Synthesis, Characterization, and Catechol Oxidase Activity of Cu (II) Complexes Derived from New Imidazolidine Derivatized Ligands

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***Abstract*—** The synthesis and full characterization of two imidazolidine derivatives and their copper complexes are described. The corresponding copper complexes generated in situ were found to be suitable catalysts for the oxidation of catechol substrate to quinone with the dioxygen specie at ambient conditions, thus mimicking the role of catechol oxidases. The complexes catalyzed the oxidation reaction with a rate varying from 1.03 μ mol L−1 min−1 for the H5L2 [Cu(CH3CO2)2] complex to 0.3 μ mol L−1 min−1 for H5L1 [CuCl2]. Finally, a theoretical study was achieved using the functional density theory (DFT), and stressing on the global and local nucleophilicity indexes.

***Keywords*—**Synthesis, Imidazole, Imidazolidine, Catechol Oxidase, DFT indexes.

I. Introduction

THE dicopper proteins hemocyanin, tyrosinase and catechol oxydase have been shown to contain a binuclear metallic center. Despite the similar geometric and electronic structures of their di-copper centers, these proteins carry out different biological functions. Indeed, hemocyanin is an oxygen transporter; tyrosinase however catalyzes the ortho-hydroxylation of phenols with further oxidation of the resulting catechol product to o-quinone. Catechol oxidases in contrast, catalyze exclusively the oxidation of catechols to the corresponding o-quinones with no action on monophenols [1, 2].

Separately, the imidazole ring of histidine residues is one of the most common ligands at the active sites of metalloproteins. The ubiquitous presence of histidine coordination has stimulated the syntheses of imidazole-containing ligand donors to perform the coordination chemistry and the biomimetic chemistry [3, 4]. As a result, it is not surprising that continuously increasing research has focused on copper complexes with the more relevant imidazole-based ligands.

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Extensive investigations focused on the synthesis of new model compounds that mimic the active sites of the catechol oxidase. These model compounds can be classified as either symmetrical with coordinating groups (pyridine, imidazole, pyrazole, benzyl thiophene, etc....) mimicking the imidazole donors from the histidine residues [5–10], or asymmetrical tripodal dicopper complexes [11–16]. The resulting catalytic properties were shown to be enhanced when the asymmetry was created. The properties of mono and binuclear Cu (II) complexes have also been compared [17, 18] and the rate for the catalysis of catechol was shown to be ligand-dependent. We wish to report herein the synthesis of two new designed ligands and the comparative reactivity of their respective complexes synthesized in situ toward catechol, in order to mimic the activity of the copper-containing enzyme tyrosinase.

Common DFT-based reactivity descriptors are consequently included in the present study for their importance during the actual study.

II. Experimental

2.1. Materials

Urocanic acid, triethylentetramine, phenylsalicylate, piperidine, thionyl chloride, hydroxylamine,

salicylaldehyde (Aldrich) were used as purchased. High-grade solvents (methanol, dimethylformamide, diethyl ether) were used without further purification.

2.2. Physical measurements

Microanalyses were performed on a Perkin–Elmer 2400 elemental analyzer. IR spectra were measured in the 4000 - 400 cm-1 range on a 9800 FTIR spectrometer (Perkin–Elmer). Samples were run as KBr pellets. UV/Vis spectra were recorded on a Perkin Elmer Lambda 800 spectrophotometer and 1H NMR spectra on a Bruker AC 250. Chemical shifts are given in ppm. All measurements were performed at the CNRST (Centre national de Recherche scientifique et technologique, Rabat, Morocco, at the exception of the UV measurements which were achieved at the COSTE (Center of the Oriental Science and Technology of Water, Oujda, Morocco).

2.3. Synthesis of ligands

The ligands were characterized by ^{1}H NMR in deuteriated DMSO unless otherwise specified.

