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Synthesis, characterization and antimicrobial properties of a Co(II)-phthalylsulfathiazolate complex

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Abstract The reaction between phthalylsulfathiazole (H_2PST), in alkaline aqueous solution, and cobalt(II) nitrate led to a pink solid, $[Co(PST)(H_2O)_4]$ **(1)**, which was characterized by elemental and thermogravimetric analysis; FT-IR, Raman and diffuse reflectance spectra. Spectroscopic data reveal that the ligand would be doubly deprotonated and that the Co(II) ion environment is a distorted octahedral one. **(1)** showed antibacterial activity similar to the ligand.

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Introduction

Aromatic sulfonamide derivatives exhibit a range of bioactivities, including anti-angiogenic (Funahashi et al. 2002; Semba et al. 2004), anti-tumor (Semba et al. 2004; Sławínski and Gdaniec 2005), anti-inflammatory and analgesic (Chen et al. 2005), anti-tubercular (Gadad et al. 2004), anti-glaucoma (Agrawal et al. 2004), anti-HIV (Yeung et al. 2005), cytotoxic (Encío et al. 2005), antimicrobial (Nieta et al. 2005) and antimalarial (Domínguez et al. 2005) agents. The synthesis of metal sulfanilamide compounds had received much attention due to the fact that sulfanilamides were the first effective chemotherapeutic agents to be employed for the prevention and cure of bacterial infections in humans (Bult 1983). N-Substituted sulfonamides are still among the most widely used antibacterial agents in the world, mainly because of their low cost, low toxicity, and excellent activity against bacterial diseases (Ajibade et al. 2006). Sulfonamides exert their antibacterial action by the competitive inhibition of the enzyme dihydropterase synthetase (DHPS) because of its similarity with *p*-aminobenzoic acid, a factor required by bacteria for folic acid synthesis (García-Raso et al. 2000). Phthalylsulfathiazole,

(2-[({4-[(1,3-thiazol-2-ylamino)sulfonyl]phenyl}amino) carbonyl]benzoic acid) is a sulfonamide used in the treatment of enteric infections (Mandell and Sande 1981). The antimicrobial activity of sulfa drugs is often enhanced by complexation with metal ions (Sharaby 2005) which is in concordance with the well-known importance of metal ions in biological systems.

The interest in metal-sulfanilamide derivatives was stimulated by the successful introduction of Ag(I) sulfadiazine complex to prevent bacterial infections during burn treatments for both human and animals (Bult 1983), which are still in current use (Vehmeyer-Heeman et al. 2006). The coordination environment around the metal is highly relevant to the biological activity (Alzuet et al. 2003).

Despite its low availability in the earth's crust (Lippard and Berg 1994), cobalt plays important roles in biological systems. Vitamin B_{12} is the cobalt only apparent biological site, which deficiency causes the severe disease of pernicious anemia in humans, indicating the critical role of cobalt in living organisms (Randaccio et al. 2007). Because of its high sensitivity to the coordination site geometry and the many experimental techniques that can be used in its characterization, cobalt has been used to replace other metal ions to gather information about changes in metal sites in proteins. In this context, the use of simple Co-containing systems with low molecular weight ligands is useful for understanding the correlation between spectroscopic and structural properties (Cowan 1997). Many complexes of Co(II) showing antimicrobial activity had been synthesized recently (López-Sandoval et al. 2008; Saghatforoush et al. 2009; Rodríguez-Argüelles et al. 2009), even with sulfonamides as ligands (Özdemir et al. 2009).

As a continuation to our work on metal complexes of sulfa drugs (Bellú et al. 2003, 2005, 2007), the present paper reports the synthesis, characterization and antimicrobiological properties of a complex of phthalylsulfathiazole (H_2PST, Fig. 1) with Co(II), Co(II)-PST.

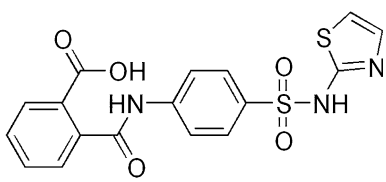

Fig. 1 Phthalylsulfathiazole (H_2PST)

Materials and methods

Chemistry and physical measurements

Phthalylsulfathiazole (FNA grade, >99%), cobalt(II) nitrate hexahydrate (Merck, GR), and all other chemicals were commercially available reagent grade. Phthalylsulfathiazole was dissolved en NaOH 0.1 M previously to react with Co(II). The molar ratio Co(II)/$H_2PST \approx 1$ in the reaction mixture was decided after several attempts where this molar ratio was finding in the complex. The use of the cobalt(II) nitrate salt instead of the chloride one was in order to exclude the chloride ion as a possible ligand.

The content of Co was determined by both complexometric back-titration with EDTA (ethylenediaminetetraacetic acid) and atomic absorption spectroscopy with a double beam Perkin Elmer spectrometer, model 3110, at UNL.

Elemental chemical analyses (C, H, N, S) were performed in a CARLO ERBA EA 1108 microanalyser at Chile University.

IR spectra of powdered samples were measured with a Bruker IFS 66 FTIR-spectrophotometer from 4,000 to 400 cm^{-1}, using the KBr pellet technique. Raman spectrum was measured with a Spex-Ramalog double monochromator spectrometer, using the 514.5 nm line of an Ar ion laser for excitation, over the region 400–2,000 cm^{-1}.

Thermogravimetric (TG) and differential thermal analysis (DTA) were performed on a Shimadzu system (models TG-50 and DTA-50 respectively), working in an oxygen flow (50 ml/min) and a heating rate of 10°C/min. Sample quantities ranged between 10 and 20 mg. Al_2O_3 were used as a DTA standard.

