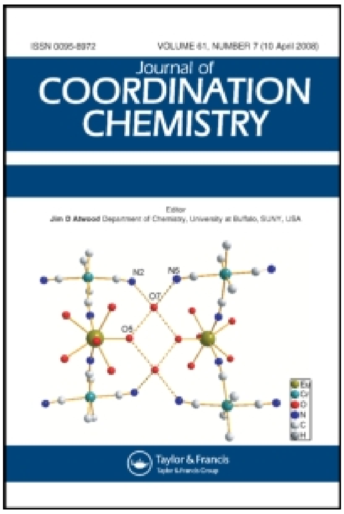

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Synthesis, crystal structure and superoxide dismutase activity of [pyrrolidinium][Co(sulfathiazole)$_3$(pyrrolidine)]

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The preparation, spectroscopic properties and crystal structure of [pyrrolidinium] [Co(sulfathiazole)$_3$(pyrrolidine)] are reported. The X-ray structure reveals tetrahedral geometry around cobalt. Three sulfathiazole ligands (each in a κ,*N*-binding mode) occupy three positions, while the remaining position is occupied by a disordered pyrrolidine. A pyrrolidinium is also disordered. The superoxide dismutase mimetic activity of the complex was investigated using the indirect xanthine-xanthine oxidase-nitroblue tetrazolium method and compared to that of the native enzyme.

Keywords: Superoxide dismutase activity; tris-Sulfathiazole pyrrolidine cobalt complex; Crystal structure

1. Introduction

Superoxide dismutase (SOD) is an enzyme that catalyses the dismutation of the superoxide radical very efficiently through a redox reaction of its copper center. The mechanism believed to be operating in the metalloproteins involves one-electron reduction of a metal ion by superoxide followed by re-oxidation of the reduced metal ion by a second superoxide anion [1, 2]. Metal complexes that can undergo such redox cycling can function as superoxide scavengers; copper is the active metal center in the best studied SOD and many copper complexes have been synthesized and tested for SOD-like activity [3–13]. The other two classes of SODs that contain iron or manganese have received less attention and their structures have only been recently described [14–16]. However, some iron [4, 17, 18], manganese [4, 17, 19], ruthenium [20] and cobalt [21] SOD mimics have been reported and some show marked SOD activity and seem to retain it in living cells [22, 23].

Complex formation between metal ions and sulfa drugs has been extensively studied to establish metal-drug interactions [24–28]. The results suggest that the different

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behavior of the ligand depends on its deprotonation and/or the metal ion, and it is difficult to generalize metal-sulfonamide structures. Three types of behavior of sulfathiazole have been observed: neutral or deprotonated, when it can act as monodentate or bidentate ligand and in $[Cu(en)_2(OH_2)_2][sulfathiazole]_2$ where the sulfathiazole behaves as a deprotonated counter ion [29]. Continuing our studies on metal drug interactions, we report here the preparation, crystal structure and superoxide dismutase activity of the title complex containing cobalt.

2. Experimental

2.1. *Materials*

$CoCl_2 \cdot 6H_2O$, sulfathiazole and pyrrolidine were purchased from Aldrich. Xanthine, xanthine oxidase, nitroblue tetrazolium chloride monohydrate (NBT) and superoxide dismutase (bovine erythrocytes) were obtained from Sigma Chemical Co.

2.2. *Synthesis*

$CoCl_2 \cdot 6H_2O$ (0.24 g, 1 mmol) was added to a solution of sulfathiazole (0.5 g, 2 mmol) in MeOH (50 cm^3) and 1.0 cm^3 (10 mmol) of pyrrolidine was added. Following the addition, the solution turned green–blue, and immediately a blue precipitate formed. The precipitate was filtered and the filtrate was left to stand at room temperature. Within a few days, prismatic red crystals were obtained from the filtrate. Anal. Calcd for $C_{35}H_{43}N_{11}S_6O_6Co$(%): C, 43.6; H, 4.5; N, 16.0; S, 19.9; Co, 6.1. Found: C, 43.8; H, 4.3; N, 15.9; S, 19.7; Co, 6.3. Yield, 62%.

