



SYNTHESIS, ANTIFEEDANT AND INSECT GROWTH-REGULATING ACTIVITY OF N-(2-NITRO) BENZYLIDINE-3 AMINOCOUMARIN COMPLEXES AND THEIR PHYSICO-CHEMICAL CHARACTERIZATION

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ABSTRACT

Synthesis, antifeedant and insect growth-regulating activity of new metal [Cr(III), Mn(II), Fe(III), Co(II), Ni(II) and Cu(II)] complexes from N-(2-nitro) benzylidene-3 aminocoumarin (NBAC) have been described. The metal complexes show appreciable antifeedant (AF) and insect growth-regulating activity (IGR) at both lower and higher doses. All the complexes have been proposed to have octahedral geometry on the basis of analytical, thermal conductivity, spectral and magnetic data.

Keywords: N-(2-nitro) benzylidene-3 aminocoumarin, Metal complexes, Anti feedant activity, Insect growth-regulating activity, Octahedral.

INTRODUCTION

Coumarins comprise a very large class of compounds found throughout the plant kingdom. The coumarin derivatives are known to have diverse applications as anticoagulants, spasmolytics, anticancer drugs or as plant growth regulating agents^{1,2}. Biological activity of coumarin nucleus and related derivatives has great important effects like antibacterial³, insecticidal⁴, antimutagenic⁵, antithrombotic and vasodilatory⁶. Several reviews summarize advances in various medicinal applications of metal complexes of coumarins^{7,8}. The biological activity of some coumarin derivatives significantly enhances by binding to metal ions^{9,10}. A broad array of medicinal applications of metal complexes of coumarins has been investigated. It was found that in some cases the metal complexes obtained revealed higher biological activity than their ligands^{11,12}. Thus, the aim of present work is to synthesize, characterize and study AF and IGR activities of transition metal complexes with newly synthesized Schiff base NBAC.

MATERIALS AND METHODS

General Procedures. All the chemicals used were either of AR or chemically pure grade. The melting points were determined in open capillaries and are uncorrected. Mass spectrum was recorded on a PerkinElmer Hitachi-6L spectrometer and ¹H NMR spectra was recorded in DMSO-d₆ at room temperature using TMS as internal standard on a VarianXL-200MHz. IR spectra were recorded using KBr discs in the 4000–400 cm⁻¹ region on a Shimadzu IR-435 and in Nujol media in the 4000–200 cm⁻¹ region on a PerkinElmer 283-B spectrometer and electronic spectra of solids were recorded on a Cary-2390 UV-Vis-NIR spectrophotometer. The elemental analysis was carried out using Heraeus-CHN-rapid analyzer. Metal contents were estimated using an AAS PerkinElmer-2380. Magnetic susceptibilities of complexes were measured on a Faraday balance CAHN-7550-03 USA at room temperature using Hg[Co(NCS)₄] as calibrant. Diamagnetic correction using Pascal's constants and temperature independent paramagnetic correction were computed. The electrical conductance measurements were recorded using 10⁻³M solutions in DMSO with an Elico conductivity bridge (Model CM-180) and dip type cell calibrated with KCl solutions. DTA was carried out using a Lead and Northup-USA instrument and TGA on a PerkinElmer model TGS-2 instrument. EPR was recorded on Jeol SE-3X spectrometer at RT and liquid nitrogen temperature.

N-(2-nitro) benzylidene-3 aminocoumarin (NBAC)

To a 0.05 mole (8 g) of 3-amino-coumarin dissolved in hot methanol, a 0.05 mole (7.6 g) of a methanolic 2-nitrobenzaldehyde was added

and refluxed for three hours. Yellow colored compound (10g) that separated out was filtered and washed with methanol, petroleum ether and dried under vacuum.

