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Synthesis and structural characterization of a cobalt(III) complex with an

(N,S,O) donor Schiff base ligand: catechol oxidase and phenoxazinone

synthase activities

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Abstract

A mononuclear Co(III) complex $[CoL_2]Cl$ (1) $[HL = (E)-2-((3-(2-hydroxyethylthio)propylimino)methyl)phenol] was synthesized and its X-ray crystallographic characterization revealed a distorted octahedral geometry around the metal centre. 1 shows catechol oxidase as well as phenoxazinone synthase activities in different solvents. The turn over numbers for the catechol oxidase activity were <math>1.18 \times 10^2 h^{-1}$ in MeOH and $1.80 \times 10^3 h^{-1}$ in MeOH, and that of phenoxazinone synthase activity were $9.72 \times 10^3 h^{-1}$ in MeOH.

Keywords: Cobalt, Schiff base, catechol oxidase activity, phenoxazinone synthase activity

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1. Introduction

Oxidation of organic molecules with molecular oxygen at mild conditions is of utmost importance for industrial and synthetic processes both from economic and environmental aspects [1]. Metalloproteins in nature can easily activate the kinetically inert triplet aerial oxygen to its active singlet form and use that for oxidising different organic substrates [2]. Metal containing active sites of those metalloproteins are now being mimicked in the form of small molecules to oxidise same/similar substrates in laboratory. Catachol oxidase, a copper containing metalloprotein having a dinuclear hydroxobridged Cu(II) active site [3], catalyses the oxidation of catechol to quinone (Scheme 1) in plant cell in presence of aerial oxygen. A number of reports with small dinuclear Cu(II) complexes as catechol oxidase mimic are there in literature [4-7]. Not only copper(II) but also some non-copper transition metals like manganese [8], nickel [9], iron [10], cobalt [11], zinc [12], vanadium [13] etc. are found to show catechol oxidase activity. Phenoxazinone synthase, a multicopper oxidase which is found in the bacterium Streptomyces antibioticus, is responsible for six electron oxidative coupling of two molecules of the *o*-aminophenol derivative, 4-methyl-3-hydroxyanthraniloyl pentapeptide to phenoxazinone chromophore (Scheme 2). In this case also along with Cu(II) [14] different non-copper complexes [15] catalyze the catechol oxidase and/or phenoxazinone synthase activities.

In continuation to our continued interest with Co(III) [11(b), 11(e), 15(f)] here we report the synthesis and X-ray structural characterization of a mononuclear cobalt(III) complex with (N,S,O) donor Schiff base ligand [CoL₂]Cl [HL = (E)-2-((3-(2hydroxyethylthio)propylimino)methyl)phenol] (1) which did catalyse aerial oxidation of 3,5di-*tert*-butyl catechol (3,5-DTBC) and *o*-aminophenol (OAPH) separately to corresponding quinone and the phenoxazinone chromophore, respectively. catechol oxidase- and/or phenoxazinone synthase-active metal complexes with (N,S,O) donor ligands are rare [16].

The catalytic results are found in methanol and acetonitrile solvents and in each case reaction rates and turn over numbers were measured. The Michealis-Menten kinetics in each solvent of each activity were measured.

2. Experimental

2.1. Materials

High purity 2-mercaptoethanol, 3-chloropropylamine, salicylaldehyde and cobalt(II) chloride hexahydrate were purchased from Sigma Aldrich, India and used as received. Ethanol, methanol and acetonitrile were used as reagent grade solvents. 3,5-DTBC and 2-aminophenol were purchased from Sigma Aldrich, India and from Loba Chemie, India, respectively.

2.2. Physical measurement

Elemental analyses (carbon, hydrogen and nitrogen) were performed on Perkin-Elmer 2400 CHNS/O elemental analyzer. UV-VIS and IR spectra (KBr discs, 4000-500 cm⁻¹) were recorded using a Shimadzu UV-VIS 1800 spectrophotometer and Shimadzu FT-IR model IR Prestige-21 spectrometer, respectively. NMR experiment was carried out in Bruker 400 MHz NMR spectrometer and mass spectral study was done in Thermo Exactive plus high resolution mass spectrometer.

