Antifeeding and insect-growth-regulating activity of certain metal complexes towards *Spodoptera litura*; F

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Metal complexes of divalent cobalt, nickel and copper and trivalent iron were synthesized using *N*-salicylidene-3-aminocoumarin as chelating agent. The ligand behaves as a monobasic ONO donor towards Co(II), Ni(II) and Cu(II) and as an ON as well as an ONO donor towards Fe(III). All the complexes have been proposed to have octahedral geometry on the basis of analytical, thermal conductivity, spectral and magnetic data. The complexes have been screened against *Spodoptera litura*; F (*Lepidoptera: noctuiidae*) for antifeeding and insect-growth-regulating activity. The results show appreciable insect-growth-regulating activity associated with metal complexation.

Keywords: metal complexes, *N*-salicylidene-3-amino-coumarin, characterization, antifeeding activity, insect-growth-regulating activity

Introduction

Several vital functions of living organisms are thought to be due to the chemistry occurring at the metal center in metal-bound biomolecules. Investigations centered on synthesis, stability, structure and reactivity of biological metal-containing compounds are well advanced. When a metal ion is bound to a biologically active organic molecule, it may result in apparent modification of the activity, generally depending on the specificity of the binding site and the stereochemistry of the metal complex.

Coumarin (2*H*-1-benzopyran-2-one) and those derivatives which are plant products have been found to exhibit a wide range of physiological activities, such as anticoagulant (Rodman 1968), antifeeding (Chaudhury *et al.* 1985), plant growth regulation (Brown Steward 1981), etc. Bharat *et al.* (1987) have reported the antibacterial and antifungal activities of Cr(III), Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes of 3-nitro-4-hydroxy-coumarin and 3-amino-4-hydroxycoumarin. Ryz-

henko *et al.* (1981) have reported the antimicrobial properties of metal-bound 3-aminocoumarin. Although coumarin is a plant product known to possess insecticidal effect (Crombie 1972, Chaudhury *et al.* 1985), so far no significant attempt has been made to study the antifeeding (AF) and insect-growth-regulating (IGR) activities with azomethine derivatives of 3-aminocoumarin and their metal complexes. The present investigation comprises a study of AF and IGR activities on *Spodoptera litura* is with certain newly synthesized and characterized metal complexes of *N*-salicylidene-3-aminocoumarin.

Materials and methods

Chemicals and instrumentation

All solvents and reagents used were of AR grade. The C, H and N contents of compounds were determined by micro-analytical methods. The metal contents in complexes were determined by atomic absorption spectrometry on a Varian spectraAA 30/40 using the necessary standards. Conductance measurements of 1 mM solutions in dimethylsulfoxide were carried out on a digital conductivity meter. Magnetic susceptibilities of the complexes were determined at room temperature on a vibrating sample magnetometer, EG and G Princeton research

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model (3). Thermal analysis of complexes was carried out on a Leed and Northup (USA) instrument at normal atmospheric pressure. Infrared spectra of the compounds were recorded on a Perkin-Elmer 783 model spectrometer, in the form of KBr pellets. Electronic reflectance spectra were recorded on a UV-Cary 2390 spectrometer. The NMR spectrum of the ligand was recorded on a VG-micro-moss 7070-H spectrometer.

Synthesis of N-*salicylidene-3-aminocoumarin and its metal complexes*

N-Salicylidene-3-aminocoumarin was prepared by earlier reported procedures (Kokotos & Tsougraki 1986). Then, to 0.01 mol, dissolved in 100 ml of hot methanol, a methanolic solution of the divalent metal chloride [Co(II), Ni(II), Cu(II)] or trivalent metal chloride [Fe(III)] (0.005 mol in 50 ml methanol) was added. The pH of the solution was adjusted to 7 using methanolic ammonia and the reaction mixture was refluxed for 4 h. The resultant colored products were filtered hot, washed with methanol and petroleum ether, and then dried in vacuum.

Rearing of the test insect and antifeeding 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 method (Wada & Manakata 1968) 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 different concentrations, i.e. 0.1, 0.5, 1.0 and 2.0%. 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 prestarved 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 method (1947) and the experiments were repeated three times.

