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Synthesis, Characterization and Antibacterial Studies of Benzylpenicillin and its Co(II) Complex

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ABSTRACT

Co(II) complex of benzylpenicillin was synthesized by the reaction between benzylpenicillin and $CoCl_2.6H_2O$. The structure and nature of bonding of the complex have been explored by conductivity, elemental analysis, continuous variation method, infrared and UV-Visible spectroscopy. The physical properties such as colour, solubility and melting point were determined for both the benzylpenicillin and its Co(II) complex. Elemental analysis of the complex agreed with the calculated value. Continuous variation method suggested metal:ligand ratio of 1:1. From the spectral data analysis, the ligand benzylpenicillin behaved as a tetradentate ligand coordinating to the metal ion through OH, C=O of amide, C=O of carboxylate, C=O of *β*-lactam and NH. A trigonal bipyramidal geometry has been proposed for the metal complex. Benzylpenicillin and its Co(II) complex have been screened for their *in vitro* antibacterial activity against four gram positive strains (*Staphyloccocus aureus, Bacillus subtilis, Bacillus cereus and Enterococcus feacalis*) and four gram negative (*Escherichia coli, Enterobacter clocae, Pseudomonas aeruginosa and Campylobacter fetus*). The Co(II) complex showed enhanced antibacterial activity when compared with pure benzylpenicillin. It was deduced that Co(II) benzylpenicillin complex have improved antibacterial activity than benzylpencillin alone and it was evident that the overall potency of benzylpencillin was enhanced on coordination with cobalt ion.

Keywords: Antibacterial, Benzylpenicillin, Complex, Tetradentate, Co(II)

INTRODUCTION

Transition metals take part in a variety of biological processes due to their characteristic electronic features, which generally involves their binding to electron-rich biological components, such as proteins and DNA. It is thus reasonable to propose that metal ions may be incorporated into drugs, with the main goal being interacting in a controlled manner with biological systems. The advances in inorganic chemistry has provided better opportunities to use metal complexes as therapeutic agents (Rafique *et al*., 2010; Thompson and Orgiv, 2004; Thompson and Orgiv, 2006). Chohan *et al*. (2003) synthesized cobalt(II) complexes of antibiotic ciprofloxacin and it was observed that metal complex showed stronger antibacterial activity than the free ciprofloxacin against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pseudomonas and Bacillus dysenteria*.
The pyrazine-2,3-dicarboxylate complexes of 1,10-phenanthroline and alkyl diamines have recently shown activity against gram (+) and gram (-) bacterial strains (Yesilel *et al*., 2010). The cobalt (II) complexes of hinikitiol, 4-isopropyltropolone, appeared to be active, in comparison to copper (II) complexes (Nomiya *et al*., 2004). Cobalt (II) complexes of benzimidazoles were synthesized and was found to have high antimicrobial activity against all the microorganisms used when compared to the standard reference compounds ciprofloxacin and gentamacin (Apohan *et al*., 2016).

Interesting biological properties have been found in the series of metal(II) oxinates of the derivative of sulfonohydrazide, belonging to the group of sulfonamides. The octahedral cobalt complex possessed good activity against both gram (+) and gram (-) bacterial strains but not higher than the free ligand alone (Dixit *et al*., 2010). Abdolmaleki *et al*. (2018) prepared cobalt (II) complexes with dipicolinic acid and imidazole derivatives which were tested against gram-negative bacteria *Escherichia coli (*ATCC25922) and *Klebsiella oxytoca* (ATCC13182) and gram-positive bacteria *Staphylococcus aureus* (ATCC33591) and *Bacillus cereus* (ATCC14579). The cobalt(II) complexes with a disordered octahedral result showed promising antibacterial activity against tested strains with the MIC values in the range <75-455 µg/mL. Similarly, Imran *et al*.

(2006) synthesized complexes of amoxicillin with some transition metal ions such as Co(II), Ni(II), Cu(II) and Zn(II). Here, amoxicillin acted as a monoanionic bidentate ligand therefore a tetrahedral geometries was suggested. The coordination to the metal ion was through carboxylate as well as the lactamic carbonyl group. These complexes were also screened for their antibacterial activity against several bacterial strains and the result showed that the metal complexes showed enhanced antibacterial activity as compared to the parent antibiotic (Imran *et al*., 2006).

Reiss *et al.,* (2015) reported Co(II) and Ni(II) complexes with Schiff base derived from amoxicillin and salicylaldehyde. They were characterized and noted as bidentate ligand that coordinated through phenolic oxygen and imino nitrogen of azomethine. The chelation was through 6-membered ring around the metal ion with an octahedral geometry. Bacteriological studies were carried out against three species, the outcomes indicated increment in antimicrobial action of metal complexes as compared to the parent antibiotics (Reiss *et al.*, 2015).

