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RESEARCH ARTICLE

Synthesis, spectroscopic characterization, dyeing properties and DNA cleavage study of a heterocyclic azo derivative and its transition metal complexes

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Abstract

Diazo-coupling reaction of 2-aminopyrimidine with 2-naphthol resulted in a novel heterocyclic azo derivative and this ligand formed a series of metal complexes with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) salts under well-defined conditions. The structural characterization of the ligand and its metal complexes was carried out on the basis of elemental analyses, molar conductance, magnetic susceptibility measurements, UV-Vis., IR, NMR and FAB mass spectral data. Analytical data revealed that all the complexes exhibited 1:2 metal-ligand ratio. Spectral studies showed that the ligand existed in the azo-enol form and coordinated to the metal ion in a bidentate fashion through the deprotonated naphtholate oxygen and one of the azo nitrogen atoms. Electronic spectral data and magnetic susceptibility measurements were used to establish the geometry of the complexes. XRD study revealed that both the ligand and its complex possessed an orthorhombic crystal lattice. The ligand and its complexes were applied to silk fabric and their fastness properties were evaluated. DNA cleavage activity of the ligand and its complexes were monitored by agarose gel electrophoresis method.

Keywords

2-Naphthol

Magnetic

IR

NMR

FAB Mass

Introduction

Heteroaromatic nitrogen-containing compounds and their transition metal complexes have received large amount of attention as a consequence of their exciting biological as well as pharmacological properties and their use in the chemistry of dyes and polymers.

The synthesis, reactivity and biological activities of pyrimidine derivatives stand as an ever-expanding area of research in heterocyclic chemistry. They are important constituents of nucleic acids and are physiologically essential for the biosynthesis of proteins. Amino derivatives of pyrimidine attract considerable interest due to their versatile biological activity and wide applications in medicine [1]. Many reports have showed that the pyrimidines can act both as a bidentate or tridentate ligand when coordinated with the metal ions and form mononuclear and binuclear complexes. In this investigation, the azo derivative formed from

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2-aminopyrimidine and 2-naphthol has been used as prospective chelating agent for some selected d-block metal ions in their common oxidation states and the resulting complexes have been examined with particular reference to the structural aspects of the ligand moieties in the metal complexes. Since pyrimidine rings have important functional roles in nucleic acids and biosynthesis of proteins, the DNA cleavage activity of the ligand and its metal complexes has also been investigated.

Experimental

Materials and methods

All the chemicals used were of Analytical grade. Commercial solvents were distilled and used for synthesis. For physico-chemical measurements, the solvents were purified by standard methods. Carbon, hydrogen, nitrogen and sulphur analyses were performed using Elementar Systeme Vario EL III CHN analyzer. The electronic spectra of the complexes were recorded on a Hitachi 320 UV-Visible spectrophotometer. Infrared spectral studies were carried out using KBr discs on a Shimadzu FT-IR 8000 spectrophotometer. Proton NMR spectra of the ligand and zinc(II) complex were recorded on a JEOL GSX 400 MHz FT-NMR spectrometer. Far IR spectra were recorded on a polytec FIR 30 Fourier spectrometer using CsI discs. Molar conductance measurements were conducted using 10-3M solutions of the complexes in DMSO using a Systronic model 304 digital conductivity meter. Magnetic susceptibility were measured at room temperature with a Magway MSB MkI magnetic susceptibility balance. X-ray diffraction were carried out on a Siemens D 5005 model X-ray spectrometer. The DNA(pUC 19) cleavage activity of the ligand and its complexes was monitored by agarose gel electrophoresis method.

Synthesis of ligand, 1-[(Pyrimidin-2-yl)azo]-2-naphthol (HPAN)

The ligand (L) was prepared by the diazotization of 2-aminopyrimidine (0.01 mol) at 5°C in concentrated sulphuric acid and subsequent coupling with 2-naphthol (0.01 mol) dissolved in 10% NaOH. The product obtained was filtered off, washed with water, dried and recrystallized from glacial acetic acid.

Synthesis of the metal complexes

An ethanolic solution (15 mL) of the metal salt (0.005 mol) was added gradually in small amounts to a hot ethanolic solution of the ligand (0.01 mol). The pH of the solution was maintained between 6.5 and 7.0 by adding 1:1 alcoholic ammonia solution. The reaction mixture was refluxed for about 3–4 h and then cooled to room temperature. The metal complex separated out was filtered off, washed successively with ethanol, ether and finally dried in vacuum.

