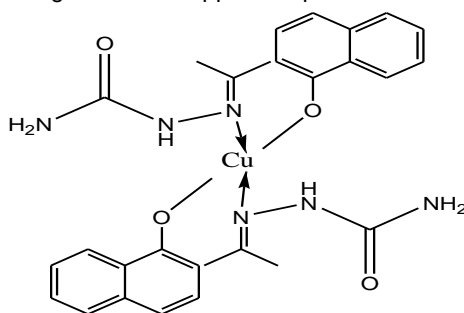


Fig. 1: Copper complexes of 2-acetyl-1-naphtholsemicarbazone

Table 3: Infrared spectral data (cm^{-1}) of 2-acetyl-1-naphtholsemicarbazone (2-Ac-1-naphtholSc)

S. No.	Assignment	Frequency (cm^{-1})
1	-C=N	1595
2	-NH ₂	3425
3	=C-H Stretching (aliphatic)	3075
4	C=O	1725
5	C=C (aromatic)	1450
6	=C-OH (Aromatic)	750
7	-OH	3475

- NMR Studies:** The ¹HNMR spectrum of the ligand displayed a singlet at δ 2.5 ppm, indicating the presence of three protons in the -CH₃ group. Additionally, a sharp multiplet was observed at δ 7.38 ppm, which can be attributed to the -CH (aromatic) group. Another singlet was observed at δ 2.0 ppm, corresponding to the -CH₃ group in the terminal aliphatic region. The peak for the -OH group was seen at 12.3 ppm. These peaks are all documented in table 4.

Table 4: ¹HNMR spectral data of 2-acetyl-1-naphtholsemicarbazone (2-Ac-1-naphtholSc)

S.No.	Assignment	δ value in ppm
1	-OH (aromatic)	12.3
2	-CH ₃ (aliphatic)	2.5
3	-CH (aromatic)	7.38
4	-CH ₃ terminal (aliphatic)	2.0

- Biochemical Studies:** The presence of the $>\text{C}=\text{N}-$ linkage is crucial for the biological effectiveness of azomethine derivatives. The activities of various fungi and bacteria on a range of compounds provided valuable insights into the Schiff base metal complexes. This prompted us to investigate the newly synthesized complex and its precursors to determine which part of the molecule is responsible for its physiological effects. By using the agar well diffusion method, we assessed the antifungal properties of 2-acetyl-1-naphtholsemicarbazone (2-Ac-1-naphtholSc) and its Cu(II) complex against *Fusarium oxysporium*.
- Determination of Antifungal Assay:** The antifungal properties of the compounds synthesized were evaluated using the agar well diffusion method (Bonjar et. al, 2005). The fungi were cultured on Sabouraud's dextrose agar, SDA (Merck, Germany) and kept at 25°C for 72 hours.

The fungal spores were diluted in sterile PBS to a **concentration** of 10⁶ cells/ml. A sterile swab was dipped into the fungal suspension and then rolled on the surface of the agar medium. The plates were left to dry for 15 minutes at room temperature. Using a sterile glass tube, wells with a diameter of 10 mm and spaced 7 mm apart were made in the culture media. Various dilutions of solutions (0.1 ml) were added to each well. The diameter of the inhibition zone (in mm) was measured after incubation to determine the bioactivity¹⁻².

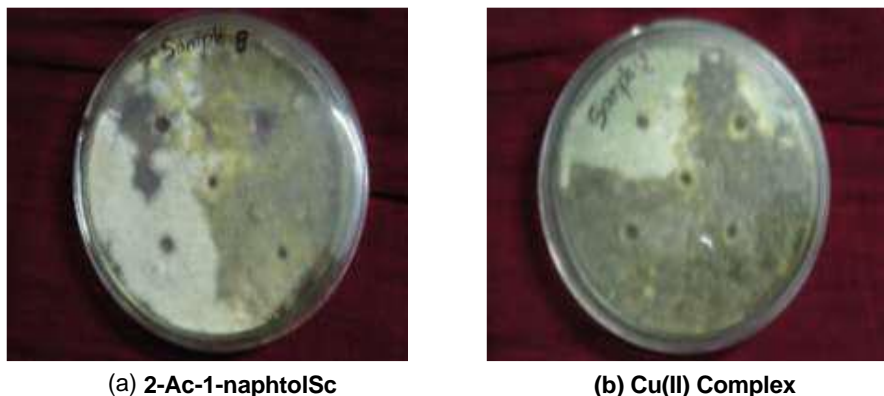


Fig. 2: Antifungal Activity of (a) 2-Ac-1-naphtolSc and (b) Cu complex against *Fusarium oxysporium*

Table 5: Antifungal Activity of 2-Ac-1-naphtolSc and its Cu complex against *Fusarium oxysporium*

S. No.	Compound	Concentration (in µg/mL)	I. Z. (In mm)	I.Z. (In mm) of Standard drug (Ketoconazole)	Activity Index
1.	2-Ac-1-naphtolSc	20	4	20mm	0.3
		40	6		0.4
		60	8		0.4
		80	12		0.6
2.	Cu complex of 2-Ac-1-naphtolSc	20	16	20mm	0.8
		40	18		1.1
		60	28		1.3
		80	30		1.5

I.Z. = Inhibition Zone, Activity Index = Sample Zone/Standard Zone

Table 5 and figure 2 present the findings of the evaluation of the newly synthesized Cu(II) complex and its corresponding Schiff base for their effectiveness against pathogenic fungi. The experimental results demonstrate that the Cu(II) complex exhibits greater efficacy in hindering the growth of microorganisms compared to the Schiff base. These results also suggest that the complex's antimicrobial activity is enhanced through the process of metallation of its ligands, as observed in previous studies³⁻⁵. The reason for the complex's increased toxicity compared to the free ligands can be attributed to Tweedy's chelation theory⁶⁻⁷.

According to this theory, chelation decreases the polarity of the central ion mainly due to the partial sharing of its positive charge with the donor groups and the potential π -electron delocalization over the entire chelate ring. This process also increases the hydrophobic properties of the central atom, making it easier for it to pass through the lipid layers of cell membranes⁸⁻⁹ and hinder the binding of metals in enzymes of microorganisms. These complexes can also disrupt the cell's respiration process and impede the synthesis of proteins, thus limiting the growth of organisms¹⁰. The effectiveness of different compounds against various organisms may vary depending on the cell's impermeability or the differences in ribosomes among microbial cells¹¹.

Conclusion

The compound Schiff base ligand 2-Ac-1-naphtolSc and its corresponding Cu(II) complex were prepared and identified through elemental analysis, infrared spectra, and ¹HNMR spectra. Results from antifungal tests against *Fusarium oxysporium* indicate that the Cu(II) complex exhibits stronger antifungal properties compared to 2-Ac-1-naphtolSc, which can be attributed to the increased lipophilicity of the Cu(II) complex.

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