2.3.1 Synthesis of 2-(2'-Hydroxyphenyl)-1,3-bis [2'-2''-hydroxybenzamido)-1'-ethyl}-1,3-imidazolidine, H_5L^1 .

The synthesis of the ligand H_5L^4 was achieved according to the Chowdhury et al. procedure [19]. The product was recrystallized from methanol to afford a yellow solid (m.p. 126 °C, yield 70%). Anal. Calc. for $C_{27}H_30N_5O_4$: C, 66.11; H, 6.16; N, 11.42; O, 16.31. Found: C, 65.98; H, 6.42; N, 11.54; O, 16.51. MS: m/z = 492.56. UV-Vis (nujol, λmax (nm)): 221.48; 241.79; 283.36; 308. 1H NMR: 4,16 (s, J = 6.4 Hz, 1H, HC imidazolidine); 4.50 (t, J = 7.1 Hz, 4H, H_2CNHCO); 4.62 (t, J = 1.5 Hz, 4H, H_2CN); 7,.2 (s, J = 7.8 Hz, 3H, Ar); 7.52 (d, J = 7.8 Hz, 6H, $H_2CC(C_6H_5)$); 6.83-7.89 (12H,-C_6H_5); 9.94 (large, J = 7.8 Hz, 2H, NH). IR (cm-1): 3366.40 (N-H amide and OH phenolic), 1642.19 (C=O amide).

2.3.2 Synthesis of N,N'-(2,2'-(2-(3-ethoxy-4-hydroxyphenyl)imidazolidine-1,3-diyl)bis(ethane-2,1-diyl))bis(2-dydroxybenzamide), H_5L^2.

The intermediate H_4L (1.44g, 0.004 mol), obtained according to the procedure of Chandra et al. [20], was reacted with a solution of 3-ethoxy-4-hydroxy benzaldehyde (0.66g, 0.004 mol) in methanol (20 ml) at 0°C for 2h then 1h at room temperature. The solvent was subsequently evaporated, and the resulting mass dissolved in methanol (5 ml). Crushed ice (200 g) was added to precipitate the ligand. The product was recrystallized from ethanol to afford a yellow solid (m.p. 142 °C, yield 70%). Anal. Calc.for $C_{29}H_{34}N_4O_6$: C, 65.15; H, 6.41; N, 10.48; O, 17.96%. Found: C, 65.48; H, 6.48; N, 10.74; O, 18.01%. MS: m/z = 536.25. UV-Vis (nujol, λmax (nm)), 210; 229.90; 271.87; 297.57; 308.03. 1H NMR: 4.16 (s, J = 6.4 Hz, 1H, HC imidazolidine), 2.31 (t, J = 7.1 Hz, 3H, $H_3CCH_2OC_6H_5$), 3.20 (q, J = 1.5 Hz, 2H, H_3C-$H_2COC_6H_5$), 4.50 (q , J = 7.8 Hz, 4H, H_2CNHCO); 4.62 (t , J = 7.8 Hz, 4H, H_2CN) , 7.42 (s , J = 7.1 Hz, 3H, HO-C_6H_5), 7.49 (s, J = 7.8 Hz, 1H, $HCCOC_2H_5$); 7.52 (d, J = 7.1 Hz, 6H, HCCOH and HCCCOH), 7.89 (dd, J = 7.8 Hz, 4H, HCCHCHCOH), 9.62 (wide, J = 7.8 Hz, 2H, NH). IR: 3425.09 (OH phenolic), 3366.10 (N-H amide) 1664.92 (C=O amide).