Diffuse reflectance spectrum was measured in the range 380 and 800 nm with a Shimadzu UV-300 instrument, using MgO as an internal standard.

Synthesis of the complex

$[Co^{II}(PST)(H_2O)_4]\cdot 2H_2O$: 2 ml of aqueous solution of cobaltous nitrate containing 0.2905 g (0.998 mmol) of $Co(NO_3)_2\cdot 6H_2O$, were added dropwise to 21.7 ml of stirring aqueous alkaline solution of

phthalylsulfathiazole, containing 0.4515 g H_2PST (1.119 mmol) (Bult 1983; Bellú et al. 2005). Immediately, the resulting mixture became a lilac one, because a lilac-colored precipitate was formed. The reaction mixture was heated during 5–6 min at 60°C into a water bath. Then, it was left to stand at room temperature, away from light. After 10 days the precipitate, which turned into a pink one, was centrifuged, washed with small volumes of ethanol and water, filtered off and dried under vacuum, away from light. Yield: 0.4976 g (87.64%).

Biological evaluation

Antibacterial assays *Microorganisms and media*: The following strains from the American Type Culture Collection (ATCC), Rockville, MD, USA, Malbrán Institute (MI), Pasteur Institute (PI) and from the Laboratorio de Microbiología (LM, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina) were used: Gram-negative bacteria: *Escherichia coli* ATCC 25922, LM_1-*Escherichia coli*, LM_2-*Escherichia coli*, *Pseudomonas aeruginosa* ATCC 27853 and Gram-positive bacteria: LM-*Staphylococcus aureus*, *Staphylococcus aureus* methicillin-sensitive ATCC 29213 and *Staphylococcus aureus* methicillin-resistant ATCC 43300 were used for the antibacterial evaluation. Bacteria were grown on Müeller–Hinton agar medium.

Preparation of inoculum: Cultures less than 30 h old were touched with a loop and transferred to sterile broth Müeller–Hinton . The broth was incubated at 37°C until the growth reached a turbidity equal to or greater than that of 0.5 McFarland standard. The culture was adjusted with sterile physiological solution to give a final organism density of 5×10^5 cfu/ml (Jorgensen et al. 1999).

Assay: The antibacterial activity was evaluated with the agar dilution method using Müeller–Hinton agar medium for Gram (+) and Gram (−) bacteria (Feresin et al. 2001). Stock solutions of the ligands and its Co(II) complexes in DMSO were diluted to give serial two-fold dilutions that were added to each medium resulting in concentrations ranging from 1,000 to 10 μg/ml. The final concentration of DMSO in the assay did not exceed 2%. The antimicrobial agent cefotaxime (Argentia Pharmaceutica) were included in the assays as positive controls. Minimal Inhibitory Concentration (MIC) was defined as the lowest compound concentration showing no visible bacterial growth after incubation time (24 h) at 37°C. Tests were done in triplicate. MICs $\leq$ 250 μg/ml were considered active.

Antifungal assays *Microorganisms and media*: The microorganisms used for the fungistatic evaluation were purchased from ATCC, or were clinical isolates from CEREMIC (identified with the capital letter C), Centro de Referencia en Micología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531-(2000)-Rosario, Argentina. Yeasts: *Candida albicans* ATCC 10231, *C. tropicalis* C131, *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264; *Aspergillus flavus* ATCC 9170, *A. fumigatus* ATCC 26934, *A. niger* ATCC 9029; Dermatophytes: *Trichophyton mentagrophytes* ATCC 9972, *T. rubrum* C 113, *Microsporum gypseum* C 115 were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30°C. Cell suspensions in sterile distilled water were adjusted to give a final concentration of 10^6 viable yeast cells/ml (Wright et al. 1983).

The strains were maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and subcultured every 15 days to prevent pleomorphic transformations. Spore suspensions were obtained according to reported procedures (Wright et al. 1983) and adjusted to 10^6 spores with colony forming ability/ml.

Assay: Minimal Inhibitory Concentration (MIC) of each compound was determined by using broth microdilution techniques according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards NCCLS) for yeasts (M27-A2) and for filamentous fungi (M 38 A) (NCCLS 2002a, b). MIC values were determined in RPMI-1640 (Sigma, St Louis, Mo, USA) buffered to pH 7.0 with MOPS. Microtiter trays were incubated at 35°C for yeasts and hialohyphomycetes and at 28–30°C for dermatophyte strains in a moist, dark chamber, and MICs were visually recorded at 48 h for yeasts, and at a time according to the control fungus growth, for the rest of fungi. For the assay, stock solutions of pure compounds were two-fold diluted with RPMI from 250 to 0.98 μg/ml (final volume = 100 μl) and a final DMSO concentration $\leq$ 1%. A volume of 100 μl of inoculum suspension was added to each well with the exception of the sterility control where sterile water was added to the well instead. Endpoints (MIC) were defined as

the lowest concentration of drug resulting in total inhibition of visual growth compared to the growth in the control wells containing no antifungal. Minimal Fungicidal Concentration (MFC) was determined by plating by duplicate 5 µl from each clear well of MIC determinations, onto a 150 mm SDA plate. After 48 h at 37°C, MFCs were determined as the lowest concentration of each compound showing no growth in the plates. Amphotericin B (Janssen Pharmaceutica, Belgium), Ketoconazole (Sigma Chem. Co. St Louis, MO, USA) and Terbinafine (Novartis, Bs. As., Argentina) were used as positive controls. Both MIC and MFC were confirmed by two replicates.

