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Copper-catalyzed *ortho*-oxidation of phenols by dioxygen (tyrosinase mimics) do yields catechols as primary products

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Abstract

Evidences are provided that catechols [as copper(II)catecholates] are the actual primary products of copper-mediated (catalysed) *ortho*-selective oxidation of phenols, on the contrary to a recent claim reporting direct generation of *ortho*-quinones.

Keywords: Copper; Phenols; Tyrosinase mimics; Catechols

1. Introduction

Tyrosinases (EC 1.14.18.1) are dinuclear copper-containing monooxygenases [1] that catalyse the selective *ortho*-hydroxylation of phenols by molecular dioxygen into *ortho*-quinones (phenolase activity) and the oxidation of *ortho*-diphenols (catechols) into *ortho*-quinones (catecholase activity). Although catechols have never been isolated in the transformation of monophenols by these enzymatic systems (Scheme 1), they were usually considered as the intermediate compounds.

Following the pioneering work of Brackman and Havinga [2] on *ortho*-oxidation of phenols into 4,5-diamino-1,2-quinones by molecular oxygen catalyzed by Cu(II)-amine complexes, we previously reported [3,4] a new and efficient chemical mimic for tyrosinases. According to this method, variously substituted phenols reacted with dioxygen in acetonitrile, in presence of a catalytic quantity of Cu(I)Cl and at least 1 eq. metallic copper powder [Cu(0)]; in the general case, this reaction yielded Cu(II)catecholates (path a), from which free catechols were obtained after acid hydrolysis or reduction of Cu(II) to Cu(0). More recently, some attempts to mimic tyrosinases led to *ortho*-quinones from phenols (paths a + b), using mononuclear [5] or dinuclear [6–8] Cu(I) complexes.

Surprisingly, it has been claimed in a recent communication [9] that the actual initial products of tyrosinase chemical models (and perhaps those of enzymatic reactions) are not catechols but *ortho*-quinones, questioning in that way the reality of monophenolase activity. This statement based on the apparent results of copper-mediated oxidation of methyl 4-hydroxybenzoate **1** by three systems:

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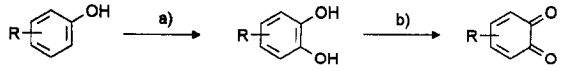


Scheme 1.

(i) the oxygenation of a Cu(I) phenolate complex: (Phen)(Ph_3P)(4-carbomethoxyphenolate)Cu(I) in experimental conditions adapted from the literature [5]; (ii) the Cu(0) corrosion method, adapted from our work [3,4], but in the presence of a tridentate amino ligand: *N,N*-bis[2-(2-pyridyl)ethyl]-benzylamine; (iii) by using Casella's dinuclear Cu(I) complex of *N,N,N',N'*-tetra-[2-(*N*-methylbenzimidazol-2-yl)ethyl]-*meta*-xylylenediamine [6] as a catalyst.

In each of the three experimental sequences, catechol **2** has never been detected by the authors but only substituted catechol methyl 2-(4-carbomethoxy phenoxy)-3,4-dihydroxybenzoate **4** (Scheme 2), reasonably supposed to result from Michael addition of phenol **1** on transient 4-carbomethoxy-1,2-benzoquinone **3**. The extremely reactive quinone **3** has never been isolated.

This last communication [9], stating that phenols are directly and exclusively oxidized into *ortho*-quinones, does not account for our previous observations. In particular, the authors deliberately disregard that Cu(I) catecholate **B** is effectively formed by the oxidation of Cu(I) 2,4-di-tertiobutyl phenolate **A** [10], yielding Cu(II) catecholate **C** and *ortho*-quinone **D** in a second step (Scheme 3). Independently prepared Cu(I) salt **B** leads also **C** and **D** under dioxygen in acetonitrile.

Such a discrepancy between the newly published experimental facts and ours imperatively required a careful reinvestigation of the copper-mediated oxidation of the precise substrate methyl-4-hydroxybenzoate **1**. We achieved in consequence the kinetic follow-up of the oxidation of **1** by two different copper-based systems: (i) oxidation by Cu(0) corrosion methods; (ii) reaction of Cu(I) phenolates with dioxygen.

