Synthesis, characterization and antimicrobial activities of metal complexes of 2-formyl thymol oxime

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Metal to ligand (1:2) complexes with molecular formula \([M(HL)_2]_n\) \( [HL = \text{anionic form of 2-formyl thymol oxime, } M = \text{metal (II) ion}] \) of 2-formyl thymol oxime \( (H_2L) \) and its manganese, iron, cobalt, nickel, copper and zinc complexes have been prepared and characterized by elemental analysis, magnetic measurements, molar conductivity, IR and UV-Visible spectroscopy. Complexes were tested for antimicrobial activities against *Escherichia coli*, *Pseudomonas putida*, *P. fluorescense* and *Bradyrhizobium japonicum* and fungal cultures *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, and *Alternaria alternata*.

**Keywords**: Antibacterial activity, Antifungal activity, Antimicrobial activity, Metal complexes, Oxime, Thymol

**IPC Code**: C07C251/32

Introduction

Oximes have received considerable attention as extractants for copper anticorrosive in protective coatings, mimics of biofunctions such as reduction of vitamin B\(_{12}\)\(^1,2\). Their metal chelates are of interest due to their fascinating chemistry as well as antimicrobial\(^3\) and anti-cancer activities\(^4\). A new ligand 2-formyl thymol oxime \( (H_2L) \) has been synthesized by derivatising a natural monoterpenoid, thymol \( (2\text{-hydroxy-3-isopropyl-6-methylbenzene}) \) and various transition metal complexes have been prepared for this purpose. The present communication describes synthesis, characterization and antimicrobial activities of \( H_2L \) ligand and its complexes.

Experimental Details

Elemental analyses were performed at micro analytical laboratory, North Maharashtra University (NMU), Jalgaon. IR spectra \((400-4000 \text{ cm}^{-1})\) were recorded on an Schimadzu FTIR spectrophotometer. Electronic spectra were recorded on Schimadzu UV/Visible spectrophotometer 1601 using 10 mm rectangular matched quartz cells. The room temperature magnetic susceptibilities of the solid samples were measured on a faraday type balance with magnetic field of 8000G using Hg \([\text{Co(SCN)}_3]_2\) as the calibrant. Molecular susceptibility was corrected using pascal’s constants.

Metal contents of the complexes were determined by known methods\(^5\). Molar conductance was measured on a systronics conductivity bridge with a dip cell using \( 10^{-3} M \) solution of complexes in DMSO.

2-Formyl Thymol

2-Formyl thymol was synthesized\(^6\) \([\text{yield-40\%}, \text{b.p.-}130^\circ\text{C (lit b.p.-}130^\circ\text{C)}]\).

2-Formyl Thymol Oxime \( (H_2L) \)

\( H_2L \) was prepared\(^7\) by adding a solution of hydroxylamine hydrochloride \((0.05 M)\) and sodium hydroxide \((0.05 M \text{ in } 25 \text{ ml aqueous methanol})\) to a boiling solution of 2-formyl thymol \((0.05 M \text{ in } 25 \text{ ml methanol})\). Mixture was refluxed for 2 h. On cooling, yellow crystalline product was obtained, which was filtered and recrystallized from ethanol \( (\text{yield, 84\%; m.p., }118^\circ\text{C})\). Crystals suitable for X-ray measurement were obtained by slow evaporation of reaction mixture. Structure of \( H_2L \) has been solved\(^8\) as: \(^1\text{H NMR (CDCl}_{3}, \delta \text{ ppm}) \): 1.22 (d, 6H, gem 2 CH\(_3\)), 2.37 (s, 3H, Ar-CH\(_3\)), 3.33 (septet, 1H, CH), 6.68 (d, 1H, Ar-H, ortho to isopropyl gr), 7.10 (d, 1H, Ar-H, ortho to methyl grade), 8.55 (s, 1H, CH = N), IR (KBr, cm\(^{-1}\)) 3420-3300 (OH), 3000-3002 (Ar 2C-H), 2990-2927 (aliphatic C-H), 1634 (C=N), 1603 (C=C), 1520, 1250, 1109, 1066 (C-O, N-O).

Metal Complexes

To a solution of \( H_2L \) \((0.002 M)\) in 40 ml ethanol, 25 ml of aqueous solution of metal salt \((0.001 M)\) was added dropwise. Reaction mixture was stirred for 30 min and precipitated compound was filtered and
washed with water and ethanol. Manganese and iron complexes were prepared in the same manner under inert atmosphere.

