SYNTHESIS AND CHARACTERIZATION OF SOME METAL COMPLEXES OF A SCHIFF BASE DERIVED FROM NINHYDRIN AND & L-ALANINE

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ABSTRACT. Complexes of Mn(II), Fe(III), Co(II), Ni(II) and Zn(II) with an intermediate Schiff base derived from ninhydrin and α ,L-alanine (indane-1,3-dione-2-imine-N-2-propionate, IDIP) were successfully synthesized. All complexes were distinctly colored and were characterized by elemental analysis, molar conductance, magnetic susceptibility, infrared and electronic spectral studies. The ligand (Schiff base) was shown to behave as a monobasic tridentate ONO donor. The Mn(II) and Fe(III) complexes contain only one ligand molecule plus water and chloride(s) per metal ion, while all the others contain two ligand molecules per metal ion. An octahedral geometry is proposed for the metal complexes.

A comparative anti-microbial study of the complexes was undertaken against two gramnegative bacteria, *Escherichia coli* and *Proteus mirabilis* and one gram-positive bacterium *Staphylococcus aureus*. It was revealed that most of the complexes and particularly Mn(II), Fe(III) and Co(II) complexes, have a much enhanced activity relative to ninhydrin against *Proteus mirabilis* and *Staphylococcus aureus*. All complexes showed a reduced activity against *Escherichia coli*.

KEY WORDS: Metal complexes, Schiff base, Ninhydrin, α ,L-Alanine, Indane-1,3-dione-2-imine-N-2-propionate (IDIP), Anti-microbial activity

INTRODUCTION

Identification and quantification of constituent amino acids in a mixture is required in the biochemical investigations of proteins and peptides. The most extensively used method is the ninhydrin reaction, in which ninhydrin (1,2,3-triketohydrindene hydrate or 2,2-dihydroxy-1,3-indandione; I and II, Scheme 1) reacts with amino acids to give a characteristically blue/purple colored compound popularly known as Ruhemann's Purple, VIII [1, 2], after Sigfried Ruhemann who first observed the color reaction [3, 4]. The blue compound, VIII, maximally absorbs at 570 nm [1, 5, 6], and this forms the basis for the spectrophotometric quantitative determination of amino acids that can detect as little as one microgram of material [1, 7]. Several workers have introduced modifications to the method [1], the most noticeable ones being those by Moore and coworkers [8-12] and Doi *et al.* [13]. The effect of metals and various other substances on this color reaction has also been studied by several workers [14-19].

The chemistry of the reaction of ninhydrin with amino acids has been extensively studied by several workers [6, 20, 21]. The mechanism, however, was not well understood until very recently and this gave rise for a series of theories as reviewed by McCaldin [20]. A simplified form of the mechanism proposed by Filippovich and McCaldin is shown in Scheme 1 [20-22]. It has a condensation step that leads to a Schiff base formation followed by decarboxylation, hydrolysis and finally further condensation with another ninhydrin molecule to give the final product, Ruhemann's purple [20-22]. The kinetic aspects have been well studied by Friedman and Sigel [5].

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Scheme 1. The reaction of ninhydrin with α -amino acids [22].

Schiff bases are good ligands for metal ions [23, 24]. The ketimine, IV, is, therefore, a potential ligand to metal ions, that can act as a tridentate ligand forming two stable five membered rings on complexation. If the side group of the amino acid is not considered for the present discussion the Schiff base can have metal binding sites as shown in Figure 1. In accordance with this proposal, Rao and Reddy [25] synthesized and characterized Co(II), Ni(II) and Zn(II) complexes of a Schiff base derived from ninhydrin and glycine (indane-1,3-dione-2imine-N-acetate, IDIA) in methyl cellosolve media. The study showed that if a metal ion is present before the reaction commences, the reaction does not proceed to the final product, but stops at the first step, the metal ion forming a highly stable colored complex with the Schiff base [25] (Figure 2 and Scheme 2). It is anticipated that this can form a basis for studying the reaction between ninhydrin and a variety of amino acids in the presence of metal ions, particularly transition metal ions. A specific correlation of metal ion-amino acid-color can thus be developed based on the results and this can form a basis for amino acid identification and determination. The present investigation was, therefore, undertaken to synthesize and characterize 3d metal complexes of a condensation product (Schiff base) of ninhydrin and α.L-alanine, indane-1,3dione-2-imine-N-2-propionate, IDIP.