2. Experimental

2.1. Materials

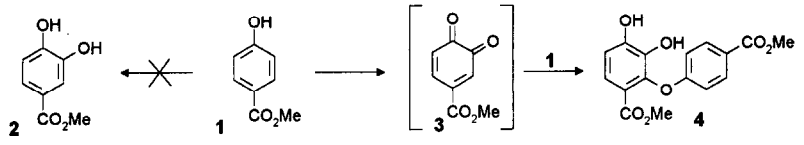
Reagents and solvents used were of commercially available reagent quality. Acetonitrile was dried over $CaCl_2$, distilled from P_4O_{10} and stored under nitrogen over molecular sieves (3 Å).

Cu(I)$(MeCN)_4BF_4$ was prepared from Cu_2O and HBF_4 as described in the literature [11].

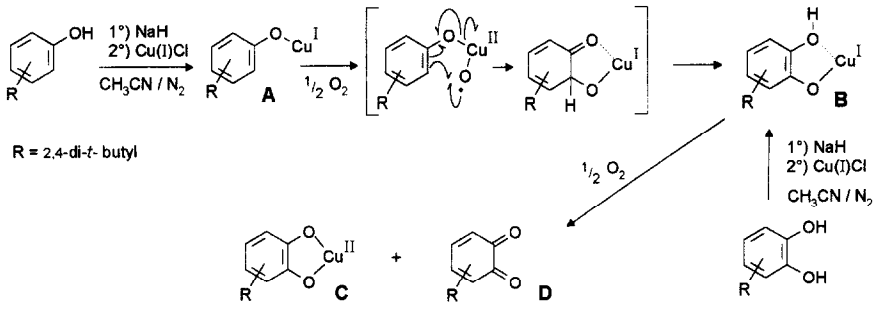
N,N-bis[2-(2-pyridyl)ethyl]-benzylamine was prepared by the reaction of 2-vinylpyridine with benzylamine in methanol, catalyzed by acetic acid [12].

2.2. Analyses

HPLC monitoring of each oxidation experiment was managed as follows: aliquots (0.15 ml) were taken from the reaction mixture, solvent was distilled off under reduced pressure, the residue was acidified with excess 15% aqueous H_3PO_4 (0.2 ml) and extracted with Et_2O (3 ml). Et_2O is removed under vacuum and the residue dissolved in MeOH (1 ml). HPLC analyses of these solutions were carried out on a Lichrospher RP-18 e, 5 μm, Merck™ (125 × 4



Scheme 2.



Scheme 3.

m m) c o l u m n , u s i n g a MeOH/H_2O/AcOH:65/35/1 mixture as an eluent; flow rate was set at 0.3 ml min^{-1}, using a Gilson solvent-delivery system. Absorbency at 254 nm was monitored. Quantifications were performed by comparison with calibration curves obtained with known amounts of authentic compounds **1**,**2** and **4**.

In each procedure, at least in one experiment, catechols **2** and **4** have been separated by column chromatography, and unambiguously characterized by ^{1}H- and ^{13}C-NMR and comparison with authentic samples.

Catechols **2** and **2'** were prepared from commercially available 3,4-dihydroxybenzoic acid by Fischer esterifications (in refluxing MeOH or EtOH with H_2SO_4 as a catalyst, respectively). Catechol **4** was purified by column chromatography, its mp and ^{1}H- and ^{13}C-NMR spectra are in complete agreement with those reported in the literature [9].

2.3. Procedures

2.3.1. Oxidation by Cu(0) corrosion methods

(a) Without additional ligand: methyl 4-hydroxybenzoate **1** (0.500 g, 3.28 mmol), a catalytic amount of cuprous chloride (0.015 g, 0.15 mmol) and excess (0.313 g, 4.92 mmol) Cu(0) powder (ca. 150 mesh) are reacted at 60°C in anhydrous MeCN (25 ml) under a dioxygen atmosphere. A stable mixture of Cu(II) salts of phenol **1** and of catechol **2** is obtained after 20 h heating. Acidification with excess aqueous 15% H_3PO_4 (after distillation of the major part of the solvent under reduced pressure) provides a mixture of **1** and **2** (79/21).

(b) In the presence of a polydentate ligand: when the same reaction is carried out without cuprous chloride but in the presence of 1 equivalent of *N*,*N*-bis[2-(2-pyridyl)ethyl] benzylamine (1.042 g, 3.28 mmol) the kinetic follow-up of the oxidation provides plots of Fig. 1 (at 60°C) and Fig. 2 (at 20°C).

