

Synthesis, characterization, and fluorescence study of phthalhydrazidylazo derivative of an unsaturated diketone and its metal complexes

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Abstract: The coupling of diazotized luminol with the unsaturated diketone 1,7-diphenylhepta-4,6-diene-1,3-dione yielded a new type of tridentate ligand (H₂L). Analytical, IR, ¹H NMR, and mass spectral data indicate the existence of the compound in the intramolecularly hydrogen-bonded keto-hydrazone tautomeric form. Dibasic tridentate coordination of the compound in its [ML(H₂O)] complexes [M = Cu(II), Ni(II) and Zn(II)] was established on the basis of analytical and spectral data. The Ni(II) and Zn(II) chelates are diamagnetic while Cu(II) complex showed normal paramagnetic moment. The fluorescence maxima of the compound in different solvents show that the emission wavelength increases with increases in the polarity of the solvent. The fluorescence intensity in the presence of Cu(II) ion shows a gradual decrease with increases in the concentration of metal ion.

Key words: Phthalhydrazidylazo derivative, unsaturated diketone, keto-hydrazone, metal complexes, spectral data, fluorescent studies

1. Introduction

The compound 5-amino-2,3-dihydro-1,4-phthalazenedione is popularly known as luminol because of its intense fluorescence and chemiluminescent properties.¹ Based on these, several analytical methods have been developed and are highly useful in the analysis of environmental and biological samples.²⁻⁴ Luminol is used by forensic investigators to detect trace amounts of blood left at crime scenes as it reacts with iron found in hemoglobin.⁵ Certain derivatives of luminol exhibit more efficient fluorescence and chemiluminescent properties.^{6,7} The diazo-coupled product of luminol with acetylacetone has been reported as a more efficient chemiluminescent acid-base and metal ion indicator.^{8,9} Curcuminoids, a group of conjugated 1,3-diketones, are also found to exhibit fluorescence and chemiluminescence properties.¹⁰⁻¹² The effect of π conjugation length on fluorescence emission efficiency is elucidated by examination of the theoretical and experimental relationship between absolute quantum yield and magnitude of the π conjugation length in the excited singlet state, which provides a novel concept for molecular design for highly fluorescent organic compounds¹³. Therefore, coupling of these 2 molecular systems can generate compounds having interesting emission characteristics and applications. Thus, an azo derivative of luminol with a synthetic curcuminoid analogue, 1,7-diphenylhepta-4,6-diene-1,3-dione, was prepared. Typical metal complexes of this ligand system were also synthesized. Their structure and fluorescence properties were studied.

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2. Experimental

Carbon, hydrogen, and nitrogen contents were determined by microanalyses (Heraeus Elemental Analyzer) and metal contents of complexes by AAS (PerkinElmer 2380). The UV spectra of the compounds in methanol (10^{-6} M) were recorded on a JASCO V-550 UV-Visible spectrophotometer, IR spectra (KBr disks) on a JASCO FT/IR 4100 instrument, ^1H NMR spectra (CDCl_3 or DMSO-d_6) on a JEOL 400 NMR spectrometer, and mass spectra on a JEOL-JMS 600H FAB mass spectrometer. Fluorescence spectra were recorded using solutions of 10^{-3} – 10^{-6} M on an Elico SL 174 spectrofluorometer. Ground state absorption measurements were carried out with a SYSTRONICS UV-Visible double beam spectrophotometer. Molar conductance of the complexes was determined in DMF ($\sim 10^{-3}$ mol/L) at 28 ± 1 °C. Magnetic susceptibilities were determined at room temperature on a SHERWOOD Scientific Magnetic susceptibility balance at room temperature (28 ± 1 °C) using $\text{Hg}[\text{Co}(\text{NCS})_4]$ as standard. The chemicals used were all of Merck and Aldrich or chemically pure grade.