Ninhydrin has been shown to exhibit strong antibacterial, antiviral, etc. activities [26]. It has also been shown that physiological activity of organic compounds is much enhanced on coordination to metals [25, 27], particularly, Schiff bases and their metal complexes have been shown to exhibit enhanced activities [25]. Thus, Schiff bases and their metal complexes have attracted a great deal of attention in biochemistry due to their vast applications as anticancer,

antitubercular, anticonvulsant, insecticidal, antibacterial, etc agents. In light of this, Rao and Reddy [25] also performed comparative anti-microbial study of their Schiff base complexes against four bacteria *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus faecalis* and *Staphylococcus aureus*. The complexes were found to be inactive. The newly synthesized complexes were also screened for anti-microbial activities against three bacteria *Escherichia coli*, *Proteus mirabilis* and *Staphylococcus aureus*.

Figure 1. Possible metal binding centers of a Schiff base derived from ninhydrin and an α -amino acid.

Figure 2. Metal complexes of IDIA [25], [M = Co(II), Ni(II) and Zn(II)].

$$\begin{array}{c} M(II) \\ -H^{+} \end{array}$$

$$\begin{array}{c} M_{12} \\ O \\ M_{2} \end{array}$$

$$Metal \ complex \\ [M(II) = Co(II), \ Ni(II) \ and \ Zn(II)] \end{array}$$

Scheme 2. Reaction of glycine and ninhydrin in the presence of M(II) ions [25].

EXPERIMENTAL

Materials and methods

Ninhydrin (Pharmacos), α ,L-alanine (Aldrich), most metal salts, other reagents and solvents used in the investigation were of AnalaR grade. Some solvents (methanol, DMSO) were purified following established methods. Ethanol was used as the solvent for synthesis.

Melting/decomposition temperatures were determined with Bock–Monoscop ('M') – Werck-NR instrument. UV-Visible spectrophotometric studies were done in the range of 200-1100 nm using a SPECTRONIC GENESYS 2PC with a 1 cm cell at a concentration of 1.5×10^{-3} – 10^{-4} M in DMSO and chloroform at room temperature. Infrared spectra (KBr disks) were recorded using a Pye-Unicam SP 2000 Infrared Spectrophotometer in the range of $4000 - 200 \text{ cm}^{-1}$ and with a Buck Scientific Infrared Spectrophotometer Model 500 in the range of $4000 - 600 \text{ cm}^{-1}$. Electrical conductivities of the complexes were studied at room temperature with freshly prepared 1 mM solutions in DMSO using a Jenway 4330 Digital Conductivity and pH meter and a Philip Harris Conductivity meter.

The complexes were analyzed for metal using a Varian SpectrAA–20 Plus Atomic Absorption Spectrometer. Chloride from some samples was determined as AgCl by the sodium fusion method using standard methods [28, 29].

Magnetic susceptibility measurements were performed at 22 °C on a MSB-AUTO Sherwood Scientific Ltd. Magnetic Susceptibility balance.

Anti-microbial studies were done by the disc-diffusion method in a nutrient agar medium against the three test organisms already noted.