**Antimicrobial Bioassay of H₂L and its Metal Complexes**

Bacterial cultures Escherichia coli, Pseudomonas putida, P. fluorescense and Bradyrhizobium japonicum and fungal cultures Aspergillus niger, A. flavus, Fusarium oxysporum and Alternaria alternata were provided by School of Life Sciences, NMU, Jalgaon. Bacterial and fungal cultures were grown on nutrient agar and czepak dox agar respectively.

**In vitro** antibacterial and antifungal activities of the metal complexes were determined by agar well diffusion method. For antibacterial activity, the nutrient agar (Hi-media, 13 g) was suspended in distilled water (1000 ml) and heated to boiling till it dissolved. The medium and petridishes were autoclaved at 15 psi for 20 min. To each petriplate, 20 ml of sterilized medium was added. After the agar was set, 10 percent of inoculum (suspension culture) was added to each petri-plate and spread thoroughly by rotary motion of the plate. After inoculation, wells were scooped out with 7 mm sterile cork borer and to each cup 100 µl of 1 % test solutions of ligand and complexes in DMSO were added. Controls were maintained with DMSO.

In case of antifungal activity, the fungal cultures were grown in czapek dox agar (Hi-media, 13 g). Three replicates were maintained for each treatment. Cultures were kept in an incubator at 28°C for both the tests and results of the antibacterial and antifungal activities were obtained after 24 h and 48 h, respectively.

**Results and Discussion**

On the basis of elemental analyses, 1:2 metal to ligand stiochiometry is assigned to all the chelates (Table 1). The low conductance of 10⁻³ mol dm⁻³ solutions of chelates in DMSO support the non-electrolytic nature of the metal complexes.

**IR Spectroscopy**

IR spectrum of free ligand exhibits a broad band (3420-3400 cm⁻¹), which can be assigned to –OH groups on the ring and of oxime functionality. On complexation, it is expected to disappear on deprotonation of OH groups, however it is not much affected indicating that the OH group of the C=N-OH chromophore, does not take part in coordination. Such mode of coordination of oximes due to coordination of imine nitrogen has been reported and confirmed through X-ray single crystal structures. This broad band supports the existence of a hydrogen bridge, a characterization feature of all transition metal complexes of salicylaldoxime ligands. The C=N band of the free ligand is shifted by 30 cm⁻¹ to lower frequencies in metal complexes supporting coordination through the nitrogen of oxime group.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Elemental analysis % Found (Cal)</th>
<th>( \mu_{eff} ) (B.M) R.T</th>
<th>Molar conductivity ( \Omega^{-1} \text{ cm}^{2} \text{ mol}^{-1} \text{ R.T} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>H</td>
<td>N</td>
<td>M</td>
</tr>
<tr>
<td>H₂L</td>
<td>69.51 (68.36)</td>
<td>8.19 (7.82)</td>
<td>7.68 (7.25)</td>
</tr>
<tr>
<td>Mn(HL)₂</td>
<td>60.49 (60.14)</td>
<td>6.81 (6.42)</td>
<td>6.81 (6.38)</td>
</tr>
<tr>
<td>Fe(HL)₂</td>
<td>60.39 (60.01)</td>
<td>6.78 (6.40)</td>
<td>6.36 (6.36)</td>
</tr>
<tr>
<td>Co(HL)₂</td>
<td>60.05 (59.59)</td>
<td>6.69 (6.36)</td>
<td>6.78 (6.32)</td>
</tr>
<tr>
<td>Ni (HL)₂</td>
<td>60.20 (59.63)</td>
<td>6.79 (6.36)</td>
<td>6.63 (6.32)</td>
</tr>
<tr>
<td>Cu(HL)₂</td>
<td>59.20 (58.80)</td>
<td>6.29 (6.30)</td>
<td>6.68 (6.25)</td>
</tr>
<tr>
<td>Zn(HL)₂</td>
<td>58.74 (58.74)</td>
<td>6.34 (6.27)</td>
<td>6.08 (6.24)</td>
</tr>
</tbody>
</table>

The results show that all the metal complexes exhibit antibacterial and antifungal activities. The metal complexes show better activities than the free ligand. The results also suggest that the type of metal ion influences the antimicrobial activity. The complexes of Co(II), Ni(II) and Cu(II) show better activities than the complexes of Mn(II) and Fe(II). The results also suggest that the type of metal ion influences the antimicrobial activity. The complexes of Co(II), Ni(II) and Cu(II) show better activities than the complexes of Mn(II) and Fe(II).
Bands (1250-850 cm\(^{-1}\)) are assigned to N-O and C–O stretching frequencies. IR band appearing around 420 cm\(^{-1}\) and 450 cm\(^{-1}\) in metal complexes are assigned to ν(M-O) and ν(M - N) vibrations respectively.

