Research Paper

Antiradical Activity and Spectroscopic Studies of [3, 5-Diamino-4-(4-Nitro-Phenylazo)-1H-Pyrazole] and Complexes

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Abstract

Azo compounds are known to be involved in a number of biological reactions such as inhibition of DNA, RNA, and protein synthesis, carcinogenesis, and nitrogen fixation. In this study, 4-[3,5-Diamino-4H-pyrazol-4-yl]diazenyl]benzoic acid (L) has been synthesized starting from 4-Phenyl-hydrazonomalononitrile-carboxylic acid, the metal complexes of the ligand 4-[3,5-Diamino-4H-pyrazol-4-yl]diazenyl]benzoic acid (L) were prepared with acetate Co(II), Ni(II) and Cu(II) in DMF as solvent. The ligand (L) and its metal complexes have been characterized by elemental analyses, IR, ¹H-NMR spectra, magnetic susceptibility, UV-Visible and thermogravimetry-differential thermal analysis (TGA-DTA). Antiradical activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH•) radical scavenging assay. The ligand and its compounds were not showed high antiradical scavenging activity when compared to standard antioxidant tocopherol. The suggested structures for the L complex of Co(II) is octahedral, for the Ni(II) complex is tetrahedral, and for the Cu(II) complex square-planar.

Keywords: Amine Compounds, Antiradical Activity, Metal Complexes, Spectroscopic Studies

1. Introduction

Complexes formed by the reactions of diamines with transition metals have a wide range of applications (Patai, 1975; Awad, 1996; Carducci et al, 1998; Zidan et al, 2000 and Mohamed et al, 2004). For instance, they are used for the determination of mercury in natural waters (Izatt et al, 1971), production of coloured thermoplastic resins and formation of crosslinked structures for epoxy resin (Bipin et al, 1984 and Sekerci, 2000). Azo compounds are known to be involved in a number of biological reactions such as inhibition of DNA, RNA, and protein synthesis, carcinogenesis and nitrogen fixation (Brunner et al, 1990; Tsai et al, 2005; Kandil et al, 2002). Furthermore, they were provided to have biological activity against bacteria and fungi (Deacon et al, 1980). Azo compounds are also used as hypnotic drugs for the nervous system (Nakamoto, 1986), in detecting cancer as chemotherapeutic agents and are involved in the structure of nucleic acids in living cells (Afrasiabi et al, 2003).

It is important to use amines containing at least two nitrogen atoms, since they produce quite stable complexes with transition metals (Figgis, 1996). Amines and their derivatives (monodental, bidental, etc.) have been known to produce stable complexes with transition metals (Iwatsuki et al, 2000). A lot of amine complex compounds have been synthesized and their properties (carcinogenic, optical and biological activities) have been established (Kasumov, 2002).

2. Experimental

The chemicals were purchased from Merck Chemicals, used without purification. The electronic spectra of the ligand (Figure 1) and the complexes in the UV-VIS region were recorded in DMF solutions using a Shimadzu UV-
1700 Spectrophotometer. The IR spectra of the ligand and the complexes were recorded with Perkin Elmer precisely Spectrum One using KBR pellets in the region of 4000-400 cm$^{-1}$. $^1$H NMR spectra were recorded on a Bruker GmbH DPX-400 MHz FT in DMSO-$d_6$. Magnetic susceptibilities were determined at room temperature on a Sherwood Scientific MKI model balance. The elemental analyses were conducted on a Leco 932 CHNS-o instrument. Thermal analyses were recorded on a Shimadzu TA60-WS.

![Figure 1. The Structure of the [3,5-Diamino-4-(4-Nitro-phenylazo)-1H-pyrazole]](image1.png)

### 2.1. 4-Phenyl-Hydrazonomalononitrile-Carboxylic Acid

A hydrochloric acid solution (6 ml) of 4-amino benzoic acid (0.01 mol) and an aqueous solution (3 mL) of sodium nitrite (0.0105 mol) were mixed and stirred at 0°C for 1 hour, followed by the addition of an aqueous solution (10 ml) of the coupling component malononitrile 2 (0.01 mol) and continued stirring at 0°C for 2 hours. The resulting product was filtered and washed with water, dried, and recrystallized from ethanol (Tsai et al, 2005).