2.3. *Physical techniques*

IR spectra of the ligand and its metal complexes (KBr pellets) were recorded from 4000–400 cm^{-1} with a Perkin–Elmer Series 2000 spectrophotometer. UV–Vis spectra were recorded using a Perkin–Elmer spectrometer. C, H, N and S were analyzed on a LECO CHNS 932 model microanalytical instrument. Metal content was estimated spectrophotometrically on an atomic absorption spectrometer. Magnetic susceptibility was measured on a Johnson Matthey Susceptibility Balance at room temperature using $HgCo(NCS)_4$ as calibrant. Corrections for diamagnetism of the complex were estimated from Pascal's constants. EPR spectrum was recorded on a Bruker ECS 106 spectrometer by the X-band method. Electrochemical measurements were recorded on a Model 170 Electrochemistry System (Princeton Applied Research). All readings were taken using a three electrode potentiostatic system in MeCN with 0.1 mol dm^{-3} $(n\text{-}Bu)_4NBF_4$ as supporting electrolyte. The potentials are referenced against a saturated calomel electrode (SCE). CV studies were carried out using Pt wires as auxiliary and working electrodes and a $Ag/AgBF_4$ reference electrode with sample concentrations of 10^{-3}–10^{-4} mol dm^{-3}. Electrolyte concentration of 0.1 M was used and the solution was degassed with argon prior to use and kept under argon throughout the experiment.

2.4. *Crystallographic data collection*

Prismatic red crystals of [pyrrolidinium][Co(sulfathiazole)$_3$(pyrrolidine)] were grown at room temperature from MeOH. Data using an oil-coated crystal were collected on a Rigaku AFC7S diffractometer using graphite-monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å) at room temperature. The initial unit cell was indexed using a least-squares analysis of a random set of reflections collected in the range of $20.98 < 2\theta < 28.78°$ that were well distributed in reciprocal space. Intensity data were collected using the ω-2θ scan technique (the crystal to detector distance was 23.5 cm) for $2.96 \leq 2 \leq 54.9$. A total of 20203 reflections were collected using XSCANS [30], while absorption correction was applied using XABS [31] with 11123 unique [$R_{\text{int}} = 0.0708$]. System symmetry, systematic absences and intensity statistics indicated the unique monoclinic space group $P2_1/c$. The structure was determined by direct methods with the location of nearly all non-hydrogen atoms using the program SIR93 [32] and refined by full–matrix least-squares on F^2 using SHELXL [33]. All non-hydrogen atoms were refined anisotropically, while hydrogen atoms were placed in calculated positions, constrained to ride on their respective atoms with U_{iso} values assigned [$U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{C,N})$ for sp^2 conformation] and [$U_{\text{iso}}(\text{H}) = 1.5U_{\text{eq}}(\text{C,N})$ for *sp*^{3} conformation]. The program PLATON/SQUEEZE [34] was used to avoid a large disordered solvent effect from the total electron density. In the crystal structure, the pairs of atoms N1, N1A; C1, C1A; C2, C2A; C3, C3A; C4, C4A in the pyrrolidine ligand have the ratio of 56 : 44% occupancies. Also, the contra-ion pyrrolidinium is disordered. All atoms of N2, N2A; C6, C6A; C7, C7A; C8, C8A; C9, C9A have a ratio of 77 : 23% occupancies. The final structure was refined to convergence [$\Delta/\sigma \leq 0.001$] with $R(F) = 0.0811$ (for 9428 data with $F > 4\sigma F$), GOF = 1.208 and $wR_2 = 0.1022$ (all data). [$R_1 = \sum|F_{\text{o}} - F_{\text{c}}|/\sum|F_{\text{o}}|$, $wR_2 = \{[w(F_o^2 - F_c^2)^2]/\Sigma wF_0^4\}^{0.5}$, $w = 1/\sigma^2(F_0^2) + (xP)^2 + yP$], $P = (F_0^2 + 2F_c^2/3)$]. The largest difference between peak and hole in the final difference map are 0.304 and $-0.342\,\text{e}^-\text{Å}^3$.