2.3. Synthesis of HL

2-Mercaptoethanol (8 mmol, 0.625 g) was added gradually in the ice cold sodium ethoxide solution which was prepared by the addition of metallic sodium in dry ethanol with continuous stirring. 3-chloropropylamine (8 mmol, 1.04 g) was then mixed slowly to this reaction mixture which was stirred for about 1 hour. During this stirring sodium chloride was precipitated that confirms the formation of the 2-(3-aminopropylthio)ethanol. Sodium

chloride which was produced as by product was separated by filtration. Salicyaldehyde (8 mmol, 0.97 g) was added to this solution of 2-(3-aminopropylthio)ethanol and this mixture was refluxed for 4 hours. This resulted the ligand HL. The synthesis of ligand (HL) is schematically shown in Scheme 3. Yield, 1.434 g (75% based on aldehyde). Anal cal. for $C_{12}H_{17}NO_2S$ (HL): C, 60.22; H, 7.16; N, 5.85 Found: C, 60.12; H, 7.23; N, 5.89. ¹H NMR δ (ppm): 2.17 (q, *J* = 6.4 Hz, 2H), 2.63 (t, *J* = 7.2 Hz, 2H), 2.73 (t, *J* = 6.0 Hz, 2H), 3.62 (t, *J* = 6.0 Hz, 2H), 6.85-6.96 (m, 2H), 7.24-7.33 (m, 2H), 13.28 (bs, 1H).

2.4. Synthesis of 1

Ethanolic solution of cobalt(II) chloride hexahydrate (0.1 mmol, 0.024 g) was slowly added to the hot ethanolic solution of HL (0.2 mmol, 0.048 g) with continuous stirring for 4 hours (Scheme 4). The brown colour of the ligand solution was changed to reddish brown on dissolving the metal salt. This reaction mixture was kept in open air for slow evaporation. After about 6 weeks reddish brown coloured block shaped crystals appeared. Yield, 0.0398 g (70% based on metal salt). Anal cal. for $C_{24}H_{32}N_2O_4S_2ClCo$ (1): C, 50.48; H, 5.65; N, 4.91 Found: C, 50.51; H, 5.68; N, 4.89.

2.5. X-ray diffraction study

X-ray quality single crystals of **1** were mounted on Bruker AXS KAPPA APEX II diffractometer using Mo-K α radiation ($\lambda = 0.71073$ Å) at 296(2) K. Identification of the space group P2₁/c for **1** were based on systematically absent reflections. Out of 9131 total reflections 2698 with I >2r(I) were used for structure solutions. The direct methods were used for structure solution, and the structure solution and refinement were based on |F2|. All non-hydrogen and hydrogen atoms were refined with anisotropic displacement parameters and were placed in calculated positions when possible and given isotropic U values 1.2 times that of the atom to which they are bonded, respectively. The maximum and minimum peak

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heights at 0.607 and -1.101 eÅ⁻³ for **1** with no chemical significance were shown by final differences Fourier map. SHELXL-2014 [17] and ORTEP-32 [18] were used to carry out all calculations. The crystal data and data collection parameters are listed in Table 1.

