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Redox-active metal(II) complexes of sterically hindered phenolic ligands: Antibacterial activity and reduction of cytochrome *c*. Part III. Copper(II) complexes of cycloaminomethyl derivatives of *o*-diphenols

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ABSTRACT

Redox-active copper(II) complexes of sterically hindered phenolic ligands have been synthesized using 5-*tert*-butyl-3-(pyrrolidinomethyl)-1,2-dihydroxybenzene (HL^{I}), 5-*tert*-butyl-3-(piperidinomethyl)-1,2-dihydroxybenzene (HL^{II}), 5-*tert*-butyl-3-(azepanylmethyl)-1,2-dihydroxybenzene (HL^{III}), 5-*tert*-butyl-3-(morpholinomethyl)-1,2-dihydroxybenzene (HL^{IV}), and 5-*tert*-butyl-3-(methylpiperazinomethyl)-1,2-dihydroxybenzene (HL^{V}). The novel compounds have been characterized by means of chemical and physico-chemical methods. The coordination core of these complexes is a square planar chromophore, $[CuO_2N_2]$, and the phenolic ligands coordinate in their monoanionic forms. The ligands and Cu(II) complexes have been screened for their antibacterial activity. The lowest MIC value (0.020 μmol ml^{-1}) has been found for $Cu(L^{III})_2$ and $Cu(L^{V})_2$ against *Mycobacterium smegmatis*, *Sarcina lutea* and *Staphylococcus aureus*, and this is comparable to the value for chloramphenicol. Their antibacterial activities were found to follow the order: (1) $HL^{I} > HL^{V} \geqslant HL^{III} \sim HL^{II} > HL^{IV}$; (2) $Cu(L^{III})_2 > Cu(L^{II})_2 \sim Cu(L^{I})_2 \sim Cu(L^{V})_2 > Cu(L^{IV})_2$; their reducing ability (determined electrochemically) followed the same order. The most bioactive complex, $Cu(L^{III})_2$, has the highest lipophilicity. A spectrophotometric investigation was carried out in order to estimate the rate of the reduction of bovine heart cytochrome *c* with the ligands and their Cu(II) complexes. HL^{I} and the complex $Cu(L^{III})_2$ have the highest reducing abilities (determined electrochemically), which are characterized by the highest Cyt *c* reduction rates respectively amongst the ligands and Cu(II) complexes.

1. Introduction

In earlier investigations, using the method of cyclic voltammetry, we have shown some mono- and di-substituted derivatives of sterically hindered phenolic ligands as well as their metal complexes to have a pronounced reducing ability, correlating with the antimicrobial activity and rate of reduction, of bovine heart cytochrome *c* (Cyt *c*) in a limited series of these compounds [1–4]. These results prompted us to extend our research to other sterically hindered phenolic ligands and their metal complexes in order to gain greater insight into what structural modifications of these compounds play an important role in their reducing ability and biological activity. For this purpose we chose the Mannich reaction because it is one of the main tools for the development of bioactive compounds [5]. The aim of modifying phenolic ligands was to produce novel redox-active ligands for complexation with transition metals and to further evaluate the changes in antibacterial activity and the rate of the reduction of Cyt *c* for both the phenolic ligands and their metal complexes.

Copper is known as an essential element, participating in many biological processes, and the role of Cu(II) complexation in enhancing the pharmacological profile of the antimicrobial activity of some drugs and bioactive compounds is known [6,7]. In this connection it is of interest to study various aspects of the coordination chemistry of Cu(II) ions interacting with phenolic ligands, and to investigate the physico-chemical and biological properties of the complexes synthesized.

In the present work, the reduction of Cyt *c* was investigated spectrophotometrically with five cycloaminomethyl derivatives of sterically hindered *o*-diphenols, namely 5-*tert*-butyl-3-(pyrro-

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lidinomethyl)-1,2-dihydroxybenzene (HL^I), 5-*tert*-butyl-3-(piperidinomethyl)-1,2-dihydroxybenzene (HL^{II}), 5-*tert*-butyl-3-(azepanylmethyl)-1,2-dihydroxybenzene (HL^{III}), 5-*tert*-butyl-3-(morpholinomethyl)-1,2-dihydroxybenzene (HL^{IV}) and 5-*tert*-butyl-3-(methylpiperazinomethyl)-1,2-dihydroxybenzene (HL^V) (see Section 2.2), as well as with their Cu(II) complexes. For the first time, the redox properties of the above-mentioned ligands and their Cu(II) complexes were determined electrochemically. As before [3,4], the results obtained are discussed in view of the presumed correlation between the capability of the compounds under study for reducing Cyt *c*, their antibacterial activity, redox properties, determined electrochemically, and lipophilicity.

