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A class of ligands designed as model for apogalactose oxidase

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Summary — Seven tripodal tetradentate ligands bearing one pyridine and two diversely substituted phenolic arms, designed as models for apogalactose oxidase, have been synthesized. Electrochemical one-electron oxidation of acetonitrile solutions of the ligands led to the formation of unstable phenoxyl radical derivatives. This study allows to determine which of the ligands are most suited for modeling the active site in galactose oxidase.

tripodal ligand / one-electron electrochemical oxidation / phenoxyl radical / galactose oxidase

Résumé — **Une classe de ligands, modèles de l'apogalactose oxydase.** Sept ligands tétradentés tripodaux comportant une pyridine et deux bras phénoliques diversement substitués, conçus comme modèles de l'apogalactose oxydase, ont été préparés. L'oxydation à un électron par voie électrochimique dans l'acétonitrile conduit à la formation d'un radical phénoxyle instable. Cette étude permet de déterminer quels sont les ligands les mieux adaptés pour préparer des modèles du site actif de la galactose oxydase.

ligand tripodal / oxydation électrochimique à un électron / radical phénoxyle / galactose oxydase

Introduction

Galactose oxidase (GOase) is a copper enzyme that catalyzes the oxidation of primary alcohols to aldehydes coupled to the reduction of molecular oxygen to hydrogen peroxide [1]. GOase is a mononuclear copper (II) protein with a N_2O_2 coordination sphere (two nitrogen from histidine imidazoles and two phenolate oxygen from tyrosines) with a little exogenous equatorial ligand (water at pH 7) completing a square pyramidal coordination environment [2] (fig 1). The one-electron oxidized active form of the enzyme harbours a stable tyrosyl free radical bound to the copper (II) center. This radical arises from the equatorial tyrosyl ligand which is covalently linked to a cysteine residue by a C–S bond *ortho* to the hydroxyl group. The major role of this thioether group is the lowering of the oxidation potential of the tyrosine, stabilizing the phenoxyl oxidation product by several hundred millivolts [1].

Some complexes, involving a copper coordination sphere of the same type as in the enzyme, have been described as structural models of the active site in GOase [3-11]. Other complexes involving a different copper coordination sphere have been described as functional models [12, 13]. The data from Whittaker et al [8] and our own results [10, 11] have emphasized the drastic influence of the substitution of the phenolic moieties on the redox properties of the models. Thus the study of diversely substituted free ligands belonging to a same series, seemed to us to be an essential prerequisite for the understanding and, consequently, the control of the redox properties of the corresponding copper complexes. In this paper, we describe studies of seven tripodal ligands (L^1H_2 to L^7H_2) bearing one pyridyl and two diversely substituted phenolic arms (fig 2). In order to make the phenoxyl radical more accessible by introducing more easily oxidizable phenolate moieties, we prepared the ligands OCH_3 and SCH_3 substituted. The introduction of *ortho-tert*-butyl groups in the phenolic arms prevents the formation in the solution of bis-μ-phenoxo dicopper complexes [11]. The C–C intercoupling of the generated phenoxyl radical and then its stabilization can be avoided by introducing additional groups in the *para* positions of the phenolic arms. To discriminate between the two phenolic arms (as is the case for the enzyme), L^1H_2 and L^7H_2 have been designed, in which one of the phenolic arms bears an electron-withdrawing group and the other an electron-donating group. The copper complexes of L^1H_2, L^2H_2 and L^3H_2 have been previously described by us [10, 11]. The ligand of the copper complex studied by Whittaker et al [8], which is of the same type as L^1–$^7H_2$, is reported for comparison (L^8H_2). On the other hand, the redox properties of simple monophenolic derivatives have been described [14-18]. Physicochemical studies (theoretical, resonance Raman and ESR spectroscopies)

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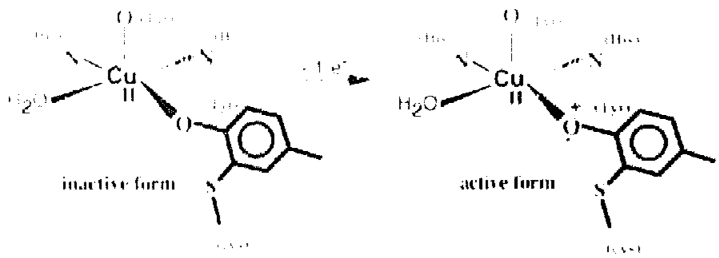