IGR activity test with juvenile hormone (Nagaraja Rao 1987)

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

Structural elucidation

All the complexes are stable to air and moisture. They decompose at high temperatures. The ligand and complexes were found to be soluble in acetone, chloroform, 1,4-dioxane, dimethylformamide and dimethylsulfoxide. Elemental analysis data of *N*-salicylidene-3-aminocoumarin and its metal complexes reveals a metal/ligand ratio of 1:1 in Co(II) and Ni(II) complexes and a 1:2 ratio in Fe(III) and Cu(II) complexes.

Conductance data reveal the Cu(II) complex to be a non-electrolyte. The other complexes show conductance in the range of 60–80 ohm^{-1} cm^2 mol^{-1} indicating the presence of a chloride ion in the ionization sphere (Angelici 1969). Thermal analysis reveals the absence of coordinated water in the Cu(II) complex. Endotherms were observed in the range of 165–225 °C for the Fe(III) complex, indicating one coordinated water molecule (Nikolaev *et al*. 1969). In the case of Co(II) and Ni(II) complexes, three distinct regions in endotherms were identified, indicating the presence of three coordinated water molecules, at 165, 180–225 and 255–315 °C in Co(II), and at 165, 210 and 240–255 °C in Ni(II), respectively. The infrared spectral characteristics of the ligand and metal complexes are presented in Table 1. The infrared spectrum of the ligand shows absorption at 1720, 1620 and 1280 cm^{-1} which are assignable to $v_{C=O}$ (lactone carbonyl), $v_{C=N}$ (azomethine) and v_{C-O} (phenolic), respectively. The v_{OH} is observed at 3400 cm^{-1}.

In all the metal complexes, except the Cu(II) one, a broad trough is observed in the region 3100–3650 cm^{-1} which indicates the presence of coordinated water as confirmed by thermal analysis. The peak observed in the region of 820–840 cm^{-1} in these complexes has been assigned to the rocking mode of coordinated water. A negative shift of about 40–50 cm^{-1} in $v_{C=O}$ is observed in all complexes. In the spectrum of the Fe(III) complex, two bands corresponding to $v_{C=O}$ are observed: one at 1710 cm^{-1} is assigned to a free carbonyl and the other at 1650 cm^{-1} to a metal-bound carbonyl. A similar downward shift of about 20 cm^{-1} was observed in the $v_{C=N}$ stretch in all metal complexes. An upward shift by ≈20–40 cm^{-1} of the phenolic (v_{C-O}) band was observed in all complexes. Isolation of complexes at neutral pH indicates coordination through the phenolic C—O to have taken place subsequent to deprotonation. New non-ligand bands identified in the range of 550–600 cm^{-1} have been

Table 1. Infrared spectral data of ligand and metal complexes

Ligand/complex	Frequencies (cm⁻¹)					
	v_{OH}	$v_{C=O}$	$v_{C=N}$	v_{C-O}	v_{C-N}	New bands
H-SAC	3400 s	1720 s	1620 s	1280 s	1350 s	—
Fe(III)SAC	—	1710 s	1600 s	1310 s	1340 s	3600–3200 b, 840 m
Co(II)SAC		1650 s				650 w, 580 w
	—	1660 s	1600 s	1300 s	1340 s	3600–3100 b, 910 m
Ni(II)SAC			1600 s			650 w, 580 w
	—	1660 s	1600 s	1300 s	1340 s	3650–3200 b, 860 m
Cu(II)SAC						650 w, 560 w
	—	1660 s	1600 s	1320 s	1340 s	660 w, 550 w

SAC = *N*-salicylidene-3-aminocoumarin; s = strong, m = medium, w = weak.

assigned to v_{M-N} and v_{M-O} (Nakamoto 1970) frequencies.

These observations show that the ligand *N*-salicylidene-3-aminocoumarin coordinates through the phenolic oxygen, azomethine nitrogen and lactone carbonyl in all the complexes, and thus acts as a monobasic tridentate ONO donor. In the Fe(III) complex, while one ligand molecule acts as an ONO donor, the other molecule acts as an ON donor, coordinating only through the azomethine nitrogen and phenolic oxygen atoms.

