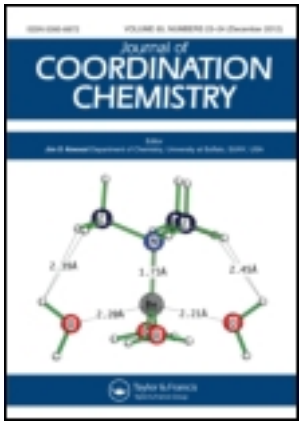

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Chromium(III) chelate of deoxyalliin and its bioactivity

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Chromium(III) chelate of deoxyalliin has been prepared and characterized using physical and spectral means and its bioactivity has been determined. FT-IR showed the coordination of amino and carboxylate groups of the amino acid to the metal. Elemental analysis, mass spectrometry, and TGA suggested its formula to be $Cr(C_6H_{10}O_2NS)_3$. UV-Vis spectroscopy and magnetic moment proved octahedral geometry. Conductivity measurement showed it to be a non-ionic compound. Bioactivity analysis showed that the complex is active against Gram-positive and Gram-negative bacteria and yeast as well.

Keywords: Chromium complex; Deoxyalliin; Antibiotic activity; Organic chromium

1. Introduction

The discovery of new therapeutic agents is an important field for scientific research. Metal complexes have been proved to be important for this research. Metals and amino acids are vital constituents of living bodies and their interaction is inevitable for the survival of living organisms [1–4]. Metal amino acid complexes are important to understand these interactions and to search for new therapeutic agents.

Deoxyalliin (figure 1) is a non-protein amino acid present in garlic and has many physiological properties which include antioxidant, antidiabetic, anticancer, antitumor, antibiotic, reno-protective, and antihypertensive effects [5–11]. Antihepatopathic, neurotropic, antilung-cancer, and protective effects against acute myocardial ischemia have also been reported for this compound [12–15].

Chromium in its oxidation state +3 is an essential micronutrient. Chromium(III) helps in glucose metabolism by binding insulin to its receptors and enhancing its effect [16]. As a part of glucose tolerance factor (GTF) and low molecular weight chromium-binding substance (LMWCr), it is thought to have antidiabetic effects [17]. The availability of chromium to the body has been suggested to be more in organic form as in GTF or LMWCr compared to inorganic chromium which is not absorbed properly by the body [17].

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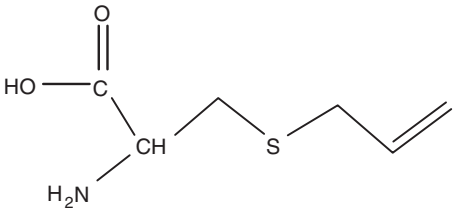

Figure 1. Deoxyalliin.

Alleviation of the severe diabetic symptoms and reduction of the exogenous insulin requirements by the use of chromium supplements has also been reported [18, 19]. Chromium(III) supplementation improves glucose and/or lipid concentration levels in children [20], in elderly people [21], in type-2 diabetic patients [22], and in patients with impaired glucose tolerance [23, 24].

Several complexes of deoxyalliin with other transition metals and their biological studies have already been published [25–29]. This work describes the preparation of a chromium(III) complex of deoxyalliin with the aim that such a complex can benefit the human body.

2. Experimental

2.1. *Material and methods*

Chromium(III) chloride hexahydrate was obtained from Riedel. Elemental analysis was performed using a CHNS-932 LECO analyzer. FT-IR spectra were recorded by using a FT-IR Nicolet 6700 of Thermoscientific Company by direct probe method. NMR spectra were recorded on a Bruker XWIN-NMR 300 MHz. Electrospray ionization-mass spectra (ESI-MS) were recorded on a Jeol MS Route and Waters micromass triple quadrupole quarto II Electrospray Ionization mass spectrometer. Cone voltages ranging from 5 to 15 V were used for best results. Electrical conductivity was determined using a TPS Model 2100 conductivity meter at 20°C. TGA and DTA were taken simultaneously by using Universal V4.2E TA instrument-SDT Q600 V8.2 Build 100. Analysis was made in nitrogen with heating rate of $10^{\circ}C\,min^{-1}$ from 25°C to 800°C.