2.5. *Superoxide assays*

Superoxide dismutase activity of the complex was determined by using its ability to inhibit the reduction of NBT by superoxide ions generated by the xanthine/xanthine oxidase system [35]. The extent of NBT reduction was followed spectrophotometrically by measuring the absorbance at 560 nm. The aqueous reaction mixture contained 2×10^{-4} M xanthine, 5×10^{-5} M NBT and 1.25×10^{-2} M sodium phosphate buffer at pH 7.5 and 25°C. For comparative purposes, we have also measured the activity of native superoxide dismutase, from bovine erythrocytes, under the same experimental conditions. Five assays were performed for each concentration of both SOD and the metal complex. We checked that the generating xanthine/xanthine oxidase system was not inhibited by our metal complex by following, at 295 nm, the uric acid produced when xanthine is oxidized by xanthine oxidase in aerobic conditions concomitantly to the production of superoxide.

3. Results and discussion

The elemental analysis agrees well with the stoichiometry of the complex. It is colored, appears to be air stable, and is soluble in methanol. The experimental conductivity value of $28\,\mathrm{S\,cm^2\,mol^{-1}}$ in DMSO solution at room temperature falls in the range usually expected for a 1 : 1 electrolyte. The corrected magnetic moment was calculated from the molar magnetic susceptibility using Pascal's constants as 4.7 B.M., which is higher than the value expected for square-planar geometry (2.10–2.80 B.M.) but is in the range expected for tetrahedral geometry (4.40–4.80 B.M.). The complex gave no EPR signal at room and liquid nitrogen temperatures.

The presence of ν(Co–N) in the 420–460 $\mathrm{cm^{-1}}$ range (absent in the free ligands) provides evidence that the sulfathiazole and pyrrolidine moieties are bonded to the metal ion through nitrogen. The usual modes of the sulfathiazole ligand are also present. The bands at 3320 and 3280 $\mathrm{cm^{-1}}$, assigned to ν_{as}(N–H) and ν_s(N–H), respectively of the NH_2, are not shifted in the complex. As expected, the bands due to $\nu(SO_2)_{as}$ (1320 $\mathrm{cm^{-1}}$), $\nu(SO_2)_s$ (1140 $\mathrm{cm^{-1}}$), scissoring and wagging SO_2 (570 and 550 $\mathrm{cm^{-1}}$) remain unchanged. There is no change of the bands at 1540, 920 and 680–640 $\mathrm{cm^{-1}}$, which have been assigned to the characteristic thiazole ring and ν(S–N) and ν(C–S) vibrations, respectively.

The UV–Vis spectrum of the metal complex in DMSO exhibits one absorption maximum at 300–320 nm, presumably due to intraligand excitation. Because of the unsaturation of the sulfathiazole, the intense UV absorption has a tail in the visible region which hampers assignment of the relatively weak d–d transitions of the cobalt(II) ion. The cobalt(II) complex presents two bands at 350–380 and 510–530 nm, which may tentatively be assigned to spin-forbidden and $^4A_2 \rightarrow {}^4T_1(\mathrm{P})$ transitions, respectively, considering $\mathrm{T_d}$ symmetry around the metal.

