

SYNTHESES, CHARACTERIZATION AND ANTIFUNGAL ACTIVITY OF TRIS(1,10-PHENANTHROLINE) IRON(II) BIS(*N*-R-SULFONYLDITHIOCARBIMATE)ZINCATE(II)

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Four new compounds with the general formula $[\text{Fe}(\text{phen})_3][\text{Zn}(\text{RSO}_2\text{N}=\text{CS}_2)_2]$, where phen = 1,10-phenanthroline, R = 4- FC_6H_4 (1), 4- ClC_6H_4 (2), 4- BrC_6H_4 (3) and 4- IC_6H_4 (4), respectively, were obtained by the reaction of the appropriate potassium *N*-R-sulfonyldithiocarbamate ($\text{RSO}_2\text{N}=\text{CS}_2\text{K}_2$) and tris(1,10-phenanthroline)iron(II) sulfate, with zinc(II) acetate dihydrate in dimethylformamide. The elemental analyses and the IR data were consistent with the formation of the expected complexes salts. The ^1H and ^{13}C NMR spectra showed the signals for the cationic iron(II) complex and dithiocarbamate moieties. The molar conductance data were consistent with the 1:1 cation:anion complexes in **1-4**. The antifungal activities of the compounds were tested *in vitro* against *Candida albicans*, *Candida tropicalis* and *Colletotrichum gloeosporioides*.

Keywords: dithiocarbamates; metal complexes; antifungal activity.

INTRODUCTION

Fungal infections in humans have increased in recent years predominantly affecting immunocompromised patients.^{1,2} Fungi from the *Candida* genus can infect the oral and vaginal cavities, skin and, more seriously, essential organs.^{1,3} *Colletotrichum gloeosporioides* is known to infect a wide variety of hosts, including vegetables, field and forage crops, fruit trees, and ornamental shrubs.⁴ *C. gloeosporioides* infections in humans can cause keratitis in healthy or immunocompromised individuals and can induce phaeohyphomycosis in patients with hematologic malignancies and iatrogenic immunosuppression.⁵⁻⁷

Several dithiocarbamate and *N*-substituted dithiocarbamate complexes and salts (Scheme 1a) have been used as agrochemicals mainly due to their high efficiency in controlling plant fungal diseases, and their relatively low toxicity.^{8,9} The literature reports that dithiocarbamate complexes are also active against *Candida* spp.¹⁰



Scheme 1. General formulae of dithiocarbamate (a) and dithiocarbimate (b) anions

In recent studies, we have demonstrated that the dithiocarbamate compounds $\text{A}_2[\text{Zn}(\text{RSO}_2\text{N}=\text{CS}_2)_2]$ and $\text{A}_2[\text{Sn}(\text{Bu})_2(\text{RSO}_2\text{N}=\text{CS}_2)_2]$ (A = tetrabutylammonium cation or tetraphenylphosphonium cation, Bu = *n*-butyl and R = 4- FC_6H_4 , 4- ClC_6H_4 , 4- BrC_6H_4 and 4- IC_6H_4) are active against *Colletotrichum gloeosporioides*.¹¹⁻¹³ It was also observed that the cations Bu_4N^+ and Ph_4P^+ were inactive.

Unlike dithiocarbamate analogues, the bis(dithiocarbimate) metal(II) complexes are anionic species (Scheme 1), offering potentially useful modulation of this activity. For example, enhanced antifungal activity should be possible either by the use of active counter ions, or by varying the solubility of the salts of the complexes through the use of different cations.

Considering these possibilities, the activities of some salts were tested in order to find an appropriate active cation to deepen the investigation into the antifungal potential of dithiocarbamates. This search revealed that tris(phenanthroline)iron(II) perchlorate is active against *Colletotrichum gloeosporioides*. Here we describe the syntheses of the new compounds $[\text{Fe}(\text{phen})_3][\text{Zn}(\text{RSO}_2\text{N}=\text{CS}_2)_2]$, where phen = 1,10-phenanthroline, R = 4- FC_6H_4 (1), 4- ClC_6H_4 (2), 4- BrC_6H_4 (3) and 4- IC_6H_4 (4), respectively. The compounds were characterized by elemental analyses, along with IR, ^1H and ^{13}C NMR spectroscopies. The antifungal activities of the new compounds **1-4** were evaluated *in vitro* against *Candida albicans*, *Candida tropicalis* and *Colletotrichum gloeosporioides*.