Synthesis

Complexes of Mn(II), Fe(III), Co(II), Ni(II) and Zn(II) were synthesized using a general procedure. The ligand (Schiff base) was prepared in the presence of a metal ion in the reaction mixture. 0.01 mole (1.78 g) of ninhydrin was dissolved in the minimum possible amount of ethanol and 0.01 mole of a metal (chloride) salt was added to the resulting solution, which was shaken and/or heated until all of it dissolved. This mixture was refluxed for about half an hour, followed by addition of 0.01 mole (0.89 g) α ,L-alanine to the hot mixture and the mixture was refluxed for a further two hours. The resulting colored precipitate was then filtered off while hot through a Whatman # 50 filter paper by suction, washed with distilled water, ethanol and finally with a 50:50 mixture of ethanol and petroleum ether (50 – 80 °C). The product was then dried and stored in a desiccator

Anti-microbial studies

This was done by the disc-diffusion method in a nutrient agar. The three bacteria were incubated on a nutrient agar slant in separate test tubes for 48 h. 0.5 mL of the exponentially growing cultures of each bacterium were then diluted further with sterile nutrient broth. These were seeded on a nutrient agar/broth media by the top and bottom technique.

The metal complexes and controls (ninhydrin, Ruhemann's purple and some metal salts) were dissolved in DMSO to make a solution of 25 mg/mL concentration. Two different concentrations of each sample were studied. 10 μL (250 mg) and 20 μL (500 mg) of each sample, controls and solvent were absorbed on paper discs (Antibiotic – Assay Disc, diam. 0.25 inch) using eppendrof. The soaked paper disks were then placed on the inoculated agar plates at relatively regular intervals and incubated in the inverted fashion at 37 °C for 24 h. Any inhibition zones were looked for and their diameters measured using a ruler.

RESULTS AND DISCUSSION

General

Ninhydrin and α.L-alanine form a deep purple colored compound known as Ruhemann's purple, which maximally absorbs at 570 and 416 nm [5]. The compound is believed to be formed via several steps; mainly condensation of the amine group of α,L-alanine with the carbonyl group of ninhydrin to give a ketimine Schiff base (indane-1,3-dione-2-imine-N-2-propionic acid, IDIPA), decarboxylation and further condensation with another ninhydrin to give the final product (Scheme 1) [20, 22]. In the present investigation, it is shown that in the presence of some first row transition metal ions [Mn(II), Fe(III), Co(II), Ni(II) and Zn(II)] the reaction does not lead to the final product but stops at the first condensation step. The ketimine thus formed, indane-1,3dione-2-imine-N-2-propionic acid, IDIPA, rather than going to the next steps, undergoes deprotonation to give indane-1,3-dione-2-imine-N-2-propionate, IDIP and forms a stable complex with the metal ions, the ligand acting as a tridentate ONO donor (Scheme 2). The presence of M(II/III) ions in the system most probably favors the condensation of ninhydrin and α,L-alanine by enhancing the polarization of the carbonyl groups, thereby promoting the nucleophilic attack resulting in the production of IDIPA [30] which in turn binds the metal ion after deprotonation. The neighboring -COOH with a replaceable hydrogen increases the basicity of the azomethine group in IDIPA [31] thus favoring the formation of a stable metal chelate.

The products obtained in good yields were distinctly colored and stable to air and sunlight (Table 1). They decompose at high temperatures and are highly soluble in solvents of high dielectric strength, like DMSO and DMF but only partially soluble in solvents like methanol, CHCl₃, etc.

Table 1. Physical properties of the metal complexes of IDIP.

Complex	Color and appearance	Yield (%)	Melting/decomposition temp. (°C)
Mn(II)	Bright red, shiny	52.5	>350
Fe(III)	Light pink, shiny	53.7	>350
Co(II)	Bright green, shiny	51.6	>350
Ni(II)	Greenish brown, powder	58.3	>360
Zn(II)	Dark brown, shiny powder	60.3	>340

Analytical studies

The analytical and molar conductance data of the complexes are given in Table 2. The complexes were analyzed for C, H, N, metal and chloride (where detected). The data shows a metal to ligand ratio of 1:2 in Co(II), Ni(II) and Zn(II) complexes and a 1:1 metal to ligand ratio with additional water and chloride(s) in Fe(III) and Mn(II) complexes and the following formulations are suggested: [MnL(H_2O_3]Cl, [FeL(H_2O_3 Cl]Cl, [CoL2], [NiL2], and [ZnL2], where L is indane-1,3-dione-2-imine-N-2-propionate, IDIP, [$C_{12}H_8O_4N$].

