

Synthesis, Crystal Structure and Polyphenol Oxidase Activities Study of [Cu(NTB) Cl] ClO₄ · 2.5CH₃ OH

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Abstract: The title complex [Cu(NTB)Cl] ClO₄ · 2.5 CH₃ OH (NTB = Tris(benzimidazolyl-2-methyl) amine) has been synthesized and characterized by UV-Vis. , elemental analysis, cyclic voltammetry and single crystal X-ray diffraction analysis. Polyphenol oxidase activities toward pyrogallol and catechol have been studied spectroscopically. Their kinetics obey the Michaelis-Menten equation. The turnover numbers of the complex toward pyrogallol are 88.2 h⁻¹ and 0.54 h⁻¹ for catechol at pH 8.0 and 30 °C. Compared with the kinetic data, it is apparent that pyrogallol oxidation is easier than catechol, and catalytic activities increase with increasing of pH values.

Key words: Synthesis; Crystal structure; Polyphenol oxidase activities

CLC number: O614; O643.32

Document code: A

Polyphenol oxidases (PPO) are widely distributed in microorganisms, plants and animals^[1]. They play an important role in plant and animal defense systems and signal transfer, which are involved in cuticular melanizations such as coloration of skin and hair, sclerotization of exoskeleton and lignin formation^[2]. Recently, it was found that these play a pivotal role in defense reactions of plants against insect, bacteria, fungi and virus attack and abiostresses^[3]. The oxidation of phenols under mild conditions is also of great interest in industry and agriculture from an economical and environmental point of view^[4~8]. Therefore, there has been sequential interest in study on PPO model compounds^[9~10], but most of them are binuclear complexes. In this paper, we report the oxidation of pyrogallol and catechol catalyzed by the model mononuclear complex [Cu(NTB) Cl] ClO₄ · 2.5CH₃OH.

1 Experimental

1.1 Physical measurements

Electronic spectra were measured on a Shimadzu UV-265 spectrophotometer. Elemental analysis for C, H and N was carried out on a Perkin-Elmer 2400. Cyclic voltammetry (CV) was performed with a BAS-100 electrochemical analyzer on a conventional three-electrode cell, i. e. , glassy carbon electrode serving as the working electrode, a platinum wire as counter electrode and Ag/AgCl electrode as reference. The supporting electrolyte tetrabutylammonium perchlorate (TBAP) was recrystallized twice from methanol and dried overnight in vacuum. The solvent N,N-dimethyl formamide (DMF) was sufficiently pure. The X-ray data for the complex was collected on a Bruker SMART APEX CCD diffractometer.

Received date: 2007-11-09; **revised date:** 2008-05-27.

Foundation item: the Key Fundamental Project of China(2002CCA00500).

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1.2 Synthesis of the title complex

The ligand Tris(benzimidazolyl-2-methyl) amine (NTB) was synthesized according to a similar method reported in literature^[11]. Aminotriacetic acid (NTA) and o-phenylenediamine (PDA) in molar ratio of 1:3 were refluxed in glycol at 180 °C for 6 ~ 8 h until no water vapor was evolved. After cooling, a reddish crude product was precipitated by adding water. And it was recrystallized a few times in ethanol, and yielded a white power or colorless crystals.

The complex [Cu (NTB) Cl] ClO₄ · 2. 5CH₃ OH was prepared by adding a solution of copper(II) chloride (0. 17 g, 1. 0 mmol) in MeOH to a solution of NTB(0. 41 g, 1. 0 mmol) and sodium perchlorate (0. 12 g, 1. 0 mmol) in MeOH. A yellow-blue precipitate was obtained after stirred at 50 °C for 5 h and was collected by filtration. This solid was dissolved in methanol and left standing for several days, yielded a blue crystal suitable for X-ray analysis. Elemental analysis(%), calculated: C 46. 35, H 4. 519, N 14. 28; found: C 46. 54, H 4. 250, N 14. 23. UV-Vis. [λ_{max}, nm (ε, M⁻¹ · cm⁻¹)] in MeOH: 271

(24900), 671(233).