Yield 65%; m.p.142^o C; UV(solid) ν : 25000, 35710, 40000 cm⁻¹; FTIR (KBr) (in cm⁻¹): 3350 (ν_{OH}), 1700 ($\nu_{C=O}$), 1620 ($\nu_{C=N}$) 1490-1510 (ν_{NO_2} sym), 1460 cm⁻¹ (ν_{NO_2} asym)

The mass spectrum of the compound exhibited the characteristic signal at m/z 294

Metal complexes

A general method was adopted for the preparation of all the complexes. A hot methanolic solution of the ligand was added to a methanolic solution of the metal chloride. After complete addition the metal to ligand mole ratio was always kept as 1:2. A distinct lowering of the pH was observed. The pH of the solution was then increased to 7 using a methanolic ammonia solution and the contents were refluxed on a hot water bath for 6-8 h. The separated solid complexes were filtered, washed with methanol, petroleum ether and dried in vacuum desiccators over P₂O₅.

Cr(III) complex: yield 45%; m.p. 165^o C; UV(solid) ν : 15380, 20400, 27770 cm⁻¹; FTIR (KBr) (in cm⁻¹): 3300(ν_{OH}), 1680 ($\nu_{C=O}$), 1600 ($\nu_{C=N}$), 1490-1510 (ν_{NO_2} sym), 1460 (ν_{NO_2} asym), 650, 620, 540, 330 cm⁻¹ (new bands); Anal. Calcd for CrC₃₂H₂₀N₄O₈Cl₃: Cr, 6.97; C, 51.44; H, 2.68; N, 7.50; Cl, 14.27 %. Found: Cr, 6.86; C, 50.72; H, 2.64; N, 7.42; Cl, 14.05%.

Mn(II) complex: yield 45%; m.p. 180^o C; UV(solid) ν : 12500-18100 cm⁻¹; FTIR (KBr) (in cm⁻¹): 3350(ν_{OH}), 1690 ($\nu_{C=O}$), 1600 ($\nu_{C=N}$), 1490-1510 (ν_{NO_2} sym), 1460 (ν_{NO_2} asym), 680, 600, 560, 460 cm⁻¹ (new bands); Anal. Calcd for MnC₄₈H₃₀N₆O₁₂Cl₂: Mn, 5.45; C, 57.15; H, 2.98; N, 8.33; Cl, 7.04 %. Found: Mn, 5.38; C, 56.40; H, 2.94; N, 8.23; Cl, 6.93%.

Fe(III) complex: yield 50%; m.p. 187^o C; UV(solid) ν : 32250-16000 cm⁻¹; FTIR (KBr) (in cm⁻¹): 1740 ($\nu_{C=O}$), 1600 ($\nu_{C=N}$), 1460-1500 (ν_{NO_2} sym), 1435 (ν_{NO_2} asym), 660, 540, 480, 450, 320 cm⁻¹ (new bands); Anal. Calcd for FeC₃₂H₂₀N₄O₈Cl₃: Fe, 7.44; C, 51.18; H, 2.66; N, 7.46; Cl, 14.19 %. Found: Fe, 7.34; C, 50.60; H, 2.62; N, 7.35; Cl, 14.00%.

Co(II) complex: yield 40%; m.p. 172^o C; UV(solid) ν : 8930, 15390, 20000 cm⁻¹; FTIR (KBr) (in cm⁻¹): 3350(ν_{OH}), 1690 ($\nu_{C=O}$), 1600 ($\nu_{C=N}$), 1490-1510 (ν_{NO_2} sym), 1460 (ν_{NO_2} asym), 700, 660, 640, 450 cm⁻¹ (new bands)FTIR; Anal. Calcd for CoC₄₈H₃₀N₆O₁₂Cl₂: Co, 5.82; C, 56.92; H, 2.96; N, 8.30; Cl, 7.01 %. Found: Co, 5.73; C, 56.11; H, 2.92; N, 8.15; Cl, 6.91%.

Ni(II) complex: yield 60%; m.p. 162^o C; UV(solid) ν : 12500, 19230, 24390 cm^{-1} ; FTIR (KBr) (in cm^{-1}): 3350(ν_{OH}), 1690 ($\nu_{\text{C=O}}$), 1600 ($\nu_{\text{C=N}}$), 1490-1510 (ν_{NO_2} sym), 1460 (ν_{NO_2} asym), 660, 620, 450, 310 cm^{-1} (new bands); Anal. Calcd for $\text{NiC}_{32}\text{H}_{20}\text{N}_4\text{O}_8\text{Cl}_2$: Ni, 8.18; C, 53.50; H, 2.79; N, 7.80; Cl, 9.89 %. Found: Ni, 8.04; C, 52.75; H, 2.75; N, 7.71; Cl, 9.75%.