Mutagenicity test

Mutagenicity activity was evaluated in a bacterial reverse mutation assay by the standard Ames test in the absence of S-9 mix, by using the *Salmonella typhimurium* histidine-requiring test with TA98 and TA100 strains (Maron and Ames 1983; Mortelmans and Zeiger 2000), which together detect the 93% of the mutagens (Jurado et al. 1993). Diagnostic mutagens, including 4-Nitro-*o*-phenylenediamine (4NOPDA) for the TA98 strain and sodium azide, NaN_3 for the TA100 one, were prepared by dissolving them in DMSO and sterile water respectively, and served as positive control chemicals. Bacteria were aerobically grown at 37°C in Oxoid nutrient broth N° 2. The test was carried out by adding 0.2 ml of sterile 0.5 mM histidine-biotin and 0.1 ml of the overnight bacterial culture (approximately 1×10^8 bacteria/ml) to 2.0 ml of molten top agar (45°C). Doses from 10 to 100 µl of the tested solution were added to top agar tubes, which were then gently vortexed and subsequently transferred to plates with minimal glucose agar (30 ml/plate). After incubation at 37°C for 48 h in darkness, the His^+ revertant colonies were manually counted.

Culture medium: nutrient broth was prepared by dissolving 25 g of Oxoid nutrient broth N° 2 in 1 l of water. Glucose minimal agar plate contained 1.5% agar, 0.02% $MgSO_4 \cdot 7H_2O$, 0.2% citric acid, 1% K_2HPO_4, 0.35% $NaHNH_4PO_4 \cdot 4H_2O$ and 2% glucose. Top agar contained 0.75% agar and 0.5% NaCl.

In a typical experience, 0.0114 g of Co(II)-PST were dissolved in 2.24 ml of DMSO, giving a 8.99 mM solution. The assayed doses were taken from this solutions, generating dose–response curves by means of the standard plate assay. As it has been proved that sulfa-drugs are not mutagenic (Zeiger et al. 1988) the activity of the ligand was not tested by us.

Plant genotoxicity test (*Allium cepa* test)

For this test, which was carried out following standard procedures (Nieva Moreno et al. 2005) equal sized young bulbs of common *Allium cepa* were used. Mother solution 4.4×10^{-4} M of Co(II)-PST was prepared dissolving 0.0625 g of (1) (1.099×10^{-4} mol) in 7 ml DMSO and commercial mineral water in sufficient quantity to 250 ml. Similarly, it was prepared a mother solution of H_2PST, the parent sulfonamide. Alicuotes from these solutions were taken in order to carried out the experiments, which were done by duplicate, with seven bulbs per dose. Onion bulbs were kept in mineral water for 48 h and then exposed to the Co(II)-PST and H_2PST solutions for 24 h. The roots were then fixed in 1:3 acetic acid–ethanol solution for 24 h, and finally stored in 70% ethanol. The roots growing in mineral water were used as a negative control, while the treatment with $K_2Cr_2O_7$ 1 mg/l in mineral water represented a positive control. Length of roots as index of toxicity and modifications in root consistency and shape (formation of tumors, hook roots, twisted roots) were observed as macroscopic parameters. Microscopic parameter was mitotic index (five slides, 1,000 cells per slide) to evaluate cellular division rate.

Chromosome preparation and staining

Root tips were hydrolyzed in 1 M HCl at 60°C for 10 min before staining in Schiff's reagent for 15 min. After the root caps were removed from well stained root tips, 1 mm of the meristematic or mitotic zones was immersed in a drop of 2% orceine in 45% acetic acid (which carried out the staining of the chromosomes) on a clean slide and squashed into single cells using the eraser end of a pencil to apply pressure. A Globe light microscope was used with ×400 magnification.

Hemolytic studies

Freshly obtained heparinized human red blood cells (RBC) were washed three times by centrifugation (2,500 rpm for 10 min) in isotonic PBS, pH 7.0 at rt.

Two ml of RBC were added to 50 ml of sterile 5% glucose to obtain a 4% suspension. Stock solutions of **(1)** solubilized in DMSO (Sigma Chemical Co, St. Louis, MO) were diluted with sterile 5% glucose to yield final test concentrations of 1,500–9 μM. One ml of RBC suspension was added, mixed, and tubes incubated at 37°C. The absorbance of the liberated hemoglobin was measured spectrophotometrically at 540 nm in a double-beam Beckman spectrophotometer. Hemolysis of erythrocytes was indicated by complete clearing (lysis) (Lee et al. 1999). DMSO alone causes no lysis. Lytic concentration (LC_{100}) is defined as the lowest concentration of **(1)** that produces complete lysis of erythrocytes. The experiment was done in duplicate.

Stability of Co(II)-PST into the aqueous media employed to make biological tests was confirmed by electronic spectroscopy, comparing its visible spectra (400–800 nm) at the beginning and the end of the experiment, at room temperature (25°C). 100 µl of DMSO solution 0.20 M of Co(II)-PST were added to 1.00 ml of commercial mineral water (pH 8.08, measured with a Chemcadet model 5986-62 pH meter), giving a pink colloidal solution. Visible spectra were recorded in a Jasco V-550 spectrophotometer, using quart cells of 1 cm of light pass at 0; 24 and 48 h. 1.00 ml of DMSO was added to each pink colloidal solution of Co(II)-PST at the moment to acquire each spectrum. Similar experiment were made in parallel with the ligand (H_2PST) and with $Co(NO_3)2{\cdot}6H_2O$ in order to compare. Besides, kinetics of Co(II)-PST 9.48×10^{-3} M in mineral water-DMSO 1:1 were recorded by 30 min at 37°C and 519 nm, giving constant absorbance during all the period of measurement.