Results and discussion

From the analytical data, it is obvious that the diazo-coupling reaction between 2-aminopyrimidine and 2-naphthol occurs in 1:1 molar ratio and the product forms well defined complexes with the metal salts. Formulation of the complexes is based on their elemental analytical data, molar conductance values and magnetic susceptibility measurements. The complexes are s at room temperature and possess good keeping qualities. They are found to be insoluble in H_2O but fairly soluble in chloroform, acetonitrile, DMF and DMSO. The elemental analyses are consistent with 1:2 metal-ligand stoichiometry for all the complexes. The low molar conductance values of all the complexes suggest that they behave as non-electrolytes [2].

Structure of the ligand

The azo-hydrazone tautomerism is the characteristic property of ortho-hydroxy azo compounds due to the presence of a labile proton in the molecule [3]. The ligand under investigation is thus capable of exhibiting azo-enol and keto-hydrazone tautomerism. The structure of the ligand is confirmed by elemental analysis, IR, UV-Vis. and ^{1}H NMR spectral data. The UV spectrum of the ligand in ethanol gives characteristic band at 280 nm for the azo form [4]. Besides, the band at 356 nm corresponds to the electronic transitions of the pyrimidine moiety. In the IR spectrum of the ligand a broad medium intensity band in the region 3600–3300 cm^{-1} centered at 3514 cm^{-1} in the spectrum is ascribed to the intramolecularly hydrogen bonded naphtholic υ(O–H) group [5]. The band due to naphtholic υ(C–O) vibration is observed at 1250 cm^{-1}. The spectrum displays a band near 1460 cm^{-1}, which can be attributed to the stretching vibration of the –N=N– group. Another band at 1593 cm^{-1} is accounted for υ(C=N) of the pyrimidine moiety. A weak band at 3062 cm^{-1} can be assigned to the stretching absorption of the pyrimidine ring. Besides, the characteristic vibrations of the pyrimidine ring have been observed at 1570 and 1490 cm^{-1}. The ^{1}H NMR spectrum of the ligand in $CHCl_3$ exhibits a low field singlet signal at 17.49 ppm due to the naphtholic- OH proton.The multiplet signal observed in the region 6.56-8.32 ppm is ascribable to the aromatic protons of

the naphthalene and pyrimidine rings. Thus, it has been found that the proton signals of the pyrimidine ring have been merged with that of the naphthalene ring.

Structure of the metal complexes

From the UV spectra of the metal complexes, it is observed that the band characteristic of the azo-enol form of the ligand persists in the complexes also. This clearly indicates that no structural alteration of the ligand occurs on metal chelation.

In the spectra of the complexes, the band due to the naphtholic –OH disappears indicating the deprotonation and involvement of naphtholic oxygen atom in coordination with the metal ion. The stretching frequency of the azo group at 1460 cm^{-1} has been lowered by about 30 cm^{-1} which suggests its coordination with the metal ion. The band due to υ(C=N) of the pyrimidine ring remains unchanged in the spectra of the metal complexes indicating the non-involvement of the ring nitrogen atoms in the bond formation. Moreover, the characteristic vibrations of the pyrimidine ring remain unaltered in the metal complexes confirming the non-participation of the heterocyclic ring nitrogen atoms in chelation. In the far infrared spectra of the complexes υ(M–O), υ(M–N) and υ(M–Cl) vibrations are observed in the region 510–520, 410–420 and 325 cm^{-1} respectively.

In the proton NMR spectra of the diamagnetic complexes, the low field signal due to the intramolecularly hydrogen bonded –OH completely disappears indicating the involvement of this group in chelation through the displacement of the naphtholic proton. All the aromatic protons resonate nearly at the same region experiencing a slight down field shift (0.10 to 0.20 ppm) compared to that of the free ligand.

On the basis of all the above observations, it is concluded that the ligand is bonded to the metal ion in a bidentate fashion through the oxygen atom of the deprotonated naphtholic group and one of the azo nitrogen atoms.