Cu(II) complex: yield 55%; m.p. 175^o C; UV(solid) ν : 14900, 15380, 22220 cm^{-1} ; FTIR (KBr) (in cm^{-1}): 1740 ($\nu_{\text{C=O}}$), 1600 ($\nu_{\text{C=N}}$), 1460-1500 (ν_{NO_2} sym), 1430 (ν_{NO_2} asym), 690, 600, 490, 450, 300 cm^{-1} (new bands); Anal. Calcd for $\text{CuC}_{32}\text{H}_{20}\text{N}_4\text{O}_8\text{Cl}_2$: Cu, 8.79; C, 53.14; H, 2.77; N, 7.75; Cl, 9.83 %. Found: Cu, 8.68; C, 52.39; H, 2.72; N, 7.64; Cl, 9.71%.

Pharmacology

Rearing of the test insect and antifeedant test

The larvae of *S. litura* were reared on the leaves of castor (*Ricinus communis*), a natural diet for the larvae. The leaf disc bioassay method¹³ was used against the fifth instar larvae to measure AF activity. This stage has been selected because the larvae feed voraciously on castor leaves. Leaf discs of uniform area (78.75 cm^2) were punched out from the castor leaves with the petioles intact. The leaf discs to be treated were dipped for 2 min in acetone solutions of various test compounds at two different concentrations, i.e. 100 and 500 ppm. The leaf discs with petioles were kept in small vials containing water which were placed in a large conical flask to facilitate easy translocation of water and to retain the freshness of leaf discs throughout the experiment. Five test insects, which were pre starved for 6 h, were introduced into the conical flask containing leaf discs and allowed to feed on them. One set of the control leaf discs were dipped in pure acetone and another set was kept without

any chemical. The results were recorded after 2, 12 and 24 h. The area of leaf discs consumed was measured by Dethier's method¹⁴ and the experiments were repeated three times.

IGR activity test with juvenile hormone¹⁵

Fifth instar larvae were fed on topically treated leaves with various compounds at different dosages. In each experiment, 10 insects were employed and the experiments were repeated three times. The observations were made in post-treatment stages for growth changes

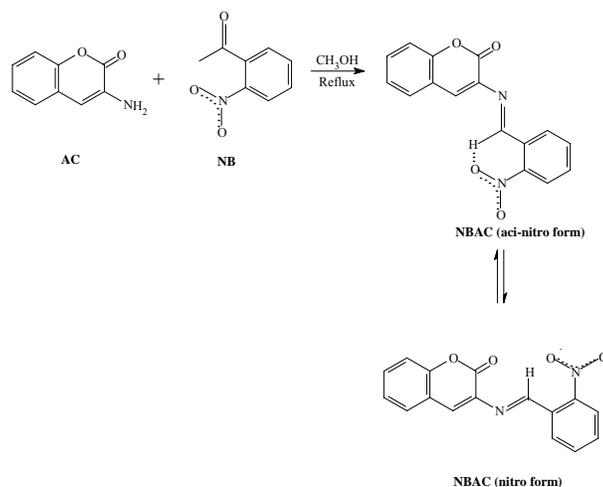
RESULTS AND DISCUSSION

Characterization of the ligand

The reaction of 3-aminocoumarin (AC) with 2-nitrobenzaldehyde (NB) resulted in N-(2-nitro) benzylidene-3 aminocoumarin (NBAC) in 65% yield which was purified by column chromatography. Elemental analysis indicates that the ligand, NBAC, has the molecular formula $\text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_4$. The mass spectrum shows parent peak at m/e 294 which is consistent with the molecular formula. The possible tautomeric forms of ligand, NBAC, are given in Scheme 1.

In IR spectrum, there was an intense band at 3350 cm^{-1} which is assignable to ν_{OH} . Such bands have been characterized for several nitro compounds with possible contribution from aci-nitro form.