Results and discussion

General physicochemical characteristics of (1)

Elemental analyses of the pink powder gave satisfactory results for $[Co^{II}(PST)(H_2O)_4]{\cdot}2H_2O$, MM: 568.4 g/mol. Found (calcd for $CoC_{17}H_{23}N_3S_2O_{11}$): C, 35.65 (35.92); H, 4.07 (4.07); N, 7.25 (7.39); S, 11.31 (11.28); Co, 10.1 (10.4). Compound **(1)** is soluble in DMSO (0.43 g/ml). In HCl 1 M solution it suffers hydrolysis, and turns brownish in NaOH 1 M solution, maybe due to oxidation by means of the atmospheric oxygen, similarly to its homologous complex between Co(II) and sulfathiazole (Bellú et al. 2005).

Vibrational FTIR and Raman spectra

FTIR and Raman spectra of phthalylsulfathiazol and its complex of Co(II) are shown in Figs. 2 and 3 respectively.

The main vibrational FTIR and Raman frequencies of both spectra are displayed in Table 1. From these data the following items can be stated: The C=O stretching of the secondary amide (Amide I mode) remains unaltered upon coordination. The *v*(COOH) band of the free ligand (at 1721 s, FTIR and 1708 m, Raman) splits into two components when the coordination occurs. The set of bands located in the range 1,600–1,510 cm^{-1} can be assigned to the N–H deformation modes of secondary amides (Amide II mode), ring stretching and the antisymmetric stretching mode of the carboxylate group (Lin Vien et al. 1991; Smith 1999). This latter vibration may probably be assigned to the band placed at 1,516 cm^{-1} taking into account that this antisymmetric mode is of stronger intensity in the infrared than in the Raman spectra. The symmetric carboxylate stretching has been established according to the presence of a new Raman band of medium intensity at 1,400 cm^{-1} and the band of the ligand present in the FTIR spectrum at 1,399 cm^{-1} (due to a CCH bend) that increases its intensity upon coordination. The assignment of these modes was tentative taking into account that there coexist different stretching modes in the same

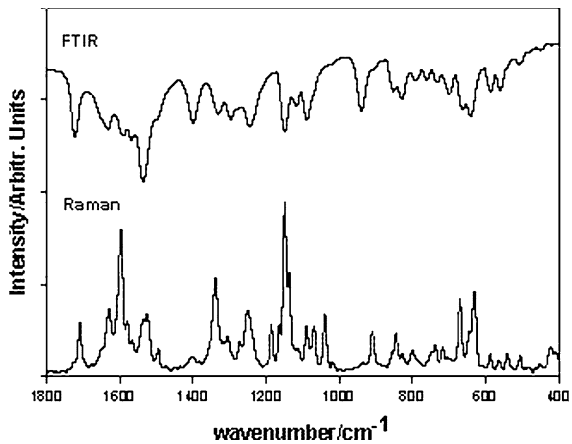

Fig. 2 FTIR and Raman spectra of H_2PST

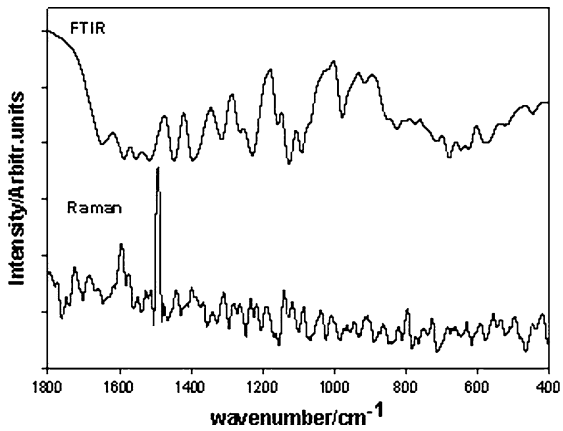

Fig. 3 FTIR and Raman spectra of (**1**)

spectral region due to the diverse groups that conforms the ligand. The only conclusion that can be unambiguously performed is that the carboxylic acid group is deprotonated in the complex but the interaction with the metal remains an open question. Evidence of the $N_{thiazole}$ deprotonation and coordination with Co(II) arises from the red shift observed for the band that is assigned to the thiazole ring vibrations (from ca. 1,530 to ca. 1,440 cm^{-1} in the complex). Besides, the interaction of the $N_{sulfonamide}$ atom with the metal center produced a blue shift in the position of the S–N stretching band from ca. 940 to ca. 978 cm^{-1}. These interactions are indicative of a bidentate behavior of the ligand, linking the metal ion through both N atoms (Pedregosa et al. 1995; Casanova et al. 1995, 2000; González-Álvarez et al. 2004). As expected, the bands at 1,315 and ca. 1,150 cm^{-1} attributed to SO_2 remain practically unchanged with respect to those of the ligand.