2.4. Synthesis of the complexes

2.4.1 $[Cu_2 (H_5L^1) (H_2O)_2]$ 0.5CH_3OH 1

1. To a stirred methanolic solution (10 mL) of the H_5L^1 ligand (0.462 g, 10^{-3} mol) was first added KOH (0.168 g, $3\cdot10^{-3}$ mol) and ten minutes later, $Cu(OAc)_2$. H_2O (0.36 g, 2 x 10^{-3} mol). The mixture was stirred while heating at 60 °C during 5 h. The formed green precipitate was filtered off and washed several times with methanol. Yield 2.32 g (70%). Anal. (Found) Calc.for $C_{27}H_{30}N_5O_4Cu_2$: C ; 49,90 (49,62) ; H ; 4,31 (4,85) ; N, 8,31(8,42) ; Cu 19,21 (19,09). m/z (100%) 664.07. Characteristic IR absorptions (KBr): 3417, 1596, 1562, 1522, 1460, 504, 410 cm^{-1}. UV–Vis of 4 (solid state): 270 (L → M), 370 (L → M), 585 (d→d) nm.

2.4.2 $[Cu_2 (H_5L^2) (H_2O)_2]$ 0,5CH_3OH 2

2. It was prepared following the procedure for **2** using the H_5L^5 ligand (0.534 g, 10^{-3} moles), KOH (0.168 g, 3x 10^{-3} mol) and $Cu(OAc)_2$. H_2O (0.36 g, 2 x 10^{-3} mol). Yield: 2.26 g (64%). Anal. Found (Calc.) for $C_{27}H_{30}N_5O_4Cu_2$: C, 49,70 (49,92) ; H, 4,96 (5,11) ; N, 7,68 (7,89) ; Cu, 17,23 (17,91). m/z (100%) 708.10. Characteristic IR absorptions (KBr): 3363, 1660, 1580, 1501, 511, 412 cm^{-1}. UV–Vis of 5 (solid state]: 260 (L → M), 310 (L → M), 360 (L → M), 645(d→d) nm.

III. Theoretical and Computational Methods

3.1. Global and local reactivity indexes

Popular qualitative chemical concepts such as hardness [21, 22] (η) have been provided with rigorous definition within the purview of conceptual DFT [23, 24].

Hardness (η) is defined [25] as:

$$\eta = I - A \tag{1}$$

Where I and A are the ionization potential and electron affinity, respectively. If EHOMO and ELUMO are the energies of the highest occupied and lowest unoccupied molecular orbitals, respectively, then the above equations can be rewritten [26] using Koopmans' theorem [27] as:

$$\eta \approx E_{HOMO} - E_{LUMO} \tag{2}$$

Softness (*S*) is the reciprocal of hardness

$$S = 1/\eta \tag{3}$$

Recently, Domingo *et al*. [28] introduced an empirical (relative) nucleophilicity index, *Nu*, based on the HOMO energies obtained within the Kohn–Sham scheme and defined as:

$$Nu = E_{HOMO(Nuc)} - E_{HOMO(TCE)} \tag{4}$$

This nucleophilicity scale uses tetracyanoethylene (TCE) as reference. This choice allows us to handle a nucleophilicity scale with only positive values [28].

The global nucleophilicity index (N) can be expressed as the sum of local nucleophilicities condensed to all atoms of the molecule:

$$N = \sum N_k \tag{5}$$

Very recently, Perez *et al.* [29] proposed a new definition of the local nucleophilicity index, Nu_k, as the product of the global nucleophilicity index, *Nu*, and the nucleophilic Fukui index, f_k^- .

$$Nu_k = Nu \,.\, f_k^- \tag{6}$$

Where f_k^- is defined as :

$$f_k^- = \left[\rho_k(N) - \rho_k(N-1)\right] \tag{7}$$

Where $\rho_k(N)$ and $\rho_k(N-1)$) are the gross electronic populations of the site k in neutral and cationic systems respectively?

3.2. Computational Détails

The quantum chemical calculations reported in this work are performed, at the HF/6-31G*// B3LYP [30] /6-311G (d,p)

level of theory , using GAUSSIAN 03 series of programs [31]. The optimization of equilibrium geometries of all reactants were performed using the Berny analytical gradient optimization method [32]. The electronic populations as well as the Fukui indexes and local nucleophilicities are computed using different population analysis NPA (natural population analysis) [33]. The cationic systems, needed in the calculation of the nucleophilic Fukui indexes are taken in the same geometry as in the neutral system.

