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RESEARCH ARTICLE

Synthesis, Characterization and Antibacterial Studies of (3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid-Cr(III) Complex

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Abstract

Benzylpenicillin, also known as (3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic, is a common bactericidal antibiotic of the betalactamin family which is mostly used to treat infections caused by gram-positive bacterial strains and few gram-negative bacterial strains. Cr (III) complex of benzylpenicillin was synthesized by the reaction of benzylpenicillin with $CrCl_3.6H_2O$. The structure of the complex was confirmed through elemental analysis, electrical conductivity, FT-IR, electronic spectra, ^{1}H-NMR, and ^{13}C-NMR spectroscopic methods. The physical properties such as solubility, color and melting point were also determined for both the ligand and the complex. The complex has a deep green color. Both the ligand and the complex are ionic (236.0 and 126.0 $Sm^2.mol^{-1}$). From the spectroscopic result, the ligand behaved as a pentadentate ligand coordinating to the metal ion through OH, NH, C=O of amide, carboxylate& β-lactam. A trigonal bipyramidal geometry has been proposed for the metal complex. The prepared complex was evaluated *in vitro* for its antibacterial activity against some pathogenic gram-positive (*Staphylococcus aureus, Bacillus substilis, Bacillus cereus, and Enterococcus faecalis*) and gram-negative bacteria (*Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa and Campylobacter fetus*). The metal complex showed enhanced antibacterial activity as compared to the uncomplexed ligand.

Keywords: Benzylpenicillin, spectroscopic, bacteria, complex, ligand

Introduction

The recent outbreak of multi-drug resistant microbial pathogens over the last three decades has rendered the already existing antibiotics ineffective. Penicillin was among the first medications to be effective against many bacterial infections. They were considered effective against many bacterial infections and among the most frequently utilized and least toxic antibiotics [1]. Furthermore, several types of research have shown significant progress in the utilization of transition metal complexes as drugs in treating several human diseases like diabetes [2], anti-inflammatory [3], neurological disorders [4]. They have also been proven to have potent anti-microbial characteristics, which include anti-bacterial, antifungal, anti-viral, anti-parasitic, and anticancer potentials [5]**.** As early as 1975, it was reported [6] that substituting the aromatic groups in the antibiotics penicillin and Cephalosporine with ferrocenyl moieties produced compounds with altered antibacterial activity compared to the starting materials. Against various strains of *Staphylococcus aureus*, ferrocenyl penicillin showed comparable inhibitory activity. Ferrocenyl penicillin consists of a metal, benzylpenicillin, and also β- lactamase, which is one of the enzymes responsible for bacterial resistance to penicillin-type antibiotics. The synthesis

of the platinum complex with tetracycline has been reported [7]. The tetracycline Pt(II) complex turned out to be as efficient as the ligand alone against *Escherichia coli* bacteria strains.

Moreover, this complex was six times more potent against *Escherichia coli* than free tetracycline. Osella and co-workers [8] inserted a ferrocenyl group into the side chain of the chloroquine, and it has been reported that the resulting compound ferroquine was much safer and more effective in mice, as well as non-mutagenic. Páez and co-workers [9] synthesized complexes of α-diimine chromium (III), and the results showed that they exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria. The study also showed that the chromium (III) complexes of α-diimine when combined with ciprofloxacin, an important synergistic effect was observed for the inhibition of *Staphylococcus aureus* and *Escherichia coli*. New fluorescent chromium (III) complexes were obtained from the coordination of the ligands derived from benzimidazole with Cr(III) cation and were tested against some bacteria strains. The results from the antimicrobial screening tests reported an improvement in the antibacterial activity of the new chromium (III) complexes against *Pseudomonas aeruginosa* and *Methicillin Resistant Staphylococcus aureus (MRSA)* species compared to ampicillin [10]. Six complexes of Cr(III) metals with triazole ligands were synthesized [11]. All the complexes showed higher antibacterial activities than the free ligands [11]. Arising from the fact that Cr complexes have shown significant performance against the microbial activity, it becomes interesting to note that to the best of our knowledge, benzylpenicillin-Cr complex and its antibacterial studies have not been reported elsewhere. Therefore, the present study is aimed at synthesizing, characterizing, and an antibacterial assay of benzylpenicillin-Cr complex.

Materials and Methods

All the chemicals used in this study were of analytical grade. Benzylpenicillin was obtained from Shanxi Federal Pharmaceutical Company limited, Shanxi, China.