Thermogravimetric behavior

The trace of the thermogravimetric analyses of the complex are shown in Fig. 4. It is characterized by five weight losses until 1,000°C. The first loss amounts to 19.0% at 115°C and is due to the loss of six water molecules (expected 19.0%). This process is accompanied by a weak endothermic DTA signal located at 93.4°C. After the release of water, a complex degradation process of the remaining material is observed. The figure then shows at least, three consecutive TG steps: (1) between 224 and 472°C (with a weak exothermic DTA signal at 389°C); (2) between 472 and 718°C (very strong exothermic DTA signal at 525°C), (3) 718 and 830°C (DTA signal is not observed). These peaks are due to the combustion of the organic ligand and result in a mass of metal oxide of Co_3O_4 at 830°C (experimental residue, 13.8%; theoretical residue, 14.1%). The last step is due to the reduction of Co_3O_4 to CoO and amounts 0.8% weight loss in good agreement with the expected values (13.0% experimental residue; 13.2% theoretical residue). The characterization of both cobalt oxides has been performed by FTIR spectroscopy (Pejova et al. 2001).

$$\left[Co(PST)(H_2O)_4\right]2H_2O \xrightarrow{\phi O_2} Co_3O_4 + \text{other volatile products}$$

Diffuse reflectance spectrum

The reflectance spectrum (Fig. 5) was measured in the range 380 and 800 nm. Considering a distorted octahedral field, due to the different Co–O and Co–N bond lengths, the broad band centered at 500 nm was assigned to ν_3 ($^4T_{1g} \rightarrow {}^4T_{1g}(P)$) and the shoulder that appeared at lower energies (ca. 610 nm) to ν_2 ($^4T_{1g} \rightarrow {}^4A_{2g}$). The ν_1 band assignable to ($^4T_{1g} \rightarrow {}^4T_{2g}$) was not observed. This band usually occurs at ca. 1,300 nm, outside our measured range (Beloso et al. 2006; Lever 1984). These assignments were in accord with previous studies for CoN_2O_4 chromophores (Barszcz et al. 2005).

About the coordination possibilities of metal ions to the sulfonamide moiety and the structure of (**1**)

Neutral sulfonamides are expected to be poor ligands because of the withdrawal of electron density from the nitrogen atom onto the electronegative oxygen atoms. However, if the sulfonamide N atom bears a dissociable hydrogen, this same electron-withdrawing effect increases its acidity, and, in the deprotonated form, the sulfonamide anions are effective sigma-donor ligands. The calculated pKa values of $–SO_2NH_2$ moiety in the most of sulfonamides are in the range of 7.3–9.7 (Balaban et al. 2005) and are characterized as weak organic acids (Remko 2010). Two protons are the ionizable ones in the ligand, H_2PST: the carboxylic one and the amidic one, which

Table 1 Assignment of the vibrational FTIR and Raman spectra (frequency: υ, cm^{-1}) of phthalylsulfathiazole and it Co(II) complex

Phtalylsulfathiazole	Co(II)-Phtalylsulfathiazole	Assignments
1721 s	*1689 m*	ν(COOH)
1708 m		
1648, sh-1632 s	1648 s–1630 sh	ν(C=O) Amide I, secondary amides
1630 m	*1614 m*	
1589 s–1569 s	1586 s–1555 s, 1516 s	$\delta(N–H)_{in\ plane}$ amide II, secondary amides; ν(ring), $\nu_{as}(COO^-)$
1596 vs, *1579 m*	*1595 s*, *1574 m*, *1512 w*	
1535 vs	1447 s	$\nu(CN)_{thiazol}$ see text
1535 sh, *1524 m*	*1442 m*	
1492 s	*1502 w*, *1492 vs*	ν(C=C)
1399 m	1395 s	$\nu_s(COO^-)$
	1400 m	
1330 m	1315 m	$\nu_{as}(SO_2)$
1338 s, *1306 w*	*1338 w*, *1309 m*	
1296 m	1263 m	δ(CH), ν(C=C)
1294 m	*1284 m*, *1266 m*	
1244 m	1229 s	δ(CH), ν(C=C)
	1233 m, *1217 m*	
1185 m	*1190 m*	Benzene ring stretching
1147 s	1158 m	$\nu_s(SO_2)$
1148 vs, *1136 s*	*1143 m*	
1116 m	1126 vs	δ(CH), ν(C=C)
1088 m	1091 s	Benzene ring stretching
1089 m 1070 m		
1038 m	*1039 m*	o-phenylene ring breathing mode (Nyquist 2001)
940 s	978 s	$\nu(S–N)_{sulfonamide}$
912 m		

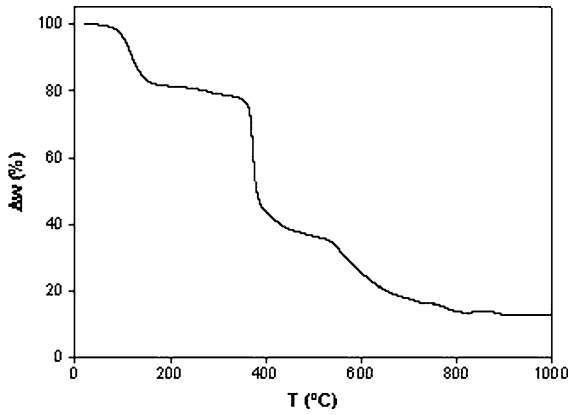

Fig. 4 TG (—) trace for the thermal decomposition of Co(II)-PST system. (O_2 flow = 60 cm^3 min^{-1}, heating rate: 10°C min^{-1}). Δw (%): % weight change

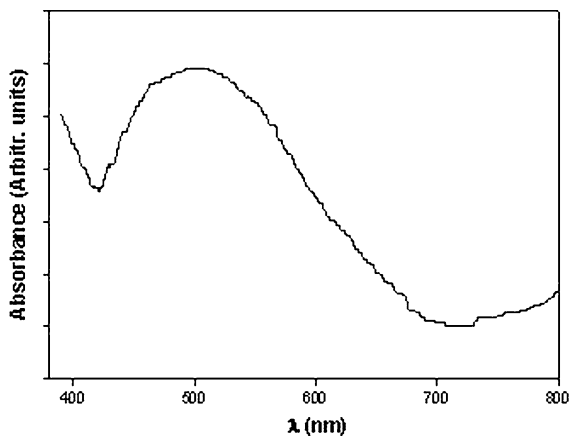

Fig. 5 Reflectance diffuse spectrum of Co(II)-PST

appear at 13.1 and 12.8 ppm respectively in the ^{1}H NMR spectrum (AIST 2010).

