

Preparation, spectral characterization and antibacterial studies of silver(I) complexes of 2-mercaptopypyridine and thiomalate

Muhammad Hanif^a, Aisha Saddiq^a, Shahida Hasnain^b, Saeed Ahmad^{a,*}, Ghulam Rabbani^a and Anvarhusein A. Isab^{c,*}

^aDepartment of Chemistry, University of Engineering and Technology, Lahore, Pakistan

^bDepartment of Microbiology and Molecular Genetics, Punjab University, Lahore, Pakistan

^cDepartment of Chemistry, King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia

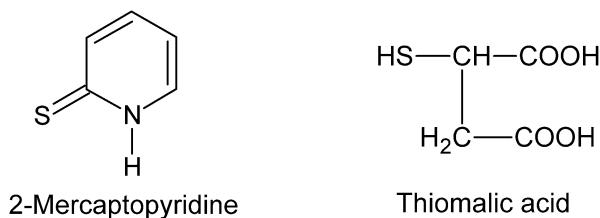
Abstract. Silver(I) complexes of 2-mercaptopypyridine (Mpy), $[\text{Ag}(\text{Mpy})]\text{NO}_3$ and $[\text{Ag}(\text{Mpy})_2]\text{NO}_3$, and the first mixed-ligand complex having a thione and thiolate coordinated to Ag(I), [Mpy–Ag–Tm] (Tm = thiomalate) have been prepared and characterized by IR and NMR spectroscopy. The ^1H and ^{13}C NMR spectra show the presence of both ligands in the mixed-ligand complex, [Mpy–Ag–Tm]. An upfield shift is observed in the $>\text{C}=\text{S}$ resonance of Mpy and $\text{C}=\text{O}$ resonances of thiomalate in ^{13}C NMR, while the other resonances are shifted downfield. The complexes showed relatively high antibacterial activity (inhibition zone of 6–11 mm) against a gram +ve bacterium, *Bacillus subtilis*, as compared to that (inhibition zone of 4 mm) against a gram –ve bacterium, *Escherichia coli*.

Keywords: Silver(I) complexes, 2-mercaptopypyridine, thiomalate, antibacterial activity

1. Introduction

Silver and its compounds are used as antimicrobial agents in medicine. Silver sulfadiazine is a broad-spectrum antibiotic ointment, used to treat skin infections [1,2]. Polymeric silver(I) complexes with weaker Ag–O and Ag–N bonds also display effective activities against bacteria, yeasts and moulds [2–7]. However, the Ag–S bonding complexes have been shown to have narrower spectrum of antibacterial activity than Ag–N or Ag–O complexes [8]. The potential target sites for inhibition of bacterial and yeast growth by silver complexes might be the sulfur containing residues of proteins (cysteine, glutathione). Thus, from biological point of view it is important to assess independently the chemical reactivities of sulfur donor ligands towards the metal ions and to identify the resulting complexes. Consequently, several silver(I) complexes containing thiones [9–15] and thiolates [16–20] have been prepared and structurally characterized. Silver(I) complexes of thiolates like thiomalic acid [16,17], thiosalalisic acid [18] and 2-mercaptoponicotinic acid [8,19] also showed remarkable antimicrobial activities for bacteria, yeast, and mold. However, there are no known reports of mixed ligand silver(I) complexes containing

*Corresponding authors: A.A. Isab, Department of Chemistry, King Fahd University of Petroleum and Minerals, Dhahran 31261, Saudi Arabia. E-mail: aisab@kfupm.edu.sa; S. Ahmad, Department of Chemistry, University of Engineering and Technology, Lahore 54890, Pakistan. E-mail: saeed_a786@hotmail.com.



Scheme 1. Structures of the ligands used in the study.

both thione and thiolate ligands. Therefore, in this study we have prepared a silver(I) complex containing both a thione (Mpy) and a thiolate (thiomalate) ligand and investigated its antibacterial activity. The structures of the ligands used in this study are shown in Scheme 1.

2. Experimental

2.1. Chemicals

AgNO_3 was a product of Panreac, Spain. 2-mercaptopypyridine (Mpy) and 2-mercaptosuccinic acid or thiomalic acid (Tm) were obtained from Acros Organics, Belgium.

2.2. Preparation of complexes

The complexes, $[\text{Ag}(\text{Mpy})]\text{NO}_3$ and $[\text{Ag}(\text{Mpy})_2]\text{NO}_3$ were prepared by adding one or two equivalents of the mercaptopyridine dissolved in 15 ml methanol to one equivalent (0.170 g) of AgNO_3 dissolved in 10 ml water. Stirring the mixture for 15–20 minutes resulted in yellowish precipitates, which were filtered, washed with methanol and air-dried.