Melting points and decomposition temperature of both benzylpenicillin and its Cr(III) complex were determined on Gallenkamp melting point apparatus. The solubility of the benzylpenicillin and its Cr(III) complex metal were tested using various organic solvents at 25 ^{o}C.

Conductometric measurements of benzylpenicillin and its Cr(III) complex (10^{-3} M) were recorded at room temperature using Jenway Conductivity Meter 4510, and DMSO was used as the solvent. The elemental analyzer for C, N, H, and S was obtained using a Perkin-Elmer 240B elemental analyzer.

Cr analyses were carried out on the AAS spectrophotometer (bulk 210). The standard solution for the calibration curve was prepared using chromium chloride hexahydrate, equivalent to 1.0 mg of Cr^{3+}. The salt was subjected to heating in a muffle furnace in a silica crucible at about 550 ℃ for 6-7 hours; the ash was then dissolved in 0.2 M sulfuric acid (pH 1 ± 0.5) in order to obtain a stock solution of 5 µg/mL. From this stock solution, suitable aliquots were taken. The absorbance was measured at 544 nm.

The spectrophotometric measurements in solution were carried out using various instruments. The liquid state UV-Vis spectra of the ligand and its metal complex were recorded on the UV-1800 series using Dimethylsulfoxide (DMSO) as the solvent in the range 200-800 nm.

The solid-state FTIR spectra of the ligand and its metal complex were recorded on a Perkin Elmer Spectrum BX FTIR spectrophotometer (4400-350 cm^{-1}) in KBr pellets. Exactly 1 mg of the complex on a microspatula and about 100 mg of KBr was mixed thoroughly in a mortar while grinding with the pestle. It was placed in press and press at 5000-10000 psi. The pressed sample was carefully removed from the die and place in the FTIR sample holder. Eight scans were performed. The NMR spectral measurements were recorded on nuclear magnetic resonance Bruker spectrophotometer using tetramethylsilane internal standard and DMSO-d6 as solvent.

Synthesis of benzylpenicillin -Cr(III) complex

The complex was prepared following the reported procedure [12]. Cr(III) solutions were prepared by dissolving 3.46 g (0.013 moles) $CrCl_3.6H_2O$ in 10 mL of water. The metal solution was added to a solution of benzylpenicillin (0.013 moles). The mixture was

stirred for 1hr, and the solid complex which separated was removed by filtration and washed with water, ethanol, and ether. The compound was dried under vacuum at room temperature for 48 h. The complex was then stored in a neatly labelled container after determining its percentage yield. The general synthesis of the metal complex is proposed in equation 1.

$$C_{16}H_{17}N_2O_4S + CrC_{13}.6H_2O \rightarrow [M(C_{16}H_{17}N_2O_4S)] + 3Cl^- + 6H_2O$$

Antibacterial activity test

The organisms used were Gram-negative *Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa,* and *Campylobacter fetus*. The Gram-positive bacterial strains were *Staphylococcus aureus, Bacillus substilis, Bacillus cereus,* and *Enterococcus faecalis*. They were obtained from the Federal Medical Centre, Umuahia, Abia State. Antibacterial activity of the sample was determined by using agar well diffusion method, and bacterial growth was subcultured on nutrient broth for their *in vitro* testing, which was prepared by dissolving (24 g) of nutrient broth. The mixture was autoclaved for 15 minutes at 120 °C. Stock solution for *in vitro* antibacterial activity was prepared by dissolving 5 mg of the compound in 9 mL of DMSO to make the stock solution of 100 g/mL. 1.15 mL of liquid nutrient agar for activation were prepared separately for tested target microorganism cultures and 1 mL of nutrient broth for antibacterial activity. Inoculation was done with the help of micropipette with sterilized tips. Exactly 100 µL of activated strain was placed onto the surface of the agar plate and spread over the whole surface. Two wells having a diameter of 10 mm were dug in media and incubated at 37 °C for 48 hours. The zone of inhibition, was measured around the disc in mm. Benzylpenicillin was used as a positive standard statistical analysis

Statistical significance was determined using Duncan multiple range test. Results were considered statistically significant at $P<0.05$ and were expressed as mean ±SD.