The situation of resistant human pathogens is critical in Africa as a result of the inexpensive drugs commonly used for the treatment of diseases, therefore there's need to encourage efforts in the development of novel metal-based drugs with the ability to increase inhibitory potential upon metal coordination to chemotherapy agents (Abd El and El Sariag, 2004). It is for this purpose that this study sought to extend the landscape of drug design for the enablement of novel mechanisms of benzylpenicillin and its Co(II) complex. Although benzylpenicillin is a common bactericidal antibiotic of the *β*-lactamin family, there is scarcity of information on its metal complexes. This brought about the researcher's investigation into the synthesis, characterization and antibacterial studies Benzylpenicillin and its Co(II) complex.

MATERIALS AND METHODS

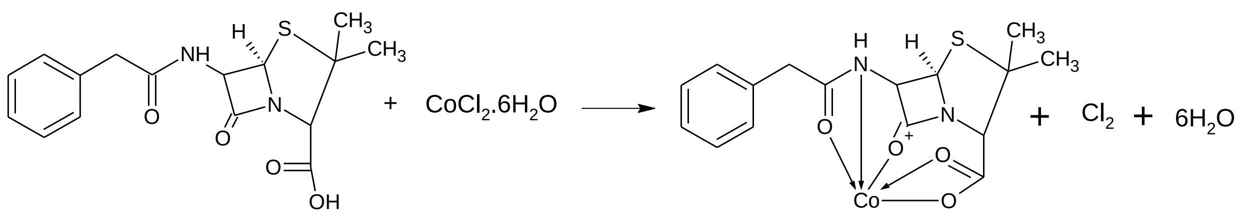
All the chemicals used were of analytical grade. Benzylpenicillin was obtained from Shanxi Federal Pharmaceutical Company Limited, Shanxi, China. The melting point and decomposition temperature of benzylpenicillin and its Co(II) complex were determined using Gallenkamp melting point apparatus. The solubility of the ligand and the metal complex were tested using various solvents such as water, methanol, ethanol, n-hexane, petroleum ether and dimethylsulfoxide (DMSO). The molar conductance of benzylpenicillin-Co(II) complex was measured at room temperature in a concentration of 10^{-3} M DMSO solution using Jenway Conductivity Meter 4510. Metal analyses were carried out using AAS spectrophotometer (bulk 210). The elemental analysis for C, N, H and S was carried out using a Perkin-Elmer 240B elemental analyzer. The stoichiometry of the complex was determined using continuous variation method as described by Tirmizi *et al.,* (2012). The UV-visible spectral measurement was obtained using UV-1800 series. IR spectra were obtained on a Perkin Elmer Spectrum BX FT-IR spectrophotometer (4400-350 cm^{-1}) in KBr pellets.

Synthesis of Benzylpenicillin - Co(II) complex

The complex was prepared following reported procedure by Anacona and Figueroa, (1999). $CoCl_2.6H_2O$ (3.09 g, 13 mmol) in 10 ml of distil water, was added to a solution of benzylpenicillin (4.35 g, 13 mmol). The mixture was stirred for 1 hour. The precipitate was filtered and washed with distil water, ethanol and diethyl ether. It was dried in a dessicator for 48 hours. The complex was then stored in a neatly labeled container after determining their percentage yield. The yield was calculated as shown in Equation 1.

$$(\%)Yield = \frac{Actual\ Yiels\ (in\ g)}{Theoretical\ Yield\ (in\ g)}\ x\ 100(\%) \quad (1)$$

The general synthesis for the benzylpenicillin - Co(II) complex is proposed in Scheme 1.



Scheme 1: Synthesis of Co(II) Benzylpenicillin complex

Antibacterial Activity Test

The organisms used were gram-negative *Escherichia coli, Enterobacter clocae, Pseudomonas aeruginosa,* and *Campylobacter fetus*. The gram-positive bacterial strains were *Staphylococcus aureus*, *Bacillus subtilis, Bacillus cereus,* and *Enterococcus faecalis*. The organisms were clinical isolates. They were obtained from Federal Medical Centre, Umuahia, Abia State. Antibacterial activity of samples were determined by using agar well diffusion method and bacterial growth were subcultured on nutrient broth for their *in vitro* testing which were 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 compound in 9 cm^3 of DMSO. Inoculation was done with the help of micropipette with sterilized tips in which 100 µL of activated strain was placed onto the surface of agar plate. It was spread over the whole surface and then two wells having diameter of 10 mm were dug in media and incubated at 37 °C for 48 hours. Activity was determined by measuring the diameter of zone showing complete inhibition and has been expressed in mm.