Fig. 1. The active site in galactose oxidase

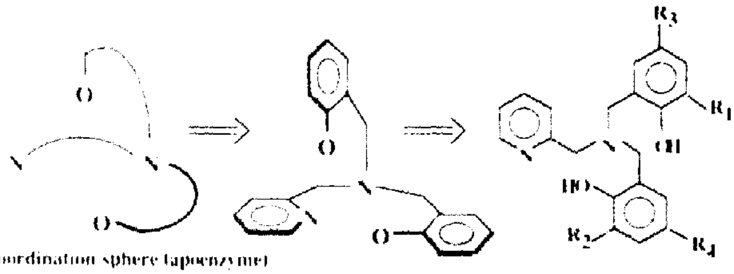

Fig. 2. Design of the ligand

L^1H_2: $R_1 = R_2 = R_3 = R_4 = H$
L^2H_2: $R_1 = R_2 = R_3 = H$; $R_4 = SCH_3$
L^3H_2: $R_1 = R_2 = R_4 = H$; $R_3 = NO_2$
L^4H_2: $R_1 = R_2 = H$; $R_3 = NO_2$; $R_4 = SCH_3$
L^5H_2: $R_1 = R_2 = tBu$; $R_3 = R_4 = H$
L^6H_2: $R_1 = R_2 = tBu$; $R_3 = R_4 = OCH_3$
L^7H_2: $R_1 = R_2 = tBu$; $R_3 = NO_2$; $R_4 = OCH_3$
L^8H_2: $R_1 = R_2 = R_3 = CH_3$; $R_4 = SCH_3$ (from [8])

have been reported for simple phenoxyl radicals including tyrosyl generated by chemical or photochemical oxidation or by pulse radiolysis [7, 19, 20]. The stable tyrosyl radical of apogalactose oxidase has been studied by Whittaker et al. [2].

Experimental section

Materials and methods

Commercial reagents (Aldrich) were used as obtained without further purification. Solvents were purified by standard methods before use. Triethylamine was distilled and stored over potassium hydroxide. Elemental analyses were performed by the CNRS Microanalytical Laboratory of Lyon, France.

Spectroscopy

¹H and ¹³C NMR spectra were recorded on a Bruker AC 200 spectrometer at 25 °C using $CDCl_3$ as solvent. Chemical shifts were measured in parts per million from internal TMS. Fast atom bombardment (FAB) mass spectra on the positive mode were recorded on a Nermag R-1010C apparatus equipped with an M-scan Wallis atom gun (8 kV, 20 μA). UV-visible spectra were obtained using a UVIKON 930 spectrophotometer operating in the range 200–900 nm with quartz cells. Infrared spectra were recorded as KBr pellets [illegible] a Nicolet 5SXC FTIR [illegible] Fourier [illegible] spectrophotometer [illegible].

Electrochemistry

Electrochemical experiments were carried out using a PAR model 273 potentiostat equipped with a Kipp-Zonen x-y recorder. All experiments were run at room temperature under an argon atmosphere. For analytical experiments in millimolar ligand solutions, a standard three-electrode cell was used. Potentials are referred to an Ag/10 mM AgNO₃ reference electrode with 0.1 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte in acetonitrile. Platinum disk electrodes (5 mm diameter) were polished with 1 μm diamond paste. A platinum plate (2 × 2 cm) was used as working electrode for electrolysis at controlled potential. Electronic absorption spectra recorded during electrolysis were obtained using a diode array spectrophotometer HP 8452 A through optic fiber in a glovebox.

Syntheses

The syntheses of L^1H_2, L^3H_2 and L^5H_2 have been previously described by us [10, 11].

- L^2H_2: [illegible]

2-(2-Hydroxybenzyl)-2-pyridylmethylamine (1.07 g, 5 mmol) prepared according to Uma et al. [?] and 2-(chloromethyl)-6-nitrophenol (0.945 g, 5 mmol) were refluxed in dry THF (25 ml) in the presence of triethylamine (0.75 g, 7.5 mmol) for 24 h under an inert atmosphere. The solution was filtered and the solvent was removed in vacuo. L^2H_2 was purified by column chromatography on silica gel and elution with ethyl

acetate hexane (yield: 91%). $\mathbf{L^1H_2}$ is a white solid (Mp: 149–150 °C).

^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 11.1 (2H, s, OH groups), 8.67 (1H, d, J = 4.9 Hz), 8.10 (1H, d, J = 8.9, 2.3 Hz), 8.04 (1H, d, J = 2.8 Hz), 7.77 (1H, ddd, J = 7.9, 7.9, 1.8 Hz), 7.34 (1H, dd, J = 5.4, 1.7 Hz), 7.18 (2H, m), 7.07 (1H, d, J = 6.6 Hz), 6.96 (1H, d, J = 8.9 Hz), 6.87 (d, 1H, d, J = 7.9 Hz), 6.81 (1H, t, J = 7.9 Hz), 3.98 (2H, s), 3.92 (2H, s), 3.90 (2H, s).