The room temperature magnetic moments data and the electronic spectral data are shown in Table 2. The former data reflect the paramagnetic nature of all complexes. These values are consistent with a spin-free configuration (Figgis & Lewis 1964). The electronic spectra of the metal complexes show bathochromic shifts in the azomethine chromophore. The absorption maxima for the complexes and the corresponding assignments of the electronic transitions are also included in Table 2. In the case

Table 2. Electronic spectral and magnetic data

Compound	Band position (cm⁻¹)	Assignment	μ_{eff} (BM)
Fe(III)SAC	35 720–12 500	—	5.9
Co(II)SAC	22 000	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$	
	15 750	$^4T_{1g}(F) \rightarrow {}^4A_{2g}$	4.0
	7 190	$^4T_{1g}(F) \rightarrow {}^4T_{2g}$	
Ni(II)SAC	23 530	$^3A_{2g} \rightarrow {}^3T_{1g}(P)$	
	14 290	$^3A_{2g} \rightarrow {}^3T_{1g}(F)$	3.17
	7 250	$^3A_{2g} \rightarrow {}^3T_{2g}$	
Cu(II)SAC	23 000	$^2B_{1g} \rightarrow {}^2E_g$	
	20 000	$^2B_{1g} \rightarrow {}^2B_{2g}$	1.93
	13 000	$^2B_{1g} \rightarrow {}^2A_{1g}$	

SAC = *N*-salicylidene-3-aminocoumarin.

of the Fe(III) spectrum, weak absorptions observed in the range of 12 500–35 720 cm⁻¹ could not be assigned due to their doubly forbidden nature (Lever 1984). On the basis of the above data, octahedral structures have been proposed for all the metal complexes (Figure 1).

Biological assay

When the larvae were exposed to various concentrations of test compounds, they showed AF activity at higher dosages and IGR with juvenile hormone activity at lower dosages.

AF activity

The experiments conducted by the leaf disc method at various concentrations and time intervals with the ligand and its metal complexes showed great variations. During the whole experimental time, the insects clung to the leaf and preferred to starve than feed on the leaves. However, in the course of time, they started feeding on the leaves, which is a typical AF character. The AF activity was found to vary and depended on duration, dosage and the type of compound administered.
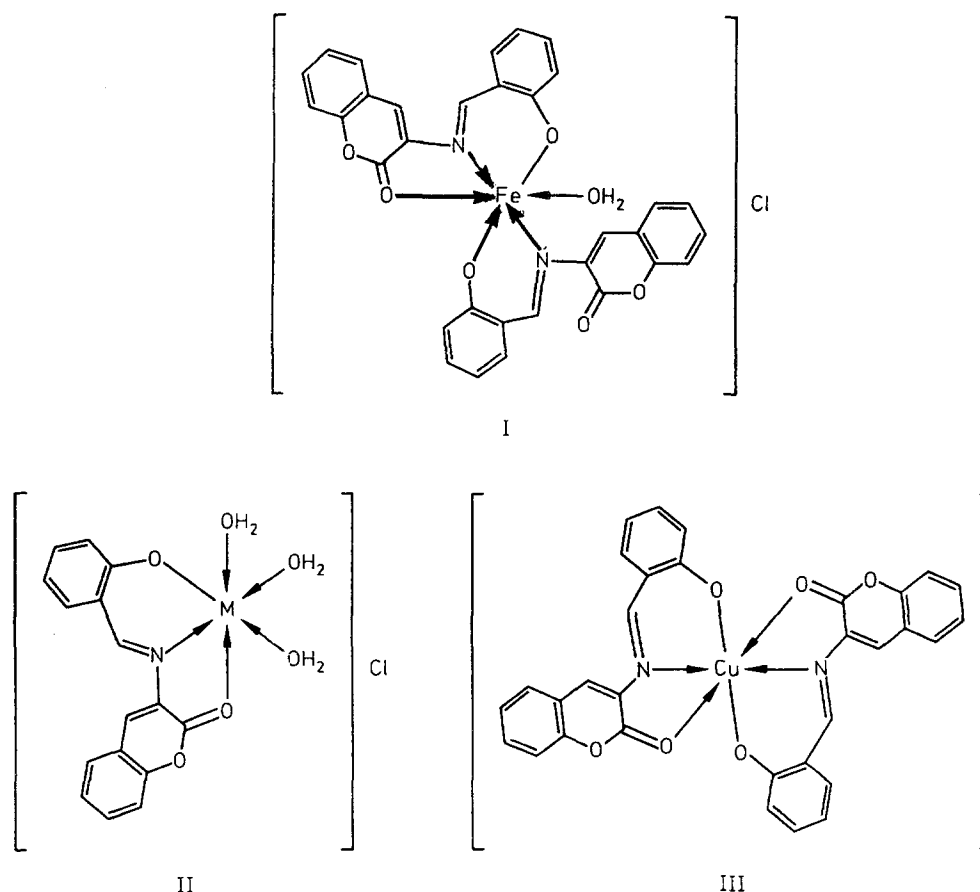
At 2 h intervals. About 60% of leaf was consumed in controls, salicylaldehyde, 3-aminocoumarin and *N*-salicylidene-3-aminocoumarin without any apparent variation. Significant changes were observed with the Ni(II) and Co(II) complexes at all concentrations with about 30–40% leaf consumption at 2% concentration. Moderate activity was recorded for the Fe(III) and Cu(II) complexes.