1.3 X-ray crystallographic analysis

A suitable crystal of the title complex was mounted on a Bruker SMART APEX CCD diffractometer. The reflection data were measured at 20 °C under graphite monochromated radiation (λ = 0. 071073 nm). The technique used was ω-scan with a θ limit of 1. 59° < θ < 22. 50°. The structure of the complex was solved by the direct methods and refined by least squares on F² by using the SHELXTL software package. Hydrogen atoms were located by using the difference Fourier map and added to the calculation with regard to the structure. The others were constrained to ride on their parent atom. The molecular graphics were plotted by using the software package. The final least-square cycle gave R₁ = 0. 0777 and wR2 = 0. 2143 for 12 810 reflections with I > 2σ(I). Atomic scattering factors and anomalous dispersion corrections were taken from the International Table for X-ray Crystallography. The crystal data and structure refinement are shown in Table 1.

Table 1 Crystallographic data of the title complex

Empirical formula	C ₅₃ H ₆₂ Cl ₄ Cu ₂ N ₁₄ O ₁₃
Formula weight	1 372
Temperature	293(2) K
Wavelength	0. 71073 Å
Crystal system, space group triclinic,	p-1
Unit cell dimensions	a = 14. 2205(17) Å, α = 90. 517(2) b = 14. 3858(17) Å, β = 108. 092(2) c = 18. 244(2) Å, γ = 115. 682(2)
Volume	3 152. 1(7) Å ³
Absorption coefficient	0. 915 mm ⁻¹
F(000)	1 404
Crystal size	0. 20 x 0. 20 x 0. 20 mm
θ range for data collection	1. 59 to 22. 50 deg.
Max. and min. transmission	0. 8383 and 0. 8383
Goodness-of-fit on F ²	1. 072
R, wR2	R1 = 0. 0777, wR2 = 0. 2143
Extinction coefficient	0. 031 (9)
Largest diff. peak and hole	0. 921 and -1. 154 × 10 ⁻³

1.4 Measurements of polyphenol oxidase activities

The kinetics of reactions was studied spectroscopically. Oxidative reactions of pyrogallol and catechol were carried out in methanol-Tris (i. e., trihydroxy-

methyl amino methane)-HCl buffer solution. The solution pH value and reaction temperature varied in the range of 7. 3 ~ 8. 0 and 18 ~ 40 °C, respectively. Stock solutions of pyrogallol and catechol were prepared in

0.15 mM phosphoric acid to prevent autooxidation. The assay wavelength of pyrogallol and catechol oxidation were chosen at 320 nm and 484 nm, respectively.

2 Results and discussion

2.1 Crystal structure of the complex

The molecular structure of the title complex is shown in Fig. 1. The selected bond lengths and bond