^{13}C NMR ($CDCl_3$), δ (ppm): 163.9 (s), 157.0 (s), 155.5 (s), 147.9 (d), 138.1 (d), 130.2 (d), 129.7 (d), 126.5 (d), 125.8 (d), 123.3 (d), 122.9 (d), 122.0 (d), 120.8 (d), 119.5 (d), 117.5 (d), 116.9 (d), 56.8 (t), 56.2 (t), 56.0 (t).

FAB$^+$ MS (NBA): m/z = 366 [M^+ + 1], 273 [M^+ – phenol], 213 [M^+ – methyl-nitrophenol] 158 [M^+ – methylphenol], 154 [methyl-nitrophenol], 107 [methylphenol], 93 [phenol].

Anal (%): calc for $C_{20}H_{19}N_3O_4$: C, 65.74; H, 5.24; N, 11.50; found: C, 65.74; H, 5.27; N, 11.58.

• L^1H_2: *(2-Pyridylmethyl) (2-hydroxy-5-nitrobenzyl) [2-hydroxy-5-(methylthio)benzyl] amine*

[2-Hydroxy-5-(methylthio)benzyl](2-pyridylmethyl)amine [10] (1.3 g, 5 mmol) and 2-(chloromethyl)-4-nitrophenol (0.935 g, 5 mmol) were refluxed in dry THF (25 mL) in the presence of triethylamine (0.56 g, 5.5 mmol) for 24 h under an inert atmosphere. The solution was filtered and the solvent was removed in vacuo. $\mathbf{L^1H_2}$ was purified by column chromatography on silica gel and elution with ethyl acetate hexane (yield: 89%). $\mathbf{L^1H_2}$ is a white solid (Mp: 174–176 °C).

^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 8.67 (1H, d, J = 5.2 Hz), 8.11 (1H, dd, J = 8.9, 2.5 Hz), 8.01 (1H, d, J = 2.5 Hz), 7.78 (1H, t, J = 7.1 Hz), 7.35 (1H, dd, J = 7.2, 5.2 Hz), 7.20 (2H, m), 7.08 (1H, d, J = 1.9 Hz), 6.96 (1H, d, J = 8.9 Hz), 6.84 (1H, d, J = 8.4 Hz), 3.98 (2H, s), 3.92 (2H, s), 3.86 (2H, s), 2.42 (3H, s)

^{13}C NMR ($CDCl_3$), δ (ppm): 163.7 (s), 155.6 (s), 155.4 (s), 147.8 (d), 139.9 (s), 138.1 (d), 130.0 (d), 130.5 (d), 127.1 (s), 126.1 (d), 125.8 (d), 123.3 (d), 122.9 (d), 121.8 (s), 121.6 (s), 117.7 (d), 117.5 (d), 56.5 (t), 56.2 (t), 55.9 (t), 18.1 (q).

FAB$^+$ MS (NBA): m/z = 412 [M^+ + 1].

Anal (%): calc for $C_{21}H_{21}N_3SO_4$: C, 61.30; H, 5.11; N, 10.21; S, 7.79; found: C, 61.91; H, 5.18; N, 10.07; S, 7.79.

• L^1H_2: *(2-Pyridylmethyl) bis(2-hydroxy-3-tert-butyl-5-methoxy)benzylamine*

The compound has been prepared according to figure 3. The aldehyde "**A**" has been obtained as a yellow solid (yield: 93 %; Mp < 50 °C) by using a known experimental procedure [28].

^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 11.50 (s, 1H), 9.83 (s, 1H), 7.17 (d, 1H, J = 3 Hz), 6.81 (d, 1H, J = 3 Hz), 3.81 (s, 3H), 1.41 (s, 9H).

^{13}C NMR ($CDCl_3$), δ (ppm): 196.5 (s), 156.1 (s), 152.0 (s), 140.0 (s), 123.7 (d), 119.7 (s), 111.6 (d), 55.6 (q), 34.8 (s), 29 (q).

FAB$^+$ MS (NBA): m/z = 208 [M^+], 193 [M^+ – CH_3].

"**B**" was obtained as a white solid (Mp: 80 °C, yield: 91%) from "**A**" by using a usual procedure

^{1}H NMR ($CDCl_3$, 250 MHz) δ (ppm): 8.57 (1H, dd, J = 8 and 1.5 Hz), 7.65 (1H, ddd, J = 8, 7.8 and 1.5 Hz), 7.20 (1H, d, J = 7.8 Hz), 7.19 (1H, d, J = 7.25 Hz), 6.81 (1H, d, J = 3 Hz), 6.41 (1H, d, J = 3 Hz), 3.96 (2H, s), 3.90 (2H, s), 3.72 (3H, s), 1.41 (9H, s).