2.2. *Preparation of deoxyalliin*

Deoxyalliin was prepared by the method of Stoll and Seebeck [30]. M.p. = 212–215°C (decomposition). FT-IR $\nu = 3151$, 2909, 2625 cm^{-1} (NH_3^+), 1585 cm^{-1} (COO–), 993, 919 cm^{-1} (allyl double bond) ^1H NMR (D_2O) $\delta = 2.77$–2.96 (2 H, *dd*, $J = 4.5$ and 7.5 Hz, SCH_2 at β-carbon), $\delta = 3.06$–3.08 (2H, *d*, $J = 7.5$ Hz, SCH_2 on allyl side), $\delta = 3.75$–3.79 (1H, *dd*, $J = 4.2$ and 4.3 Hz, CH at α-carbon), $\delta = 5.03$–5.10 (2H, *dd*, $J = 10.4$ and 17.0 Hz, vinylic CH_2), $\delta = 5.62$–5.76 (*m*, 1H, vinylic CH). MS; EI: $m/z = 161$ (M+, 13.9%),

$m/z = 73.9$ (100%), $m/z = 87$ (94.51%). Anal. Calcd for ($C_6H_{11}O_2NS$) (%): C, 44.71; H, 6.88; N, 8.69; S, 19.87. Found (%): C, 44.33; H, 6.42; N, 8.41; S, 19.69.

2.3. *Synthesis of complex*

Chromium(III) chloride hexahydrate (0.01 mol) dissolved in 50 mL of distilled water was added into a solution of deoxyalliin prepared by dissolving 0.03 moles of deoxyalliin in 100 mL of distilled water. This solution was stirred overnight during which it changed from green to purple. This solution was concentrated to saturation and left covered for a month in a closed container. Reddish pink precipitates settled from the solution which were filtered and washed with distilled water and ethanol. Yield 49%, m.p. = decomposition > 221°C. FT-IR $\nu = 3075$, 2979 cm^{-1} (NH_2), 1647, 1380 cm^{-1} (COO–). UV-Vis $\nu = 32258$, 26525, 19305 cm^{-1}. μ_{eff} (B.M.) = 3.79. Anal. Calcd for $Cr(C_6H_{10}O_2NS)_3$ (%): C, 40.60; H, 5.63; N, 7.89; S, 18.04. Found (%): C, 40.48; H, 5.77; N, 7.68; S, 17.88.

3. Results and discussion

3.1. *FT-IR spectroscopy*

Coordination of amino group of an amino acid is observed in the form of two well-resolved peaks at 3000–3400 cm^{-1}. This coordination also removes $NH_3^+{}_{\delta}$ which appears around 1505. Similarly, coordination of carboxylate is shown by the displacement of carboxylate band as compared to carboxylate band of the ligand or its potassium salt. The difference between asymmetric and symmetric stretching frequencies of the carboxylate ($\Delta\nu = \nu_{asy.} - \nu_{sym.}$) gives the measure of the metal–oxygen (M–O) bond strength and mode of coordination of carboxylate. Higher value of $\Delta\nu$ indicates a stronger M–O bond. $\Delta\nu$ value close to $\Delta\nu$ of potassium salt of carboxylate of the amino acid (as in *S*-allyl-L-cysteine potassium salt) indicates bidentate carboxylate, while more difference indicates monodentate coordination [31].

As compared to *S*-allyl-L-cysteine potassium salt where a broad band is observed for NH_3^+, two well-resolved peaks at 3075 and 2979 cm^{-1} are an indication for the coordination of the amino NH_2. Furthermore, the absence of band at 2080 and 1505 cm^{-1}, as compared to the ligand, is also a proof of amino group coordination.

The increase in the asymmetric frequency of carboxylate in this complex is a proof that it is coordinated to the metal. In this complex $\Delta\nu = 267$ cm^{-1} is larger than the *S*-allyl-cysteine potassium salt, where $\Delta\nu = 173$ cm^{-1}, indicating that carboxylate has coordinated unidentate to the metal. For this complex the Cr–N and Cr–O vibrations were detected at 541 and 436 cm^{-1}, respectively.