The electronic spectrum of NBAC reveals in addition to a multiple band in the region of 40000 cm^{-1} to 35710 cm^{-1} , two more bands at 33330 cm^{-1} and 25000 cm^{-1} . The bands between 40000 cm^{-1} and 33330 cm^{-1} are attributed to the various $\pi-\pi^*$ and $n-\pi^*$ transition involving lactone carbonyl and the nitro group. The band at 25000 cm^{-1} is consistent with transitions involving the azomethine group¹⁶.



Scheme 1

The NMR spectrum of the ligand reveals the presence of -CH proton of the coumarin moiety at δ 6.64. The ring protons of the coumarin are identified in the region of δ 7.36 to δ 7.88. The ring protons of nitrobenzaldehyde are identified in the region of δ 7.98 to δ 8.68. The CH - proton of the azomethine group is appearing at δ 9.37.

Characterization of metal complexes

The complexes are found to be stable under atmospheric conditions. They are soluble in methanol, acetone, benzene, 1, 4 - dioxane, DMF, THF and DMSO.

The analytical data show metal to ligand ratio of 1:2 in Cr(III), Fe(III), Ni(II) and Cu(II) complexes and a ratio of 1:3 in Mn(II) and Co(II) complexes. The presence of two chlorides per every divalent metal ion and three chlorides per each trivalent metal ion is also evidenced by analytical data. The molar conductance measurements show that Cr(III) and Fe(III) complexes are 1:1 electrolytes and the rest are non-electrolytes¹⁷. Hence chlorides in Mn(II), Co(II), Ni(II)

and Cu(II) complexes are in the coordination sphere. In Cr(III) and Fe(III) complexes two chlorides are in the coordination sphere and one chloride in the outer sphere are confirmed by analytical and conductance data. The absence of coordinated water was established by TGA and DTA analysis.

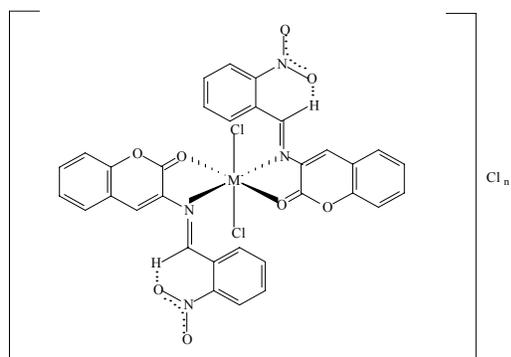
There was sharp modification between the IR spectra of the metal complexes and the ligands. The IR spectra of the complexes indicate two distinct patterns of metal binding by NBAC. In Cr(III), Mn(II), Co(II) and Ni(II) complexes the aci-nitro form of NBAC is intact and is behaving as a neutral bidentate ON donor, binding to metal ions through azomethine nitrogen and lactone oxygen. Stabilization of aci-nitro form in metal complexes is a new feature. The spectra of Fe(III) and Cu(II) complexes do not show the band characteristics of ν_{OH} . This is a direct indication of the transformation of the aci-nitro tautomer to nitro tautomer and the preference of the ligand to bind in the latter form to Fe(III) and Cu(II) ions. In these complexes, the ligand behaves as a neutral bidentate ON donor, binding to metal ions through the azomethine nitrogen and the oxygen of nitro group¹⁸.

The room temperature magnetic moments reflect the paramagnetic nature of all the complexes. These values are consistent with a spin free configuration. The absorption maxima for the complexes and

the corresponding assignments of the electronic transitions are given in Table 1. On the basis of the data octahedral geometries (Figures 1-3) have been proposed¹⁹.