An ORTEP drawing including the atomic numbering scheme is given in figure 1. Crystal data and selected bond distances and angles are listed in tables 1 and 2. The X-ray structure shows tetrahedral geometry around cobalt with three sulfathiazole ligands (each in a κ,*N*-binding mode) and a disordered pyrrolidine moiety. A contra-ion of pyrrolidinium is also disordered. The bond distances between cobalt and nitrogen range from 1.917(3)–2.007(2) Å, relatively shorter compared to similar bond distances (2.202 Å) retrieved from Cambridge Structural Data Base [36]. All the geometric parameters for the structure lie within their expected ranges [36]. The final position, thermal parameters and all distances and angles of the complex structure have been deposited under the deposition number CCDC 667492 at the Cambridge Crystallographic Data Centre.

In order to further explain superoxide dismutase activity of the complex, we have tested its electrochemical behavior by cyclic voltammetry. Two well-resolved redox waves were observed for the cobalt(II) complex, a reduction wave at −1.22 V, assigned to the cobalt(II)/cobalt(I) reduction and an oxidation wave at +0.91 V attributed to the Co(II)/Co(III) couple [37–39]. The separation between the anodic and cathodic peaks in the cyclic voltammograms is near 60 mV, which corresponds to one-electron reduction and suggests that the electrode reactions approached Nernstian behavior.

The superoxide dismutase activity of the title complex was assayed by their ability to inhibit the reduction of nitroblue tetrazolium. Figure 2 shows the percentage inhibition with increase in concentration of the cobalt(II) complex. In the SOD-like activity test, the metal complexes compete with NBT for oxidation of the generated superoxide ions. The more efficient the complex, the lower the concentration that corresponds to 50% inhibition of NBT reduction; this concentration is termed IC_{50} for comparative purposes. On a molar basis the catalytic activity of the cobalt(II) complex ($IC_{50} = 580\,\mu M$) corresponds to 0.17% of that of native SOD ($IC_{50} = 1\,\mu M$). An activity of

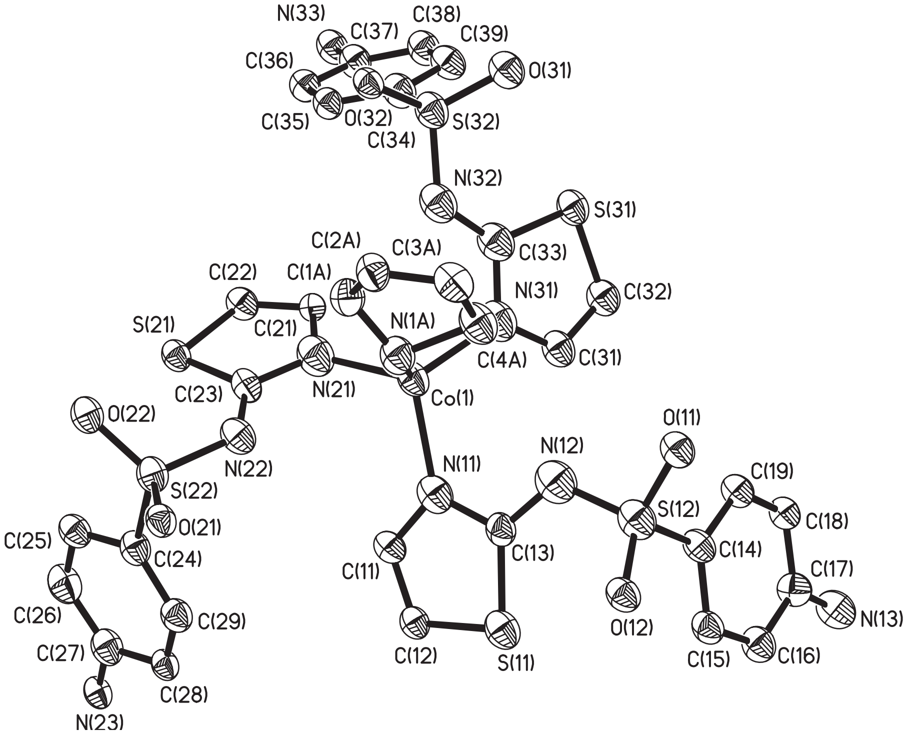

Figure 1. Molecular structure of [Co(sulfathiazole)$_3$pyrrolidine] anion. Displacement ellipsoids are drawn at the 30% probability level. All hydrogen atoms have been omitted.