2.3.2. Reaction of Cu(I) phenolates with dioxygen

(a) Without additional ligand: to a stirred suspension of the sodium salt of **1** (0.174 g, 1 mmol) in anhydrous MeCN (10 ml) under argon at room temperature, Cu(I)$(MeCN)_4BF_4$ (0.314 g, 1 mmol) in MeCN (5 ml) is added. The

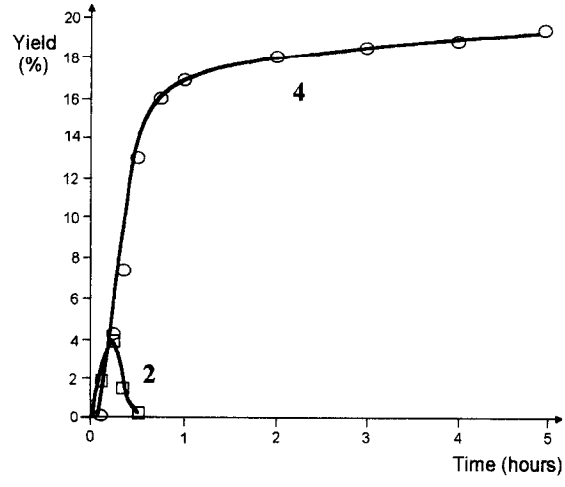


Fig. 1. Oxidation of **1** at 60°C.

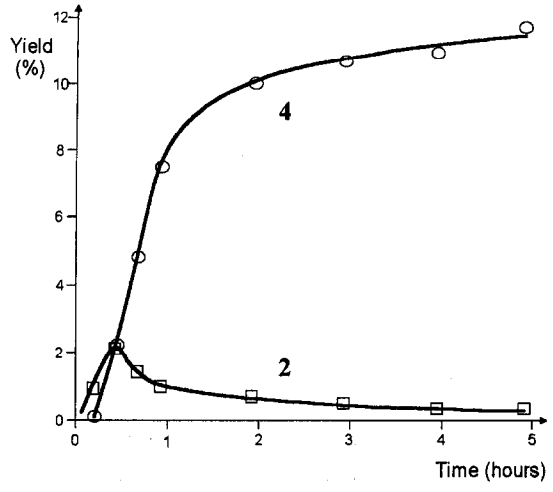


Fig. 2. Oxidation of **1** at 20°C.

orange–yellow reaction mixture is stirred for 3 additional h and turns progressively green when the flask is flushed with dioxygen at normal pressure. The kinetic follow-up of the reaction (Fig. 3) is performed as described above.

(b) With Phen and PPh_3 ligands: a mixture of triphenylphosphine (0.262 g, 1 mmol) and 1,10-phenanthroline (0.180 g, 1 mmol) in anhydrous MeCN (6 ml) is added to the same orange–yellow Cu(I) phenolate prepared under argon as above, the colour immediately turns brown red. The reaction medium is stirred for 3 more h. The flask is flushed with dioxygen at the beginning of the kinetic study (Fig. 4).

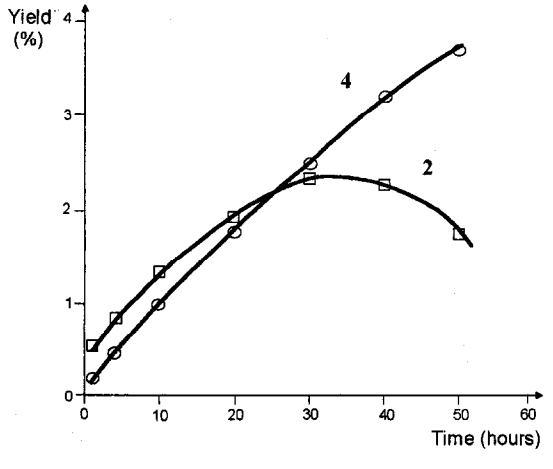


Fig. 3. Reaction of **1**–Cu(I) with O_2.