Table 2. Analytical and conductance data for metal complexes of IDIP*.

Complex	%Metal	%Chloride	%Hydrogen	% Carbon	%Nitrogen	$\Lambda_{\rm m}(\Omega^{\text{-}1}$
	calc (found)	calc (found)	calc (found)	calc (found)	calc (found)	cm ² mol ⁻¹)
						DMSO
[MnL(H ₂ O) ₃]Cl	14.67 (14.08)	9.51 (9.70)	3.74 (3.72)	38.45 (38.97)	3.74 (3.84)	50.78
[FeL(H ₂ O) ₂ Cl]Cl	14.90 (14.12)	18.07 (17.84)	3.05 (3.01)	36.64 (36.45)	3.56 (3.69)	50.10
[CoL ₂]	11.34 (11.03)	_	3.08 (3.15)	55.50 (54.83)	5.40 (5.32)	30.06
[NiL ₂]	11.30 (10.70)	_	3.09 (2.98)	55.52 (55.65)	5.40 (5.42)	26.72
$[ZnL_2]$	12.49 (11.77)	_	3.06 (2.97)	55.01 (55.97)	5.35 (5.27)	30.06

 $^{^*}L = [C_{12}H_8O_4N]^T$ or indane-1,3-dione-2-imine-N-2-propionate, IDIP.

The molar conductance data show that Fe(III) and Mn(II) complexes are 1:1 electrolytes while the others are non electrolytes [32]. This also shows the presence of one chloride, counter ion, in the 1:1 electrolytes. The other chloride determined in the Fe(III) complex must then be in the inner coordination sphere.

Infrared spectral studies

The Infrared data are given in Table 3. The sharp NH stretching band of the free amino acids at 3200 – 3000 cm⁻¹ and the OH stretching frequency of ninhydrin are not observed, indicating the derivatization of these groups. The nitrogen may have been converted in to a tertiary nitrogen that does not show any peak in the region.

Table 3. Infrared spectral data of the metal complexes of IDIP and starting materials* (KBr pellets).

Compound	vOH	vC=O	v _a COO	vC=C (arom)	vC=N	δ CH, δ CH ₃	v _s COO ⁻	vM-O-C	Other non-ligand bands v(M-N, M-O, M- Cl,), ρ _r H ₂ O,
	3200 – 2920(b,s)	1768(s), 1754(s), 1720(s)	_	1590(s)	_	_	_		_
,	3300 – 2550(b,s)		1595(s)			1458(s), 1355(s)	1410(m)		
() · · · I ·	3620 – 2640(b,s)		1661(vs)	1588(s)	1505(vs)	1445(m), 1350(s)	1314(vs)	1232(vs)	715(s), 680(m), 590(m), 519(m), 470(m)
Fe(III) complex	3600 – 2700(b,s)		1630(vs)	1592(s)	1503(vs)	1468(m), 1320(s)	1318(vs)	1210(vs)	705(m), 660(m), 540(m), 519(m), 255(s)
Co(II) complex		1730(s), 1710(m)	1635(vs)	1598(s)	1508(vs)	1449(m), 1350(m)	1302(vs)	1200(vs)	660(m), 595(m), 530(m), 492(m)
Ni(II) complex		1735(s), 1714(s)	1631(vs)	1592(s)	1516(vs)	1447(m), 1350(s)	1327(vs)	1245(vs)	698(m), 672(m), 600(m), 540(s)
Zn(II) complex		1733(m), 1710(m)	1652(vs)	1595(s)	1508(vs)	1450(m), 1350(m)	1320(vs)	1232(vs)	687(w), 670(w), 594(m), 545(m)

^{*}Intensities in parenthesis: b-broad, vs-very strong, s-strong, m-medium, w-weak, a-asymmetric, s-symmetric, arom-aromatic. Assignments based on Nakamoto [35], v - stretching vibration, δ - bending or deformation.