^{13}C NMR ($CDCl_3$), δ (ppm): 157.8 (s), 151.5 (s), 149.36 (s), 147.94 (s), 136.6 (d), 122.8 (s), 122.63 (d), 122.3 (d), 112.65 (d), 111 (d), 55.6 (q), 53.01 (t), 52.18 (t), 34.8 (s), 29.36 (q).

FAB$^+$ MS (NBA): m/z = 300 [M^+].

"**C**" was obtained from "**A**" as a colourless oil (yield: 89%).

^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 7.35 (1H, s), 6.83 (1H, d, J = 3 Hz), 6.39 (1H, d, J = 3 Hz), 4.76 (2H, s), 3.71 (3H, s), 2.52 (1H, broad), 1.40 (9H, s)

^{13}C NMR ($CDCl_3$), δ (ppm): 153.06 (s), 149.3 (s), 148.6 (s), 125.1 (s), 113.6 (d), 110.1 (d), 65.1 (t), 55.7 (q), 34.8 (s), 29.1 (q)

FAB$^+$ MS (NBA): m/z = 210 [M^+], 192 [M^+ – 18].

"**D**" was obtained from "**C**" as a yellow oil (yield: 98%)

^{1}H NMR ($CDCl_3$, 250 MHz) δ (ppm): 6.94 (1H, d, J = 3 Hz), 6.61 (1H, d, J = 3 Hz), 5.10 (1H, broad s), 1.65 (2H, s), 3.76 (3H, s), 1.41 (9H, s).

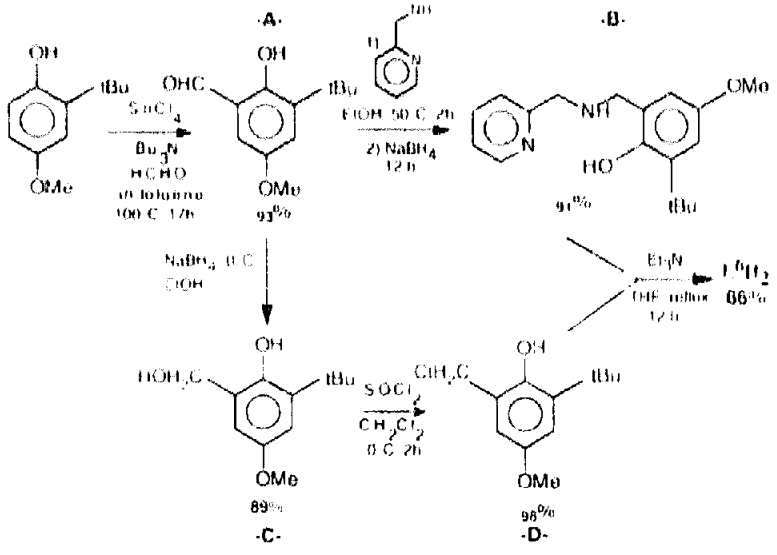

Fig 3. Synthetic pathway to L^1H_2

^{13}C NMR (CDCl$_3$ [illegible]) δ (ppm): 172.9 (s), 147.6 (s), 140.6 (s) [illegible] 127.5 (d), 77.5 (q) [illegible] 115.9 (d), 31.8 [illegible]

[illegible] 228 [illegible] 152 [illegible] 177 [illegible] CH_2Cl [illegible]

L'H was synthesized according to the procedure used for **L'H** and **L'H$_2$** by reacting **B** (2.3 g, 7.6 mmol), triethylamine (1.54 g, 15.3 mmol) and **D** (1.74 g, 7.6 mmol) in THF (100 mL). It was recrystallized in hexane/CH_2Cl_2 (90/10) as a white solid (yield 66%, Mp 170–172 °C).

^{1}H NMR (CDCl$_3$, 200 MHz) δ (ppm): 10.27 (2H, broad s), 8.68 (1H, ddd, *J* = 5.11, 1.03 and 0.7 Hz), 7.70 (1H, ddd, *J* = 7.7, 7.7 and 1.72 Hz), 7.29 (1H, ddd, *J* = 7.6, 5.11 and 1.03 Hz), 7.12 (1H, d, *J* = 7.8 Hz), 6.82 (2H, d, *J* = 3.05 Hz), 6.50 (2H, d, *J* = 3.05 Hz), 3.79 (2H, s), 3.72 (3H, s), 3.71 (6H, s), 1.39 (18H, s).