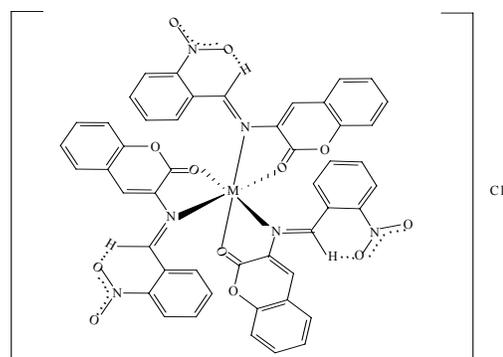
Table 1: Electronic spectral data of metal complexes

Complex	Band position (cm ⁻¹)	Assignment
Cr(III)	15380, 20400, 27770	${}^4A_{2g} \rightarrow {}^4T_{1g}(P)$, ${}^4A_{2g} \rightarrow {}^4T_{1g}$, ${}^4A_{2g} \rightarrow {}^4T_{2g}$
Mn(II)	12500-18100	-
Fe(III)	32250-16000	-
Co(II)	8930, 15390, 20000	${}^4T_{1g} \rightarrow {}^4T_{2g}$, ${}^4T_{1g} \rightarrow {}^4A_{2g}$, ${}^4T_{1g} \rightarrow {}^4T_{1g}(P)$
Ni(II)	12500, 19230, 4390	${}^3A_{2g} \rightarrow {}^3T_{2g}$, ${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$, ${}^3A_{2g} \rightarrow {}^3T_{1g}(P)$
Cu(II)	14900, 15380, 22220	${}^2B_{1g} \rightarrow {}^2A_{1g}$, ${}^2B_{1g} \rightarrow {}^2B_{2g}$, ${}^2B_{1g} \rightarrow {}^2E_g$



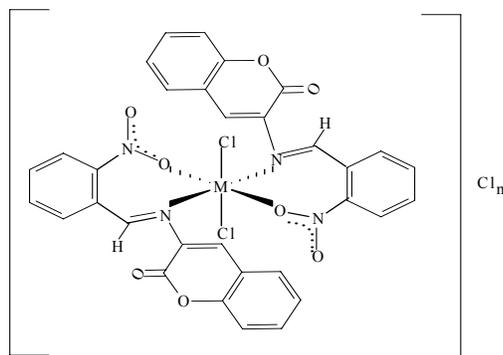
M = Cr(III), n = 1; M = Ni(II), n = 0

Fig. 1: Structure of Cr(III) & Ni(II) complexes



M = Mn(II) or Co(II)

Fig. 2: Structure of Mn(II) & Co(II) complexes



M = Fe(III), n = 1; M = Cu(II), n = 0

Fig. 3: Structure of Fe(III) & Cu(II) complexes

The EPR spectrum of copper (II) complex at liquid nitrogen temperature has been evaluated to give g_{\parallel} and g_{\perp} values as 2.12 and 2.16 respectively. The difference in g_{\parallel} and g_{\perp} values indicates the octahedral distortion in the complex. Absence of hyperfine interaction reveals that the M-L interaction is far higher and more prominent in comparison with the possible hyperfine interaction in free Cu(II) ion.

Antifeedant and IGR activity

The ligand, NBAC, its starting materials and its metal complexes were evaluated for antifeedant and IGR activity against *Spodoptera litura*. It was observed that during the total experimental period, the insects did not show any repellent behaviour towards the treated leaf surfaces. The metal salts, AC did not exhibit any antifeedant activity either at a lower or at a higher dosage. At higher dosages, NB and NBAC were found to exhibit negligible antifeedant activity.

The metal complexes of NBAC exhibited antifeedant activity at both lower and higher dosages, with the said activity being remarkably

enhanced at higher dosages. The activity orders of the metal complexes are as follows:

At 500 ppm dosage: Ni>>Co>Cr>Fe=Cu>NBAC>Mn=NB
At 100 ppm dosage: Ni>>Co>Cr=Fe=Cu>Mn

IGR activity was not recorded at both higher and lower dosages in the case of metal salts, AC, NB and the ligand NBAC. The metal complexes of NBAC were observed to exhibit IGR activity at lower dosages itself and by increasing the dosage to 500 ppm, enhanced activity was observed. The activity order of the metal complexes is as follows:

At 100 ppm dosage: Ni=Co>Cr=Fe=Cu=Mn>NBAC
At 500 ppm dosage: Ni>Co>Cr=Fe=Cu>Mn>NBAC

However, further detailed study is required regarding the activity enhancement of the test compounds with structural modifications, identification of the site of action of the compound.

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