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SYNTHESIS, X-RAY ANALYSIS AND ANTIBACTERIAL STUDY OF SILVER COMPLEX WITH ETHYL-*5*-HYDROXY-*2*-OXO-*2H*-CHROMENE-*3*-CARBOXYLATE

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ABSTRACT. A novel silver complex $[Ag(C_{12}H_9O_5)_2]$ was synthesized by the reaction of silver salt ($AgNO_3$) and coumarin based ligand (ethyl-*5*-hydroxy-*2*-oxo-*2H*-Chromene-*3*-carboxylate) at room temperature. The synthesized complex was characterized by using different analytical techniques like melting point (mp), infrared (IR) spectroscopy, powder X-ray diffraction (PXRD), thermo gravimetric analysis (TGA), atomic absorption spectroscopy (AAS) and mass spectrometry (ESI-MS). Ligand showed activity with MIC 20 µg/mL, 15 µg/mL, 15 µg/mL for *S. aurues*, *E. coli* and *S. typhi*, respectively whereas MIC values of Ag-complex for above mentioned bacterial strains were found to be 15 µg/mL, 10 µg/mL, 10 µg/mL, respectively. Ligand could not inhibit the growth of *B. Subtilis*, *P. auruginosa*, MRSA but Ag-complex showed MIC 30 µg/mL, 25 µg/mL for *B. Subtilis* and *P. auruginosa*. It also remained ineffective against MRSA.

KEY WORDS: Silver complex, Ethyl-*5*-hydroxy-*2*-oxo-*2H*-Chromene-*3*-carboxylate, Antibacterial study

INTRODUCTION

Coumarin and its derivatives form an important class of benzopyrones and found in nature. Many complex natural compounds contain them as structural subunits and have various numerous biological activities, such as antitumor [1], anti-HIV (NNRTI) [2], antioxidation [3], antimicrobial activity [4] and anticancer activity [5]. The extensive biological activities of coumarin derivatives have gained their prominent position in the area of synthetic chemistry and pharmacology.

Transition metals like silver have been used for years as anti-microbial agents because it has low toxicity than other transition metals. A silver compound which is frequently used is silver(I) sulfazine; it is used in the treatment of severe burns to prevent from bacterial infections [6]. Chlorhexidine, silver sulfadiazine is an anti-infective metal complex against catheter infections in vivo [7]. It is well established that only silver in its ionic or complexed forms is antimicrobially active, while the elemental silver, even in the so-called "nanocrystalline" state, is not [8]. Silver-containing compounds are attractive because of the fact that in the range of the applicable concentrations, silver ions do not exhibit toxicity and carcinogenic activities [9]. There is an increased interest in the potential use of silver(I) as a therapeutic agent for different antimicrobial applications.

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By keeping in view the above intriguing applications, it's highly attractive and fascinating to synthesize different type of Ag(I) complexes with biomedicinally active ligands. In this paper, the synthesis, characterization, and antibacterial properties of Ag(I) complex with ethyl-*5*-hydroxy-*2*-oxo-*2H*-chromene-*3*-carboxylate ligand are being reported.

EXPERIMENTAL

Synthesis of ethyl-5-hydroxy-2-oxo-2H-chromene-3-carboxylate

According to the procedure followed by Teizo Sugino *et al.* [10] a mixture of salicylaldehyde (1.22 g, 10.0 mmol), diethyl malonate (1.60 g, 10.0 mmol) and a few drops of piperidine was mixed and ground well for 5 min at room temperature. The reaction mixture was neutralized with dil. HCl and then the crystalline product was isolated by filtration to give 3-ethoxycarbonylcoumarin (2.07 g) in 95% yield (Figure 1). The crude crystals thus obtained were recrystallized from EtOH to give white crystalline solid. The proposed structure of silver complex with ethyl-5-hydroxy-2-oxo-2H-chromene-3-carboxylate is given in Figure 2.