Moreover, these ligands are very versatile in terms of their coordination modes and offer several points for binding metals (Otter et al. 1998). The 4-amino-*N*-(5-methyl-1,3,4-thiadiazole-2-yl)sulfanilamide (sulfamethizole) (Hsmtz), has several groups with donor atoms that are able to interact with metal ions too. The structure of $Co(smtz)_2(py)_2(OH_2)_2$ were determined by X-ray diffraction. The metal ion presents a distorted octahedral environment, and the deprotonated sulfamethizole acts as monodentate ligand coordinating through the thiadiazole N atom (Borrás et al. 2000). In the Co complex of sulfamethoxazole [4-amino-*N*-(5-methyl-3-isoxazolyl) benzenesulfonamide] the metal atom coordinates through the sulfonamide and amino nitrogens (Kesimli and Topacli 2001). Cobalt(II) complexes of sulfadiazine formulated as $[Co(C_{10}H_9N_4O_2S)_2(CH_3OH)_2]$ and $[Co(C_{10}H_9N_4S)_2(H_2O)_2]$, with sulfadiazine acting as a bidentate ligand. Cobalt is coordinated to two sulfonamide nitrogen and the pyrimidine nitrogen of the sulfadiazine. Two molecules of solvent complete the octahedral geometry around the cobalt atom (Ajibade et al. 2006).

Relating to sulfathiazole metal complexes, different compounds were reported in which the sulfa moiety acts with a high versatility in its coordination ability. For example, with Zn(II) the drug acts as a bridging ligand through both the N_{amino} and $N_{thiazole}$ atoms (Casanova et al. 1993). As a neutral ligand, HST acts as a monodentate ligand, binding the metal ion through the N_{amino} atom (Casanova et al. 1994). As a deprotonated ligand, the sulfathiazolato (ST^-) has a variety of coordination behaviors, e.g., besides the Zn(II)–ST complex (Casanova et al. 1993), in Cu(II) complexes coordination through the $N_{thiazole}$ atom could be seen, and in another case the sulfathiazolato exhibits bidentate behavior linking the metal ion through the $N_{thiazolic}$ and the $N_{sulfonamido}$ atoms (Casanova et al. 1997). More recently, we have analyzed the interaction of mercury(II) (Bellú et al. 2003) and cobalt(II) with sulfathiazole (Bellú et al. 2005). In both cases the $N_{thiazolic}$ atom was one of the binding site.

In spite of the steric bulk of the PST moiety, which could induce tetrahedral geometry (Otter et al. 1998), Co(II) presents an octahedral environment in the Co(II)-PST complex, but in this complex only one ligand coordinates to the metal and the coordination sphere is completed using water molecules.

Spectroscopic and chemical data, and the comparison with its homologous complexes, let us suggest that the $N_{thiazolic}$ and the $N_{sulfonamide}$ atoms could be the binding sites for the Co(II) ion to the phthalylsulfathiazole moiety.

Antimicrobial properties

Emergence of resistance in bacterial strains has become as one of the prime concerns of the twenty-first century (Mishra et al. 2008), while the increased incidence of invasive mycoses and the emerging problem of antifungal drug resistance have encouraged the search for new antifungal agents or effective combinations of existing drugs (Navarro-Martínez et al. (2006).

Several cobalt(II) complexes recently reported have shown antibacterial and/or antifungal activities, which, in many cases, were better than the ligand one (Rodríguez-Argüelles et al. 2009). However, the reported MICs are into wide ranges, with values between, such as, from 0.3 (against *Haemophilus influenzae*) to 200 μg/ml (against *Bacillus subtilis*) for bacteria and >200 μg/ml for fungi (Rodríguez-Argüelles et al. 2007).

Results of the antibacterial assay with H_2PST, NaST and its Co(II) complexes, and of the antifungal assay with H_2PST and its Co(II) complex are shown in Tables 2 and 3 respectively. Examples of some cobalt(II) complexes against the same or similar to our tested strains were included for comparative purposes.

Apart from Co(II)-ST against both *S. aureus* (methicillin-sensitive and methicillin-resistant), and NaST against *P. aeruginosa*, all the tested sulfa drug (NaST, H_2PST) and its Co(II) complexes have shown a moderate activity against the tested bacteria (MICs from 20 to 30 μg/ml). Looking at the molecular composition of the complexes, Co(II)-ST has two sulfathiazole moieties, so, its antibacterial activity could be due to this part of the molecule, except for the case of *Pseudomonas aeruginosa*, in which the metal ion could play an important role in the activity. On the other hand, the activity of NaST was lost after complexation against both tested *Staphylococcus aureus* strains. Alternatively, all the tested bacteria were sensitive to both H_2PST and its Co(II) complex.