Popular qualitative chemical concepts such as hardness [21, 22] have been provided with rigorous definitions within the purview of conceptual DFT [23-27]. The global nucleophilicity was calculated using the empirical correlation of Domingo et al. [28] and local nucleophilicity according to Pérez et al. [29].

IV. Catecholase Activity Measurements

Kinetic measurements were carried out spectrophotometrically, following the appearance of o-quinone over time at 25 °C (390nm absorbance maximum, ε=1600M^{-1} cm^{-1} in methanol). The metal complex (prepared *in situ*: 0.3mL of a 10^{-3}M methanol solution) and a solution of catechol (2 mL of a 10^{-1}M solution in methanol) were mixed in the spectrophotometric cell [17].

4.1. Catecholase Studies

The progress of the catechol oxidation reaction was conveniently and closely monitored, by following the strong absorbance peak of quinone in the UV/Vis spectrophotometer. In all cases, catecholase activity was noted. Figs. (1,2) show the absorbance *versus* time for the first 60 min of the reaction of the copper (II) complexes while the oxidation rates are shown in Table 5. As shown in these data, the complexes catalyzed the oxidation reaction of catechol to quinone with a rate varying from 1.03 μ mol L^{-1} min^{-1} for the H_5L^2 [$Cu(CH_3CO_2)_2$] complex to 0.3 μ mol L^{-1} min^{-1} for H_5L^1 [$CuCl_2$] complex.

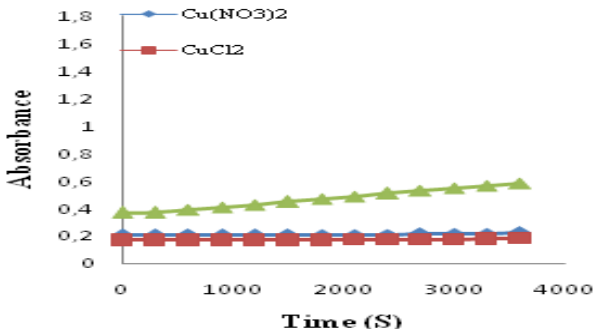


Fig. 1: Catechol oxidation with H_5L^1 complex

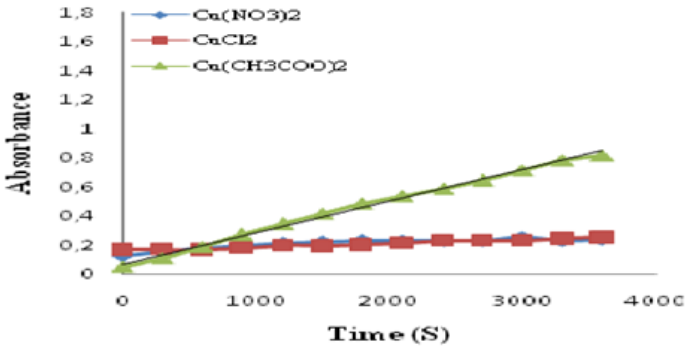
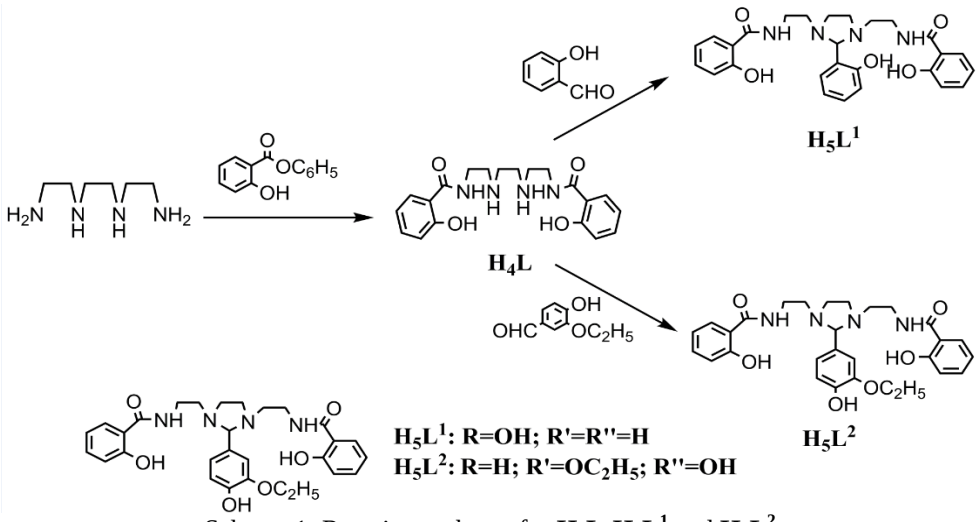