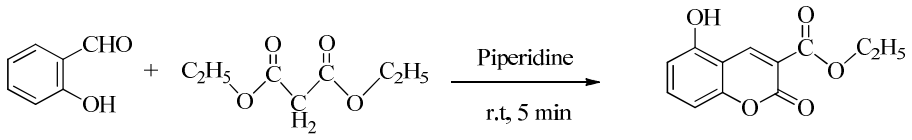
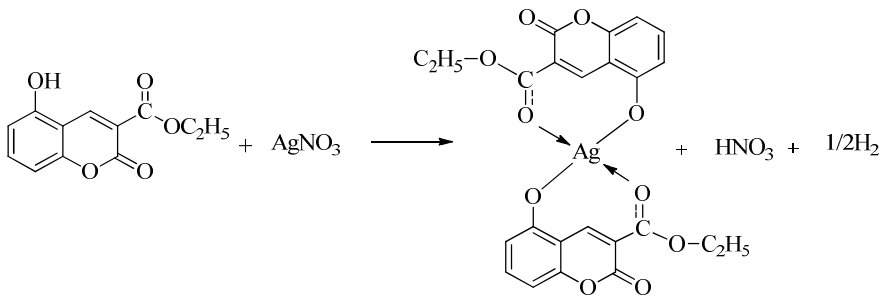


Figure 1. Synthesis of ethyl-5-hydroxy-2-oxo-2H-chromene-3-carboxylate.



Proposed structure I

Figure 2. Proposed tentative structure of silver complex with ethyl-5-hydroxy-2-oxo-2H-chromene-3-carboxylate.

Equipment

Melting point was determined using Scientific Electrothermal melting point apparatus (1401Q). The vibrational studies of the complex were performed on Nicolet 6700P Infrared Spectrometer (USA). The structural parameters were calculated using PAN analytical (X'Pert PRO) diffractometer (using Cu-Kα radiation). Thermal measurements were performed on TGA/DSC (Q600) purging nitrogen gas using alumina crucible. Mass spectrum of complex was obtained on Mass spectrometer (1200 series, Agilent technology, with scanning speed up to 5250 μ/s,

quadrupole, mass range m/z = 2-3000) while AAS experiments were performed on Atomic Absorption Spectrometer (Varian AA240).

RESULTS AND DISCUSSION

Melting point

Melting point of the complex was determined by using melting point apparatus. The silver complex showed melting point in a range of 168-175 ^{o}C which was not comparable with that of reactants, i.e. $AgNO_3$ (212 ^{o}C) and ethyl-*5*-hydroxy-*2*-oxo-*2H*-Chromene-*3*-carboxylate (94 ^{o}C) which indicated the completion of the reaction and establishment of new phase as a result of a chemical reaction.

Infrared studies

IR study of synthesized complex verified the presence of ethyl-*5*-hydroxy-*2*-oxo-*2H*-chromene-*3*-carboxylate. The stretching vibration of C-H corresponding to alkene was recorded at 3010 cm^{-1} while a peak due to symmetric stretch of C-H of alkane was observed at 2888 cm^{-1} [12]. Two strong bands of alkane C-H showed the symmetrical deformation and antisymmetric deformation at 1386 cm^{-1} and 1454 cm^{-1}, respectively. An overtone band of 980 cm^{-1} corresponding to alkene appeared at 1981 cm^{-1}. A strong band at 1761 cm^{-1} is also recorded which belongs to carbonyl (C=O) group [13, 14]. Since carbonyl group is directly attached to an electronegative atom, i.e. oxygen, that is why peak was appeared on higher field side. The spectrum exhibited a strong band at 1606 cm^{-1}, this could be attributed to the stretching vibration of C=C (aromatic ring). A number of bands for alkenes without plane stretching frequencies are shown in the range of 1000 cm^{-1} to 650 cm^{-1}. The peaks appeared in the range of 1300 cm^{-1} to 1000 cm^{-1} corresponds to C-O-C [15, 16]. It is reported that stretching frequency of Ag-O bond lies in the range of 253-205 cm^{-1} [17, 18] which is out of limit of this IR spectrum. In this spectrum no –OH band could be seen which indicated the reaction progress and complex formation. In this study, all the observed vibrations are comparable with the literature.