3.2. *Thermal analysis*

The thermogram (figure 2) leads to the formula $Cr(C_6H_{10}O_2NS)_3$. The decomposition occurs in two major steps. First mass loss indicates loss of carbon chain –CH–CH_2–S–CH_2–CH=CH_2, corresponding to 57.51%. This step starts at 160°C and ends at 260°C.

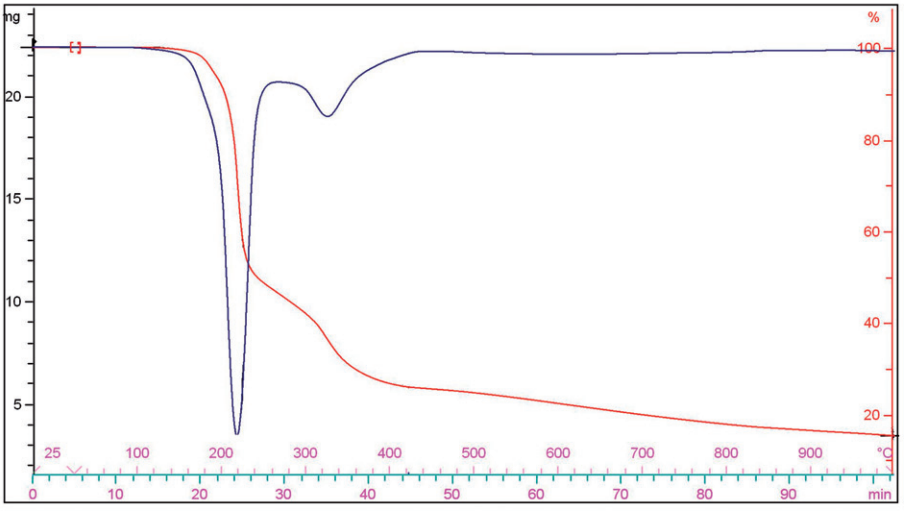

Figure 2. TGA and DTG of Tris(*S*-allyl-L-cysteinato)Cr(III).

Second decomposition is relatively small and immediately follows the first step, from gradual loss of carboxylate and amino group. The final decomposition product was detected as CrO_2.

DTG of the thermogram shows a very sharp strong peak at 220°C and a small sharp peak at 320°C corresponding to above two mass loss steps. Similarly, DTA shows a sharp absorption at 220°C and a broad absorption peak starting from 270°C and ending at 450°C. Both of these are due to decomposition of the ligand.

3.3. *Mass spectrometry*

The molecular ion peak $[M+H]^+$ is found at 533.6 amu. All the isotopic patterns for chromium and organosulfur compound come immediately after it. Other peaks of monomeric ligand at 162.1 and dimeric ligand at 323.4 are also present. Peaks for the complex with allyl groups removed from cysteine residue are present at 406.0 and 450.5. Peak for the complex with one ligand removed is present at 372.4 amu.

3.4. *Electronic spectra*

Solution of this complex in DMSO shows peak maxima, absorption coefficients and band assignment given in table 1 that indicate octahedral geometry [32]. Identical electronic data has been observed for other octahedral chromium amino acid complexes like $Cr(Thr)_3$ and $Cr(His)_3$ [33].

3.5. *Magnetic moment*

The spin only magnetic moment of this complex was 3.79, indicating three unpaired electrons and an octahedral geometry with d^3 configuration around the metal [32].

Table 1. UV-Vis data for Tris(*S*-allyl-L-cysteinato)Cr(III).

Complex	λ_{max} (cm^{-1})	ε ($L\,mol^{-1}\,cm^{-1}$)	Band assignment
Tris(*S*-allyl-L-cysteinato) Cr(III)	19305	4	$^4A_{2g} \rightarrow {}^4T_{2g}$
	26525	6	$^4A_{2g} \rightarrow {}^4T_{1g}$
	32258	17	$^4A_{2g} \rightarrow {}^4T_{1g}$ (P)

3.6. *Conductivity measurement*

The conductivity value in DMSO for the complex is $18.62\,\Omega^{-1}\,cm^2\,mol^{-1}$, far below a 1 : 1 electrolyte which is $118–131\,\Omega^{-1}\,cm^2\,mol^{-1}$. So we can safely say that the complex is non-ionic or very weakly ionic.