**Magnetic Susceptibility Measurements**

Magnetic susceptibility measurements provide additional information about structure of metal complexes. \(\mu_{\text{eff}}\) values for all complexes were found to vary from normal values indicating a possibility of association of M (II) centres (Fig. 1) having weak metal-metal interactions through phenolic oxygen atoms in adjacent units. Similar metal complexes containing square pyramidal or tetragonally distorted M (II) centres have been reported for substituted oximes\(^2\).

**Electronic Absorption Spectra**

The spectral data of the complexes in chloroform are presented in Table 2. There are two absorption peaks and a shoulder, in spectrum of ligand, which can be assigned to \(\pi-\pi^*\) and two n-\(\pi^*\) transitions. These bands are also observed in complexes but they are shifted towards lower or higher frequencies confirming the coordination of the ligand to metallic ion. Apart from these bands, complexes exhibit comparatively, weak absorption ascribed to charge transfer transition. The electronic spectrum of manganese complex shows a broad band at 15556 cm\(^{-1}\) attributable to the \(6A_{1g} \rightarrow 4T_{1g} (G)\) transition for octahedral structure around Mn (II) ion. Iron complex shows absorption peak at 19011 cm\(^{-1}\), which can be assigned to \(5T_{2g} \rightarrow 5E_g\) transition of six coordinate complexes. Cobalt complex exhibits a weak band at about 15408 cm\(^{-1}\), which can be assigned to \(4T_{1g} \rightarrow 4A_{2g}\) transition of Co (II) octahedral structure. Electronic spectrum of nickel complex exhibits a band at 16286 cm\(^{-1}\) assignable to \(3A_{2g} \rightarrow 3T_{1g}\) (F) transition. The d- d transitions are characteristic of metal in six coordinate environments and the \(\mu_{\text{eff}}\) value is an additional evidence for octahedral structure. Copper complex exhibits broad bands at 14948 and 11834 cm\(^{-1}\) as observed in elongated octahedral Cu (II) centre\(^1\). The complexes under study are six coordinate, where ligand behaves as bidentate chelating agent in uninegatively charged form and remaining apical sites of metal centre associate with phenolic oxygens on adjacent units, (Fig. 1).

**Biological Activity**

Inhibitory activity of \(H_2L\) as well as its metal complexes were examined against bacterial (\textit{E. coli, P. putida, P. fluoroscens, B. japonicum}) and fungal

<table>
<thead>
<tr>
<th>Compound</th>
<th>Interligand and charge transfer (CT)</th>
<th>CT</th>
<th>d-d</th>
</tr>
</thead>
<tbody>
<tr>
<td>(H_2L)</td>
<td>41152  32362  28490</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mn(HL)(_2)</td>
<td>42735  37037  30864</td>
<td>23041</td>
<td>15556</td>
</tr>
<tr>
<td>Fe(HL)(_2)</td>
<td>41667  36900  30864</td>
<td>23973</td>
<td>19011</td>
</tr>
<tr>
<td>Co(HL)(_2)</td>
<td>42735  37037  30769</td>
<td>22371</td>
<td>15408</td>
</tr>
<tr>
<td>Ni (HL)(_2)</td>
<td>42735  37878  31250</td>
<td>22152</td>
<td>16286</td>
</tr>
<tr>
<td>Cu(HL)(_2)</td>
<td>40984  35336  27778</td>
<td>21142</td>
<td>14948, 11834</td>
</tr>
<tr>
<td>Zn(HL)(_2)</td>
<td>42735  37037  30864</td>
<td>20661</td>
<td>—</td>
</tr>
</tbody>
</table>

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Fig. 1 — Probable structure

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Table 2 — Electronic spectra of 2-formyl thymol oxime and its metal complexes in cm\(^{-1}\)
(A. niger, A. flavus, A. alternata, F. oxysporum) cultures. No zone of inhibition was observed for the control. The compounds possess moderate antibacterial activity however, insignificant increase in activities has been observed for Mn and Zn complexes (Tables 3 and 4). Inhibitory activities of metal complexes against bacterial species are of the same order as that of free ligand indicating that complexation is unable to modulate or alter antibacterial property of H$_2$L. While complexation was found to be effective in increasing antifungal activity of most of the complexes for all the species except F. oxysporum.

Conclusions
Metal complexes of 2-formyl thymol oxime were prepared. IR spectra indicate the coordination through phenolic oxygen and oximino nitrogen. Magnetic moment values and electronic spectra indicate formation of distorted octahedral complexes. The complexes of studied metals have been found to be ineffective to increase the antibacterial activities against the test bacteria species, while the complexation of 2-formyl thymol oxime was found to be effective to boost the antifungal activities with all the selected metals except copper.

Acknowledgment
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References
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