Synthesis of unsaturated diketone, 1,7-diphenylhepta-4,6-diene-1,3-dione: The compound was prepared by the condensation of cinnamaldehyde with benzoylacetone-boric oxide complex in ethylacetate medium in the presence of tri(*sec*-butyl)borate and *n*-butyl amine as reported earlier.^{14,15}

Synthesis of phthalhydrazidylazo derivative of unsaturated diketone (H_2L): Luminol was diazotized as reported.^{8,16} The azo compound was synthesized by the coupling of diazotized luminol with the unsaturated 1,3-diketone.

To a stirred suspension of luminol (0.885 g, 0.005 mol) in 6 N HCl (3 mL) kept cold below 5 °C in an ice-salt bath, an aqueous solution of NaNO_2 (0.0345 g in 1 mL of distilled water) was added dropwise. The cold mixture was stirred further for 1 h and then filtered quickly. The filtrate, after destroying excess nitrous acid with urea, was added slowly to a stirred suspension of unsaturated diketone (1.38 g, 0.005 mol) dissolved in methanol (10 mL) at 0 °C. Sodium acetate was added to maintain the pH of the medium at around 6. The precipitated azo dye was filtered, washed with water, and recrystallized from hot benzene.

Synthesis of Cu(II), Ni(II), and Zn(II) complexes: A concentrated aqueous solution of metal(II) acetate (0.01 mol, 15 mL) was added to a hot methanolic solution of the ligand (0.01 mol, 20 mL). The mixture was refluxed on a water bath for ~ 3 h. Sodium acetate was added to maintain pH at around 6. The precipitated complex on cooling to room temperature was filtered, washed with water, recrystallized from hot methanol, and dried in a vacuum.

2.1. Fluorescence studies

Effect of solvents on the absorption and fluorescence maxima: Solutions of the compound (10^{-6} M) in different solvents (methanol, ethanol, acetone, DMSO) were prepared. The absorption and fluorescence spectra were recorded. The fluorescent emission was measured at 400 V.

Emission maxima of the metal complexes: Solutions of the metal chelates (10^{-6} M) in DMSO were prepared. The absorption and emission spectra were recorded. The fluorescent emission was measured at 550 V.

Effect of metal ions on fluorescence: The effect of metal ions was studied by measuring the fluorescence intensity after adding the metal ions. Different concentrations of methanolic solution of metal salts were added to 1 mL of 10^{-6} M solution of H_2L in methanol and the total volume was made up to 10 mL. The fluorescence intensities of these solutions were measured at 500 V.

3. Results and discussion

3.1. Structural characterization of H₂L and its metal complexes

The observed elemental analytical data (Table 1) of H₂L indicated that a diazo-coupling reaction occurred in the 1:1 ratio. The compound was crystalline in nature and was soluble in common organic solvents. It formed stable complexes with Ni(II), Cu(II), and Zn(II) ions. The analytical data (Table 1) together with their nonelectrolytic nature in DMF (specific conductance $< 10 \Omega^{-1} \text{ cm}^{-1}$; 10^{-3} M solution) suggested $[\text{ML}(\text{H}_2\text{O})]$ stoichiometry of the complexes. The Ni(II) and Zn(II) chelates were diamagnetic while Cu(II) complex showed normal paramagnetic moment (1.76 BM). The observed IR, ¹H NMR, and mass spectral data were in conformity with structure **1** (Figure 1) of H₂L and structure **2** (Figure 2) of the complexes.

Table 1. Physical and analytical data of H₂L and its metal complexes.

Compound/ molecular formula	Color	Yield (%)	MP (°C)	Elemental analysis: found (calculated)%			
				C	H	N	M
H ₂ L C ₂₇ H ₂₀ N ₄ O ₄	Orange red	72	105	69.66 (69.83)	4.32 (4.31)	12.02 (12.07)	-
[NiL(H ₂ O)] C ₂₇ H ₂₀ N ₄ NiO ₅	Greenish brown	61	> 300	60.26 (60.14)	3.69 (3.71)	10.42 (10.40)	10.82 (10.90)
[CuL(H ₂ O)] C ₂₇ H ₂₀ CuN ₄ O ₅	Coffee brown	73	> 300	59.54 (59.61)	3.66 (3.68)	10.22 (10.30)	11.62 (11.69)
[ZnL(H ₂ O)] C ₂₇ H ₂₀ N ₄ O ₅ Zn	Cream	58	> 300	59.52 (59.41)	3.66 (3.67)	10.12 (10.27)	12.02 (11.99)

