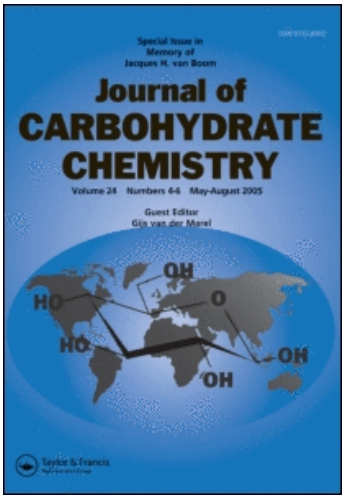

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SUPEROXIDE DISMUTASE MIMETIC ACTIVITY OF THE METAL (II) COMPLEXES OF A DITHIOCARBAMATE DERIVATIVE OF ß-CYCLODEXTRIN[1]

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ABSTRACT

The synthesis of a new ß-cyclodextrin derivative containing a dithiocarbamate group (**3**) by condensation of mono-2-methylamino-2-deoxy-ß-CD (**2**) with CS_2 in the presence of NEt_3 is reported. SOD-mimetic activity was found for the Mn(II) and Cu(II) complexes of **2** and **3** (IC_{50} = 0.76-7.4 µM). To study the influence of the cyclodextrin residue on the catalytic activity of these complexes, a comparison was made with diethylamine and diethyldithiocarbamate complexes. Complexes of **2** and **3** resulted in 1.3 to 11 fold higher activities. An explanation for this observation, in terms of a possible cooperation of the cyclodextrin residue with the catalytic center of the complex, is given.

INTRODUCTION

Cyclodextrins (CDs) are a class of cyclic oligosaccharides composed of α(1→4)-linked D-glucopyranose units in the $^4C_1$ chair conformation. The commonly available CDs have 6, 7 and 8 glucopyranose units and are named α-, ß- and γ-CD, respectively. The overall form of the molecules is a truncated cone with an essentially hydrophobic cavity. Such a structure allows them to form stable inclusion complexes with a wide variety of guests.[2-4]

The synthesis of modified CDs[5] provides new structures with molecular recognition properties and catalytic behavior over several substrates.[6] Some modified CDs have been explored as ligands for transition metal ions in transacylation reactions.[7,8]

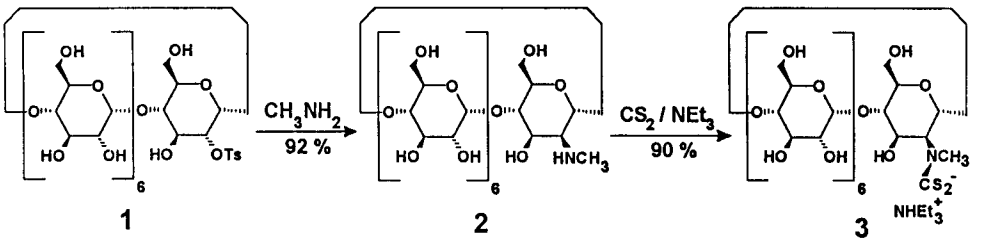
They can act as both recognition sites and catalytic centers, and some examples of cooperativity between the hydrophobic CD cavity and the metal ion have been recently reported.[9,10] The catalytic activity in the disproportionation of superoxide radical ($O_2^{\cdot-}$) has also been studied for the copper (II) complexes of isomeric ß-CD dihistamine derivatives and a dependence of the scavenger activity on the geometry of the complex was found.[11] Such a complex tends to resemble the active site of Cu-Zn-superoxide dismutase (SOD), where a Cu(II) ion is coordinated at the active site to four imidazole nitrogen atoms, coming from His-44, 46, 61 and 118.[12] It was observed that the more tetrahedral complex, as indicated by the EPR parameters, showed the highest catalytic activity. This fact was interpreted by considering that the cyclodextrin moiety seems to aid the dismutation process providing a flexible coordination geometry of the two histamine residues around the Cu(II) ion.

We report here on the synthesis of a new ß-CD derivative containing a dithiocarbamate group appended to its secondary hydroxyl side (**3**) and on the SOD-like activity of its metal(II) complexes. Dithiocarbamates are a class of compounds that show a wide spectrum of biological properties such as antibacterial, [13] cytostatic,[14] antifungal,[15] immunoregulatory,[16] etc. activity. They form stable hydrophobic neutral complexes with transition metals and such a property is related to their biological activity since they can interact with traces of metals that, complexed to bioligands, exist in physiological fluids.