For the preparation of mixed-ligand complex [Mpy–Ag–Tm], a solution of 1 mmol of thiomalic acid in 15 ml water was added to an aqueous solution of 1 mmol (0.17 g) of AgNO₃. A yellow-colored solution formed immediately. While stirring, a solution of 1 mmol of mercaptopyridine in 10 ml methanol was added to it and the mixture was stirred for half an hour. As a result yellowish precipitates were formed, which were filtered, washed with methanol and air-dried. The product yield is about 50–60%. The melting points of the complexes are given in Table 1.

2.3. IR measurements

The solid-state IR spectra of the ligands and their thiocyanato silver(I) complexes were recorded on a Perkin–Elmer FTIR 180 spectrophotometer using KBr pellets over the range 4000–400 cm^{−1}.

2.4. 1H and ^{13}C NMR measurements

The ^1H NMR spectra of the complexes in DMSO- d_6 were obtained on Jeol JNM-LA 500 NMR spectrometer operating at a frequency of 500.00 MHz at 297 K using 0.10 M solution. The ^{13}C NMR spectra were obtained at the frequency of 125.65 MHz with ^1H broadband decoupling at 298 K. The spectral conditions were: 32 K data points, 0.967 s acquisition time, 1.00 s pulse delay and 45° pulse angle. The ^{13}C chemical shifts were measured relative to TMS.

Table 1
Melting points and selected IR absorptions (cm^{-1}) of silver(I) complexes

Species	m.p. ($^{\circ}\text{C}$)	$\nu(\text{C=S})$	$\nu(\text{NH}_2)$	$\nu(\text{C-N})$	$\nu(\text{C=O})$
Tm	—	—	—	—	1715
Mpy	—	613, 745	3176	1487	—
$[\text{Ag}(\text{Mpy})\text{NO}_3]$	202	615, 757	3155	1508	—
$[\text{Ag}(\text{Mpy})_2]\text{NO}_3$	143	623, 749	3175	1501	—
$[\text{Mpy-Ag-Tm}]$	179	621, 756	3153	1507	1718

2.5. Biological activities of the complexes

The complexes were screened for biological activities against two bacteria, *Bacillus subtilis* (Py. 79 [21]) and *Escherichia coli* (ATCC 14169). Antibacterial activities were estimated by agar well diffusion method [22,23]. The liquid medium for bacteria was Lauria Bretaini (1.2% agar [24]). The test samples (complexes) were dissolved in DMSO (10 $\mu\text{g}/\mu\text{l}$) and 35 μl of the sample solution (containing 350 μg of the complex) was used per well. Bacteria were cultured for 24 hours at 37°C and then the growth of microorganisms was observed. The diameter of inhibition zone was recorded as the excess radius (mm) from a 6 mm (diameter) disc.

3. Results and discussion

3.1. IR and NMR studies

The reaction of AgNO_3 with mercaptopyridine and thiomalate in a 1:1:1 molar ratio resulted in a mixed-ligand complex $[\text{Mpy-Ag-Tm}]$, which to our knowledge is the first example of silver(I) complexes having both a thione and a thiolate ligand coordinated to silver(I). The selected IR frequencies of the ligands and their silver(I) complexes are given in Table 1. In IR spectrum of Mpy, the characteristic bands are expected in three frequency regions; $\nu(\text{C=S})$ appears around 600 cm^{-1} , $\nu(\text{C-N})$ bands at about 1500 cm^{-1} and $\nu(\text{N-H})$ is observed near 3200 cm^{-1} . N-H bending vibration is also observed around 1580 cm^{-1} . The presence of N-H vibrations indicate the existence of thione form of Mpy in the solid state. A sharp band around 1718 cm^{-1} was observed for the C=O stretch in $[\text{Mpy-Ag-Tm}]$ indicating the binding of thiomalate with silver(I). The $\nu(\text{S-H})$ at 2530 cm^{-1} was not observed showing the replacement of S-H hydrogen of thiomalic acid by silver(I) ions.