3.7. *Bioactivity*

The complex was dissolved in DMSO to prepare 0.1% W/V solution. Aqueous solutions of $CrCl_3$ and deoxyalliin corresponding to the metal and the ligand concentrations in the complex solution were used. Commercially available disks (OXOID, 6 mm) of standard antibiotics, i.e., streptomycin, vancomycin, and rifampicin, were used as reference. DMSO was used as control. Antimicrobial activity was assessed by employing *Staphylococcus aureus*, *Escherichia coli*, and *Saccharomyces cerevisiae* as representatives of Gram positive, Gram negative, and eukaryotic microorganisms, respectively.

A given solution (10 μL) was dispersed on autoclaved Whatman filter paper No.1 disks of 6 mm diameter. Growth of overnight incubated culture of a given test microbe in nutrient broth (10 μL) was spread over solidified nutrient agar plate containing 15 mL of the medium with the help of a sterilized glass spreader. Five minutes were allowed to absorb the fluid of inoculum before applying the disks. Then the complex and the control (10 μL of DMSO) loaded disks were picked up with the help of sterilized forceps and placed at least 15 mm apart from the edge of the inoculated nutrient agar plate and 2 cm apart from each other. Each disk was then gently pressed with the help of a blind-ended sterilized steel probe. The plates were then incubated at 37°C for 18–24 h. After incubation, diameters of growth inhibition zones were measured in millimeters. Results of bioactive analysis are shown in table 2.

Tris(*S*-allyl-L-cysteinato)Cr(III) showed reasonable growth retardation against the test organisms as compared to the standard drugs. The metal and ligand did not show any effect independently in the used concentration ranges against the test organisms. Hence, it can be concluded that the complex showed strong antibiotic effects against Gram-negative and Gram-positive bacteria and yeast as well.

4. Conclusion

Chromium chelate of deoxyalliin was prepared. The ligand coordinates to the metal through amino and carboxylate groups. UV-Vis and magnetic moment indicate

Table 2. Zones of growth inhibition (mm) of different compounds against the test microorganisms.

Sr. No.	Compound	*E. coli*	*S. aureus*	*S. cerevisiae*
1	Streptomycin	10.0	16.5	13.0
2	Vancomycin	R	17.0	16.0
3	Rifampicin	R	16.0	12.0
4	Tris(*S*-allyl-L-cysteinato)Cr(III)	8.5	7.0	7.0
5	$CrCl_3$	R	R	R
6	Deoxyalliin	R	R	R
7	DMSO	8	R	R

R = The microbe was resistant to the applied substance

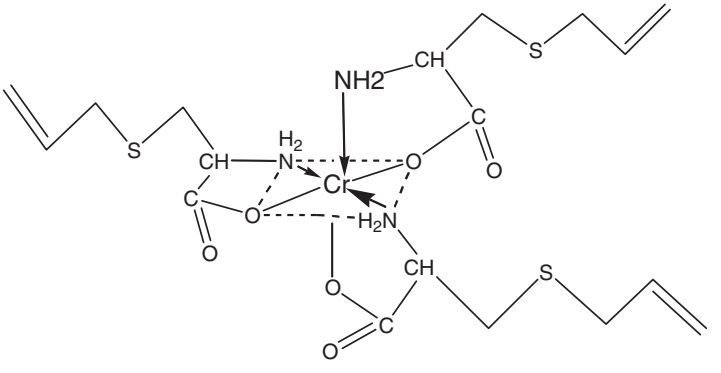

Figure 3. Proposed structure for Tris(*S*-allyl-L-cysteinato)Cr(III).

octahedral geometry. Hence, the structure of the complex is proposed (figure 3). The complex is bioactive against Gram-positive, Gram-negative bacteria, and yeast.

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