Fig. 2: Catechol oxidation with H_5L^2 complex

V. Results and Discussion

5.1 Ligands synthesis

The ligands H_5L^1 and H_5L^2 belong to a family of μ-bis (tetradendate) hydroxybenzamido imidazolidine ligands. Unlike the previous ones, these closely related ligands are characterized by an imidazolidine ring carrying two bridging spacers and a pendent phenolic group inside the hexadentate hydroxyl benzamido ligand framework. The ligands were prepared through a two-step synthesis that includes first the condensation of triethylenetetramine with 2 equivalents of phenyl salicylate derivative and a subsequent reaction with one equivalent of salicylaldehyde (Scheme 1).



Scheme 1: Reaction pathway for H_4L, H_5L^1 and H_5L^2

The intermediate hexadendate benzamido ligand H_4L is synthesized by gently heating (60°C) of phenyl salicylate with triethylenetetramine in a 2:1 mole ratio in isopropanol (Scheme 2). The imidazolidine ring in compounds H_5L^1 and H_5L^2 is subsequently obtained by reaction of the intermediate H_4L with salicylaldehyde.

All compounds were characterized by elemental analysis, UV- Vis, FTIR, ^{1}H RMN and mass spectroscopy. The IR spectra of the ligands (Table 1) show strong bands corresponding to the vibration of C=O amide groups at 164 and 1684 cm^{-1} in H_5L^1 and H_5L^2 respectively.

TABLE I
COMPARATIVE IR AND ^{1}HNMR DATA OF THE LIGANDS AND THEIR COMPLEXES

	IR□ cm^{-1}						^{1}H NMR		
Product	ν(C=O)	ν(OH)/H_2O/phenolic	δ(N-H) amide	ν(C=C)	ν(Cu-O)	ν(□Cu-N)	δ (HO)	δH imidazolidine	δ (N-H) amide
H_5L^1	1642	3386	1593	1531	—	—	7,32	4,16	9,94
(1)	1596	3417	1562	1522	504	410	—	—	—
H_5L^2	1684	3366	1643	1591	—	—	7,42	4,72	9,62
(2)	1660	3393	1580	1501	511	412	—	—	—

The absorption peak at 3420-3480 cm^{-1} is assigned to the vibrational mode of N-H in imidazole. The proton NMR spectra of the imidazolic ligands reveal signals at 11.32-11.42 ppm corresponding to the imidazolic protons (N-H). The H_5L^1 and H_5L^2 ligands display distinct proton signals at 9.62–9.94 ppm for the amide proton, 4.16 ppm for the imidazolidinic proton and 7.32-7.42 ppm for the phenolic proton.

5.2 Complexes synthesis

The dinuclear complexes **1** and **2** were synthesized by treating a methanolic solution of copper (II) acetate dihydrate with the H_5L^1 and H_5L^2 ligands respectively in the presence of excess sodium hydroxyde in the ratio 2.1. The formation of this complex is supported by endogenous phenolato and etheroato bridging respectively. These bridges involve the introduction of two metal ions within the ligand cavity as shown in scheme 2.