In ^1H NMR spectra of the complexes, a slight downfield shift (of ~ 0.5 ppm) was observed in the aromatic protons of Mpy. For example, in $[\text{Ag}(\text{Mpy})_2]\text{NO}_3$ the aromatic protons at C-3, C-4, C-5 and C-N appear at 7.706, 7.243, 7.754, 8.203 ppm respectively (for the free ligand; 7.338, 6.808, 7.467 and 7.705 ppm). The protons at C-3 and C-N appear as doublets, while the other two appear as triplets. The deshielding is related to an increase in π electron density in the C-N bond upon coordination. The N-H signal of Mpy was not observed. The ^1H NMR spectrum of free thiomalate ligand shows an ABX system consisting of a doublet of doublets for methyne protons and two doublets of doublets for geminal protons (^aH and ^bH) of the neighboring methylene group. The chemical shift of methyne proton is ~ 3.8 ppm, while the methylene protons resonate at ~ 3.0 and 2.9 ppm respectively [16]. In $[\text{Mpy-Ag-Tm}]$ it has been observed that -CH signals are shifted upfield by 1 ppm, whereas the chemical shifts of methylene protons are almost unchanged. This observation suggests that thiomalate is binding to silver(I) through sulfur atom.

The ^{13}C chemical shifts of the ligands and complexes are summarized in Table 2. In ^{13}C NMR, the $>\text{C}=\text{S}$ resonance of Mpy in the complexes is shifted significantly upfield (by about 6–10 ppm) as compared to its position in free ligand in accordance with the data observed for other silver(I) complexes of heterocyclic thiones [11–15]. A shift of this magnitude is diagnostic for S-bonded thiones, ascribed to back-bonding of the metal d-orbitals to the antibonding π -orbitals of sulfur in the $>\text{C}=\text{S}$ bond, which will not only reduce the $>\text{C}=\text{S}$ bond order but also shield the carbon atom of $>\text{C}=\text{S}$ group resulting in a high field shift [11–15]. A small deshielding effect is observed in other carbon atoms, which is due to an increase in π character of the C–N and C–C bonds. The assignment of Mpy resonances is based on the electronegativity relationships and the splitting pattern of aromatic protons in ^1H NMR. The changes in chemical shifts are slightly larger for the bis complex, $[\text{Ag}(\text{Mpy})_2]\text{NO}_3$ than in $[\text{Ag}(\text{Mpy})]\text{NO}_3$ showing that Mpy ligands are strongly bound in the former.

The ^{13}C NMR spectrum of $[\text{Mpy}-\text{Ag}-\text{Tm}]$ shown in Fig. 1 displayed peaks indicating the presence of both (Mpy & Tm) ligands. The ^{13}C NMR spectrum of uncoordinated thiomalate ligand shows four resonances due to two carboxyl carbons around 180 ppm, methylene carbon at ~ 42 ppm and methyne carbon at ~ 39 ppm [16]. Upon complexation with silver(I), the C=O signals are shifted upfield, while the C–H resonances are shifted downfield (Table 2). However, in the reported silver(I) and gold(I) com-

Table 2
 ^{13}C chemical shifts (in ppm) of the ligands and their silver(I) complexes in DMSO-d_6

Species	C=S	C-N	C-3	C-4	C-5	CH & CH ₂	C=O
Tm	—	—	—	—	—	38.9, 41.9	177.1, 178.9
Mpy	177.69	137.91	132.99	112.78	137.49	—	—
$[\text{Ag}(\text{Mpy})]\text{NO}_3$	167.36	141.82	131.29	118.22	140.90	—	—
$[\text{Ag}(\text{Mpy})_2]\text{NO}_3$	167.02	141.81	131.26	118.33	140.65	—	—
$[\text{Mpy}-\text{Ag}-\text{Tm}]$	167.41	141.10	132.08	118.47	141.10	43.70	172.28, 176.13