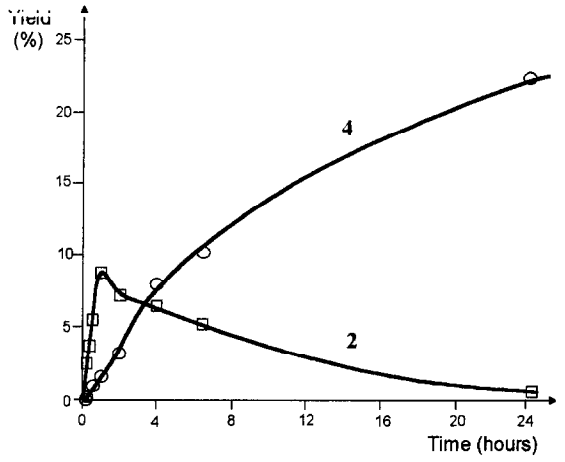


Fig. 4. Reaction of **1**–Cu(I)(Phen)(PPh_3) with O_2.

After 20 h, MeCN is evaporated under reduced pressure, the crude product is acidified with aqueous 15% H_3PO_4, extracted with Et_2O, dried over $MgSO_4$ and separated by preparative TLC on a silica gel plate with a mixture of cyclohexane and AcOEt (70/30) as an eluent. Catechol **4** (28,6 mg, 0.09 mmol, 18% from phenol **1** is separated from recovered phenol **1** (117.1 mg, 0.77 mmol, 77%).

2.4. Copper / dioxygen-mediated conversion of ethyl-3,4-dihydroxybenzoate (2') to ethyl-2-(4-carbomethoxy phenoxy)-3,4-dihydroxybenzoate (4')

In a corrosion experiment as described in Ref. [9]: [methyl-4-hydroxybenzoate (**1**) (0.228 g, 1.5 mmol), *N,N*-bis-[2-(-2-pyridyl)ethyl] benzylamine (0.475 g, 1.5 mmol), copper powder (0.143 g, 2.25 mmol), 20 ml anhydrous MeCN, at 60°C in an oxygen atmosphere], ethyl-3,4-dihydroxybenzoate (**2'**) (182.2 mg, 1 mmol) is added 1 h after the beginning of the reaction. After 20 h, MeCN is evaporated; the residue is acidified with aqueous 15% H_3PO_4, extracted with Et_2O, dried over $MgSO_4$ and purified by preparative TLC, on 4 silica gel plates (200 × 200 × 1 mm) using a cyclohexane/AcOEt (70/30) mixture as an eluent, to yield 0.110 g (41%) of catechol ethyl ester **4'**, besides a small amount (4%) of catechol methyl ester **4** and recovered phenol **1**.

2.5. Ethyl-2-(4-carbomethoxy phenoxy)-3,4-dihydroxybenzoate (4′)

$C_{17}H_{16}O_7$, colourless crystals, mp = 165°C (Et_2O), ^{1}H-NMR ($CDCl_3$) δ(ppm) = 1.0 (t, 3 H, ethyl Me), 3.86 (s, 3 H, OMe), 4.05 (q, 2 H, ethyl CH_2), 6,80 and 7,82 (2 d, 2 H each, J = 8.8 Hz), 6,83 and 7,50 (2 d, 1 H each, J = 8.8 Hz); ^{13}C-NMR ($CDCl_3$) δ(ppm) = 13.69, 52.01, 60.80, 112.20, 114.51, 116.10, 123.65, 124.17, 131.49, 137.17, 140.93, 141.23, 149.75, 161.81, 166.93.

2.6. Copper(II)-mediated conversion of ethyl 3,4-dihydroxybenzoate (2′) to ethyl-bis-2-6-(4-carbomethoxy phenoxy)-3,4-dihydroxybenzoate (5′)

Cuprous chloride (0.550 g, 5.5 mmol) is autoxidized in anhydrous MeCN (20 ml) during 12 h in a dioxygen atmosphere, with stirring, at room temperature, to provide a cupric complex as previously described [15]. Sodium sulfate (2 g) (drying agent) is added and the flask is then flushed with argon. Ethyl-3,4-dihydroxybenzoate (**2′**) (0.182 mg, 1 mmol) and methyl-4-hydroxybenzoate (**1**) (0.608 g, 4 mmol) are then added and the mixture is stirred for 2 h at 60°C under argon. MeCN is evaporated, the residue is acidified with aqueous 15% H_3PO_4, extracted with Et_2O, dried over $MgSO_4$ and the crude product purified by column chromatography on silica gel (cyclohexane/AcOEt, 70/30 as an eluent). Disubstituted catechol **5′** is the main oxidation product isolated (0.130 g, 0.27 mmol, 27%). Only small amounts of intermediate catechol **4′** are detected (HPLC and TLC).