RESULTS AND DISCUSSION

The synthesis of **3** was achieved as shown in Scheme 1.

When the tosyl group substitution by CH_3NH_2 was first attempted in a refluxing solution of mono-2-*O*-tosyl-ß-CD (**1**) in concentrated aqueous methylamine, the formation of mono-3-methylamino-3-deoxy-ß-CD via the 2,3-*manno*-epoxide competed with tosyl group substitution, according to TLC. However, the methylamino-derivative **2** (CDAM) was obtained almost quantitatively by simply stirring the solution overnight at room temperature. The inversion of the C-2 configuration, due to S_N2 substitution, was confirmed by NMR. The condensation of **2** with carbon disulfide was first carried out in the presence of several alkaline catalysts such as NaOH and $Ba(OH)_2$. The resulting products were, in all cases, highly hygroscopic and, therefore, difficult to purify. The use of triethylamine overcame these problems.



Scheme 1

Although **3** shows a protective action against pyrogallol auto-oxidation at pH 7.4 (IC_{50} = 0.16 mM), it does not catalyze the disproportionation of $O_2^{\cdot-}$ even at a 10^{-3} M concentration, presumably due to its anionic character that produces an electrostatic repulsion between $O_2^{\cdot-}$ and **3**. The catalytic activity of its M(II) complexes in the disproportionation of $O_2^{\cdot-}$, commonly expressed in terms of the concentration that inhibits in 50 % the reduction of nitroblue tetrazolium chloride (IC_{50})[11], is summarized in Table 1. The IC_{50} value of SOD was also determined for comparison.

The SOD-like activity of the complexes under study involves a cyclic two-step redox process. For a M(II) complex, catalysis might occur according to one of the following mechanisms:

$$O_2^{\cdot-} + M^{2+} \rightarrow O_2 + M^{+}$$
$$O_2^{\cdot-} + M^{+} + 2H^{+} \rightarrow H_2O_2 + M^{2+}$$

$$O_2^{\cdot-} + M^{2+} + 2H^{+} \rightarrow H_2O_2 + M^{3+}$$
$$O_2^{\cdot-} + M^{3+} \rightarrow O_2 + M^{2+}$$

These processes are thermodynamically governed by the redox potential of the M^{2+}/M^{+} and M^{2+}/M^{3+} pairs, which should have an appropriate value for the dismutation to take place. For our systems, this condition is mostly satisfied by the Cu(II)/Cu(I)[17] and Mn(II)/Mn(III) pairs. Therefore, Cu(II) and Mn(II) complexes should have the highest catalytic activity (see Table 1).

Because CDTC acts as a π-acceptor ligand, their complexes should have a higher catalytic activity than both M-CDAM complexes and 'uncomplexed' metal. An interesting exception for this rule is Cu-CDAM, which appeared to be about 6 fold more active than Cu-CDTC. The SOD-like activity of copper complexes is associated to the Cu(II)/Cu(I) redox potential which is strongly dependent on the nature of the coordinated ligands. Thus,

Table 1. IC_{50} Values[a] for the Studied Metal (II) Complexes.

		LIGAND			
METAL	'uncomplexed' metal	CDAM[b,f]	CDTC[c]	DEAM[d,f]	DDTC[e]
Mn	21	10	7.1	73	11
Co	140	35	90	81	n.d.
Ni	430	100	54	130	n.d.
Cu	2.2	0.76	4.6	8.4	>50[g]
SOD			0.002		

a. Values in μmol/L b. Mono-2-methylamino-2-deoxy-ß-CD (**2**) c. Triethylammonium mono-2-methylamino-2-deoxy-ß-CD-dithiocarbamate (**3**) d. Diethylamine. e. Sodium diethyldithiocarbamate. f. M:amine molar ratio = 1:6. g. The complex precipitates at higher concentrations. n.d. not determined due to low water solubility.

Cu(II) is stabilized by anionic sulfur containing ligands such as mercaptans and dithiocarbamates decreasing the Cu(II)/Cu(I) redox potential to negative values.[18] The Cu(II) reduction is accompanied by a change in coordination geometry from square planar to tetrahedral. In Cu-CDTC, the Cu(II) stabilization by dithiocarbamate binding plus the π -character of the Cu-S bonds provokes a sterically less favorable geometric rearrangement. Therefore, a decrease in catalytic activity is to be expected. The change in the total charge of the complexes from the cationic Cu-CDAM to the neutral Cu-CDTC might also be invoked to explain that the former is more active than the latter in terms of the electrostatic interaction between $O_2^{\cdot-}$ and the complex. These could be the reason why Cu-CDTC is less active than Cu-CDAM.