^{13}C NMR (CDCl$_3$) δ (ppm): 155.6 (s), 151.6 (s), 150.08 (s), 148.00 (d), 138.7 (s), 137.1 (d), 123.9 (d), 122.7 (s), 113.1 (d), 112.2 (d), 56.1 (q), 55.6 (t), 51.8 (t), 35.0 (s), 29.1 (q).

FAB$^+$ MS (NBA) [illegible] 592 [illegible]

Anal. [illegible] for [illegible]N_2O_3: C 73.11, H 8.18, N 5.69 [illegible] found C 72.96, H 8.29, N 5.76.

• ***L'H$_2$*** *[illegible] 2-Pyridylmethyl [illegible] 2-hydroxy-5-tert-butyl-5-methoxyphenyl [illegible] 2-hydroxy-5-tert-butyl [illegible] amine*

[illegible] compound has been prepared according to Figure 4. Nitration [illegible] by a classical procedure of 2-methyl-6-tert-butylphenol **E** gave the 4-nitro derivative **F** (yield 70%, Mp 137–138 °C).

^{1}H NMR (CDCl$_3$, 200 MHz) δ (ppm): 8.10 (1H, d, *J* = 2.7 Hz), 7.96 (1H, d, *J* = 2.7 Hz) [illegible] 3H [illegible] 9H [illegible]

[illegible] δ (ppm): [illegible]

FAB [illegible] MS (NBA) [illegible]

F [illegible] derivative **G** [illegible] with Ac_2O in the presence of triethylamine (yield 85%, Mp 85 °C).

^{1}H NMR (CDCl$_3$, 200 MHz) δ (ppm): 8.15 (1H, d, *J* = 2.7 Hz), 8.0 (1H, d, *J* = 2.7 Hz), 2.40 (3H, s), 2.21 (3H, s), 1.38 (9H, s).

^{13}C NMR (CDCl$_3$) δ (ppm): 168.0 (s), 153.1 (s), 145.0 (s), 143.3 (s), 124.1 (d), 120.7 (d), 35.1 (s), 30.1 (q), 21.2 (q), 17.1 (q).

Then, 9.22 g (36.7 mmol) of **G** and 6.51 g (36.7 mmol) of *N*-bromosuccinimide (in the presence of traces of benzoyl peroxide) in CCl_4 (250 mL) were refluxed under argon for 38 h; after filtration, removing of the solvent gave an oil which was crystallized in diethyl ether and then recrystallized in methanol leading to pure **H** (yield: 56%, Mp 124–125 °C).

^{1}H NMR (CDCl$_3$, 200 MHz) δ (ppm): 8.29 (1H, d, *J* = 2.5 Hz), 8.21 (1H, d, *J* = 2.5 Hz), 4.32 (2H, s), 2.46 (3H, s), 2.21 (3H, s), 1.40 (9H, s).

^{13}C NMR (CDCl$_3$) δ (ppm): 166.1 (s), 153 (s), 145.2 (s), 144.6 (s), 133 (s), 124.4 (d), 123.1 (d), 35.1 (s), 30.1 (q), 26.6 (t), 21.3 (q).

FAB$^+$ MS (NBA) *m/z* = 331 [M$^+$ + 1].

1.64 g (4.96 mmol) of **H** in dry THF (40 mL) was added dropwise to a solution of 0.54 g (4.96 mmol) of (2-pyridylmethyl)amine in 80 mL of dry THF containing 0.85 mL (6 mmol) of triethylamine. The solution was refluxed for 15 h; after filtration and removal of the solvent, the residue was dissolved in a pentane/ether mixture. **I** precipitated as a yellow powder which was filtered (yield 96%, Mp 147 °C).

^{1}H NMR (CDCl$_3$, 200 MHz) δ (ppm): 11.12 (1H, broad s), 8.60 (1H, ddd, *J* = 4.46, 1.47 and 0.69 Hz), 8.15 (1H, d, *J* = 2.75 Hz), 7.81 (1H, d, *J* = 2.75 Hz), 7.70 (1H, ddd, *J* = 7.88, 7.88 and 2.05 Hz), 7.26 (1H, dd, *J* = 7.54 and 0.69 Hz), 7.20 (1H, m, *J* = 7.54 Hz), 4.08 (2H, s), 4.54 (2H, s), 2.27 (3H, s), 1.42 (9H, s).

^{13}C NMR (CDCl$_3$) δ (ppm): 174.6 (s), 164.8 (s), 154.5 (s), 150 (d), 138 (s), 138.7 (s), 137 (d), 124.4 (d), 123.5 (d) [illegible] 121.2 (d) [illegible] (q), 29 (q), 21 (q).