Electronic spectra and magnetic moment measurements

The magnetic moment value [6,7] of the manganese(II) complex is found to be 5.90 B.M indicating a high-spin complex with five unpaired electrons.These observations support the assumption of a tetrahedral geometry for the manganese(II) complex. An absorption band at 16,800 cm^{-1} in the cobalt complex corresponds to $^4A_{2g} \rightarrow {}^4T_{1g}$ transition, which is characteristic of a tetrahedral geometry around the cobalt(II) ion. The magnetic moment value of the cobalt(II) complex is 4.38 B.M which is in good agreement with the tetrahedral geometry. Nickel(II) complex is diamagnetic and it exhibits two absorption bands at 13,500 and 18,830 cm^{-1} assignable to $^1A_{1g} \rightarrow {}^1A_{2g}$ and $^1A_{1g} \rightarrow {}^1B_{1g}$ transitions respectively which are consistent with a square-planar geometry. The electronic spectrum of the copper(II) complex exhibits a broad band centered at 13,800 cm^{-1} corresponding to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition and its magnetic moment value of 1.83 B.M suggest a distorted square-planar geometry around the metal ion. Zinc(II) complex is diamagnetic and a tetrahedral geometry is most preferable for four coordinated zinc(II) complex.

X-ray diffraction

The diffractogram of the ligand has recorded 14 reflections of 2θ ranging from 19° to 63°. The maximum recorded is at 26.2420°, which corresponds to a d-spacing of 3.3931A°. The indexing of the X-ray diffraction powder photograph of an orthorhombic crystal lattice.The unit cell dimensions have bee calculated to be: a = 5.4164 Å, b = 4.0026 Å, c = 8.0430 Å and unit cell volume = 174.3696 $Å^3$. From the XRD pattern of the complexes, it is clear that the crystallinity of the ligand is completely lost on complexation with the metal ions.

DNA cleavage activity

The ability of HPAN and its metal complexes to affect oxidative DNA cleavage in the presence of H_2O_2 has been investigated and the result obtained is shown in **Fig 1** (Lane 1-7). It is evident from the figure that the different metal complexes exhibit different cleavage efficiency for the plasmid DNA and it may be due to the different binding affinity of the complexes to the DNA. Control experiment using DNA alone (lane 1) fails to show any apparent cleavage of pUC 19 DNA.

From the result, we conclude that the copper(II) complex (lane 5), due to its labile coordination sphere, catalyzes the cleavage of DNA more efficiently than the other complexes under physiological conditions. Ligand and zinc complex (lane 2 and 7) do not cleave DNA in the presence of H_2O_2.Cobalt(II), manganese (II) and nickel(II) complexes (lane 3, 4 and 6) show less cleavage efficiency as compared to the copper(II) complex. Nuclease activity exhibited by the complexes is modulated by metallo-complexes bound hydroxyl radical or peroxo species generated from the

co-reactant H_2O_2. Probably this may be due to the formation of redox couple of the metal ions. This results in the formation of hydroxyl radical which further generates a deoxyribose-centered radical by hydrogen abstraction and finally cleaves the DNA. Thus, the metal complexes can promote redox mediated cleavage of sugar-phosphate backbone of DNA. Hence, the chemical nuclease activity of the complexes in the presence of H_2O_2 follows hydroxyl radical pathway. Further, the presence of a smear in the gel diagram indicates the presence of radical cleavage.

To know the involvement of hydroxyl radical in oxidative DNA cleavage, the experiment is also carried out by adding radical scavenger (DMSO) to complex-DNA mixture. Addition of DMSO causes significant reduction in the DNA cleavage efficiency of the samples. This observation suggests that the cleavage is oxidative and hydroxyl radical generated from the complexes in the presence of H_2O_2 mediates the cleavage reaction [8].

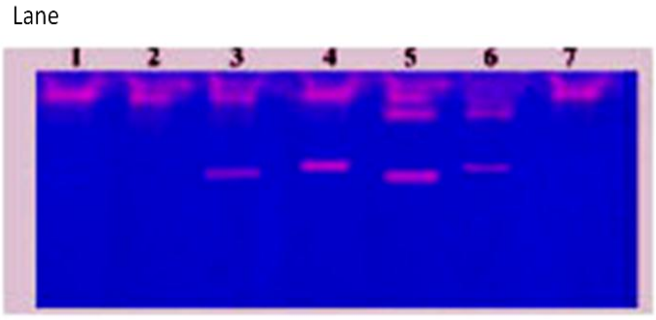

Fig 1 Gel electrophoresis diagram of DNA cleavage.
1-Control DNA; 2-DNA+Ligand+H_2O_2;
3-DNA+CoL_2+H_2O_2; 4-DNA+MnL_2+H_2O_2;
5-DNA+CuL_2+H_2O_2; 6-DNA+NiL_2O_2;
7-DNA+ZnL_2+H_2O_2;

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