As has been pointed out for histamine derivatives,[11] the CD residue plays an important role in the catalytic activity of their Cu(II) complexes defining a slightly distorted square planar coordination geometry. This effect favors the geometric rearrangement that occurs during the $O_2^{\cdot-}$ oxidation step and is reflected in the catalytic activity.[11,19] To investigate the possible influence of the CD residue in the catalytic activity of the CDAM and CDTC complexes, we studied the same process under the same conditions but catalyzed by diethylamine (DEAM) and diethyldithiocarbamate (DDTC) complexes. CDAM complexes were in all cases more active than the corresponding DEAM complexes, especially for Mn(II) and Cu(II). In the presence of the latter metal the catalysis was found to be about 11 fold more rapid. Unfortunately, the same comparison but for dithiocarbamate

complexes can only be made for Mn(II) and Cu(II) complexes due to the low water solubility of DDTC complexes. They were 1.5 and more than 11 fold more active than Mn-DDTC and Cu-DDTC, respectively. These results suggest that the CD moiety may assist the catalytic process by fixing the substrate to the active site via hydrogen bonding with its secondary hydroxyl groups (Figure 1). Since $O_2^{\cdot-}$ can deprotonate very weak acids such as aliphatic alcohols,[20] the proton transfer step, that determines the disproportionation rate, may be favored. Besides, in the case of CDTC it is possible that the CD residue provides a hydrophilic surrounding to the essentially hydrophobic active site of the complexes, promoting the interaction of $O_2^{\cdot-}$ with the divalent metal and the proton diffusion. Such effect, together with the π-acceptor character of the dithiocarbamate group might explain the activity of the CDTC complexes.

Although the proposed interaction between $O_2^{\cdot-}$ and the CD-containing complexes requires further confirmation, our results illustrate the role of CD in enhancing the catalytic activity of metal complexes with CD-containing ligands. Such systems may be useful as models for studying hydrogen bonding in biological systems and its role in the biological activity of metal complexes. New experiments are in progress to complement the present results.

EXPERIMENTAL

General Methods. ^{1}H NMR spectra were recorded on a Bruker AC250 spectrometer in D_2O at 250.13 MHz and were referenced to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate. FAB mass spectra were recorded on a Jeol HX-110 spectrometer using glycerol as matrix. TLC was run on glass sheets precoated with silicagel $60F_{254}$ using the mobile phases specified bellow. Detection was effected by charring the plates after spraying with 20% aq H_2SO_4. Cation exchange chromatography was performed on CM-Sephadex C-25 on a Pharmacia XK 16/70 column using water and 0.5% aq NH_3 as eluents. Mono-2-*O*-tosyl-ß-CD (**1**) was synthesized as reported by Rong and D'Souza.[21]

Mono-2-methylamino-2-deoxy-ß-CD (2). A solution of **1** (600 mg) in 40% aq methylamine (20 mL) was stirred overnight at rt. After evaporation of the solvent under reduced pressure, the resulting colorless solid was redissolved in water (5 mL), applied to a cationic exchange column, eluted with water (500 mL) and 0.5% aq NH_3 (1 L). The appropriate ammonia fractions were collected and concentrated to give **2** (490 mg, 92%): mp 230-235 ^{o}C (dec.); TLC (*n*-BuOH/EtOH/H_2O = 5:4:3) R_f 0.19; ^{1}H NMR δ 2.39 (s, 3H, NCH_3), 2.69 (dd, 1H, H-2'), 3.45-3.85 (m, 41H, H-2, H-3, H-4, H-5, H-6), 4.76 (d, 1H, H-1'), 4.96-5.11 (m, 6H, H-1). FABMS *m/z* 1150.8 $(M+H)^+$, 1169.4 $(M+Na)^+$, 1191.2 $(M-H+2Na)^+$.

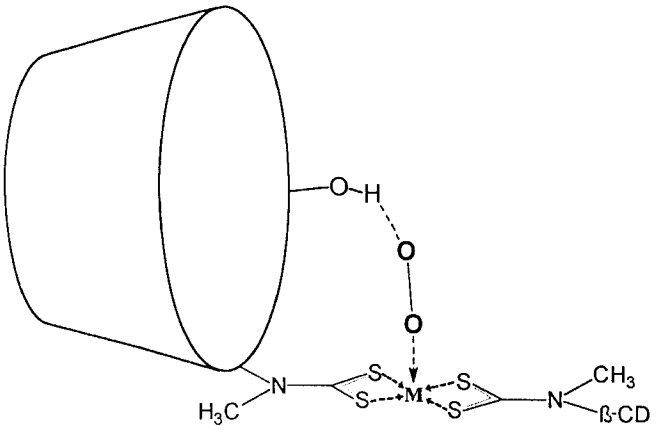


Figure 1. Suggested binding of superoxide radical to the metal in CDTC complexes.