3. Results and discussion

3.1. Synthesis and formulation

The two-step synthesis of the organic ligand HL was carried out, firstly, by abstracting proton from the 2-mercaptoethanol by in situ built sodium ethoxide in dry ethanol. The ethanolic solution of 3-chloropropylamine was added dropwise with the mixture and stirred for an hour to get 2-(3-aminopropylthio)ethanol. As a result precipitated sodium chloride was filtered off. Next to this, treatment of salicyaldehyde with this reaction mixture and consequent stirring for four hours or so leads to the final compound solution which on evaporation of the solvent in rotary evaporator resulted the solid brown coloured ligand HL (Scheme 3). The ¹HNMR spectrum of HL in CDCl₃ showed the characteristic peaks of the protons (Figs. S1 and S2; supporting information). The quintet signal for the two methylene protons which are β to C-S bond and β to imine bond of HL appeared at δ 2.17 (q, J = 6.4 Hz, 2H). The two triplet signals for four protons of the two methyl groups were found at δ 2.63 (t, J = 7.2 Hz, 2H) for one methylene group α to C-S bond and δ 2.73 (t, J = 6.0 Hz, 2H) for another methylene group β to the OH group. The signal appeared at δ 3.62 (t, J = 6.0 Hz, 2H) due to the methylene group which is α to the imine bond. A triplet signal appears at δ 3.90 (t, J = 6.0 Hz, 2H) for another methylene group α to the OH group. The signals of the four aromatic protons were appeared at the range between δ 6.85 ppm to δ 7.33 ppm (m, 4H). In IR spectrum some characteristic frequencies are at 2926 cm⁻¹ is due to O-H bond vibration, 1629 due to imine bond stretching and 754 cm⁻¹ for C-S bond stretching [19]. Absorption bands of the ligand were found at 401, 313, 254, 214 (π - π *) nm in MeOH solution in the 200-

800 nm range. The IR bands for complex **1** at 1595 cm⁻¹ was attributed to the asymmetric $\bar{\nu}$ (C=N), band at 1062 cm⁻¹ was due to the metal-oxo bond stretching and band at 752 cm⁻¹ was detected as $\bar{\nu}$ (C-S) stretching [19]. Absorption bands of the complex in MeOH solution displays the spectral pattern of the complex. The absorption band at 223, 260 nm is due to the $(\pi - \pi^*)$ transition from the ligand side and band at 394 nm for ${}^1A_{1g} \rightarrow {}^1T_{1g}$ transition and 555 nm due to ${}^1A_{1g} \rightarrow {}^1T_{2g}$ transition of octahedral cobalt(III) centre [20].

3.2. X-ray structure

The thermal plot of **1** which is a centrosymmetric mononuclear cobalt(III) complex is presented in Fig. 1. Considering bond angle bond distance data (Table 2) the cobalt(III) centre is found to be hexa coordinated and the geometry around the cobalt(III) is distorted octahedral. Again from the bond angle-bond distance parameters, phenolic oxygens (O1 and $O1^{i}$) and imine nitrogens (N1 and $N1^{i}$) of the ligand are in the square plane and thio ether sulfurs (S1 and S1ⁱ) from the ligand network are situated at the axial position. The bond distance range in the basal plane is 1.889(3) to 2.2776(10) Å (Table 2) which are consistent with some of the reported cobalt(III) complexes [11a, 11b, 11d, 11e].

3.3. Catechol oxidase activity of 1: spectrophotometric study

3,5-DTBC was taken as a substrate for the investigation of catechol oxidase activity of **1**. The reason for this choice was that it (3,5-DTBC) could easily be oxidised to respective 3,5-di-*tert*-butylquinone (3,5-DTBQ) which is electrochemically stable having low quinone-catechol reduction potential. The formation of quinone was studied by repetitive UV-Vis scanning for 6 hours by mixing **1** (1×10^{-4} M) and 100 equivalent of 3,5-DTBC separately in methanol (Fig. 2) and acetonitrile (Fig. 3) media. Pure 3,5-DTBC shows bands at 282 nm (inset to Fig. 2) and 278 nm (inset to Fig. 3) respectively in methanol and acetonitrile.

Characteristic peak of the oxidation product 3,5-DTBQ appears at around 400 nm in both the solvents [21]. The newly generated 3,5-DTBQ was purified by column chromatography (yield = 68%) and characterized by measuring its melting point at $110^{\circ}C$ [22].

During the measurement of rate of this reaction it was found out that absorbances at ~400 nm in both methanol and acetonitrile were time dependent. The gradual conversion of colour from light yellow to deep brown of the mixture of **1** and **3**,5-DTBC in both the solvents in air is indicative of the slow conversion of **3**,5-DTBC to **3**,5-DTBQ. The absorbance differences at ~400 nm were plotted against time to calculate the initial rate in methanol (Fig. 4) and acetonitrile (Fig. 5), respectively. In both the solvents first order kinetics were found with initial rate $5.08 \times 10^{-3} \text{ min}^{-1}$ (methanol) and $3.25 \times 10^{-5} \text{ min}^{-1}$ (acetonitrile).