EXPERIMENTAL

General

Uncorrected melting points were measured with an MQAPF-302 device. Microanalyses for C, H and N were done using a Perkin-Elmer 200 CHN Elemental Analyzer. Zinc and iron were analyzed by atomic absorption with a Hitachi Z-8200 Atomic Absorption Spectrophotometer. Molar conductance (Λ_m) was measured in DMSO at 25 °C with a Jenway 4010 Conductivity Meter. IR spectra were recorded with a Perkin-Elmer FT-IR 1000 infrared spectrophotometer using CsI pellets. The ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded with a Varian (Mercury 300) spectrophotometer in DMSO- D_6 with TMS as the internal standard. The solvents, carbon disulfide, concentrated ammonia aqueous solution, ammonium iron(II) sulphate hexahydrate, 1,10-phenanthroline, magnesium perchlorate and potassium hydroxide were purchased from Vetec. The *N*-4-bromophenylsulfonyl and *N*-4-iodophenylsulfonyl chlorides, 4-fluorobenzenesulfonamide, 4-chlorobenzenesulfonamide and zinc acetate dihydrate were purchased from Alfa Aesar. The tris(phenanthroline) iron(II) perchlorate was precipitated from an aqueous solution of ammonium iron(II) sulphate hexahydrate and 1,10-phenanthroline (1:3 molar ratio) after the addition of magnesium perchlorate. Its formation was confirmed by IR and comparison with published data.¹⁴ The 4-bromo- and 4-iodobenzenesulfonamides were prepared by the

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— NCCLS — (1997). A 0.1 mL aliquot of over-night culture of this microorganism strain corresponding to 0.5 turbidity on the McFarland scale was placed onto 10 mL of Sabouraud dextrose agar (SDB plus 1.5% agar). The commercial antifungal agent nystatin and DMSO were used as a positive control. The DMSO employed as a solvent in the experiment was tested as a negative control. The inhibition zone for the commercial nystatin was 14 mm.

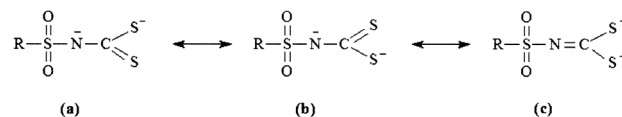
RESULTS AND DISCUSSION

Chemistry

The compounds **1-4** are stable under ambient conditions, soluble in DMSO and DMF but insoluble in water and in most of the organic solvents. The elemental analyses of C, H, N, Fe and Zn were consistent with the proposed formulae. The molar conductance values were consistent with a proportion of 1:1 between cations and anions.²¹

Three intense bands characteristic for the tris(1,10-phenanthroline)iron(II) cation were observed at 1400-1600 cm⁻¹ in the IR spectra of the compounds **1-4**.¹⁴ Two additional strong bands were observed in the 1300-1400 cm⁻¹ range in the spectra of **1, 2** and **4**. The spectrum of **3** showed a broad band in this region. These bands are in the same region as in the spectra of other metal(II)-dithiocarbamate complexes, and were assigned to the νCN vibration of the RSO₂N=CS₂ group. They are shifted to higher wavenumbers with respect to the spectra of the ligands.¹⁸⁻²⁰ The spectral region of 1000-900 cm⁻¹ is characteristic for disulfuric chelation.²² Two medium bands at 930-950 cm⁻¹ were observed in the spectra of **1, 2** and **4**. The spectrum of **3** showed a broad band in this region. These bands were assigned to the ν_{as}CS₂ and are shifted to lower wavenumbers with respect to the spectra of the ligands.¹⁸⁻²⁰ The positions observed for the ν_{as}CS₂ and νCN bands in the spectra of the compounds studied here were consistent with the complexation of the dithiocarbamate group by two sulfur atoms. The higher wavenumber values observed for νCN and the lower values observed for ν_{as}CS₂ compared with the related dithiocarbamate salts indicate a greater contribution of the canonical form (c) for the resonance hybrid in the complexes (Scheme 3).¹⁹ The presence of two bands in the mentioned regions is indicative of asymmetric ZnS bonds.²³ The spectra of the compounds also showed the expected band of medium intensity in the 300-400 cm⁻¹ range assigned to the Zn-S stretching vibration, also indicating chelation by two sulfur atoms.²⁴

The NMR spectra showed all the expected signals for the compounds **1-4**. The ¹H NMR spectra showed the signals for the hydrogen



Scheme 3. Three canonical forms for *N-R-sulfonyldithiocarbamate anion*

atoms of the tris(1,10-phenanthroline)iron(II) cation while the remaining signals were assigned to the zinc(II) anionic complexes. The region from δ 9.0 to 7.5 is complex with signals of the cationic and anionic moieties frequently superimposed. However, the signals of the hydrogen atoms 3 and 7 in the compounds **1-3** are isolated from the other aromatic hydrogens signals. Their integration curves were consistent with a 1:1 proportion between the cationic and anionic complexes. The ¹³C NMR spectra showed the expected signals for the tris(1,10-phenanthroline)iron(II) cation and most of the carbon atom signals of the anionic complexes. Only the spectrum of **1** exhibited the signal of C=N at δ 207.4. This signal is typically difficult to observe due to its very low intensity.²⁵ The signal is shifted to a higher field compared with the spectra of the ligands (ca. 225 ppm).¹⁸ This fact is also in accord with the increase in the contribution of the canonical form (c) after the formation of the complexes (Scheme 3).