### 2.2. Synthesis of the [3,5-Diamino-4-(4-Nitropheno...]

Hydrazine hydrate (10 mmol) was added to a solution of 2-(4-Nitro-phenylazo) malononitrile (10 mmol) and pyridine 0.5 mL in 30 mL ethanol. The reaction mixture was heated under reflux for 3-4 hours, and then cooled at room temperature. The separated solid was filtered, washed with water, dried, and recrystallized from ethanol (1.48g, 60%) (Tsai et al, 2005).

### 2.3. Synthesis of the Co(II), Ni(II), Cu(II) Complexes

1.6 mmol of metal acetates [Cu(Ac)$_2$.H$_2$O (0.319 g), Co(Ac)$_2$.4H$_2$O (0.398 g) and Ni(Ac)$_2$.4H$_2$O (0.398 g)] was dissolved in 25 mL of hot DMF at 110°C and added gradually to a stirred hot solution of the ligand [3,5-Diamino-4-(4-Nitro-phenylazo)-1H-pyrazole] (0.5 mmol, 0.1 g) dissolved in 25 mL of hot DMF at 110°C. The reaction mixture was refluxed for about 3-4 hours. The solid complexes (Figures 2 and 3) which separated out at room temperature were filtered and washed with ethanol and dried. Yields, colours, spectral data and magnetic measurements results are given in Tables 1, 2 and 3.

![Figure 2. Suggested Structure of the Cu(II) And Ni(II) Complexes](image2.png)

![Figure 3. Suggested Structure of the Co (II) Complex](image3.png)

### 2.4. DPPH$^\bullet$ Free Radical Scavenging Activity

The free radical scavenging activity of complexes was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH$^\bullet$) using the method described by Shimada et al (1992). Briefly, 0.1 mM solution of DPPH$^\bullet$ in ethanol was prepared. 3 ml of the solution was added to 1000 μg/mL of complexes solution in DMF. The mixture was stand at room temperature for 30 minutes. Absorbance was then measured at 517 nm by using a spectrophotometer (Shimadzu UV-1700
Spectrophotometer). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH• scavenging effect was calculated using the following equation:

\[
\% \text{ DPPH}^\bullet \text{Scavenging Activity} = \left[ \frac{A_C - A_S}{A_C} \right] \times 100
\]

Where \( A_C \) was the absorbance of the control reaction and \( A_S \) was the absorbance in the presence of the complexes.

### Table 1. Elemental Analyses of The Ligand and its Compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>F.W. g/mole (%)</th>
<th>Yield Elemental Analyses Calculated (Found) %</th>
<th>( \mu \text{ eff} ) (B.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand (L) (purple)</td>
<td>247</td>
<td>C 43.72</td>
<td>H 3.64</td>
</tr>
<tr>
<td>((\text{C}_9\text{O}_2\text{N}_7\text{H}_9))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{CoL}(\text{Ac})_2.4\text{H}_2\text{O}]) (Brown)</td>
<td>531.93</td>
<td>C 29.32</td>
<td>H 5.06</td>
</tr>
<tr>
<td>((\text{C}_{13}\text{O}_7\text{N}_3\text{H}_2\text{Co}))</td>
<td>-</td>
<td>C (28.38)</td>
<td>H (5.08)</td>
</tr>
<tr>
<td>([\text{NiL}(\text{Ac})_2.4\text{H}_2\text{O}]) (Dark Red)</td>
<td>672.42</td>
<td>C 30.35</td>
<td>H 4.31</td>
</tr>
<tr>
<td>((\text{C}<em>{17}\text{O}</em>{12}\text{N}_7\text{H}_2\text{Ni}_2))</td>
<td>-</td>
<td>C (31.22)</td>
<td>H (3.93)</td>
</tr>
<tr>
<td>([\text{Cu}_2\text{L}(\text{Ac})_4.4\text{H}_2\text{O}]) (Black)</td>
<td>682.08</td>
<td>C 29.91</td>
<td>H 4.25</td>
</tr>
<tr>
<td>((\text{C}<em>{17}\text{O}</em>{12}\text{N}_7\text{H}_2\text{Cu}_2))</td>
<td>-</td>
<td>C (28.95)</td>
<td>H (4.88)</td>
</tr>
</tbody>
</table>