Results and Discussion

Some physicochemical parameters of benzylpenicillin and its Cr(III) complex are presented in Table 1. Solubility data of benzylpenicillin and its Cr(III) complex in some selected solvents are shown in Table 2. The percentage zone of inhibition (mm) of benzylpenicillin and its Cr(III) complex on the bacterial population are shown in Table 3. The infrared, UV-Visible, ^{1}H NMR, and ^{13}C NMR spectra of benzylpenicillin and its Cr(III) complex is shown in Figures 1-8, respectively.

Some physicochemical parameters of benzylpenicillin and its Cr(III) complex is shown in Table 1. The % yield of benzylpenicillin -Cr(III) complexwas 39. The decomposition temperature of benzylpenicillin was 209 °C, while the complex decomposed at 220 °C. This indicates that the complex is more photostable than benzylpenicillin. The color change from white to deep green also suggested that complexation occurred [13]. The molar conductance of the ligand was 236.0 $Sm^2.mol^{-1}$ and its Cr(III) complex was 126.0 $Sm^2.mol^{-1}$. The molar conductance indicated that the ligand and complex are electrolytes [14]. The micro-analytical data obtained in percentage were compared with the calculated percentage and were found to be in good agreement. The elemental analysis suggested 1:1 metal-ligand ratio.

Table 1: *Some physicochemical parameters of benzylpenicillin and its Cr(III) complex*

Ligand/Complex	Colour	M.P. (dec.) (°C)	Yield %	Conductance $Sm^2.mol^{-1}$	C % Found (Calc)	H % Found (Calc)	N % Found (Calc)	S % Found (Calc)	M % Found (Calc)
Bpen	White	209	-	236.0	57.42 (57.47)	5.41 (5.43)	8.37 (8.38)	9.54 (9.58)	-
[Cr(Bpen)]	Deep green	220	39	126.0	50.03 (50.00)	4.20 (4.20)	7.26 (7.29)	8.53 (8.34)	13.54 (13.53)

The conductivity of DMSO which was used as solvent is 8.37$Sm^2.mol^{-1}$; dec. = decomposition; Bpen =Benzylpenicillin

The solubility of benzylpenicillin and its Cr(III) complex in various solvents is shown in Table 2. Benzylpenicillin was found to be soluble in distilled water, n-hexane, ethanol, methanol, petroleum ether, and DMSO. Similarly, the complex was found to be insoluble in distilled water, n-hexane, and petroleum ether. It was slightly soluble in ethanol and methanol but completely soluble in DMSO. Solubility data suggested that Cr(III) complex is a highly polar compound.

Table 2: *Solubility data of benzylpenicillin and its Cr(III) complex in some selected solvents*

Ligand/Complex	n-Hexane	Distilled water	Petroleum ether	Ethanol	Methanol	DMSO
Bpen	S	S	S	S	S	S
[Cr(Bpen)]	IS	IS	IS	SS	SS	S

Key: S-Soluble, SS-Slightly Soluble, IS-Insoluble; Bpen = Benzylpenicillin

Selected infrared spectral data of benzylpenicillin and its Cr(III) complex is shown in Table 3. The FT-IR spectra of the benzylpenicillin exhibited a strong band at 1697.66 cm^{-1} due to the presence of carbonyl of amide and was observed at 1646.76 cm^{-1} in the spectrum of Cr(III) complex. This shift suggested that complexation occurred through the C=O of amide [15]. The wavenumber 1778.04 cm^{-1} was assigned to C=O of the β-lactam carbonyl group, which was absent in the spectrum of the complex. This suggested the involvement of the β-lactam carbonyl group in complex formation [15]. The wavenumber for C-O was observed at 1136.13 cm^{-1} in the spectrum of Cr(III) complex. This indicated that C=O was converted to C-O during complex formation.

Similarly, vibration frequency 3542.26 cm^{-1} was assigned as OH of carboxylic acid in the spectrum of benzylpenicillin. This wavenumber was absent in the spectrum of Cr(III) complex. This suggested deprotonation during complexation. The N-H stretch vibration frequency was observed at 3351.50 cm^{-1} in the spectrum of the ligand. This wavenumber was shifted to 3290.00 cm^{-1} in the spectrum of the complex. This shift suggested that NH was involved in complications to Cr(III) ion.