Table 1. Crystal data, data collection parameters and details of the structure refinement for [pyrrolidinium][Co(sulfathiazole)$_3$(pyrrolidine)].

Crystal description	Red block
Crystal size (mm^3)	$0.42 \times 0.38 \times 0.28$
Empirical formula	$C_{35}H_{43}CoN_{11}O_6S_6$
M_w	965.25
T (K)	298(2)
Crystal system	Monoclinic
Space group	$P2_1$/c
Unit cell dimensions (Å,°)	
a	12.388(3)
b	22.662(5)
c	18.236(4)
α and γ	90
β	108.11(3)
U (Å^3)	4866(2)
Z (Z')	4(1)
D_{Calcd} (Mg m^{-3})	1.317
(Mo-Kα) (mm^3)	0.661
F(000)	2004.1
Reflections collected/unique	20203/11123 [$R_{(\mathrm{int})} = 0.0708$]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	9428/95/608
Goodness-of-fit on F^2	1.208
Final R indices [$I > 2(I)$]	$R_1 = 0.0811$, $wR_2 = 0.1005$
R indices (all data)	$R_1 = 0.0817$, $wR_2 = 0.1022$

Table 2. Selected bond lengths (Å) and angles (°).

Co(1)–N(1A)	1.917(3)
Co(1)–N(11)	2.007(2)
Co(1)–N(21)	1.989(3)
Co(1)–N(31)	1.981(2)
N(1A)–Co(1)–N(11)	109.75(9)
N(1A)–Co(1)–N(21)	109.51(11)
N(1A)–Co(1)–N(31)	115.81(11)
N(11)–Co(1)–N(21)	106.79(11)
N(11)–Co(1)–N(31)	107.12(9)
N(21)–Co(1)–N(31)	107.48(9)
C(1A)–N(1A)–Co(1)	122.23(17)
C(4A)–N(1A)–Co(1)	109.92(14)

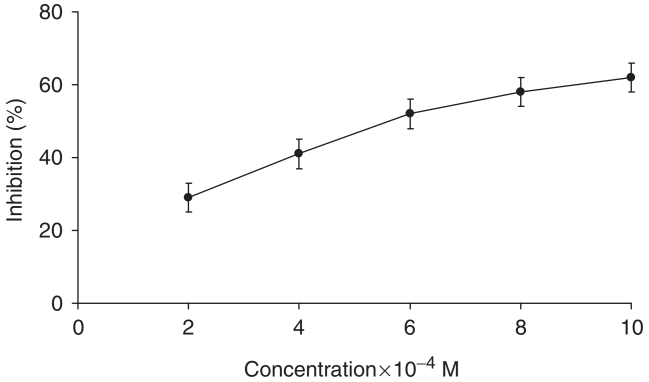

Figure 2. Percentage of inhibiting NBT reduction with an increase in the concentration of the complex.

ca. 0.1% of that of the enzyme is considered quite interesting, considering the high molecular weight of SOD. Higher percentages of SOD activity have been observed for some copper(II) complexes [3, 8–10, 40–42]. Although the cobalt(II) complex has an interesting IC_{50} value, it does not go beyond 72% inhibition, whereas other complexes tested reach 80% inhibition or more. This kind of flattening of the curves below 100% inhibition have already been observed for other metal complexes, but the kinetic reasons for such limitation have not been explained [5, 6].

4. Conclusion

The cobalt(II) complex tested in this work has been shown to compete with NBT for superoxide. Although the SOD activity is not higher than some already reported, it presents higher percentages of SOD activity than the $[Co(quinoline)_2(SH)_2]$ complex [22] and appears to be a promising SOD mimic.

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