### Table 2. IR Analyses Results of Ligand and its Compounds

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>(\nu(\text{N}=\text{N}))</th>
<th>(\nu(\text{NH}_2))</th>
<th>(\nu(\text{N}-\text{N}))</th>
<th>(\nu(\text{M}-\text{N}))</th>
<th>(\nu(\text{M}-\text{O}))</th>
<th>(\nu(\text{O-H}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand (L)</td>
<td>1493</td>
<td>1627–1631</td>
<td>1059</td>
<td>-</td>
<td>-</td>
<td>3428</td>
</tr>
<tr>
<td>([\text{CoL}(\text{Ac})_2.4\text{H}_2\text{O}]),6\text{H}_2\text{O}</td>
<td>1513</td>
<td>1559-1567</td>
<td>1104</td>
<td>465-450</td>
<td>510-513</td>
<td>3434</td>
</tr>
<tr>
<td>([\text{NiL}(\text{Ac})_2.2\text{H}_2\text{O}])</td>
<td>1511</td>
<td>1569-1575</td>
<td>1096</td>
<td>471-445</td>
<td>507-513</td>
<td>3445</td>
</tr>
<tr>
<td>([\text{Cu}_2\text{L}(\text{Ac})_4.4\text{H}_2\text{O}]) &amp; 1499            &amp; 1580-1621</td>
<td>1104</td>
<td>474-460</td>
<td>513-515</td>
<td>3428</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. DPPH Radical Scavenging Activity of Samples, and \(\alpha\)-tocopherol by 1,1-diphenyl-2-picrylhydrazyl Radicals (%)

<table>
<thead>
<tr>
<th>Samples (1000 (\mu)g/mL)</th>
<th>Scavenging Activity DPPH(^\bullet) (%)</th>
<th>% DPPH(^\bullet) Scavenging Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>[Ac-A_3 / Ac] x 100</td>
</tr>
<tr>
<td>Ligand (L)</td>
<td>11.19</td>
<td>% DPPH(^\bullet) Scavenging Activity</td>
</tr>
<tr>
<td>L-Ni</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>L-Co</td>
<td>22.98</td>
<td></td>
</tr>
<tr>
<td>L-Cu</td>
<td>5.29</td>
<td></td>
</tr>
<tr>
<td>(\alpha)-Tocopherol</td>
<td>89.45</td>
<td></td>
</tr>
</tbody>
</table>

3. Results and Discussion

The colours, metal salts and molar ratios of preparation, magnetic susceptibilities, stoichiometries, and elemental analyses of the prepared complexes are listed in Table 1. The analytical data show that the reaction of ligand with different salts; Cu(II), Ni(II) and Co(II) ions in different appropriate molar ratios give mono and binuclear complexes with different stoichiometries 1:1 and 2:1 (M:L). The

3.1. \(^1\)H-NMR and IR Spectra

The \(^1\)H-NMR spectra of ligand (L) has been carried out in DMSO-\(d_6\) at room temperature. The spectra of the ligand show signals within 6.00-6.52, 7.73-8.21, 10.95 ppm range assigned to \(\text{NH}_2\), aromatic –CH and -NH, respectively.

Table 2 presents the most important IR spectral bounds for ligand and all the metal complexes. The bands at 1627-1631 cm\(^{-1}\) are assigned to the stretching vibration of the azomethine group of the ligand (L). This band is shifted in the complexes toward the lower frequencies because of the coordination of the nitrogen to the metal ion. The infrared spectra of the complexes exhibited broad bands at 3450-3325 cm\(^{-1}\) that attributed to OH of the crystalline water molecules. Coordination of the metal ion results with upward shifts of \(\nu(\text{N}=\text{N})\) bounds by the 6-20 cm\(^{-1}\), in all complexes implying coordination of the \(\text{NH}_2\) – \(\text{N}=\text{N}\)- to the metal ion (Kandil et al, 2002). These conclusi-
ions are further supported by the appearance of medium-weak bands at 445-474 cm$^{-1}$ in the spectra of the complexes.