2.7. Ethyl-bis-2,6-(4-carbomethoxy phenoxy)-3,4-dihydroxybenzoate (5′)

$C_{25}H_{22}O_{10}$, colourless crystals, mp = 178–180°C (isopropyl ether), ^{1}H-NMR ($CDCl_3$) δ(ppm) = 0.80 (t, 3 H, ethyl Me), 3.86 (s, 6 H, 2 OMe), 3.90 (q, 2 H, ethyl CH_2), 6.56 (s, 1 H, 5-aromatic), 6.89 and 6.94 (2 d, 2 H each, J = 8.8 Hz), 7.90 and 7.95 (2 d, 2 H each, J = 8.8 Hz). ^{13}C-NMR ($CDCl_3$) δ(ppm) = 13.40, 51.96, 52.05, 61.25, 106.25, 113.26, 115.00, 116.43, 124.18, 131.47, 131.52, 134.22, 139.58, 146.66, 148.13, 161.10, 161.85, 163.35, 166.71.

3. Results and discussion

3.1. Oxidation of monophenol (1)

3.1.1. Oxidation by Cu(0) corrosion methods

We checked at first that phenol **1** is effectively oxidized by dioxygen in our previously described conditions [3,4], at 60°C in acetonitrile, in presence of catalytic Cu(I)Cl (< 0.05 eq.) and excess powdered Cu(0) (1.5 eq.). Since the presence of the ester electron withdrawing substituent renders **1** less oxidisable than alkyl phenols such as 2,4-ditertiobutyl phenol, complete oxidation of **1** is not reached and a stable mixture of Cu(II) salt of phenol **1** (79%) and Cu(II) salt of catechol **2** (21%) is obtained after 20 h heating under O_2. At this stage, binuclear catechol **4** is not detected from the crude material.

On the other hand, when the tridentate ligand *N,N*-bis[2-(2-pyridyl)ethyl] benzylamine is added at 60°C to the preceding stable reactional medium [mixture of Cu(II) salts in a dioxygen atmosphere], binuclear catechol **4** arises slowly, to the detriment of **1** and **2**; the ratio **4/2** reaches 3/7 after 30 h in these conditions.

Such a **2** → **4** transformation is also observed when 1,10-phenanthroline (three-fold excess) is added to this reactional medium, either in an oxygen atmosphere or after deoxygenation by Ar bubbling.

A careful time-course reinvestigation of the corrosion experiment described in the literature [9] (60°C, with 1 equivalent of *N,N*-bis[2-(2-pyridyl)ethyl]-benzylamine) clearly indicates that catechol **2** is the major reaction product during the first 5 min; it rapidly vanishes while the amount of binuclear catechol **4** increases in the opposite way (Fig. 1). After 30 min the

concentration of **2** is already too reduced to allow its bona fide detection. It is thus not surprising that catechol **2** has not been detected after 6 h under these conditions! Intermediate **2** is more easily observed when the experiment is conducted at room temperature, since it remains detectable for several hours (Fig. 2).

A very similar behaviour can be observed in Cu(0) corrosion-based oxidation of **1** when bidentate ligands such as 1,10-phenanthtroline (Phen) or 2,2′-bipyridine (Bipy) are used in place of the former tridentate benzylamine in presence (and without) of Cu(I) salts in catalytic amount.

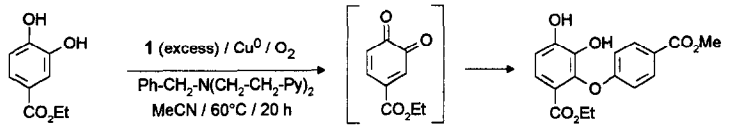
3.1.2. Reaction of Cu(I) phenolates with dioxygen