Triethylammonium Mono-2-methylamino-2-deoxy-ß-CD-dithiocarbamate (3). CS_2 (50 μL) was added to a solution of **2** (400 mg) in 2% aq NEt_3 (5 mL) and the resulting emulsion was stirred for 3 h at rt, then concentrated to dryness. The crude product was dissolved in water (5 mL) and precipitated by addition of acetone (250 mL). The resulting white solid was collected by filtration, redissolved in water (5 mL) and precipitated with acetone to give **3** (415 mg, 90%): mp 196-200 °C (dec.); TLC (*n*-BuOH/DMF/EtOH/H_2O = 5:1:1:1) R_f 0.65; UV (H_2O) λ_{max} 259 ($\varepsilon = 2.3 \times 10^4$), 291 ($\varepsilon = 2.9 \times 10^4$); ^1H NMR δ 3.19 (s, 3H, NCH_3), 3.40 (dd, 1H, H-2'), 3.43-3.93 (m, 41H, H-2, H-3, H-4, H-5, H-6), 4.85 (d, 1H, H-1'), 4.91-5.00 (m, 6H, H-1); FABMS *m/z* 1267.0 $(M\text{-}NHEt_3+2Na)^+$, 1324.4 $(M+H)^+$.

Inhibition of Pyrogallol Auto-oxidation. The inhibition of pyrogallol auto-oxidation was performed as reported by Puget,[22] on solutions containing pyrogallol (0.4 mM) and producing slopes of ~ 0.020 ΔOD/min throughout 10 min at 440 nm.

SOD-like Activity. The SOD-like activity was studied using superoxide radical generated by the xanthine-xanthine oxidase system under conditions similar to those described by Fridovich,[23] that is: phosphate buffer pH 7.8 (10 mM), nitroblue tetrazolium chloride (NBT) (2.5 μM), xanthine (10 μM) and the amount of xanthine oxidase required for slopes of ~0.025 ΔOD/min. EDTA was not used in order to avoid competition with the chelating agents submitted to study. NBT reduction by superoxide radical was spectrophotometrically monitored at 560 nm.

The complexes of CDTC were prepared *in situ* in an Eppendorf tube due to their insolubility in almost all solvents which increases with aging. An aqueous solution of the

corresponding metal(II) sulfate was mixed with 200 µL of DMSO and to this solution the dithiocarbamate was added dropwise with constant shaking, the final volume being 1 mL. A M(II):CDTC molar ratio of 1:2.5 was used in all cases. Under such conditions the divalent metal was totally coordinated. The resulting mixture was then centrifuged in order to detect the possible formation of an insoluble product; if so, the mixture was neglected. The final DMSO concentration was much less than 20 %, the permissible limit that does not affect the enzymes, as has been reported [20] and also confirmed by us. For the inhibition determinations of the sulfates, DMSO was also used in order to maintain the same conditions.

Kinetic Determinations. The kinetic measurements were performed on an Ultrospec III (Pharmacia-LKB) spectrophotometer using its *Enzyme Kinetics (EK)* software. The assay time was fixed to no less than 10 min, with measurements each minute. In several cases, the OD vs time curve tended asymptotically to a constant value of OD after the first 10 min but such variation was not considered in the slope determination. The Δ OD/min slope obtained had, in all cases, a linearity greater than 0.993. Each series of experiments was carried out with 4 cuvettes, synchronized in time by the *EK* program, and where the first cuvette was always the xanthine oxidase system without the assay. The % of inhibition was calculated related to the slope of this first cuvette (ref. slope) using the equation:

$$\% \text{ inhibition} = (\text{assay slope} - \text{ref. slope}) \times 100 / \text{ref. slope}$$

The IC_{50} values were determined by regression analysis and interpolation of the % inhibition vs assay concentration curve, for no less than five experimental points for each system, with inhibition values within the range of 10 to 75 %. Inhibitions out of this range tend to have a nonlinear behavior. In all cases, a linearity greater than 0.970 was achieved.

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