Scheme 2: Reaction pathway for **1** and **2** synthesis

All compounds were characterized by elemental analysis, ESI Mass, IR and UV–Vis spectroscopy. In the mass spectra of complexes **1-5**, the molecular peak at 664.07 and 708.10 respectively, could be assigned to their corresponding precursors. The amidic ν(N-H) stretching bands in **1** and **2** shift to a lower frequency by 31, 22 and 43.52cm^{-1} in IR spectra**,** clearly showing their participation in coordination. The presence of water and methanol molecules in complexes **1** and **2** were inferred from IR spectral (3500-3400 cm^{-1} region) and weight loss method. New bands were observed at (504-530) cm^{-1} an (400- 412) cm^{-1}, which may be attributed to ν(Cu-O) and ν(Cu-N) vibrations.

The electronic spectra for **1** and **2** complexes either in solution or in the solid state give the typical absorption band characteristic of square-pyramidal coordination environnement Cu(II) methanol solution (Fig. 3): λmax = 500 – 750 nm (ε = 163 M^{-1} cm^{-1}), which is in agreement with the literature [19, 16].

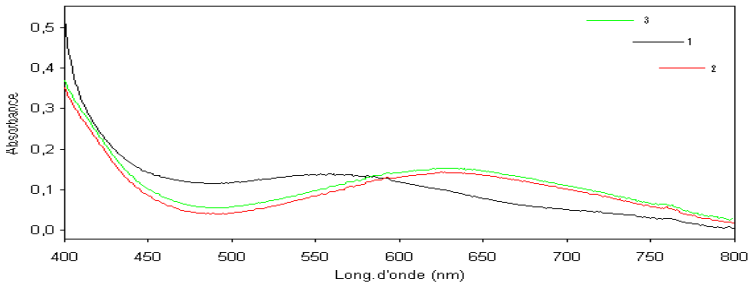


Fig. 3: UV-Visible spectra of 1, 2 and 3([9]) complexes

After analysis and spectral comparison of the ligands with those of their corresponding complexes, the categorie of complexes is represented by the bis-nuclear copper (II) complexes formed by the heptadentate ligands H_5L^1 and H_5L^2. Each metal is bonded in a square pyramidal environement by one terminal phenoxo, one amido and imidazolidino nitrogen atom and the pendent phenolic oxygen in the case of the complex $[Cu_2(H_5L^1)(H_2O)_2]$ and ether oxygen in the case of the complex $[Cu_2(H_5L^2)(H_2O)_2]CH_3OH$ (schemes 6-7). The apical donor is a H_2O oxygen atom, used as cristallization solvent.

5.3 Theoretical studies

The conceptual Functional Density Theory (DFT) allowed for the expansion of local and total reactivity indexes. Global softness and Fukui functions are used in this work to describe the reactivity of the synthesized H_5L^1 and H_5L^2 ligands toward Copper (II), considered as the electrophile in each case.

5.3.1 Nucleophilic Attack Sites:

The condensed Fukui function was used to analyze the local reactivity which contains the information regarding nucleophilic power of a given atomic site in a molecule. The values of the Fukui functions fk- for a nucleophilic attack are given for the two compounds H_5L^1 and H_5L^2 in Table 2 (only for the oxygen and nitrogen atom). The results of the condensed Fukui function show that, for the nucleophilic attack, the most reactive sites are on the imidazolic nitrogen and carbonyl oxygen.