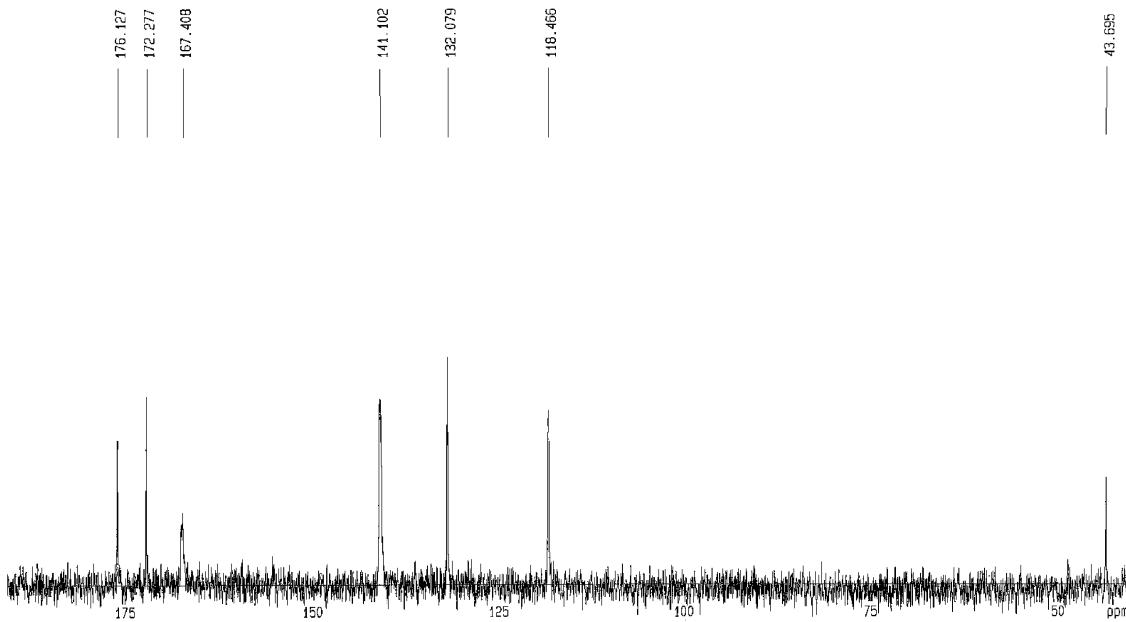


Fig. 1. $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of $[\text{Mpy}-\text{Ag}-\text{Tm}]$.

Table 3
Antibacterial activities of silver(I) complexes^{*}

Complex	Activity (in terms of zone of inhibition in mm)	
	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>
[Ag(Mpy)]NO ₃	9 ± 0.5	Not detectable
[Ag(Mpy) ₂]NO ₃	11 ± 1.2	4 ± 0.3
[Mpy–Ag–Tm]	6 ± 0.7	Not detectable
Control (DMSO)	Not detectable	Not detectable

*Concentration used was 10 µg/µl in DMSO per well.

plexes of thiomalate both types of carbons were observed at downfield position and it was inferred from this observation that thiomalate ligands are coordinated to silver(I)/gold(I) ions through sulfur atom only [16,25,26]. The upfield shifts in the carboxylic carbons in the present investigation indicates the involvement of oxygen atoms of carboxyl groups, in addition to sulfur in the binding of thiomalate to silver atom.

3.2. Antibacterial activities

The biological activities of the complexes (average of three measurements) are summarized in Table 3. The complexes showed moderate activities against the gram +ve bacterium, *B. subtilis*, while only one complex exhibited activity against gram –ve bacterium, *E. coli*. The activity of Ag-thiomalate complex in terms of minimum inhibitory concentration (MIC) against the same two bacteria has been reported previously [16]. The complex showed remarkable and superior activity against *E. coli* compared to *B. subtilis*. The activity (MIC) of the complex against the gram +ve bacterium was 2000 µg/ml [16]. In the present case, 350 µg/35 µl of the complexes yielded about 1 cm zone of inhibition in the gram +ve bacterium, which represents their significant activity against this bacterium. The oxygen bonded silver(I) complexes usually exhibit superior activities compared to the presently tested sulfur bound complexes [2–7]. The antibacterial activities of these complexes are due to a direct interaction of silver(I) ion with biological ligands such as proteins, enzymes and membranes. The coordinating ligands usually play a role of carrier for metal ion to the biological system. The strongly bound ligands result in the decreased activity [2].

The spectroscopic investigation of the Ag-thiomalate complex showed that the complex is oligomeric with the repeating linear Ag–S₂ units (i.e., [Ag(Tm)]_n) [16]. We have found that the addition of Mpy to this complex results in the breakage of the polymeric structure of Ag–Tm complex forming a ternary complex, [Mpy–Ag–Tm]. There are several studies describing that the interaction of thiones with gold(I) thiomalate ([Au(Tm)]_n) results in the formation of ternary complexes, [Thiones–Au–Tm] [27–30]. However, this is the first study describing the formation of such a complex of silver(I) from the reaction of a thione with the silver(I)–thiolate.

Acknowledgements

Financial support from Pakistan Council for Science and Technology, Islamabad, Pakistan and from King Fahd University of Petroleum & Minerals, Dhahran, Saudi Arabia is gratefully acknowledged.

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