As it is well known [13] that *ortho*-benzoquinones can react with metallic Cu(0) to yield Cu(II) catecholates, one was allowed to suspect this reaction to occur in our corrosion systems by trapping transient quinone **3** to yield Cu(II) salt of **2**. This reaction would have to be extremely fast, even with a solid [Cu(0)] component, since **3** is extremely sensitive to nucleophilic reagents and to Diels–Alder type dimerizations [14]. We have checked this hypothesis with a Cu(0) depleted system, starting from the stoichiometric Cu(I) salt of phenol **1**, which is usually considered to be the initial intermediate in copper-mediated oxidations of phenols with dioxygen. We performed at first a quite simple experiment avoiding the use of possibly unnecessary supplementary ligands such as phenanthroline and PPh_3: Cu(I) salt of phenol **1** [**1**-Cu(I)] was prepared, in acetonitrile, from Na^+ salt of **1** (independently obtained by reaction of phenol **1** with sodium hydroxide in methanol, evaporation of the solvent and drying) according to the procedure by which we have previously prepared Cu(I) salt of 4,5-ditertiobutylphenol [10]. The addition of $Cu(I)(MeCN)_4$ BF_4 under inert atmosphere yields a solution of **1**-Cu(I) which is then reacted with dioxygen at 20°C. Catechols **2** and **4** are the only products one can isolate after acidification of the crude mixture. Kinetic follow-up (Fig. 3) points out that catechol **2** is quite preponderant at the very start of the reaction, remains the principal product for the first 25 h and is slowly transformed into substituted catechol **4**. This pattern is characteristic of successive reactions: **1** → **2** → **4**.

When complex [**1**-Cu(I)(Phen)(PPh_3)] is reacted with dioxygen in these conditions (same concentration, 20°C), it appears (Fig. 4) that catechol **2**, the only detectable product for the first 15 min, is transformed afterwards into catechol **4** at a higher rate than in the absence of Phen and PPh_3 ligands; the intersection of the two plots occurs in fact after only 3 h (to be compared with 25 h, Fig. 3). Overall transformation is also greater with Phen and PPh_3 ligands; it is noticeable that PPh_3 yields progressively its oxide $POPh_3$, detected along with **2** and **4** by HPLC.

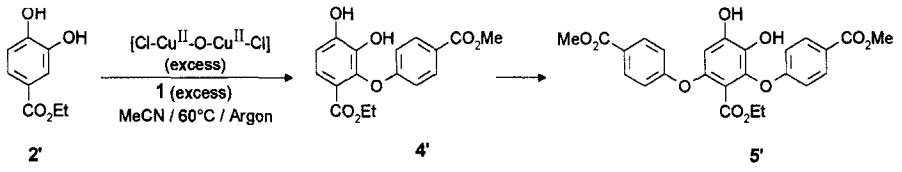
3.2. Oxidation of catechols

The principal argument in Ref. [9] leading to refute the intervention of catechol **2** consists in the observation that its sodium salt is not oxidized by dioxygen in the presence of Casella's Cu(I) dinuclear complex (prepared from a N6-dinucleating ligand) [6]. This observation may perhaps be restricted to such particular dinuclear copper complexes since we have independently



2' **3'** **4'**

Scheme 4.



Scheme 5.

observed [1] that some related dinuclear Cu(II) catecholates are stable.

However, we do observe that catechol **2** is transformed into substituted catechol **4** in the presence of phenol **1** either in Cu(0) corrosion experiments or by reaction with appropriate oxidizing Cu(II) species.

'Labelling' of presumed intermediate catechol in the form of its corresponding ethyl ester **2'** and careful analysis of reaction yields unambiguously demonstrate that quinones **3**, **3'** result from oxidation of catechols **2**, **2'**, according to the three following experiments:

(i) Labelled catechol ethyl ester **2'** is added to the reaction medium 1 h after the beginning of a corrosion experiment in the conditions of Ref. [9] (phenol **1**, *N,N*-bis[2-(2-pyridyl)-ethyl]-benzylamine, excess Cu(0), in an oxygen atmosphere at 60°C in MeCN, 20 h). The reaction provides mainly ethyl 2-(4-carbomethoxy phenoxy) 3,4-dihydroxybenzoate (**4'**) (41%) resulting from addition of phenol **1** onto ethyl ester quinone **3'** (Scheme 4), besides 'classical' methyl ester catechol **4** (4%).