The Fe(III) and Mn(II) complexes show a broad strong band in the range 3700 – 2500 cm⁻¹ assigned to OH stretching vibration of coordinated water. The presence of coordinated water in

these complexes is supported by other water bands in the regions $1630 - 1600 \text{ cm}^{-1}$ (HOH bending vibration) and $600 - 200 \text{ cm}^{-1}$.

Ninhydrin shows three bands in the C=O stretching region: 1768, 1754 and 1720 cm⁻¹ [33]. The 1754 and 1720 cm⁻¹ bands are characteristic of its 1,3-dicarbonyl functional group [34] and the 1768 cm⁻¹ band is characteristic of the intermediate carbonyl in the tricarbonyl species, which is in equilibrium with the dihydroxy species. In the products there are two carbonyl stretching peaks at decreased wave numbers, which shows the derivatization of the higher frequency (1768 cm⁻¹) carbonyl group of ninhydrin. All complexes show two strong bands in the carbonyl stretching region, which is characteristic of the 1,3-dicarbonyl functionality. There is, however, a decrease of their positions (15 – 50 cm⁻¹), which indicates the involvement of one of the carbonyls in coordination [34]. The 1768 cm⁻¹ C=O stretching band of ninhydrin is lost, and along with the loss of the NH stretch band of the amino acids shows the condensation reaction of these functional groups and this is consistent with the formation of the Schiff base.

 α ,L-Alanine and all free amino acids show a strong carboxyl anti symmetric stretching peak at $1600 - 1590 \text{ cm}^{-1}$ and a weaker symmetric stretching peak at $\sim 1400 \text{ cm}^{-1}$ [35-38]. In the complexes, the anti symmetric stretching mode appears between $1665 - 1630 \text{ cm}^{-1}$, while the symmetric stretching mode appears in the range $1330 - 1302 \text{ cm}^{-1}$. The observed positive shift of the anti symmetric stretching peak and the negative shift of the symmetric stretching peak is strong evidence of coordination through an ionized carboxyl group via one of the oxygens [25, 35]. The separation between the two frequencies varies with the metal ion involved and the pattern observed with both Schiff bases is consistent with literature reports [25, 35, 36].

A new strong and sharp band is observed in the spectra of the products, which is absent in the starting materials, assignable to the azomethine (C=N) group. Azomethine group absorbs between 1640 - 1620 cm⁻¹ in aromatic ketimines [24, 34-37]. In the complexes the C=N group shows a very strong, sharp peak between 1520 - 1500 cm⁻¹. This negative shift, by as much 100 cm⁻¹, is strong support for the coordination of the azomethine nitrogen [25].

The complexes show M-N and M-O bands in the region 750 – 200 cm⁻¹. The M-Cl stretching band is also observed for Fe(III) complexes near 250 cm⁻¹. Other supporting bands include a M-O-C stretching vibration that appears between 1245 – 1200 cm⁻¹, CH bending vibrations of CH and CH₃ groups that occur in the range 1480 – 1350 cm⁻¹, an aromatic C=C stretch near 1590 cm⁻¹ and a strong band near 750 cm⁻¹ due to ortho disubstitution of an aromatic ring.

In conclusion, the IR data clearly showed that in the complexes the Schiff base (IDIP) behaves as a tridentate monobasic ONO donor ligand involving a carbonyl, azomethine nitrogen and carboxyl oxygen.