Biological assay

The magnesium perchlorate was not active, while the tris(1,10-phenanthroline)iron(II) perchlorate showed considerable activity towards *C. Gloeosporioides* (80.6% growth inhibition). These results indicated that this activity was due to the cation only, making it a candidate for enhancing the recently discovered activity of the dithiocarbamates.^{11,12}

The new compounds **1-4** containing both the tris(1,10-phenanthroline)iron(II) cation and the zinc-dithiocarbamate anions were active against *C. Gloeosporioides* and inactive against both *Candida albicans* and *Candida tropicalis*. These results indicate that the mode of action of the dithiocarbamates is specific for filamentous fungi.

Table 1 shows the inhibition percentages in the *C. Gloeosporioides* colony on the 10th day of incubation in comparison with analogous tetrabutylammonium salts.¹¹ Compound **1** was the most active of the new salts.

Interestingly, the tris(phenanthroline)iron(II) perchlorate and the tetrabutylammonium zinc-*N-R-sulfonyldithiocarbamate*¹¹ complexes were more effective separately against *C. Gloeosporioides* than the complexes **1-4** containing both active ions (Tab 1). These

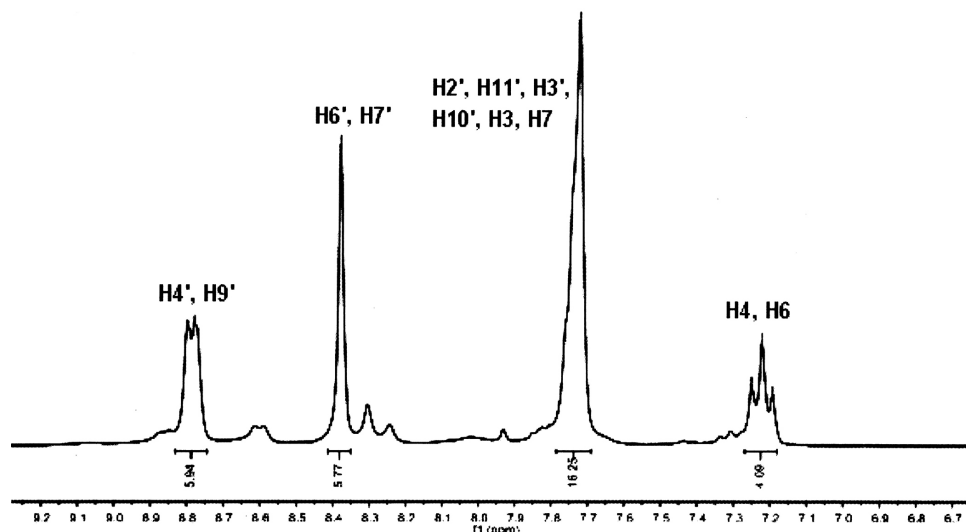


Figure 1. ¹H NMR spectrum of compound **1** (300 MHz, DMSO-d₆)