FAB$^+$ MS (NBA) [illegible]

[illegible] of **I** [illegible] HCl [illegible] reflux [illegible] followed by extraction with ethyl acetate led after removing of the solvent to a black oil which was purified by column

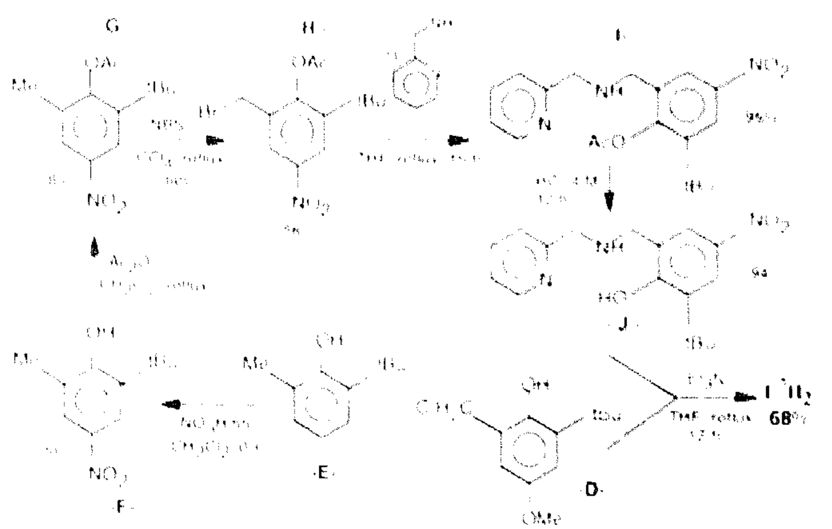

Fig. 4. Synthetic pathway [illegible] L'H

chromatography on silica gel (elution with ethyl acetate/hexanes) and recrystallized to afford pure **J** (yield: 91%, Mp: 85–86 °C).

^{1}H NMR (CDCl$_3$, 200 MHz) δ (ppm): 8.60 (1H, d, J = 1.1 Hz), 8.11 (1H, d, J = 2.75 Hz), 7.80 (1H, d, J = 2.75 Hz), 7.69 (1H, ddd, J = 7.7, 7.6 and 1.07 Hz), 7.25 (2H, m, J = 7.7, 6.5 Hz), 4.06 (2H, s), 3.93 (2H, s), 1.43 (9H, s).

^{13}C NMR (CDCl$_3$), δ (ppm): 164.2 (s), 156.7 (s), 149.5 (d), 139.4 (s), 137.7 (s), 136.8 (d), 122.6 (d), 122.4 (d), 122.1 (s), 52.4 (t), 51.6 (t), 31.9 (s), 29.0 (q).

FAB$^+$ MS (NBA): m/z = 316 (M^+ + 1).

L^7H_2 was synthesized according to an adapted procedure used for **L^6H_2**, **L^8H_2** and **$L'H_2$** by reacting **J** (2.5 g, 8 mmol), triethylamine (0.858 g, 8.5 mmol) and **D** (1.86 g, 8.9 mmol) in THF (130 mL) which were refluxed overnight under an argon atmosphere. It was purified on a silica gel column (elution with ethyl acetate/hexane) leading to a yellow powder (yield: 68%, Mp: 175 °C) as a chlorhydrate. Free **L^7H_2** was obtained upon washing with KOH 1 M, extraction with ether and removal of the solvent (Mp: 90–110 °C).

^{1}H NMR (CDCl$_3$, 200 MHz) δ (ppm): 11.21 (2H, broad s), 8.70 (1H, ddd, J = 5.11, 1.7 and 0.7 Hz), 8.15 (1H, d, J = 2.8 Hz), 7.91 (1H, d, J = 2.8 Hz), 7.71 (1H, ddd, J = 7.7, 7.7 and 1.70 Hz), 7.32 (1H, ddd, J = 7.5, 5.1, 1.03 and 0.7 Hz), 7.11 (1H, dd, J = 7.8 and 1.03 Hz), 6.81 (1H, d, J = 3.0 Hz), 6.51 (1H, d, J = 3.0 Hz), 3.89 (2H, s), 3.87 (2H, s), 3.82 (2H, s), 3.75 (4H, s), 1.41 (9H, s), 1.41 (9H, s).

^{13}C NMR (CDCl$_3$, δ (ppm): 163 (s), 155.5 (s), 151.7 (s), 149.8 (s), 147.8 (d), 139.1 (s), 138.8 (s), 138.3 (s), 137.8 (d), 123.5 (d), 123.2 (s), 123 (d), 122.7 (s), 122.2 (s), 121.7 (s), 113.6 (s), 112.2 (d), 56.7 (t), 56.1 (t), 55.6 (q), 55.4 (t), 45.1 (s), 44.9 (s), 29.2 (q), 29 (q).