Magnetic susceptibility and electronic spectral studies

The magnetic susceptibility and electronic spectral data of the metal complexes are given in Table 4. The magnetic susceptibility data show that all measured complexes, except the zinc complex, are paramagnetic. The values show that the manganese and cobalt complexes are high spin, the iron complex is low spin and the zinc complex is diamagnetic. The data are consistent with an octahedral geometry, which is further supported by electronic spectral data.

The complexes show some common bands characteristic of the ligand. The bands near 42,000 and 40,000 cm⁻¹ are assigned to the $\pi \to \pi^*$ transition of the benzene moiety [39, 40] and that near 35,500 cm⁻¹ is assigned to the $n \to \pi^*$ transition of the carbonyl group [39, 40]. A very strong band near 20,000 cm⁻¹ ($\epsilon_{max} > 10,000$) is assigned to a combination of $\pi \to \pi^*$ and $n \to \pi^*$ transitions of the azomethine chromophore [25, 41], which in the free state absorbs between 25,000 – 23,256 cm⁻¹. The observed bathochromic shift gives strong support for the coordination of the azomethine nitrogen [25, 41]. This band, because it is very strong, is thought to obscure

some d-d bands of the complexes in the region, which could otherwise be useful for structure elucidation.

The electronic spectrum of the Co(II) complex shows two d-d bands at 14,837 cm⁻¹ and 12690 - 12547 cm⁻¹ assignable to ${}^4T_{1g} \rightarrow {}^4A_{2g}$ (P) and ${}^4T_{1g} \rightarrow {}^4T_{2g}$ (F) transitions, respectively, in an octahedral geometry [25, 41–47]. The third d-d band expected near 20000 cm⁻¹ (${}^4T_{1g} \rightarrow {}^4T_{2g}$ (P)) must have been obscured by the very strong band of the azomethine group at 20120 cm⁻¹.

The electronic spectrum of the Ni(II) complex shows three d-d bands at 27,174 cm⁻¹, 15,385 cm⁻¹ and 10,460 cm⁻¹ assignable to ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (P), ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (F) and ${}^3A_{2g} \rightarrow {}^3T_{2g}$, respectively, in octahedral geometry [25, 41–47].

The Mn(II) and Fe(III) complexes show a number of very weak d-d bands, consistent with a high spin d^5 octahedral system. Thus, based on elemental, IR, magnetic susceptibility, conductance and other data these and the Zn(II) complex are also assumed to have an octahedral geometry.

Table 4. Electronic spectral and magnetic susceptibility data of metal complexes of IDIP and their assignments.

Complex	Non-ligand electronic spectral bands (cm ⁻¹)	Assignment	$\mu_{eff}\left(BM\right)$
	 	6 4 6 4	
Mn(II)	35, 587; 31,250 and	Mix. ${}^{6}A_{1g} \rightarrow {}^{4}E_{g}(D)$, ${}^{6}A_{1g} \rightarrow {}^{4}T_{1g}(P)$,	4.93
	27,397 (very broad)	$^{6}A_{1g} \rightarrow {}^{4}T_{2g}(D)$ and $^{6}A_{1g} \rightarrow {}^{4}A_{1g}(G)$	
Fe(III)	28,571;	Mix. ${}^{6}A_{1g} \rightarrow {}^{4}E_{g}(G)$, ${}^{6}A_{1g} \rightarrow {}^{4}A_{1g}$,	2.76
	27,397 and	${}^{6}A_{1g} \rightarrow {}^{4}E_{g}(D), {}^{6}A_{1g} \rightarrow {}^{4}T_{1g}(D),$	
	12,739	${}^{6}A_{1g} \rightarrow {}^{4}T_{2g}(G) \text{ and } {}^{6}A_{1g} \rightarrow {}^{4}T_{1g}(G)$	
Co(II)	14,837	$^{4}\text{T}_{1g} \rightarrow ^{4}\text{A}_{2g}(P)$	
	12690 – 12547	$^{4}T_{1g} \rightarrow ^{4}T_{2g}$	4.12
Ni(II)	27,174	$^{3}A_{2g} \rightarrow ^{3}T_{1g}(P)$	
	15,385	$^{3}A_{2g} \rightarrow ^{3}T_{1g}(F)$	2.91
	10,460	$^{3}A_{2g} \rightarrow ^{3}T_{2g}$	
Zn(II)	_	_	Diamagnetic

Thus, the following structures (Figures 3-5) are proposed for the metal complexes based on the available data.