At 12 h intervals. Total (100%) leaf consumption was recorded in all the control tests with salicylaldehyde, 3-aminocoumarin and *N*-salicylidene-3-amino-

Figure 1. Structures of metal complexes of *N*-salicylidene-3-aminocoumarin. M = Co(II) or Ni(II).

coumarin at all concentrations. The insects resisted feeding on leaves treated with the Ni(II) and Co(II) complexes. The leaf consumption was about 81.03–71.00% in Ni(II), 86.53–78.00% in Co(II) and ≈90.00% in Fe(III) and Cu(II) complexes.

At 24 h intervals. Total (100%) leaf consumption was recorded in the controls, salicylaldehyde, 3-aminocoumarin and *N*-salicylidene-3-amino coumarin at all concentrations.

These observations indicate the absence of repellence by the insects towards the test compounds, a necessary prerequisite in developing an AF compound, as is clear by the insect behavior, i.e. the larvae clung to the leaves throughout the experimental period. In such cases, the larvae prefer to starve and die instead of feeding on the leaves (Dethier 1947, Gorz *et al*. 1972). However, at 12 and 24 h intervals, the percentage of leaf damage was enhanced, probably due to force feeding or to a fall in the efficacy of the compounds. In such cases, only a few of the larvae were dead and most of them pupated but without adult emergence, indicating the

need to test the compounds for IGR activity. The AF activity of the free ligand, metal complexes, etc., was in the following sequence: Ni(II) > Co(II) > Fe(III) > Cu(II) > *N*-salicylidene-3-aminocoumarin > 3-aminocoumarin > salicylaldehyde. Similar results were noticed when many synthetic or natural products were tested for AF activity against different insect pests (Wada & Manakata 1968, Ishaaya *et al*. 1980).

IGR activity

In these experiments, when the larvae were allowed to feed on the treated leaves, developmental inhibitions were noticed with certain compounds. Most of the abnormalities were observed during development mainly at lower concentrations. Larval life was found to be prolonged in those treated with the Ni(II) and Co(II) complexes. Later, all the larvae pupated normally. However, prominent changes in certain areas were noticed during adult emergence: (i) prolongation of larval life; (ii) formation of apparently normal pupae; (iii) mortality during

pupal formation; (iv) adult mortality during emergence as a consequence of pupal case attachment; (v) emergence of abnormal adults with crumpled wings and malformed legs; (vi) though some normal adults had emerged, they were dead soon after emergence; (vii) non-emergent pupae; (viii) emergence of few normal adults.

All these changes are commonly recorded with exogenous juvenile hormone activity. Similar results were also noticed when the plant originated antifeedant azadirhychtin was tested for juvenile hormone activity (Schmutlerer *et al*. 1980). Many earlier workers noticed similar changes in different insects when they were tested for IGR with juvenile hormone activity (Deb & Chakravorthy 1985, Nagaraja Rao 1987). This was considered as due to interference by the test compounds with the neuroendocrine system of the test insects (Pratt & Brooke 1981, Nagaraja Rao 1987) or possibly changes brought about in the general metabolism which, in turn, affected the growth of the insect through the neuro-endocrine system (Deb & Chakravorthy 1985, Krocke *et al*. 1985).

Thus, from the above studies, it can be concluded that the metal complexes used in the present study have AF properties at higher dosages and IGR juvenile hormone activity at lower dosages. Hence, it is clear that these metal complexes may be used as a new group of pesticides which may have multiple benefits over the conventional pesticides like DDT, Malathion, etc.

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, bioassay against various insects and their toxicity evaluation against different vertebrate models, etc., at laboratory and field levels.

Acknowledgments

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