The IR spectra of the Ni(II), Co(II) and Cu(II) complexes exhibits new bands, which are not present in the ligand. These bands at 507-513 cm$^{-1}$, 510-513 cm$^{-1}$ and 513-515 cm$^{-1}$ are assigned to $v$(M-O) of coordinated acetate groups. The spectrum of [Ni(2-L(AcO)$_2$)2H$_2$O and [CuL(3-AcO)$_2$]4H$_2$O shows another two bands due to $v$(OAc) and $v$(OAc) of an acetate group at 1385-1415 cm$^{-1}$ and 1385-1419 cm$^{-1}$, respectively. The $\Delta v$= 30-34 cm$^{-1}$ can be taken as evidence for the existence of a bridging bidentate acetate group in this complex (Deacon & Philips, 1980; Nakamoto, 1986 and Kandil et al, 2002).

3.2. Magnetic Moment and UV Spectrum

The electronic spectrum of the ligand L exhibits absorption bands at 42000 cm$^{-1}$ and 30500 cm$^{-1}$ attributable to $\pi$→$\pi^*$ and $n$→$\pi^*$ transitions within the molecule.

The magnetic moment of 2.01 B.M observed for the cobalt complexes falls in the range reported for many low-spin octahedral Co(II) compounds (Figgis, 1996; Iwatsuki et al, 2000 and Afrasiabi et al, 2003). The electronic spectrum of this compound shows an absorption at 19551 cm$^{-1}$ which can be assigned to $^3E_g$→$^3T_{1g}$ transition in low spin Co(II) octahedral compound (Nakamoto, 1986 and Kandil et al, 2002). For the tetrahedral Ni(II) complex, observed magnetic moment is 2.49 B.M . The observed reduction in the magnetic moment of Ni(II) can be caused by intermolecular antiferromagnetic interactions between neighboring molecules (Everett et al, 1965; Holm et al, 1971 and Kasumov, 2002). The electronic spectrum of the Ni(II) complex exhibits two bands at 9267 cm$^{-1}$ and 17400 cm$^{-1}$ due to tetrahedral geometry (Shashikala, 1985 and Satyaranayana et al, 2004). They are assigned to $^2T_1g(F)$→$^2A_2g$ ($v_3$) and $^3T_1g(F)$→$^2T_{1g}(P)$ ($v_1$) transitions. The low values of the room temperature magnetic moment of Cu(II) complex 1.59 B.M, indicate molecular association. This may occur either through Cu-Cu bonding and/or through a bridging anion. The appearance of vibrational frequencies due to bridged OAc and the absence of an electronic transition band in the 21691 cm$^{-1}$ region due to Cu-Cu bonding suggests that the molecular association of Cu(II) ions via bridged aceto is the factor responsible for lowering of $\mu_{\text{eff}}$ values at room temperature. The electronic spectrum of the Cu(II) complex show an absorption band at 600 nm due to the $^3B_{1g}$→$^3A_{1g}$ transition, observed for a square-planar structure (Shashikala, 1985; Lever, 1968).

3.3. Thermal Studies

The thermal stability of the complexes was investigated using TGA. The TGA curves were obtained at a heating rate at 15 °C / min in N$_2$ atmosphere over the temperature range of 25-1000 °C.

All of the complexes started to decompose at the same temperature ranges. The Ni(II) complex is thermally stable up to 52°C. In the TGA curve of the Ni(II) complex 5.38 % weight loss was observed at 180°C, corresponding to 2 mole of water of the crystallization. The Co(II) complex was stable up to 36 °C and its decomposition started at this temperature, 12.50 % weight loss was observed at 175 °C corresponding to 4 mole of water of the crystallization and 6.73% weight loss was observed at 260 °C, this shows that the complex contains 2 mole of coordinated water molecule. In the TGA curve of the Cu(II) complex 10.53 % weight loss was observed at 170 °C corresponding to 4 mole of water of the crystallization.

3.4. Antiradical Activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compare with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Talaz et al, 2009).

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discolouration from purple to yellow. Hence, DPPH$^*$ is usually used as a substrate to evaluate antioxidative activity of antioxidants. DPPH scavenging activity of all complexes was lower than standard antioxidant α-tocopherol (Table 3). This result showed that there is not high antiradical activity of synthesized complexes.

References