Powder X-ray diffraction analysis

The complex was grinded and its X-ray powder diffraction analysis was done on X-ray powder diffractometer under 45 kV/40 mA X-ray, $2\theta/^{o}$ scanning mode, fixed monocharomator and with a range from $2\theta/^{o}$ = 10 to 90 with a step of 0.02 degree for a period of 30 min as shown in Figure 3. Synthesized complex was analyzed through powder diffractometer for half an hour with a step of 0.03º along fixed monochromator for 2θ value (10º-90º). A total ten peaks were targeted and calculated their miller indices shown in Table 1.

In order to observe the novelty of synthesized silver complex, a comparison was made between the calculated pattern and reported patterns using peak search method. The measurement showed that peaks present at 10.2111, 10.4900, 12.0240, 22.4693, 25.3374, 27.1114, 28.7865, 37.0110, 44.9786, 64.4516 ($2\theta/^{o}$) exhibit miller indices as 111, 111, 200, 321, 222, 421, 422, 532, 642, 952, respectively that confirm the uniqueness of the pattern. The material parameters like particle size, dislocation line density and strain of the synthesized product were also calculated as shown in Table 2.

Table 2 showed that the grain size was found to be in the range of 6.8005 nm at $2\theta/^{o}$ = 10.21 to 4.0050 nm at $2\theta/^{o}$ = 64.45. Less variation in dislocation density indicated the purity of the substance.

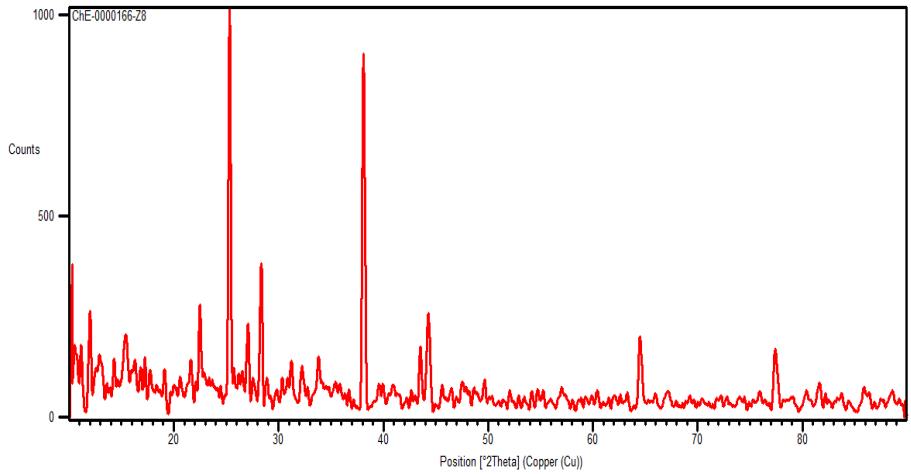


Figure 3. X-ray diffraction spectrum of silver complex with ethyl-*5*-hydroxy-*2*-oxo-*2H*-chromene-*3*-carboxylate.

Table 1. Claculation of miller indices through xrd diffraction pattern.