TABLE II
FUKUI FUNCTIONS VALUES F_K^-, LOCALS NUCLEOPHILICITY INDEXES N_K OF H_5L^1 AND H_5L^2 SITES

H_5L^1	f_k^-	N_k	H_5L^2	f_k^-	N_k
N8	0,639	2,403	**N8**	0,640	2,627
O9	0,701	2,636	**O9**	0,633	2,593
N12	0,540	2,032	**N12**	0,543	2,229
N15	0,562	2,115	**N15**	0,256	1,050
N19	0,634	2,384	**N19**	0,285	1,170
O21	0,671	2,526	**O21**	0,633	2,596
O23	0,706	2,656	**O23**	0,303	1,244
O29	0,711	2,703	**O29**	0,726	2,977
O61	0,725	2,730	**O61**	0,721	2,958
			O36	0,562	2,302

The H_5L^2 system carries more reactive sites than the corresponding H_5L^1 and the local indexes values are as well higher in the H_5L^2 system (Table 2). Consequently, the H_5L^2 ligand is likely to show a better reactivity and an easier complexation of copper than the analogous H_5L^1. The reactivity is thus in favor of H_5L^2.

5.3.2 Global Softness

The global softness is a reactivity index that has proven its usefulness oftentimes (eq (3)) when predicting relative reactivity of various analogs. Table 3 shows that H_5L^2 has a higher softness than that of H_5L^1, thus more reactive toward copper (Figures 4, 5).

TABLE III
HOMO, LUMO ENERGIES, GLOBAL INDICE OVERALL HARDNESS (H) AND SOFTNESS (H)

Ligand	HOMO	LUMO	η	S
H_5L^4	-0,20589	-0,03369	0,1722	5,80
H_5L^5	-0,1932	-0,03064	0,16256	6,15

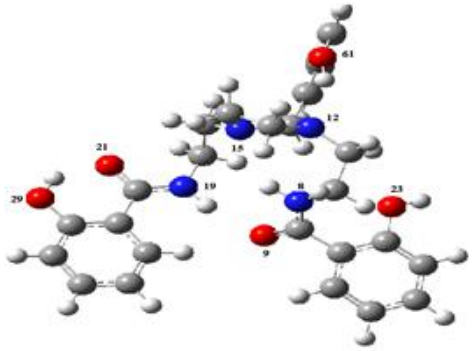


Fig. 4 : Chemical structure of H_5L^1

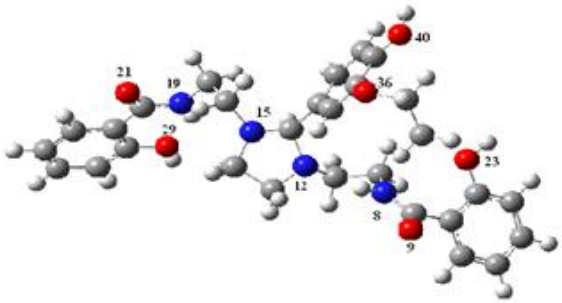
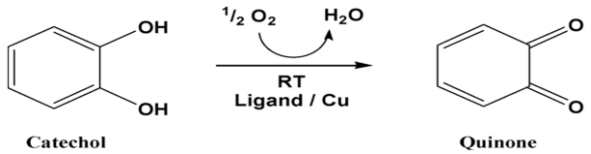


Fig. 5 : Chemical structure of H_5L^2

5.4 Catalytic properties

The two-electron oxidation of catechol to o-quinone was investigated in this study since this is one of the reactions that thyrosinase catalyzes (scheme 3). Studies of copper (II) complexes reactivity towards catechols have shown that both the geometry around the copper ions and their electronic features are important in determining the catalytic activities of these complexes [17].



Scheme 3: Oxidation of catechol to o-quinone

5.4.1 Ligand nature effect on the oxidation of the catechol to o-quinone kinetics

- The NO_3^- anion in complex **2** gives an oxidation rate of 0.1341 mol L^{-1} min^{-1} which is higher than that of complexe **1**, with rate of 0.0135 µ mol L^{-1} min^{-1}.
- The same observation is valid in the case of Cl^- anion. 0.1198 mol $^{L-1}$ mi^{n-1} for complex **2** by comparison to 0.0106 µ mol. L^{-1} $min^{-1,}$ corresponding to complexes **1**.
- In both cases, the ligand H_5L^1 gives the worst result and the H_5L^2 the best.