The electronic spectral data of benzylpenicillin and its Cr(III) complex is shown in Table 4. The electronic data of the ligand show λ_{max} = 197.50, 203.50, 209.50, 215.50, 226.50, 238.50, 255.50, 259.50, 270.50, 278.50, 284.50 and 317.50 nm and these bands are as a result of the chromophores present in the benzylpenicillin. These bands have been assigned π–π* and n–π* and are known as intra-ligand charge transfer (ILCT), in which the metal complex also absorbed at the same range. The electronic data of the

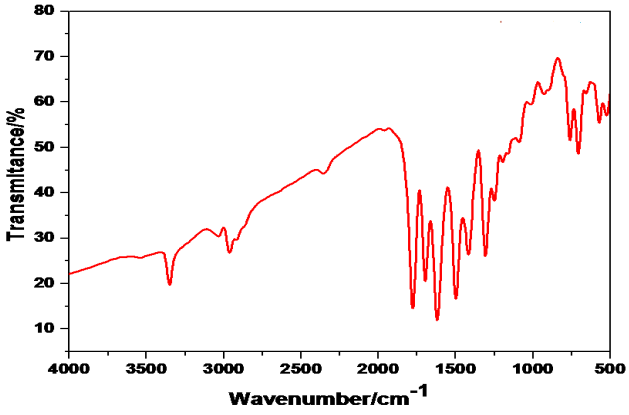

Figure 1*: IR spectrum of benzylpenicillin (Ligand)*

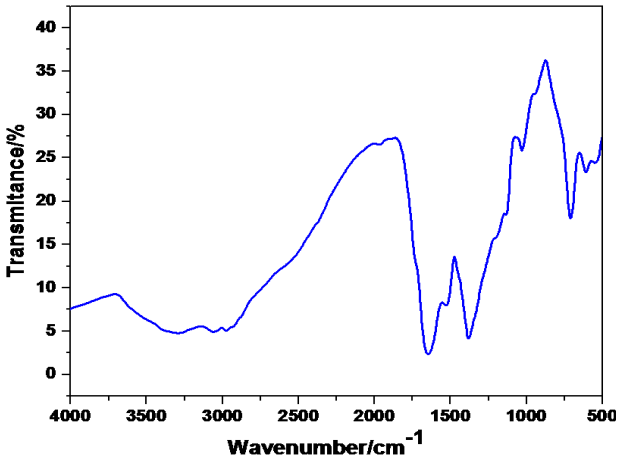

Figure 2*: IR spectrum of [Cr(Bpen)]*

Cr(III) complex show λ_{max} = 565.50 nm assignable to the ligand to metal charge transfer (LMCT) [15].