2θ/°	θ/°	$Sin^2\theta$	$1x\frac{Sin^2\theta}{Sin^2\theta_{min}}$	$2x\frac{Sin^2\theta}{Sin^2\theta_{min}}$	$3x\frac{Sin^2\theta}{Sin^2\theta_{min}}$	Whole integers	*Hkl*
10.2111	5.1055	0.00791	1	2	3	3	111
10.4900	5.2450	0.00835	1.0556	2.1112	3.1668	3	111
12.0240	6.0120	0.01096	1.3856	2.7712	4.1568	4	200
22.4693	11.2346	0.03795	4.7977	9.5954	14.3931	14	321
25.3374	12.6687	0.04809	6.0796	23.1722	12.1592	12	222
27.1114	13.5557	0.05494	6.9456	13.8912	20.8368	21	421
28.7865	14.3933	0.06179	7.8116	15.6232	23.4348	24	422
37.0110	18.5055	0.10074	12.7357	25.4714	38.2073	38	532
44.9786	22.4893	0.14631	18.4968	36.9936	55.4904	56	642
64.4516	32.2258	0.28436	35.9494	71.8988	107.8482	108	952

Table 2. Claculation of material parameters with the help of 2θ/° value.

2θ/°	FWHM [°2Th.]	Intensity counts	d-spacing [Å]	Grain size (D) (nm)	Dislocation density (δ) ($lines/cm^{-2}$)	Strain (S) ($lines^{-2}cm^{-4}$)
10.2111	0.2047	26.61	8.66309	6.8005	0.02162	0.0509
10.4900	0.1535	17.25	8.43340	9.0742	0.01214	0.0382
12.0240	0.2814	24.55	7.36069	4.9081	0.04151	0.0706
22.4693	0.2303	23.12	3.95703	6.1405	0.02652	0.0564
25.3374	0.2814	100.00	3.51523	5.0512	0.03919	0.0686
27.1114	0.2814	18.37	3.28911	5.0696	0.03890	0.0683
28.7865	0.2558	33.83	3.14528	5.5954	0.03194	0.0619
37.0110	0.3582	89.50	2.36146	4.0828	0.05999	0.0849
44.9786	0.3838	22.85	2.04406	3.9101	0.06540	0.0886
64.4516	0.4093	17.44	1.44571	4.0050	0.06234	0.0865

Thermal gravimetric analysis

Sample was analysed through TGA analyzer in inert atmosphere and decomposition pattern is given in Figure 4. The major weight loss was observed in 1st step with sharp decomposition in the range of 180 °C to 200 °C. Further weight loss was observed in 2nd step in the range of 250 °C to 590 °C. In 1st step one ligand out of two may be removed and in the 2nd step the second ligand might be separated from the metal atom. The final residue in this analysis was undetermined.

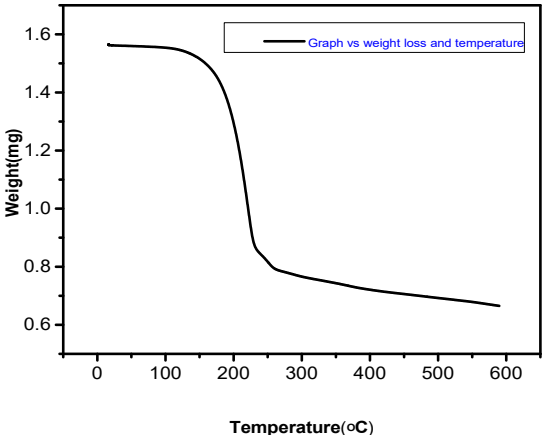


Figure 4. TGA curve of Ag metal with ethyl-*5*-hydroxy-*2*-oxo-*2H*-chromene-*3*-carboxylate.

Electrospray ionization-mass spectrum (ESI-MS)

Mass spectra were done to check the composition and purity of the synthesized complex. Mass spectrometer for metal complexes with an ESI source is normally employed in an acidic media. In this analysis molecular ion peak is normally recorded as $[M+H]^+$ ion cluster and due to the presence of sodium salt results a peak as $[M+Na]^+$. A molecular ion peak was recorded at m/z 574.2334 which is associated to the $[C_{24}H_{18}AgO_{10}+H]^+$. A sharp peak was found at m/z 596.0131 which was attributed to the ionic specie $[C_{24}H_{18}AgO_{10}+Na]^+$. Some extra peaks were also seemed in the spectra, which might be due to the presence of some impurities or due to the smaller fragments formed during decomposition.