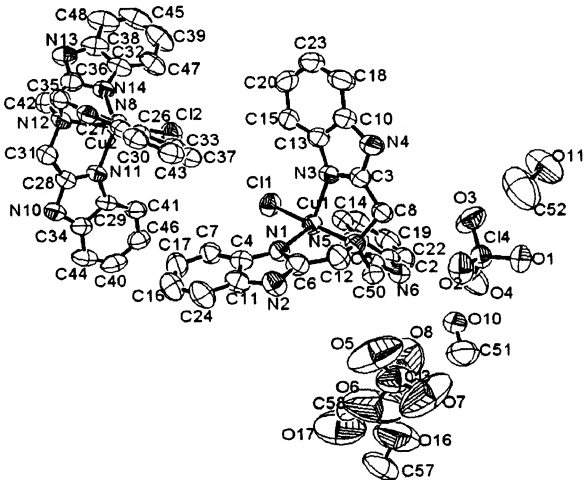


Fig. 1 ORTEP view of the title complex CCDC 244762

Table 2 Selected bond lengths(Å) and bond angles(°) for the title complex

Cu1-N7	2.029(4)	Cu2-N12	2.042(5)
Cu1-N5	2.056(5)	Cu2-N8	2.062(5)
Cu1-N3	2.130(4)	Cu2-N14	2.102(4)
Cu1-N1	2.164(4)	Cu2-N10	2.194(5)
Cu1-Cl1	2.2731(15)	Cu2-Cl2	2.2630(18)
N7-Cu1-N5	133.09(18)	N12-Cu2-N8	127.76(18)
N7-Cu1-N3	111.56(18)	N12-Cu2-N14	112.21(18)
N5-Cu1-N3	106.56(18)	N8-Cu2-N14	110.76(17)
N7-Cu1-N1	80.30(17)	N12-Cu2-N10	80.32(19)
N5-Cu1-N1	81.05(17)	N8-Cu2-N10	79.83(17)
N3-Cu1-N1	79.18(18)	N14-Cu2-N10	79.32(17)
N7-Cu1-Cl1	99.12(13)	N12-Cu2-Cl2	100.75(16)
N5-Cu1-Cl1	99.90(13)	N8-Cu2-Cl2	98.56(14)
N3-Cu1-Cl1	100.35(14)	N14-Cu2-Cl2	101.30(13)
N1-Cu1-Cl1	179.03(14)	N10-Cu2-Cl2	178.39(13)

steric match between substrate and the complex is believed to desire enough close in typical catalysis of cop-

per polyphenol oxidase, because it is first the nuclear binding with hydroxyl group of polyphenol. angles are listed in Table 2.

The coordination geometry around the copper atom is distorted trigonal bipyramid formed by three nitrogen atoms from ligand NTB in the basal plane, a NTB nitrogen atom and one chlorine atom in the axial coordination sites. The axial and planar bond angles are respectively light departure from 90° and 120°, so the coordination geometry of copper is distorted trigonal bipyramid. And the average bond lengths of Cu-N and Cu-Cl are 2.097 and 2.2680 Å, respectively. The axial binding chlorine ion is weaker, and it could be easy to leave to change the coordination structure, which would play an important role in catalysis^[12]. Moreover, the complex can bind and activate oxygen molecules based on the appearance of an ultraviolet absorption peak at 316 nm, when O₂ was bubbled through the complex-DMF solution for 10 min at room temperature. This peak is assigned to π_{σ}^* orbit of peroxide ion O₂²⁻ → Cu(II) d_{x²-y²} LMCT (ligand to metal charge transfer) transition. However, the complex can not directly bind and activate oxygen before reduction. A

per polyphenol oxidase, because it is first the nuclear binding with hydroxyl group of polyphenol.

2.2 Cyclic voltammogram data

As is shown in Fig. 2, the cyclic voltammogram

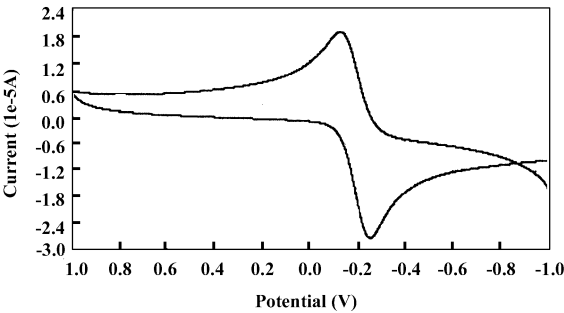


Fig. 2 Cyclic voltmetry of the complex in DMF
($c = 1.0 \times 10^{-3}$ mol/L, 0.1 mol/L TBAP) at a platinum
electrode (scan rate = 50 mV s^{-1})

of the complex contains a cathodic peak at -0.255 V and an anodic peak at 0.123 V , which are assigned to a one-electron redox process of $\text{Cu}(\text{II}) \rightleftharpoons \text{Cu}(\text{I})$. Catechol and pyrogallol are sensitive to dioxygen in alkaline solution ($E = -0.23 \text{ V}$ and -0.20 V versus SCE, respectively). They are easily oxidized to semi-quinone and quinone^[13]. The anodic potential of the complex is higher than that of the two substrates, therefore, the complex can oxidize catechol and pyrogallol.