FAB$^+$ MS (NBA): m/z = 507 (M^+), 506 (M^+ − 1).

Anal. Calcd for $C_{29}H_{38}N_4O_2$: C, 68.62; H, 7.55; N, 11.04. Found: C, 68.15; H, 7.71; N, 7.96.

Results and discussion (one-electron oxidation studies)

The electrochemical oxidation of phenolic derivatives has been widely investigated [18]. It has been established that important intermediates in the anodic oxidation such as phenoxyl radicals are highly reactive species whose diverse chemical reactions complicate the electrochemical response from the point of view of the mechanistic aspects as well as the electrosynthetic products. However, there seems to be general agreement on the scheme depicted in figure 5, for the first steps in the anodic oxidation of the phenolic compounds [18].

Phenolic compounds having free *ortho* or *para* positions (as **$L^{1-7}H_2$**) undergo upon anodic oxidation a C–C coupling quickly leading to dimers or polymers. When blocking substituents are present in the 2, 4 and 6 positions, a more stable solution of the one-electron oxidized species, a phenoxyl radical, can be obtained under suitable conditions.

The electrochemical study described in this paper focuses on the first one-electron oxidation of the phenolic arms of **$L^{1-7}H_2$** in view of studying the modulation of this property by the various substituting groups. This study may allow to predict the ease of the one-electron oxidation of the corresponding copper (II) complexes owing to the fact that the oxidation potential of the protonated free ligand is close to that of the corresponding copper (II) complex (protonation parallels metallation) [8, 11].

The first step of the anodic oxidation of **$L^{1-7}H_2$**, studied by cyclic voltammetry in CH_3CN + TBAP 0.1 M, is characterized by an irreversible peak (see fig. 6 (left) for **L^7H_2** as an example). The anodic peak potentials, E_{pa}, are reported in table I.

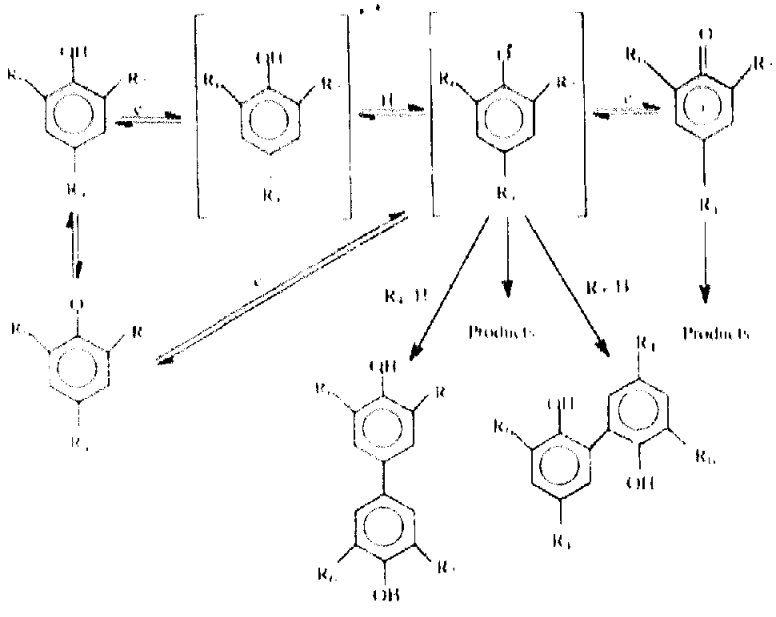

Fig. 5. Anodic mechanism for the oxidation of phenolic derivatives [18].

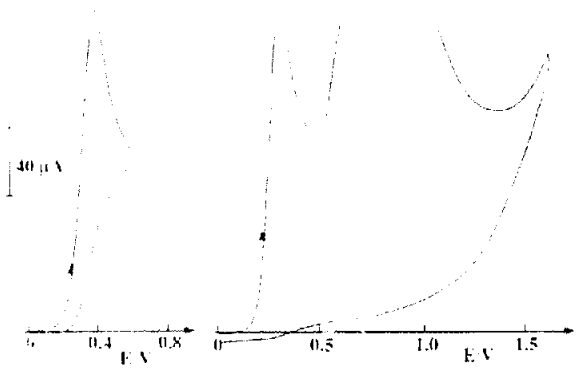

Fig. 6. Cyclic voltammetry of L^5H_2, 8.7 mM in CH_3CN + TBAP 0.1 M on a Pt electrode (5 mm diameter); scan rate: 0.1 V s^{-1}; E vs. $Ag/AgNO_3$ 10 mM + CH_3CN + TBAP 0.1 M.