Atomic absorption measurement

Atomic absorption analysis was done to find out metal to ligand ratio (by weight %) in the synthesized complex. The sample and standard solutions were prepared according to standard procedure and following result was found.

Table 3. Concentration of silver metal complexes obtained experimentally.

Sr. No.	Sample description	Amount of metal estimated by AAS (ppm)
1	Sample (Z8)	18.19

Table 4. Expected metal to ligand ratio.

S. No.	Ag metal	Ligand	Total weight	Metal (%)
1	1	1	343.86	31.42
2	1	2	579.92	18.51
3	1	3	815.98	13.21
4	1	4	1052.04	10.24
5	1	5	1288.10	8.36

To determine the exact formula of the complex, experimental result was compared with theoretical one. Atomic absorption analysis of the complex showed the metal concentration (weight %) in the synthesized complex is 18.19% which is comparable with the theoretical data (18.51) of complex as shown in Table 4. The above results showed that metal/ligand ratio was 1:2. Thus the proposed formula of the complex was $[Ag(C_{12}H_9O_5)_2]$.

Antibacterial activity of silver complexes

Ligand and silver metal complex were screened to study their ability to inhibit the growth of a number of Gram-positive and Gram-negative bacterial strains. To study the effectiveness of ligand and metal complex three gram-positive strains i.e. *S. aureus*, *B. subtilis* and *MRSA* and three gram-negative strains i.e. *E. coli*, *S. typhi* and *P. auruginosa* were selected. It is reported that number of coumarin-based compounds show good antimicrobial activity [19-21]. Results of this study are shown in Table 5.

Table 5. Antibacterial study of silver complex.

Name of compound	Diameter of inhibition zone (mm)					
	Gram positive			Gram negative		
	S. aureus	*B. subtilis*	MRSA	*E. coli*	*P. auruginosa*	*S. typhi*
Ligand	18	-	-	15	-	25
Ag-complex	20	12	-	22	10	30

Note: Mean inhibition zones are measured in mm.

Ligand and Ag-complex did not show any activity against the growth of *MRSA* strain. The complex was found to be more effective against *S. aureus*, *E. coli* and *S. typhi* and reluctant their growth to a greater extent but ligand showed less activity against these strains. Another interesting result was observed in this study that ligand could not inhibit the growth of *P. auruginosa* and *B. Subtilis* but Ag-complex displayed moderate activity against both strains. Now it is clear from above discussion that activity of the ligand was considerably enhanced by introducing Ag metal atom.

The MIC values were the minimum concentration of the ligand and the complex required to inhibit 80% of the microbe's growth. The MIC values are given in Table 6.

Table 6. MIC results of ligand and silver metal complex.

Name of compound	MIC values against various pathogens (µg)					
	Gram positive			Gram negative		
	S. aureus	*B. Subtilis*	MRSA	*E. coli*	*P. auruginosa*	*S. typhi*
Ligand	20 µg	-	-	15 µg	-	15 µg
Ag-complex	15 µg	30 µg	-	10 µg	25 µg	10 µg

The MIC value of ligand and Ag-complex for *S. aureus*, *E. coli and S. typhi* were 20 µg, 15 µg, 15 µg and 15 µg, 10 µg, 10 µg, respectively. Similarly for *B. subtilis* and *P. auruginosa*, ligand proved inactive but Ag-complex inhibited their growth and showed MIC values of 30 µg and 25 µg, respectively. Ag-complex showed lower MIC values than ligand and it is a big clue of enhanced antibacterial activity of the silver complex against pathogens.

CONCLUSION

A new complex $[Ag(C_{12}H_9O_5)_2]$ with coumarin based ligand was synthesized. Structural studies were done by using atomic absorption spectroscopy, TGA and X-ray measurements. The material parameters of complex including grain size, dislocation density, and stress have been calculated. Antibacterial study proved the enhancement in activity of ligand by introducing silver metal against various pathogens.

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