In the CH_3COO^- case, The H_5L^2 complex gives an oxidation rate of 1.0368 µ mol.L^{-1} min^{-1}, more important than that of H_5L^1 (table 5).

TABLE IV

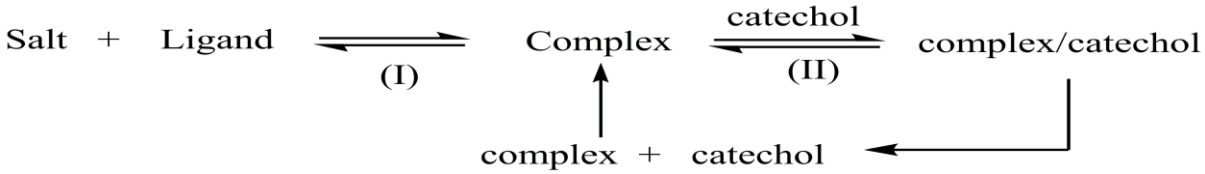
OXIDATION RATES (_MOL L−1 MIN−1) OF CATECHOL.

Ligand	NO_3^-	Cl^-	CH_3COO^-
H_5L^1	0,0135	**0.0106**	0,3066
H_5L^2	0,1341	0,1198	1,0368

The oxidation rate depends strongly on both the nature of the ligand and the type of the inorganic anion. The nature of the anion affects the geometry of the copper. This factor can contribute to the explanation of the oxidation rate dependence in the oxidization of catechol in quinone [34].

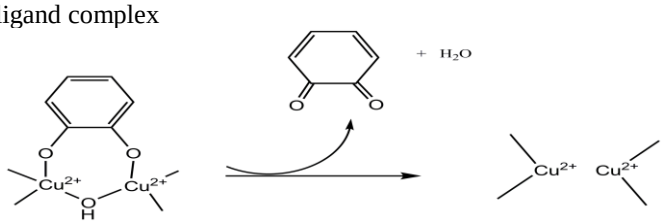
The order of reactivity for the oxidation of catechol by $Cu(CH_3COO)_2$ complexes is $H_5L^2 > H_5L^1$. The nature of the R radical (schemes 1), and particularly the electronic effect are factors that assigned the copper complex geometry.

The coordination sites as previously verified by local indexes are amidic nitrogen and the phenolic oxygen in the case of the H_5L^1 and H_5L^2 ligands. In solution, the formed complex is in equilibrium with the copper salt and the ligand (scheme 4).



Sheme 4: Balance ligand complex

However, the catalytic activity of the complex formed « *in situ* » during the catechol oxidation depends on its concentration and its geometry. This reaction requires the presence of a vacant site within the metallic ion in order to allow the catechol anchoring. It has been demonstrated with various models that the electronic transfer between the catechol and copper proceeds only after the catecholate formation (scheme 10) [35].



Sheme 10

As previously mentioned, copper (II) acetate complexes **1** and **2** are bis-nuclear complexes. This favors the catechol

anchoring trough a bridge mode and consequently a two-electron transfer process, necessary for the oxidation to occur. On the other side, data in Table 5 clearly show that the complex **2** catalyzes the catechol oxidation into o-quinone 4 to 10 times faster than the complex **1**.

By considering the complexes structures, the distance between the copper ion and the etherate oxygen of the central phenyl (*meta* position) in **2** is greater than the distance between the copper and the phenolic oxygen (*ortho* position) in **1**. This makes the latest less stable and less reactive.

VI. Conclusion

The present study deals with the catalytic activity evaluation of copper complexes prepared *in situ* in the oxidation of catechol into o-quinone. The ligands susceptible to form binuclear complexes produced good results.Moreover, the conceptual Functional Density Theory (DFT) predictions are in agreement with the experimental results. Thus, new tools have been developed to mimic and to better study the tyrosinase activity.

Acknowledgement

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