(ii) Cuprous chloride is first reacted with O_2 in MeCN (12 h) to provide a μ-oxo cupric complex with Cl–Cu(II)–O–Cu(II)–Cl structure [15]. In the presence of an excess of phenol methyl ester **1** under an inert atmosphere (Ar), this complex oxidizes catechol ethyl ester **2'** (60°C, 2 h) mainly (27%) into ethyl bis-2,6-(4-carbomethoxy phenoxy)-3,4-dihydroxybenzoate (**5'**) (Scheme 5). A small quantity of the intermediate monosubstituted catechol **4'**, readily oxidized in these conditions, is detected in the crude product.

(iii) The reaction products of the oxidation of [**1**-Cu(I)(Phen)(PPh_3)] complex by O_2 in MeCN (20°C, 20 h) are carefully separated by preparative TLC. 1 mmol of complex provides 0.09 mmol of isolated catechol **4** (from 0.18 mmol phenol **1**) and 0.77 mmol of **1** is recovered unchanged. Thus, real global yield is superior to 95%. Since Fig. 4 indicates that catechol **2** reaches an instant yield of 9% (after 2 h of reaction), it becomes evident that the major part of final catechol **4** necessarily stems from intermediary **2**.

4. Conclusion

It can be mainly stated from the whole of these results that catechol **2** is actually the primary product of the copper-catalysed selective *ortho*-oxidation of phenol **1**. This behaviour seems to be common to phenols as a whole, because electron-depleted ester phenol **1** appears here to be oxidized by the same mechanism than the very more reactive, since dialkyl-substituted, electron-enriched 2,4-di-t-butyl phenol we previously studied [4,10].

One can suppose that particular experimental conditions (temperature, ligand environment, excessive reaction time, analytical method, · · ·) unfortunately precluded the observation of catechol **2** by the authors of Ref. [9].

The subsequent **2** → **4** transformation may be particularly attributed to the presence of additional polydentate amino ligands such as 1,10-phenanthroline or *N,N*-bis[2-(2-pyridyl)ethyl]-benzylamine, inducing an internal redox decomposition of the Cu(II) catecholate into reactive quinone **3**.

[1] M. Maumy and P. Capdevielle, unpublished results.

Moreover, catechol **2'** is effectively oxidized into substituted catechol **4'** under reaction conditions where phenol **1** itself is converted into the Michael addition product **4**. By the way, it is also established that catechol **2'** can be oxidized by an appropriate Cu(II) complex in the presence of phenol **1** into substituted catechols **4'** and **5'**, according to a similar reactivity pattern.

Further investigations are in progress, in order to fully substantiate the mechanism of these reactions.

References

[1] D. A. Robb, in: Copper Proteins and Copper Enzymes, ed. R. Lontié, Vol. 2 (CRC Press, Boca Raton, 1984) p. 207.
[2] W. Brackman and E. Havinga, Rec. Trav. Chim. 74 (1955) 937, 1021, 1070, 1100, 1107.
[3] M. Maumy, P. Capdevielle, P. Dostert and M. Langlois French patent 2504917 (5/4/1981) to Delalande SA.
[4] P. Capdevielle and M. Maumy, Tetrahedron Lett. 23 (1982) 1577.
[5] F. Chioccara, P. Di Gennaro, G. La Monica, R. Sebastiano and B. Rindone, Tetrahedron 47 (1991) 4429.
[6] L. Casella, M. Gullotti, R. Radaelli and P. Di Gennaro, J. Chem. Soc., Chem. Comm. (1991) 1611.
[7] M. Réglier, C. Jorand and B. Waegell, J. Chem. Soc, Chem. Comm. (1990) 1752.
[8] F. Chioccara, G. Chiodini, F. Farina, B. Rindone, R. Sebastiano, J. Molec. Catal. 97 (1995) 187.
[9] L.M. Sayre and D.V. Nadkarni, J. Am. Chem. Soc. 116 (1994) 3157.
[10] P. Capdevielle and M. Maumy, Tetrahedron Lett. 23 (1982) 1573.
[11] E. Heckel, German Patent 1230025 (8/12/1966), Chem. Abstr. 66 (1967) 46487e.
[12] E. Amadei, Thesis, Univ. Aix-Marseille III (1991) 149.
[13] M.M. Rogic and T.R. Demmin, J. Am. Chem. Soc. 100 (1978) 5472.
[14] A.A. Patchett and B. Witkop, J. Org. Chem. 22 (1957) 1477.
[15] P. Capdevielle and M. Maumy, Tetrahedron Lett. 24 (1983) 5611.