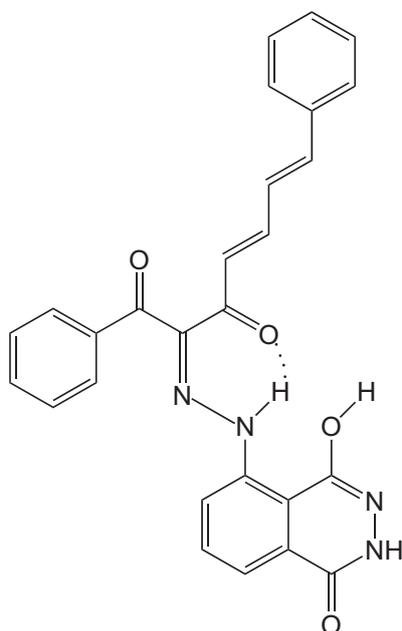


Figure 1. Structure of H₂L.

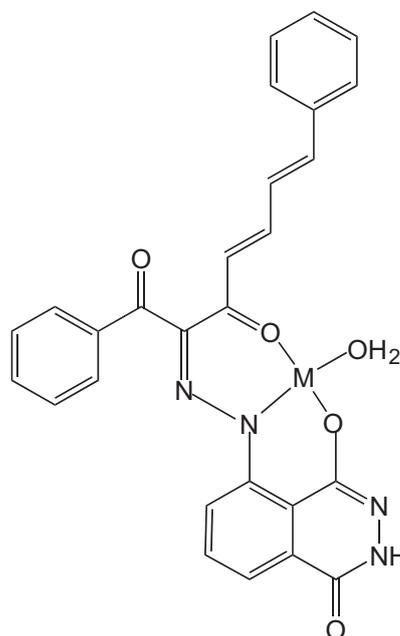


Figure 2. Structure of the metal complexes of H₂L.

Infrared spectra: The IR spectrum of H₂L in the 1600–1800 cm^{-1} region showed strong bands at 1648 and 1635 cm^{-1} due to the stretching of free amide and benzoyl carbonyls, respectively. The spectrum also

showed a strong band at 1610 cm^{-1} and a medium intensity band at 1603 cm^{-1} assignable to the stretching of intramolecularly hydrogen bonded α, β -unsaturated carbonyl and $\text{C} = \text{N}$ vibrations^{17,18} of structure **1**. The broad band in the range $2500\text{--}3500\text{ cm}^{-1}$ indicated the existence of strong intramolecular hydrogen bonding in the compound.¹⁹

In the spectra of all the complexes the free carbonyl and $\text{C} = \text{N}$ bands remained almost unaffected, indicating their noninvolvement in complexation. However, the band due to hydrogen bonded α, β -unsaturated carbonyl at 1610 cm^{-1} of the ligand disappeared and instead a new strong band appeared at $\sim 1560\text{ cm}^{-1}$ assignable to the stretching of a metal bonded carbonyl group^{18–20} as in structure **2**. A prominent band present at 1525 cm^{-1} of the ligand due to $\nu\text{N-H}$ vibration disappeared in the spectra of all the complexes, indicating the replacement of the hydrazone NH proton by metal ion.²⁰ The broad free ligand band at $2500\text{--}3500\text{ cm}^{-1}$ disappeared in the spectra of all the complexes and several medium intensity bands due to various $\nu\text{C-H}$ vibrations appeared in the region. This strongly supports the replacement of the chelated protons of the ligand by metal ion as in structure **2**. The spectra of the complexes also show new bands at ~ 860 and 3450 cm^{-1} due to coordinated water molecule.^{21,22} That the hydrazone nitrogen, intramolecularly hydrogen bonded carbonyl oxygen, and enolic oxygen are involved in complexation is clearly evident from the appearance of 3 additional medium intensity bands at $\sim 540, 460,$ and 420 cm^{-1} assignable to $\nu\text{M-N}$ and $\nu\text{M-O}$ vibrations.²⁰ Important bands that appeared in the spectra are given in Table 2.