3.3.1. Enzyme kinetics study

Spectrophotometric measurements thermostated at 25°C with **1** and the substrate 3,5-DTBC were used to collect the enzyme kinetic data in both methanol and acetonitrile. 0.04 ml 1×10^{-4} M solution of **1** was added to 2 ml of 3,5-DTBC of concentration varying from 1 $\times 10^{-3}$ M to 1×10^{-2} M. 3,5-DTBC to 3,5-DTBQ conversion was monitored with time at the wavelength of ~400 nm in methanol and in acetonitrile solvents. Initial rate method was used to determine the rate for each concentration of the substrate.

The rate versus substrate concentration data were found to follow the Michaelis-Menten approach of enzyme kinetics to get the double reciprocal Lineweaver-Burk plot and the values of the V_{max} (maximum velocity), K_M (Michaelis-Menten constant) and K_{cat} (Turn over number). The observed rate vs. [substrate] plot along with Lineweaver-Burk plot in methanol and acetonitrile solutions are given respectively in Figs. 6 and 7. The K_{cat} values (turnover numbers) are calculated as 1.18×10^2 h⁻¹ in methanol and 1.80×10^3 h⁻¹ in acetonitrile (Table

3) whereas the highest K_{cat} reported till date, to the best of our knowledge, is 1.32×10^6 h⁻¹ [23].

3.3.2. Mechanism of catechol oxidase activity

The catalytic cycle of the process is given in Scheme 5. Probably, in the first step, the 1:1 adduct of catechol and the cobalt complex is formed. HRMS spectrum of **1** in methanol and of a 1:100 mixture of **1** and 3,5-DTBC in methanol solvent were recorded. The peak at m/z = 243.56 can be assigned to sodium aggregate of quinone [3,5-DTBQ]-Na⁺ (Fig. S3; Supporting information). The complex **1** exhibits a peak at m/z = 572.65 (Fig. S4; Supporting information). The formation of a complex-substrate adduct intermediate species (**III**, Scheme 5) is identified by the peak at m/z = 517.22 (Fig. S5; Supporting information). The catalytic process is shown in Scheme 4 and the each reaction intermediates (**II-V**, Scheme 5) are identified by HRMS (Figs. S6 to S8; Supporting information). The catechol derivative, 3,5-DTBC gets oxidised to quinone in presence of aerial oxygen. The aerial oxygen that oxidises 3,5-DTBC to 3,5-DTBQ in this process is converted to H₂O₂. H₂O₂ thus liberated was identified and characterized spectrophotometrically (S1; Supporting information) [24].

3.4. Phenoxazinone synthase activity of 1: Spectrophotometric study

In case of phenoxazinone synthase activity OAPH was used as the substrate. **1** catalyzed the aerial oxidation of OAPH to the corresponding 2-aminophenoxazinone-3-one. Repetitive UV-Vis scan of the mixture of methanolic solution of **1** and 100 equivalent of OAPH was recorded. The plot for the representative scan is given in Fig. 8. OAPH absorbs at 232 nm and 286 nm in methanol (inset to Fig. 8). The characteristic peak of 2-aminophenoxazine-3-one (APX) grows smoothly at 427 nm in MeOH [14a].

To monitor the reaction kinetics and find out the initial rate between OAPH and **1**, time dependent change of absorbance for the catalytic reaction was performed at 427 nm for 60

min in the above solvent. The difference in absorbance at 427 nm is plotted against time in minute in methanol solvent. The plot is given in Fig. 9. The initial rate for the particular catalyst and substrate is obtained from the slope. The initial rate is found to be 2.50×10^{-3} min⁻¹ in MeOH.