2. Experimental

2.1. Materials and methods

All the reagents used for the synthesis of the cycloaminomethyl derivatives of sterically hindered *o*-diphenols HL^I–HL^V and their complexes are commercially available and were used without further purification. The synthesis of the above-mentioned ligands and their Cu(II) complexes was performed as described in Sections 2.2 and 2.3. The purity of the *o*-diphenol derivatives HL^I–HL^V was checked by Thin Layer Chromatography (TLC). Elemental analyses were carried out with a Vario EL (CHNS mode) instrument. Copper was determined using an atomic emission spectrometer with an inductively coupled plasma excitation source (Spectroflame Modula). Infrared spectra of solids were recorded with a Nicolet 380 spectrometer in the wavelength range 4000–400 cm^{-1} at room temperature, using «Smart Performer». Thermal analysis was performed with a Simultaneous Thermal Analyzer STA 449 C. X-ray Diffraction (XRD) analysis was carried out with an HZG 4A diffractometer (Co Kα radiation, MnO_2-filter). ESR (Electron Spin Resonance) spectra of polycrystalline samples were measured with an ERS-220 X-band spectrometer (9.45 GHz) at room temperature and at 77 K, using 100-kHz field modulation; *g* factors were quoted relative to the standard marker 2,2-diphenyl-1-picrylhydrazyl (DPPH). ^1H NMR spectra were recorded with a Varian Unit Plus 300 MHz spectrometer in $CDCl_3$; chemical shifts were reported in parts per million (ppm) relative to an internal standard of Me_4Si. Mass spectra (EI) were recorded on Shimadzu GCMS-QP 2010 Plus spectrometer. Ultraviolet–Visible (UV–Vis) absorption spectra were recorded with a SPECORD M500 spectrophotometer. The molar conductance of 10^{-3} mol l^{-1} solutions of the Cu(II) complexes in acetonitrile was measured at 20 °C using a TESLA BMS91 conductometer (cell constant 1.0). The lipophilicity test was made by determining the *n*-octanol/water partition coefficient (P_{ow}) [8]. Electrochemical measurements were performed under dry nitrogen in a three-electrode two-compartment electrochemical cell using a glassy-carbon (GC) working electrode, Pt auxiliary electrode and Ag|AgCl|0.1 mol l^{-1} $(C_2H_5)_4NCl$ reference electrode. The supporting electrolyte was 0.1 mol l^{-1} $(C_2H_5)_4NClO_4$. The Ag|AgCl|0.1 mol l^{-1} $(C_2H_5)_4NCl$ reference electrode was calibrated with the ferrocenium|ferrocene redox couple located at $E_{1/2}$ = +0.54 V. Anhydrous acetonitrile was used as the solvent. The $(C_2H_5)_4NClO_4$ and $(C_2H_5)_4NCl$ used to prepare solutions were dried respectively at 80 and 100 °C under vacuum for 3 h. Preparation of solutions and the subsequent filling of the electrochemical cells were carried out in a glove box under dry nitrogen.

2.2. Synthesis of the ligands – cycloaminomethyl derivatives of *o*-diphenols (HL^I–HL^V)

The compounds HL^I–HL^V were synthesized according to the methodology described in the literature [9]. 4-*Tert*-butylcatechol HL (Sigma) (10 mmol), paraformaldehyde (10 mmol), a secondary amine (pyrrolidine, piperidine, hexamethylene amine, morpholine or methylpiperazine) (11 mmol) and isopropyl alcohol (20 ml) were mixed, and the mixture was refluxed for 2–4 h. Then the solution was boiled dry under vacuum, and the residue was recrystallized from petroleum ether (or ethyl acetate) and dried under vacuum.

2.2.1. 5-Tert-butyl-3-(pyrrolidinomethyl)-1,2-dihydroxybenzene (HL^I)

Yield: 72%; M.p.: 132–134 °C. M.wt.: 249.35. *Anal.* Calc. for $C_{15}H_{23}O_2N$: C, 72.25; H, 9.30; N, 5.62. Found: C, 72.17; H, 9.18; N, 5.57%. Mass spectrum (*m/z*, *I*%): 249.25 (M^+, 27), 234.20 (M–CH_3, 14). Prominent IR absorption bands (ν, cm^{-1}): 3409w (O–H), 1608w and 1490m (C=C arom), 1320m, 1301m and 1189s (C–O), 1120m and 1102m (C–N). ^1H NMR δ_H(100 MHz, $CDCl_3$): 7.70 [s, 2H, 2OH], 6.89 [dd, 1H, aromatic H, *J* 0.4, 2.3 Hz], 6.52 [m, 1H, aromatic H], 3.82 [s, 2H, CH_2], 2.67 [m, 4H, $N(CH_2)_2$], 1.87 [m, 4H, $(CH_2)_2$], 1.28 [s, 9H, Me_3C].