Figure 3. Proposed structure of metal complexes of IDIP [[ML_2], M = Co(II), Ni(II) and Zn(II)].

$$\begin{array}{c} OH_2 \\ O \\ O \\ O \\ O \\ O \end{array}$$

$$\begin{array}{c} OH_2 \\ OH_2 \\ O \\ O \\ O \end{array}$$

Figure 4. Proposed structure of Mn(II) complex of IDIP [[ML($H_2O)_3$]+Cl⁻, M = Mn(II)].

$$CI$$
 O
 OH_2
 OH_3
 OH_2
 OH_3
 OH_2
 OH_3
 OH_3
 OH_3
 OH_4

Figure 5. Proposed structure of Fe(III) complex of IDIP [[ML($(H_2O)_2CI)^+CI$, M = Fe(III)].

Anti-microbial studies

The diameter of the inhibition zones around each sample, controls and solvent were measured. These data were then converted into a conventional way of expressing bacterial activity (+ and -) by taking some approximate ranges and comparing them to the activity of the starting material, ninhydrin.

Ninhydrin was found to be strongly active against the three bacteria studied. The activities of the metal complexes, ninhydrin and Ruhemann's purple against the three test organisms are given in Table 5. Many of the complexes are active against the one gram-negative bacterium, Proteus mirabilis and the gram-positive bacterium, Staphylococcus aureus. Some of the metal complexes, particularly the Co(II), Fe(III) and Mn(II) complexes, showed a greater enhanced activity than the starting material, ninhydrin. However, all complexes exhibited a highly reduced activity against the gram-negative bacterium, Escherichia coli when compared to ninhydrin's activity. The corresponding metal salts and the solvent have not shown any anti-microbial activity. Thus, the observed enhancement of activity of those complexes that were found to be more active than ninhydrin must be due to a synergistic or combination effect associated with the derivatization and complexation of the ninhydrin and the presence of the side group of the amino acid. These effects may also be associated with the electronic distribution and stereochemistry of the ligand due to complexation. Compared to the inactivity of the metal complexes of the Schiff base made with glycine, IDIA, against these three bacteria [25], the activity of the current new complexes may be mainly due to the side groups and/or their structural variations.

Test organism Compound/complex Escherichia coli Proteus mirabilis Staphylococcus aureus Ninhydrin ++ ++ ++ Ruhemann's purple + + Mn(II) + ++++ +++ Metal Fe(III) ++++ +++ complexes of Co(II) ++++ ++++ IDIP Ni(II) ++++ ++ Zn(II)

Table 5. Results of anti-microbial studies on ninhydrin, the metal complexes of IDIP and Ruhemann's $purple^*$.

CONCLUSION

Ninhydrin and α ,L-alanine undergo a condensation reaction to give a Schiff base, indane-1,3-dione-2-imine-N-2-propionate, IDIP, which forms distinctly colored complexes with the metal ions Mn(II), Fe(III), Co(II), Ni(II) and Zn(II). The Schiff base ligand (IDIP) was shown to behave as a monobasic tridentate ONO donor and an octahedral geometry is assumed for all the metal complexes.

Many of the metal complexes were shown to have enhanced anti-microbial activities towards two bacteria, *Proteus mirabilis* and *Staphylococcus aureus* relative to the starting material nihydrin. Thus, the metal complexes can be studied further for any possible anti-microbial or medical application.

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^{*++++ =} very high activity; +++ = high activity; ++ = moderate activity; + = some activity; -- = no activity.

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