Table I. Anodic peak potential[a], E_{pa}, for the first oxidation of L^1–$^7H_2$ in CH_3CN + TBAP 0.1 M.

Ligand	L^1H_2	L^2H_2	L^3H_2	L^4H_2	L^5H_2	L^6H_2	L^7H_2
E_{pa}, V	0.72	0.42	0.91	0.46	0.65	0.48	0.45

[a] 0.1 V s^{-1}; V vs. Ag, $AgNO_3$ 10 mM in CH_3CN + TBAP 0.1 M.

Exhaustive potentiostatic electrolysis at a potential slightly higher than the E_{pa} of these ligands's give one exchanged electron per molecule, in agreement with the formation of a phenoxyl radical in a first step. However, one electron exchanged per molecule seems a surprising result for symmetrical ligands such as L^1H_2, L^3H_2 or L^4H_2. Thus, the first step of the oxidation of one phenolic arm leading to the corresponding radical cation perturbates the redox properties of the second identical phenolic arm of these ligands. In addition, the resulting solutions are EPR-silent, confirming the instability of the phenoxyl radicals in our experimental conditions and the irreversibility of the electrochemical process.

The E_{pa} values of table I are in agreement with the electron-donating or -withdrawing character of the substituents on the phenolic arms. Compared to L^1H_2, the substitution by electron-donating groups such as SCH_3 (L^2H_2), L^3H_2 or OCH_3 (L^6H_2, L^7H_2) decreases E_{pa} by 0.3 V and, to a smaller extent, by 0.1 V after substitution by a *tert*-Bu group (L^4H_2). The substitution by an electron-withdrawing group such as NO_2 (L^5H_2) increases E_{pa} by 0.2 V. In the case of the unsymmetrical ligands (L^2–$^7H_2$, L^3H_2), the first one-electron transfer is presumably undergone by the more oxidizable phenolic arm, *i.e.* by the non-substituted phenolic arm in L^5H_2 or by the phenolic moiety substituted by the electron-donating group in L^2H_2, L^3H_2 and L^7H_2.

Further oxidations of L^1–$^7H_2$ occur at higher potentials (see fig. 6, right) for L^5H_2 as an example). For all these ligands, this anodic process corresponds to a multi-step electron transfer, corresponding to the overoxidation of the first oxidized phenolic moiety, in addition to the oxidation of the second phenolic arm and of the substituent.

The instability of the one-electron oxidized species does not allow the obtention of well-resolved EPR spectra. A change in the electronic spectra has been observed during the exhaustive oxidation of the ligands (see fig 7 for L^5H_2 as an example). The emergence of a new and transient strong band in the range from 419 nm for L^7H_2 to 428 nm for L^5H_2 is in accordance with the formation of a phenoxyl radical [17, 20].

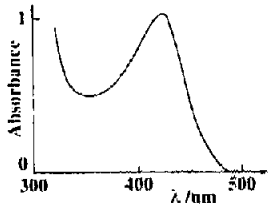

Fig. 7. UV-visible spectrum of L^5H_2 (0.35 mM in CH_3CN + TBAP 0.1 M) recorded during electrolysis (0.75 electron consumed per molecule); the *y* scale is arbitrary due to the instability of the oxidized L^5H_2.

Even at low temperature (UV irradiation of the ligands at 77 K as described in [7]), the instability of the radicals does not allow the obtention of well-resolved EPR spectra.

Conclusion

We have previously shown that *ortho*-*tert*-butyl groups prevent the formation of binuclear complexes in solution [11]. The most easily one-electron oxidizable ligands are L^2H_2, L^4H_2, L^6H_2 and L^7H_2, ie. ligands bearing an electron-donating group on at least one of the phenolic arms. Among them, L^6H_2 and L^7H_2 are *o*-, *p*-disubstituted on the phenolic arms, with a *tert*-butyl group in the *ortho* position. These two ligands clearly seem to be the most suited for the modelling of the oxidized active form of galactose oxidase. Moreover, the substitution of the *ortho* and *para* position of the hydroxyl group of the phenolic arms prevents the carbon-carbon intercoupling of the resonance forms of the radical. L^7H_2, which involves two different phenolic arms, allows the discrimination of the two phenolic arms: if the methoxysubstituted one, which is more readily oxidizable, is in an equatorial position in the copper (II) complex, an EPR-silent spectrum is expected (antiferromagnetic coupling) and if it is in the axial position, a ferromagnetic coupling is expected (see [11]). Further investigations are now in progress concerning the copper (II) complexes from L^6H_2 and L^7H_2.

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