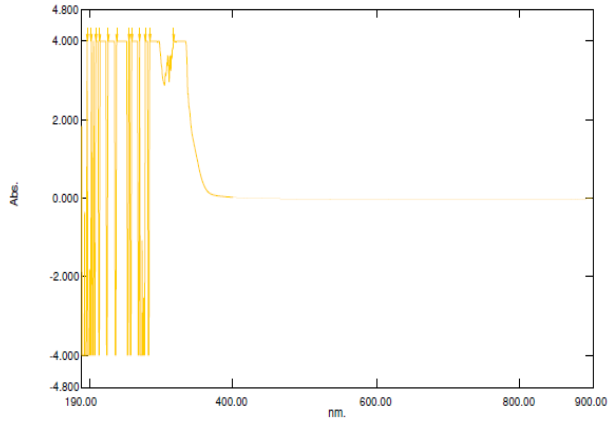

Figure 3: *UV-Vis spectrum of benzylpenicillin (Ligand)*

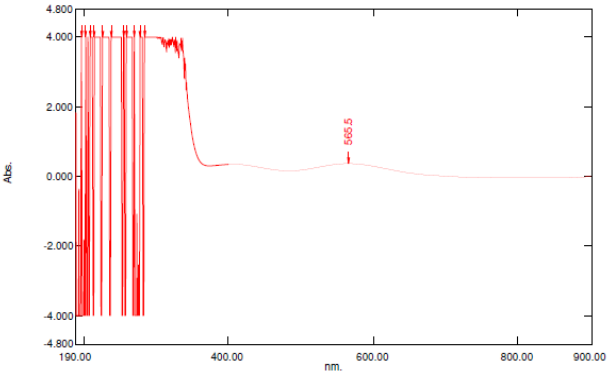

Figure 4: *UV-Vis spectrum of [Cr(Bpen)$_n$] (Complex)*

Figure 5 shows the ^{1}H NMR spectral data of benzylpenicillin and its Cr(III) complex. The ^{1}H NMR spectrum of the ligand displayed a singlet at 11.00 ppm. This was assigned to the hydroxyl group. This chemical shift was absent in the spectrum of Cr(III) complex. This suggested deprotonation of OH during coordination. A doublet observed at 8.72 ppm in ^{1}H NMR spectrum of the ligand was assigned NH proton of amide. This chemical shift was observed at 8.35 ppm in the spectrum of Cr(III) complex. This suggested NH was involved in complexation to Cr ion. Multiplets observed in the ^{1}H NMR spectrum of the ligand at 7.12-7.32 ppm were assigned to aromatic protons, and it was also observed at 7.35 ppm in the ^{1}H NMR spectrum of the Cr(III) complex. A doublet observed at 3.89 ppm in the ^{1}H NMR spectrum for the ligand was assigned N-CH proton on the thiazolidine ring. It was observed at 3.58 ppm in the ^{1}H NMR spectrum of the complex. Multiplets observed at 3.39 ppm in the ^{1}H NMR spectrum of the ligand were attributed to CO-CH_2 of thiazolidine ring and was observed at 3.54 ppm in the spectrum of the metal complex. A singlet observed at 1.46-1.59 in the spectrum of benzylpenicillin was assigned to protons of the methyl group on the thiazolidine ring. This was observed at 1.35-1.50 ppm in the ^{1}H NMR spectrum of the Cr(III) complex.

Figure 6 shows the ^{13}C NMR (DEPT 135) spectral

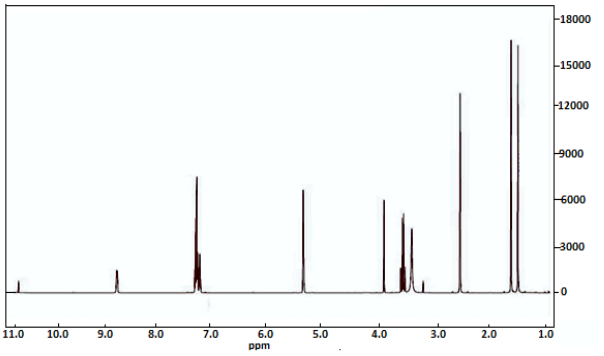

Figure 5: *^{1}H NMR Spectrum of Benzylpenicillin (Ligand)*

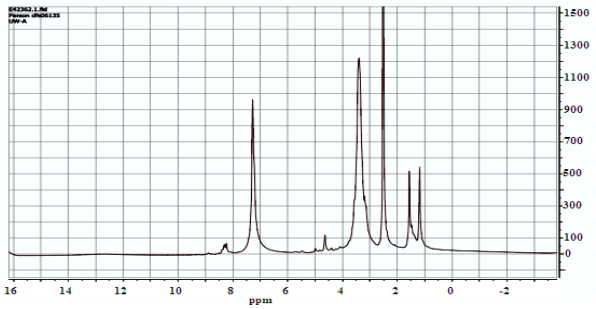

Figure 6: *^{1}H NMR Spectrum of [Cr(Bpen)]*

data of benzylpenicillin and its Cr(III) complex. The chemical shift observed at 170.86, and 173.73 ppm in the spectrum of benzylpenicillin was assigned as C=O of amide and β-lactam. In the spectrum of the complex, the C=O of β-lactam was absent, while a slight shift was observed in the carbonyl of amide. This suggested that β-lactamic C=O was converted to C-O during complexation while the carbonyl of amide was involved in complexation. This functionality was absent in the spectrum of the complex. This suggested the conversion of C=O to C-O during coordination to the Cr ion. The spectra further indicated the presence of the number of carbons in agreement with the expected number.