3.4.1. Enzyme kinetics study

UV-Vis spectrophotometric measurements thermostated at 25°C were used for the enzymatic kinetic experiments with the complex and the substrate (OAPH) in MeOH. 0.04 ml of complex solution (with a constant concentration of 1×10^{-4} M) was added to 2 ml of OAPH of a particular concentration varying from 1×10^{-3} M to 1×10^{-2} M to make the final concentration of the complex as 1×10^{-4} M. The conversion of OAPH to APX was monitored with time at a wavelength of 427 nm. Initial rate method was used to determine the rate for each concentration of the substrate by the initial rate method.

The rate versus concentration of substrate data were used (on the basis of Michaelis-Menten approach of enzymatic kinetics) to get the Lineweaver-Burk (double reciprocal) plot as well as the values of the various kinetic parameters V_{max} , K_M and K_{cat} in MeOH (Table 4). Fig. 10 demonstrates the plot in MeOH and the turnover number (K_{cat}) is found to be 9.72 × 10^3 h⁻¹ in that solvent.

3.4.2. Mechanism of phenoxazinone synthase activity

The sodium aggregate of APX was also identified by HRMS at m/z value 235.54 (Fig. S9; Supporting information). To get the reaction intermediate that is **1**-OAPH adduct, HRMS study was carried out with m/z value 440.11 (OAPH-**1**) (Fig. S10; Supporting information). This adduct generates 2-amoinophenoxazine-3-one through some intermediate reaction with

molecular oxygen which get reduced to H_2O_2 . H_2O_2 was detected spectrophotometrically (S1; Supporting information) [24].

4. Conclusion

Synthesis and X-ray crystallographic characterization of a new cobalt(III) complex **1** with an (N,S,O) donor Schiff base were reported. Catalytic behaviour of **1** towards aerial oxidation of 3,5-di-*tert*-butyl catechol to its corresponding quione and *o*-aminophenol to phenoxazinone chromophore separately. The catalyst **1** shows better turn over numbers (K_{cat}) in methanol and acetonitrile than that of the other reported cobalt complexes [11(a), (d)]. Similarly K_{cat} values for phenoxazinone synthase activity by **1** in methanol is also higher than some of the reported ones [15(a), (d), (e)].

5. Supplementary data

CCDC 1846518 contains the supplementary crystallographic data for **1**. This data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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Emperical formula	$C_{24}H_{32}N_2O_4S_2ClCo$
Formula weight	571.04
<i>T</i> (K)	296(2)
Wavelenght (Å)	0.71073
Crystal system	Monoclinic
Space group	P2 ₁ /c
Unit cell dimensions	
A (Å)	9.3982(11)
<i>B</i> (Å)	17.578(3)
<i>C</i> (Å)	8.4820(10)
β(°)	110.654(8)
<i>V</i> (Å ³)	1311.2(3)
Z	2
$D_{\text{calc}} (\text{mg m}^{-3})$	1.536
Absorption coefficient (mm ⁻¹)	1.052
F(000)	630
Crystal size (mm ³)	$0.87 \times 0.42 \times 0.22$
Theta range for data collection (°)	2.316 - 26.453
Index ranges	$-11 \le h \le 11$, $-21 \le k \le 19$, $-10 \le l \le 10$
Reflections collected	9131
Independent reflections	2698 [Rint= 0.0371]
Completeness of theta	99.9 % [θ=25.242]
Absorption correction	Multi-scan