Table 2 MIC values in μg/ml and (nmol/ml) of phthalylsulfathiazole (H_2PST), its Co(II) complex (Co(II)-PST), sodium sulfathiazole (NaST) and its Co(II) complex (Co(II)-ST), acting against human pathogenic bacteria

Bacteria	H_2PST	Co(II)-PST	NaST	Co(II)-ST	Co(II)[a]	Cf	Another Co(II) complexes
Staphylococcus aureus methicillin-sensitive ATCC 29213	20 (49.6)	25 (44.0)	20 (65.8)	>30 (>46.9)	25 (91.2)	0.500	64[b]
Staphylococcus aureus methicillin-resistant ATCC 4330	20 (49.6)	20 (35.2)	20 (65.8)	>30 (>46.9)	25 (91.2)	0.500	64[b] 100[c]
Escherichia coli ATCC 25922	30 (74.4)	30 (52.8)	20 (65.8)	20 (31.2)	25 (91.2)	0.500	>64[b]
LM_1-*Escherichia coli*	25 (62.0)	30 (52.8)	25 (82.2)	25 (39.0)	25 (91.2)	5.000	
LM_2-*Escherichia coli*	25 (62.0)	25 (44.0)	20 (65.8)	25 (39.0)	25 (91.2)	0.500	
Pseudomonas aeruginosa ATCC 27853	20 (49.6)	25 (44.0)	>30 (>98.7)	20 (31.2)	25 (91.2)	7.500	>64[b]

[a] As $CoCl_2 \cdot 6H_2O$; [b]Lv et al. (2006); [c]Rodríguez-Argüelles et al. (2009); Cf: cefotaxim

Since Co(II)-PST has only one moiety of the parent sulfa drug per molecule, it seems be a synergic action between the sulfa and the cobalt ion.

In contrast to antibacterial agents, few antimetabolites are available for use against pathogenic fungi. In spite of fungal organisms, as well as bacteria, posses a pathway for folate, inhibitors of folate metabolism [like sulfonamides, as shown above (García-Raso et al. 2000)] are not effective in the treatment of *C. albicans* infections. Among the reasons for this fact it could be mentioned differences between the nature of key enzymes in folic acid biosynthetic pathways of fungus and bacteria (Baccanari et al. 1989) and the impermeability of the membrane to same drugs (Navarro-Martínez et al. (2006). With respect to metal complexes of sulfa drugs, in addition to its well-known antibacterial activity, silver sulfadiazine was recently reported to possess strong antifungal properties, which makes it a clinically widely used topical agent for the treatment of wound and burnt infection (Vehmeyer-Heeman et al. 2006).

In all cases, the complex Co(II)-PST showed better antifungal activity than the ligand (H_2PST), which was inactive against the tested fungi, and better o similar antifungal activity than Co(II)-ST, except for *A. fumigatus*. Co(II)-PST displayed a moderate activity against *C. albicans*, fact that could be of interest considering the increasing antifungal drug resistance.

In view of the synergistic effect reported between cerium nitrate and silver sulfadiazine in the treatment of wound infections (Rosenkranz 1979; Wassermann et al. 1989), work is in progress in this sense with the aim of improve the antifungal effect of Co(II)-PST.

Mutagenicity test

Results of the Ames test for Co(II)-PST are shown in Table 4

The Ames *Salmonella typhimurium* assay has been a classic bioassay to determine the potential genotoxicity and mutagenicity of compounds since 1970s (Maron and Ames 1983). The reversion coefficient (Maron and Ames 1983) has been defined as follows: revertant number per tested plate/ revertant number per control plate (spontaneous). i.e.: dividing the average revertants/plate of the tested substance by the spontaneous mutation rate. A non-statistical procedure has been established to evaluate the results of *Salmonella* experiments (Mortelmans and Zeiger 2000) In agreement with this procedure, a substance (pure or mixture) is considered a mutagen if it produces a reproducible, dose-related increase in the number of revertant colonies in one or more strains (i.e.: R.C. $\geq$ 2.0). Consistent with this (**1**) did not show direct mutagenicity in the assayed range.

Plant genotoxicity test

Allium cepa species has been frequently used to determine the cytotoxic, mutagenic and genotoxic effects of several substances, being considered the

Table 3 MIC/ MFC values in μg/ml and (μmol/ml) of phthalylsulfathiazole (H_2PST) and its Co(II) complex (Co(II)-PST), acting against human opportunistic pathogenic fungi

	H_2PST	Co(II)-PST	NaST[b]	Co(II)-ST[b]	Co(II)[a]	Amp	Ket	Terb	Another Co(II) complexes
C. albicans	>250 (>0.62)	125/250 (0.22/0.44)	>250 (>0.82)	>250 (>0.39)	250/>250 (0.86/>0.86)	0.78	6.25	1.56	32[c]
C. tropicalis	>250 (>0.62)	250/250 (0.44/0.44)	>250 (>0.82)	>250 (>0.39)	250/>250 (0.86/>0.86)	1.56	6.25	0.78	>200[d]
S. cerevisiae	>250 (>0.62)	>250 (>0.88)	>250 (>0.82)	>250 (>0.39)	250/>250 (0.86/>0.86)	0.78	3.12	3.12	100[d]
C. neoformans	>250 (>0.62)	250/250 (0.44/0.44)	>250 (>0.82)	>250 (>0.39)	125/>250 (0.43/>0.86)	0.78	1.56	0.39	16[c]
A. fumigatus	>250 (>0.62)	250/250 (0.44/0.44)	200 (0.66)	200 (0.31)	250/>250 (0.86/>0.86)	3.12	12.5	0.78	105[c]
A. flavus	>250 (>0.62)	>250 (>0.88)	>250 (>0.82)	100 (0.16)	>250 (>0.86)	0.78	6.25	0.78	105[c]
A. niger	>250 (>0.62)	>250 (>0.88)	>250 (>0.82)	>250 (>0.39)	>250 (>0.86)	0.78	6.25	1.56	105[c], >200[d, e]
M. gypseum	>250 (>0.62)	250/250 (0.44/0.44)	>250 (>0.82)	>250 (>0.39)	250/250 (0.86/0.86)	6.25	12.5	0.006	
T. rubrum	>250 (>0.62)	250/>250 (0.44/>0.88)	>250 (>0.82)	>250 (>0.39)	250/>250 (0.86/>0.86)	6.25	12.5	0.003	
T. menta-grophytes	>250 (>0.62)	>250 (>0.88)	>250 (>0.82)	>250 (>0.39)	>250 (>0.86)	6.25	12.5	0.006	