Table 2. Characteristic IR stretching bands (cm^{-1}) of H_2L and its metal complexes.

Compound	Amide ($\text{C} = \text{O}$)	Benzoyl ($\text{C} = \text{O}$)	Chelated ($\text{C} = \text{O}$)	($\text{C} = \text{N}$)	(M-N)	(M-O)
H_2L	1648 s	1635 s	1610 s	1603 m	–	–
$[\text{NiL}(\text{H}_2\text{O})]$	1642 s	1632 s	1558 s	1605 m	538 m	460 m 422 m
$[\text{CuL}(\text{H}_2\text{O})]$	1646 s	1636 s	1560 s	1604 m	535 m	470 m 424 m
$[\text{ZnL}(\text{H}_2\text{O})]$	1644 s	1630 s	1562 s	1606 m	542 m	462 m 420 m

s = strong, m = medium

^1H NMR spectra: The hydrazone proton resonance signal of arylazo derivatives of 1,3-diketones that exist in the keto-hydrazone form generally appear in the $\delta 14\text{--}17$ ppm region of the spectra.^{23,24} The ^1H NMR spectrum of H_2L displayed a low field one proton signal at $\delta 16.30$ ppm and another one proton signal at $\delta 9.80$ ppm assignable respectively to the intramolecularly hydrogen-bonded hydrazone and enol protons.^{18,19} The amide proton signal was observed at $\delta 6.45$ ppm. The integrated intensities of various signals agreed well with the structure **1** of the compound.

In the ^1H NMR spectra of the diamagnetic Ni(II) and Zn(II) complexes the signals of the free ligand due to hydrazone and enol protons disappeared, indicating their replacement by metal ion during complexation.²⁵ That the amide NH group is not involved in complex formation is clearly indicated in the spectra of the complexes where the signal remains unaltered.¹⁹ The integrated intensities of all the protons agree well with the structure **2** of the complexes. The aryl and alkenyl protons appear in the range $\delta 6.60\text{--}8.50$ ppm as a complex multiplet. The assignments of various proton signals observed are compiled in Table 3.

Mass spectra: The mass spectrum of H_2L showed an intense molecular ion peak at $m/z 464$, thereby confirming the formulation of the compound as in structure **1**. Peaks due to the elimination of ArN_2 ,^{26,27} a characteristic feature of the azo tautomer, were not observed in the mass spectrum, indicating the existence of the compound in hydrazone form. The formation of other important peaks was due to the elimination of one or more phenyl groups, PhCO , $\text{PhCH} = \text{CH}$, etc. from the molecular ion or subsequent fragments.²⁶

Table 3. ^1H NMR spectral data (δ , ppm) of H_2L and its Ni(II) and Zn(II) complexes.

Compound	Hydrazone NH	Enolic OH	Amide NH	Alkenyl/aryl
H_2L	16.30 s	9.80 s	6.45 s	6.60–8.22 m
$[\text{NiL}(\text{H}_2\text{O})]$	-	-	6.42 s	6.74–8.50 m
$[\text{ZnL}(\text{H}_2\text{O})]$	-	-	6.44 s	6.83–8.36 m

s = singlet, m = multiplet

The FAB mass spectrum of the Cu(II) complex showed a molecular ion peak corresponding to $[\text{CuL}(\text{H}_2\text{O})]$ stoichiometry. Peaks corresponding to $[\text{CuL}]^+$, L^+ , and fragments of L^+ were also present in the spectrum. The spectrum also showed a number of fragments containing copper¹⁸ in the 3:1 natural abundance of ^{63}Cu and ^{65}Cu isotopes (Table 4).

Table 4. Mass spectral data of H_2L and its Cu(II) complex.