2.3 Study of polyphenol oxidase activities

Assuming V_i is the initial velocity, it is found that V_i follows saturation kinetics with respect to substrate concentration $[S]$. At sufficiently low $[S]$, Plot of V_i versus $[S]$ is linear. When $[S]$ becomes sufficiently high, catalyst (here is model compound or mimetic en-

zyme, M) is saturated, the rate of reaction is zero-order and tends toward a limiting value, maximum velocity V_{\max} . The kinetics is expressed quantitatively in Michaelis-Menten equation. Where k_{cat} is turnover number, that is the number of moles of substrate converted to product (P) per minute per mole of model compound. It is a pseudo-first-order rate constant of catalyst-substrate complex MS conversion to products.

$$V = \frac{k_{\text{cat}}[M][S]}{k_m + [S]} = \frac{V_{\max}[S]}{k_m + [S]} \text{ here } V_{\max} = k_{\text{cat}}[M]$$

By applying traditional Michaelis-Menten approach^[14], values of kinetic parameters k_{cat} and V_{\max} were obtained from the corresponding plot of V_i vs. $[S]$. As is shown in Fig. 3, Polyphenol oxidase activities toward pyrogallol and catechol obey the Michaelis-Menten equation. The results listed in Table 3 indicate that the turnover numbers of the complex

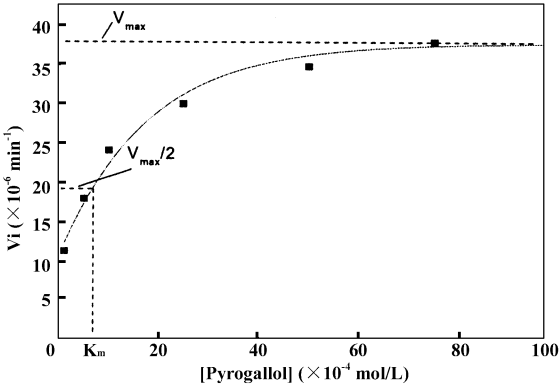


Fig. 3 Plot of initial ratio V_i vs
 $[S] : [\text{complex}] = 2.5 \times 10^{-5} \text{ mol/L}$,
 $T = 30 \text{ }^\circ\text{C}$, $\text{pH} = 8.0$

Table 3 The kinetic data of the complex

Substrate	Temperature ($^\circ\text{C}$)	pH	$k_{\text{cat}} (\text{h}^{-1})$	$V_{\max} (10^{-6} \text{ mol} \cdot \text{L}^{-1} \cdot \text{h}^{-1})$
Catechol	30	8.0	0.54	54.0
		7.6	0.42	42.2
		7.3	0.18	18.1
	40	8.0	1.68	168
		7.6	1.08	108
		7.3	0.48	48.0
Pyrogallol	18	8.0	75.1	1 875
		7.6	56.7	1 417
		7.3	42.0	1 050
	30	8.0	88.2	2 205
		7.6	59.4	1 485
		7.3	46.2	1 155

toward pyrogallol are much higher than those of catechol under the same conditions, which has been reported previously^[15].

All of the turnover numbers increase with increasing pH values. It is considered that the rate-determining step of the reactions is the first proton of a polyphenol to be abstracted to form the corresponding semiquinone^[16], hence, in the range of pH 7.3–8.0, the higher the pH value, the better the activities. Moreover, when the catalytic reaction temperature increases, the reactivities for catechol increase obviously, while un conspicuous for pyrogallol.

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$[\text{Cu}(\text{NTB})\text{Cl}]\text{ClO}_4 \cdot 2.5\text{CH}_3\text{OH}$ 的合成、晶体结构和多酚氧化酶活性研究

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摘要:合成了配合物 $[\text{Cu}(\text{NTB})\text{Cl}]\text{ClO}_4 \cdot 2.5\text{CH}_3\text{OH}$ (NTB为N, N, N-三(2-(*p*-苯并咪唑甲基)胺), 并进行了紫外可见光谱、元素分析、循环伏安和单晶X射线衍射分析等表征. 用UV-Vis. 研究了以邻苯二酚、邻苯三酚为底物时配合物的多酚氧化酶活性, 结果表明:配合物的多酚氧化酶活性符合米氏方程曲线;在pH为8.0和30℃的条件下, 配合物催化邻苯三酚和邻苯二酚反应的转化数分别为88.2 h⁻¹和0.54 h⁻¹. 通过动力学数据比较发现, 邻苯三酚比邻苯二酚更容易氧化, 而且它们的催化氧化速率都随着pH的升高而增大.

关键词:合成;晶体结构;多酚氧化酶活性