Table 1. Crystal data and structure refinement parameters for $\boldsymbol{1}$

T and T	0.7454 and 0.4620
$I_{\rm max}$ and $I_{\rm min}$	0.7454 and 0.4620
Refinement method	Full matrix
Data/restrains/parameters	2698/0/ 164
Goodness-of fit (GOF) F^2	1.068
Final <i>R</i> index $[l > 2\sigma(l)]$	$R_1 = 0.0587$ and $wR_2 = 0.1682$
R index (all data)	$R_1 = 0.0704$ and $wR_2 = 0.1772$
Largest difference between peak and hole (e Å ⁻³)	0.607, -1.101
$\mathbf{R} = \Sigma \mathbf{F}\mathbf{o} - \mathbf{F}\mathbf{c} / \Sigma \mathbf{F}\mathbf{o}, \ \mathbf{w}\mathbf{R} = [\Sigma \mathbf{w}(\mathbf{F_o}^2 - \mathbf{F_c}^2) / \Sigma \mathbf{w}(\mathbf{F_o}^2) / \Sigma \mathbf{w}(\mathbf{F_o}$	$(F_o^2)^2$] ^{1/2} , calcd w = 1/[$\sigma^2(F_o^2)$ + (0.1311P) ² +
9.1582P]; where $P = (F_o^2 + 2F_c^2)/3$.	
Table 2. Bond lengths [Å] and angles [°] for 1	
Bond lengths	
Co(1)-O(1)	1.889(3)
Co(1)-N(1)	1.953(3)
Co(1)-S(1)	2.2776(10)
Bond angles	
N(1)-Co(1)-O(1)	91.40(13)
O(1)-Co(1)-S(1)	91.61(9)
N(1)-Co(1)-S(1)	89.89(10)
N(1)-Co(1')-N(1')	180.0
S(1)-Co(1')-S(1')	180.0
O(1)-Co(1')-O(1')	180.0

Symmetry transformation: 'x, y, z', '-x, y+1/2, -z+1/2', '-x, -y, -z', 'x, -y-1/2, z-1/2'

Solvents	$V_{max} (M s^{-1})$	Standard	$K_{M}(M)$	Standard	$K_{cat} (h^{-1})$
		error		error	
MeOH	3.28×10^{-6}	5.62×10^{-7}	1.8×10^{-3}	$7.50 imes 10^{-4}$	1.18×10^{2}
					<u></u>
MeCN	5.00×10^{-5}	7.64×10^{-6}	2.36×10^{-3}	1.50×10^{-4}	1.80×10^{3}

Table 3. Kinetic parameters for t	e oxidation of 3,5-DTBC cataly	zed by 1
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 Table 4. Kinetic parameters for the oxidation of OAPH catalyzed by 1

Solvents	$V_{max} (M s^{-1})$	Standard error	$K_{M}(M)$	Standard error	$K_{cat} (h^{-1})$
MeOH	$2.70 imes 10^{-4}$	1.05×10^{-4}	1.22×10^{-2}	2.04×10^{-3}	9.72×10^{3}
	CLP				



Fig. 1 – ORTEP of 1 with 30% ellipsoid probability (H atoms are omitted for clarity)



Fig. 2 – Change in spectral pattern of **1** after addition of 3,5-DTBC and allowing the reaction for 6 h in MeOH [inset: spectrum of pure 3,5-DTBC in MeOH]



Fig. 3 – Change in spectral pattern of **1** after addition of 3,5-DTBC and allowing the reaction for 6 h in MeCN [inset: spectrum of pure 3,5-DTBC in MeCN]



Fig. 4 – Plot of the difference in absorbance (ΔA) vs time to evaluate the rate of catalysis

of 3,5-DTBC by 1 in MeOH



Fig. 5 – Plot of the difference in absorbance (ΔA) vs time to evaluate the rate of catalysis of

3,5-DTBC by 1 in MeCN



Fig. 6 – Plot of rate vs. [substrate] (3,5-DTBC) in presence of 1 in MeOH [inset:

Lineweaver-Burk plot]



Fig. 7 – Plot of rate vs. [substrate] (3,5-DTBC) in presence of 1 in MeCN [inset: Lineweaver-

Burk plot]



Fig. 8 – Change in spectral pattern of **1** after addition of OAPH and allowing the reaction for 6 h in MeOH [inset: spectrum of pure OAPH in MeOH]



Fig. 9 – Plot of the difference in absorbance (ΔA) vs time to evaluate the rate of catalysis of

OAPH by **1** in MeOH



Fig. 10 – Plot of rate vs. [substrate] (OAPH) in presence of 1 in MeOH [inset: Lineweaver-

Burk plot]







Scheme 5. Proposed catalytic cycle for catechol oxidase activity