Compound	Mass spectral data (m/z)
H_2L	464, 387, 361, 359, 335, 310, 258, 256, 230, 129, 105, 103
$[\text{Cu}(\text{L})(\text{H}_2\text{O})]$	545, 543, 527, 525, 462, 440, 438, 416, 414, 387, 335, 258, 129, 103

Electronic spectra: The UV spectrum of H_2L showed 2 broad bands with maxima at 380 and 280 nm due to various $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. In complexes these absorption maxima shifted appreciably to low wave numbers. The Cu(II) complex showed a broad visible band, λ_{max} , at $15,000\text{ cm}^{-1}$. This, together with the measured μ_{eff} values (1.76 BM), suggests square-planar geometry. The observed diamagnetism and broad medium-intensity band at $17,500\text{ cm}^{-1}$ in the spectrum of the Ni(II) chelate suggests its square-planar geometry. In conformity, the spectrum of the chelate in pyridine solution (10^{-3} M) showed 3 bands corresponding to configurational change to octahedral due to the association of pyridine.²⁸ The 3 well-separated absorption bands at λ_{max} 8200, 13,500, and $24,300\text{ cm}^{-1}$ correspond to the transitions $^3\text{A}_{2g} \rightarrow ^3\text{T}_{2g}$, $^3\text{A}_{2g} \rightarrow ^3\text{T}_{1g}(\text{F})$, and $^3\text{A}_{2g} \rightarrow ^3\text{T}_{1g}(\text{P})$, respectively.

3.2. Fluorescence studies

Effect of solvents: The fluorescence maxima of H_2L in different solvents showed that the emission wavelength increased with increases in polarity of the solvent (Table 5). The absorption spectrum of the compound showed a strong intense band in the 750–800 nm wavelength region. This suggests the interaction of solvent molecules with the excited state of the molecule.²⁹

Table 5. Absorption and emission maxima of H_2L in different organic solvents.

Solvent	λ_{ab}^* (nm)	λ_{fl} (nm)
Methanol	384	769
Ethanol	382	764
Acetone	406	757
DMSO	390	788

*Absorption and excitation wavelength

Effect of metal ions: In the presence of metal ions the emission remained almost unaffected (Table 6). However, the fluorescence intensity in the presence of Cu(II) ion showed a gradual decrease with increases in concentration of the metal ion. The fluorescence intensity in the presence of Ni(II) and Zn(II) showed a marginal

Table 6. Effect of metal(II) ions on the fluorescence intensity of H₂L.

Concentration metal(II) solution (M)	Cu(II)			Ni(II)			Zn(II)		
	λ_{fl} max (nm)	Shift in λ_{fl} max (nm)	Fluorescence intensity	λ_{fl} max (nm)	Shift in λ_{fl} max (nm)	Fluorescence intensity	λ_{fl} max (nm)	Shift in λ_{fl} max (nm)	Fluorescence intensity
1×10^{-6}	762	-7	528	802	+33	785	762	-7	709
2×10^{-6}	760	-9	342	802	+33	780	764	-5	704
3×10^{-6}	761	-8	315	801	+32	775	763	-6	700
4×10^{-6}	761	-8	272	801	+32	761	763	-6	700
5×10^{-6}	760	-9	226	801	+32	749	764	-5	697
6×10^{-6}	761	-8	64	801	+32	740	762	-7	690
7×10^{-6}	761	-8	46	802	+33	734	762	-7	689
8×10^{-6}	761	-8	20	802	+33	731	763	-6	689

Concentration of H₂L is 1×10^{-6} M
Absorption and excitation wavelength = 384 nm

decrease with increases in metal ion concentration. In the measurement of fluorescence intensity, when all 3 metal ions were present simultaneously, it was found that Cu(II) ions can be quantitatively determined in the presence of Ni(II) and Zn(II) ions fluorimetrically.

Most paramagnetic metal ions are effective quenchers of fluorescence and cause a decrease in fluorescence intensity. This is due to the formation of an excited state charge transfer process between the fluorescent molecule and the metal ion and also due to the spin-orbit coupling between the unpaired electron on the metal ion and the excited state of the molecule, which increases the rate of intersystem crossing.³⁰ Based on the above observations, it can be stated that the decrease in fluorescence intensity in the presence of Cu(II) ion was due to the formation of a nonfluorescent complex and the emission profile was entirely due to the ligand molecule that was not involved in complexation.

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