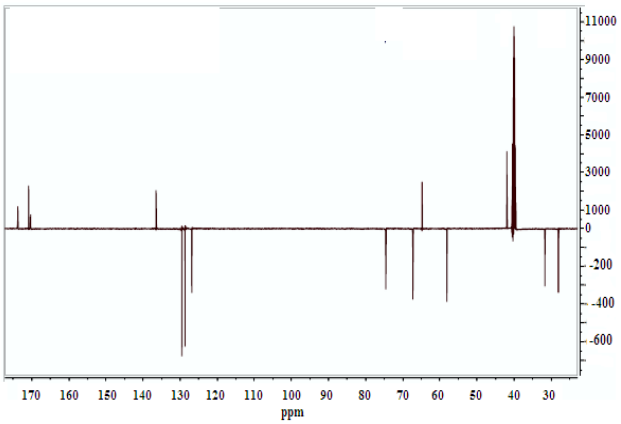

Figure 7: *^{13}C NMR spectrum (DEPT 135) of benzylpenicillin (Ligand)*

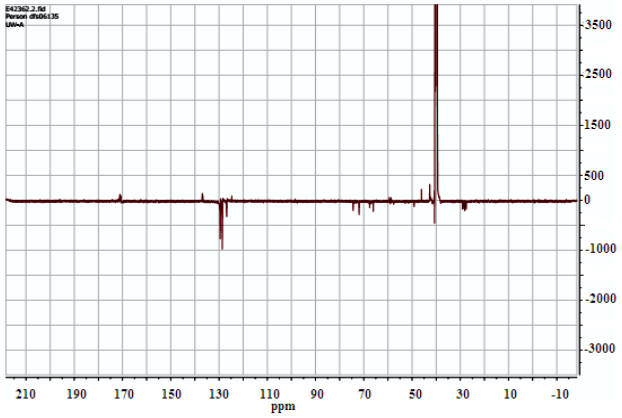

Figure 8: *^{13}C NMR Spectrum (DEPT 135) of [Cr(Bpen)]*

Table 3 shows the antibacterial activity of benzylpenicillin and its Cr(III) complex against four-gram negative bacterial strains (*Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa,* and *Campylobacter fetus*) and four gram-positive bacterial strains (*Staphylococcus aureus, Bacillus substilis, Bacillus cereus,* and *Enterococcus faecalis*). The results showed that the zone of inhibitions of Cr(III) complex was significantly higher ($p < 0.05$) compared to benzylpenicillin against all the bacterial strains used. This may have been as a result of the facilitated diffusion of the drug through the cell membranes, presumably by increasing the lipophilicity of the drug [16].

Table 3: *Percentage zone of inhibition (mm) of the Bpen and its Cr(III) complex on the bacterial population*

Ligand/ Complex	Bacteria							
	Gram-Positive				Gram-Negative			
	S. aureus	*B.substilis*	*B. cereus*	*E. faecalis*	*E.coli*	*E. cloacae*	*P. aeruginosa*	*C. fetus*
Bpen	2.52±0.03[a]	6.12±0.03[a]	4.96±0.01[a]	2.12±0.03[a]	10.43±0.03[a]	1.32±0.03[a]	6.82±0.03[a]	7.32±0.02[a]
[Cr(Bpen)]	7.12±0.02[b]	6.43±0.04[b]	16.12±0.03[e]	3.51±0.01[b]	18.13±0.04[e]	4.97±0.21[b]	21.16±0.08[e]	16.37±0.04[b]

Bpen = Benzylpenicillin (Positive standard)

Based on spectroscopic methods, a tentative structure has been proposed for benzylpenicillin Cr(III) complex (Figure 9).

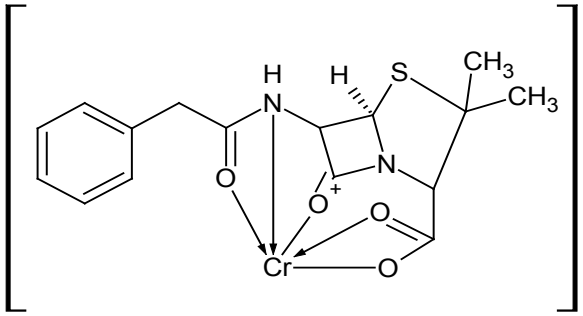

Figure 9: *Proposed structure of benzylpenicillin-Cr(III) complex*

Conclusion

Benzylpenicillin Cr(III) complex was synthesized. Physical characteristics, elemental analysis, conductivity measurement, atomic absorption spectroscopy, FTIR, UV, and NMR spectral data confirmed the suggested coordination mode of the ligand as tetradentate. Coordination occurred through OH, NH, C=O of amide, β-lactam, and carboxylate, forming stable chelate. The data suggested a trigonal bipyramidal geometry for the complex. The antibacterial activity of benzylpenicillin and its Cr(III) complex against four-gram negative bacterial strains (*Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa,* and *Campylobacter fetus*) and four gram-positive bacterial strains (*Staphylococcus aureus, Bacillus substilis, Bacillus cereus,* and *Enterococcus faecalis*) showed that the Cr(III) complex exerted a higher inhibitory activity against the bacterial strains used.

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