Previous MICs values of sodium sulfathiazole (NaST) and its Co(II) complex (Co(II)-ST), are shown for comparative purposes

[a] As $Co(NO3)_2{\cdot}6H_2O$ [b] Bellú et al. (2005); [c] Lv et al. (2006); [d] Rodríguez-Argüelles et al. (2009); [e] Rodríguez-Argüelles et al. (2007)

Co(II)-PST: $[Co^{II}(PST)(H_2O)_4]{\cdot}2H_2O$, Co(II)-ST: $[Co^{II}(ST)_2(H_2O)_4]$, *Amp* amphotericin B; *Ket* ketoconazole; *Terb* terbinafine

Table 4 Mutagenic activity of (1) with *S. typhimurium* TA98 and TA100 strains

Co(II)-PST (1) μg/plate (nmol/plate)	TA98 No. rev./plate[a] ± SD	R.C.[b]	TA100 No. rev./plate[a] ± SD	R.C.[b]
0[c] (0)	24.83 ± 5.67	1.00	151.67 ± 8.71	1.00
102.2 (179.8)	26.17 ± 3.37	1.05	157.83 ± 9.64	1.04
306.6 (539.4)	25.33 ± 8.29	1.02	140.20 ± 10.23	0.92
511.0 (899.0)	30.50 ± 6.77	1.23	145.83 ± 7.73	0.96
715.4 (1258.6)	24.67 ± 6.98	0.99	132.40 ± 8.08	0.87
1022 (1798.0)	24.17 ± 6.85	0.97	151.80 ± 11.43	1.00

[a] Mean of duplicate experiments with three replicates per experiment ± standard deviation (SD). [b] R.C.: reversion coefficient = revertants with tested substance/spontaneous revertants. [c] Negative control, without tested compound: spontaneous revertants/plate. Positive controls with respective diagnostic mutagens: TA98, 4NOPDA 1.25 μg/plate, no. rev./plate, 76.5 ± 0.5, R.C.: 3.19; TA100, NaN_3 0.15 μg/plate, no. rev./plate, 490 ± 2, R.C.: 3.11

standard organism for quick tests, since it shows a high correlation with mammal test systems (Casimiro Fernandes et al. 2009). Another advantage of this test system is the presence of an oxidase enzyme system, which is essential for promutagen evaluations (Morais Leme and Marin-Morales 2009).

The results of phytotoxicity and mitotic index (MI) of Co(II)-PST and H_2PST with comparative purposes, evaluated with the *Allium cepa* test are shown in Table 5.

Root lengths showed similarity between the Co(II)-PST and the parent sulfa drug. MI was not affected at concentrations in which both sulfa drugs showed antibacterial effects. The inhibition of the MI was similar for both sulfa drugs at higher concentration.

Hemolytic activity

Erythrocyte lysis has been used for many years as a measure of general membrane disruption and perusal of recent literature indicates that the use of such assays continues today. One direct benefit of a red blood cell lysis assay is in predicting unacceptable toxicity, since compounds that disrupt mammalian cell membranes are unlikely to prove interesting for further pharmaceutical development (Chapman and Buxser 2002). **(1)** showed not hemolytic effects to human erythrocytes at concentrations in which it is active.

Table 5 Phytotoxicity (as root length) and mitotic index (MI) of Co(II)-PST and H_2PST evaluated with the *Allium cepa* test

M × 10^6	Root length (% of control)		MI (%)[c] ± SD	
	Co(II)-PST	H_2PST	Co(II)-PST	H_2PST
0	100[a]	100[b]	4.0 ± 0.9	
4.4	72	83	5.2 ± 0.7	6.7 ± 0.6
5.9	73	–	4.8 ± 0.9	7.1 ± 0.2
8.8	68	62	4.3 ± 0.5	5.1 ± 0.7
17.6	48	50	4.3 ± 0.5	4.1 ± 0.7
440	21	19	2.6 ± 0.6	1.5 ± 0.5

Δ (cm): [a]1.45 ± 0.52; [b]1.20 ± 0.14. [c]Mean of duplicate experiments with seven replicate per each dose. SD: standard deviation. Positive control: $K_2Cr_2O_7$ 3.4×10^{-6} M (1 mg/l): root length: 58% of negative control; MI % ± SD: 5.9 ± 0.9

Conclusion

The reaction between phthalylsulfathiazolate and cobalt(II) aqueous solutions leads to a stable complex compound, $[Co^{II}(PST)(H_2O)_4]\cdot 2H_2O$ **(1)**. Reflectance diffuse spectrum is in agreement with a distorted octahedral environment of the Co(II) ion. Vibrational FTIR and Raman spectroscopic data reveal that the ligand would be doubly deprotonated. Spectroscopic and chemical data let us suggest that the $N_{thiazolic}$ and the $N_{sulfonamide}$ atoms could be the binding sites for the Co(II) ion to the phthalylsulfathiazole moiety. **(1)** showed antibacterial activity similar to the ligand. Activity against *Candida albicans*, if moderate, was better than the ligand one.

(1) did not show direct mutagenicity with the Ames test in the range of assay doses nor hemolytic effects to human erythrocytes in vitro at concentrations in which it is active. The phytotoxicity of **(1)**, evaluated with the *Allium* test, was similar to the phthalylsulfathiazole one in the whole tested range.

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