2.2.2. 5-Tert-butyl-3-(piperidinomethyl)-1,2-dihydroxybenzene (HL^{II})

Yield: 87%; M.p.: 164–166 °C. M.wt.: 263.38. *Anal.* Calc. for $C_{16}H_{25}O_2N$: C, 72.96; H, 9.57; N, 5.32. Found: C, 73.09; H, 9.79; N, 5.29%. Mass spectrum (*m/z*, *I*%): 263.25 (M^+, 24), 248.20 (M–CH_3, 16). Prominent IR absorption bands (ν, cm^{-1}): 3403 m (O–H), 1609w and 1490m (C=C arom), 1326w, 1307s, 1197s and 1188s (C–O), 1098m and 1029w (C–N). ^1H NMR δ_H(100 MHz, $CDCl_3$): 7.10 [br s, 2H, 2OH], 6.61 [m, 1H, aromatic H], 6.45 [m, 1H, aromatic H], 3.75 [s, 2H, CH_2], 2.60 [m, 4H, $N(CH_2)_2$], 1.67 [m, 6H, $(CH_2)_3$], 1.35 [s, 9H, Me_3C].

2.2.3. 5-Tert-butyl-3-(azepanylmethyl)-1,2-dihydroxybenzene (HL^{III})

Yield: 88%; M.p.: 160–162 °C. M.wt.: 277.41. *Anal.* Calc. for $C_{17}H_{27}O_2N$: C, 73.60; H, 9.81; N, 5.05. Found: C, 73.52; H, 9.64; N, 4.97%. Mass spectrum (*m/z*, *I*%): 277.40 (M^+, 30), 262.35 (M–CH_3, 14). Prominent IR absorption bands (ν, cm^{-1}): 3403 m (O–H), 1606w and 1489 m (C=C arom), 1331w, 1307m, 1224w and 1188s (C–O), 1119w, 1102m and 1063m (C–N). ^1H NMR δ_H(100 - MHz, $CDCl_3$): 7.20 [br s, 2H, 2OH], 6.77 [d, 1H, aromatic H, *J* 2.3 Hz], 6.55 [d, 1H, aromatic H, *J* 2.3 Hz], 3.78 [s, 2H, CH_2], 2.53 [m, 4H, $N(CH_2)_2$], 1.67 [m, 8H, $(CH_2)_4$], 1.27 [s, 9H, Me_3C].

2.2.4. 5-Tert-butyl-3-(morpholinomethyl)-1,2-dihydroxybenzene (HL^{IV})

Yield: 85%; M.p.: 150–152 °C. M.wt.: 265.35. *Anal.* Calc. for $C_{15}H_{23}O_3N$: C, 67.89; H, 8.74; N, 5.28. Found: C, 68.03; H, 8.92; N, 5.17%. Mass spectrum (*m/z*, *I*%): 265.15 (M^+, 49), 250.20 (M–CH_3, 12). Prominent IR absorption bands (ν, cm^{-1}): 3375m (O–H), 1605w and 1493m (C=C arom), 1312m, 1300s, 1228m, 1192m and 1107s (C–O), 1127w, 1071m and 1031m (C–N). ^1H NMR δ_H(100 MHz, $CDCl_3$): 6.77 [d, 1H, *J* 2.3 Hz, aromatic H], 6.56 [d, 1H, *J* 2.3 Hz, aromatic H], 3.71 [m, 4H, $O(CH_2)_2$], 3.66 [c, 2H, CH_2], 2.55 [m, 4H, $N(CH_2)_2$], 1.23 [s, 9H, Me_3C].

2.2.5. 5-Tert-butyl-3-(methylpiperazinomethyl)-1,2-dihydroxybenzene (HL^V)

Yield: 84%; M.p.: 157–158 °C. M.wt.: 278.39. *Anal.* Calc. for $C_{16}H_{26}O_2N_2$: C, 69.03; H, 9.41; N, 10.06. Found: C, 68.94; H, 9.27; N, 9.84%. Mass spectrum (*m/z*, *I*%): 278.35 (M^+, 20), 263.30 (M–CH_3, 4). Prominent IR absorption bands (ν, cm^{-1}): 3038w (O–H), 1594w and 1497w (C=C arom), 1326m, 1299w, 1168m and 1135m (C–O), 1203w, 1082w, 1050w and 1036w (C–N). ^1H NMR δ_H(100 MHz, $CDCl_3$): 6.89 [d, 1H, aromatic H, *J* 2.4 Hz,], 6.82 [br s, 2H, 2OH], 6.52 [d, 1H, aromatic H, *J* 2.4 Hz,], 3.70 [s, 2H, CH_2], 2.55 [m, 8H], 2.32 [s, 3H, NMe], 1.26 [s, 9H, Me_3C].