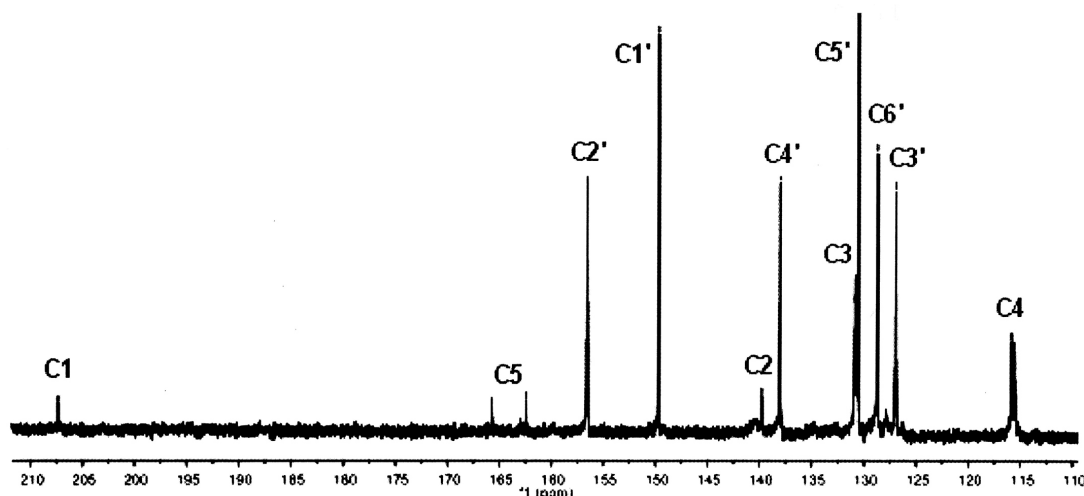


Figure 2. ^{13}C NMR spectrum of compound **1** (300 MHz, DMSO-d_6)

Table 1. Inhibition of *C. gloeosporioides* growth by complexes **1-4** at 2.0 mM on 10th day of incubation at 25°C compared with analogous tetrabutylammonium salts¹¹

Substances	Inhibition (%)
(1)	74.7
$(\text{Bu}_4\text{N})_2[\text{Zn}(4\text{-FC}_6\text{H}_4\text{SO}_2\text{N}=\text{CS}_2)_2]$	86.0
(2)	64.2
$(\text{Bu}_4\text{N})_2[\text{Zn}(4\text{-ClC}_6\text{H}_4\text{SO}_2\text{N}=\text{CS}_2)_2]$	86.5
(3)	63.1
$(\text{Bu}_4\text{N})_2[\text{Zn}(4\text{-BrC}_6\text{H}_4\text{SO}_2\text{N}=\text{CS}_2)_2]$	82.6
(4)	67.9
$(\text{Bu}_4\text{N})_2[\text{Zn}(4\text{-IC}_6\text{H}_4\text{SO}_2\text{N}=\text{CS}_2)_2]$	84.6
$[\text{Fe}(\text{phen})_3](\text{ClO}_4)_2$	80.6

results are clearly due to the low solubility of the new compounds both in water and in most organic solvents, probably causing low dispersion rates in the aqueous medium and possibly less effective interactions with the fungi cellular walls. Nevertheless, the results indicate that this activity can be improved by choosing the right pair of counter ions and that the new compounds are selective in their antifungal activities.

CONCLUSIONS

Four salts of anionic bis(N-R-sulfonyldithiocarbamate)-zinc(II) complexes with the tris(1,10-phenanthroline)iron(II) cation were obtained (**1-4**). The compounds were characterized by elemental analyses, conductivity measurements, in addition to IR, ^1H , and ^{13}C NMR spectroscopies. The conductivity values (c.a. $60 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) and the ^1H NMR integration curves were consistent with a proportion of 1:1 for cation:anion. The IR spectra showed the expected bands for the tris(1,10-phenanthroline)iron(II) cation. The wavenumbers for the $\nu\text{C}=\text{N}$ vibration corresponding to the dithiocarbamate ligand were greater than those observed for the free ligands while the νCS_2 bands were shifted to lower wavenumbers after complexation. Both $\nu\text{C}=\text{N}$ and νCS_2 bands were split in the spectra of **1**, **2** and **4**, and broadened in the spectrum of **3**. These findings point to an asymmetric coordination of the dithiocarbamate to the zinc(II) by the two sulphur atoms forming a ZnS_4 distorted tetrahedral environment as observed for $(\text{Bu}_4\text{N})_2[\text{Zn}(4\text{-XC}_6\text{H}_4\text{SO}_2\text{N}=\text{CS}_2)_2]$.¹¹ All the new substances

were active against *C. Gloeosporioides* whereas no activity was observed against *C. albicans* or *C. tropicalis*. These findings point to selective biological activity considering that *Candida* spp. are yeasts and *Colletotrichum* are filamentous fungi. Yeasts are unicellular micro-organisms, having similarities with human cells, and do not grow as hyphae as do the filamentous fungi. The results against *C. Gloeosporioides* were similar to those presented by the analogous tetrabutylammonium bis(N-R-sulfonyldithiocarbamate)-zinc(II) salts.¹¹ The change of the tetrabutylammonium by the active tris(1,10-phenanthroline)iron(II) cation did not produce the expected results, with the new salts being less active than their precursors. The lower solubility of the compounds **1-4** compared to the tetrabutylammonium bis(N-R-sulfonyldithiocarbamate)-zinc(II) analogues is the probable cause for this reduction in antifungal activity. Further studies are being carried out in order to determine the mode of action of the dithiocarbamates and modulate their activities.

SUPPLEMENTARY MATERIAL

The supplementary material includes infrared spectra of the compounds **1-5** and NMR spectra of the compounds **2-5**.

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