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 properties of benzylpenicillin and its Co(II) complex are presented in Table 1. [Co(Bpen)] complex has a purple colour with a melting of 210 ºC. The complex percentage yield of 77 % was highly commendable. The elemental analysis results were in good agreement with the calculated value. The conductivity measurements results in DMSO revealed that the complex is an electrolyte. Geary (1971) reported that coordination compounds with molar conductance above 100 Ohm^{-1} mol^{1} cm^{-1} are electrolyte. Based on the continuous variation method, metal: ligand ratio is 1:1 was proposed.

Table 1: Some physicochemical properties of benzylpenicillin and its Co(II) complex

Ligand/ complex	Colour	M.P. (ºC)	Yield (%)	Metal: ligand ratio	Conductance Ohm^{-1} mol^{1} cm^{-1}	C (%) Found (Calc)	H (%) Found (Calc)	N (%) Found (Calc)	S (%) Found (Calc)	Co (%) Found (Calc)
Bpen	White	209	-	-	236.0	57.39 (57.47)	5.41 (5.43)	8.37 (8.38)	9.54 (9.58)	-
[Co(Bpen)]	Purple	210	77	1:1	107.6	49.06 (49.11)	4.13 (4.12)	7.13 (7.16)	8.13 (8.19)	15.02 (15.06)

Bpen = Benzylpenicillin

Table 2 showed the solubility profile of Benzylpenicillin and its Co(II) complex in various solvents. Benzylpenicillin was found to be soluble in distilled water, n-hexane, ethanol, methanol, petroleum ether and DMSO. 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 result suggested that the complex is polar.

Table 2: Solubility profile of Benzylpenicillin and its Co(II) complex in some selected solvents

Ligand/ complex	Hexane	Distilled water	Ethanol	Methanol	Petroleum ether	DMSO
Bpen	S	S	S	S	S	S
[Co(Bpen)]	IS	IS	SS	SS	IS	S

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

Table 3 showed some selected infrared spectral data of the ligand and its Co(II) complex. The infrared spectra of benzylpenicillin and its Co(II) complex are presented in Figures 1 and 2 respectively. In the FTIR spectrum of benzylpenicillin, the absorption band at 1697. 66 cm^{-1} was assigned to carbonyl of amide. In the spectrum of [Co(Bpen)] this wavenumber shifted to 1642.12 cm^{-1}. This suggested coordination through the carbonyl of amide. Increase in electron density will increase the C=O bond length and consequently slow down the vibration frequency. Malecki and Maron, (2012) also reported a decrease in the vibration frequency of carbonyl stretch in $[RuHCl(CO)(PPh_3)_3$ complex. Elena, (2017) also reported a shift of 11 – 20 cm^{-1} for C=O vibration frequency in the spectra of hydrazone metal complexes. The absent of absorption band in the spectrum of the complex characteristic of β-lactam carbonyl group which was observed at 1778.04 cm^{-1} for benzylpenicillin suggested the involvement of β-lactam carbonyl group in complex formation, hence, the formation of C-O→M bonding system. This also suggested that the β-lactam C=O was converted to C-O during complexation and was observed at 1134.77 cm^{-1} in the complex. This was in agreement with the report of Elena, (2017) where C-O stretching was observed at 1157 cm^{-1}. The absorption band 3542.26 cm^{-1} in the IR spectrum of benzylpenicillin was not found in the spectrum of the complex which indicated that OH of the carboxylic acid deprotonated during coordination to the Co (II) ions. The absorption band at 3351.48 cm^{-1} in IR spectrum of benzylpenicillin shifted to a lower wavenumber in the complex 3301.00 cm^{-1}. This suggested that N-H was involved in complexation to cobalt ion.

Table 3 Selected Infrared Spectral Data of the Ligand and its Co(II) Complex

Compound	OH of Carboxylic (cm^{-1})	N-H (cm^{-1})	C=O of lactam (cm^{-1})	C=O of amide (cm^{-1})	V_{assym} (COO) (cm^{-1})	V_{sym} (COO) (cm^{-1})	Δv (cm^{-1})	C-O (cm^{-1})
Bpen	3542.26 3034.43	3351.48	1778.04	1697.66	1620.54	1418.40	202	1161.61
[Co(Bpen)]	-	3301.00	-	1642.12	-	1395.54	-	1134.77

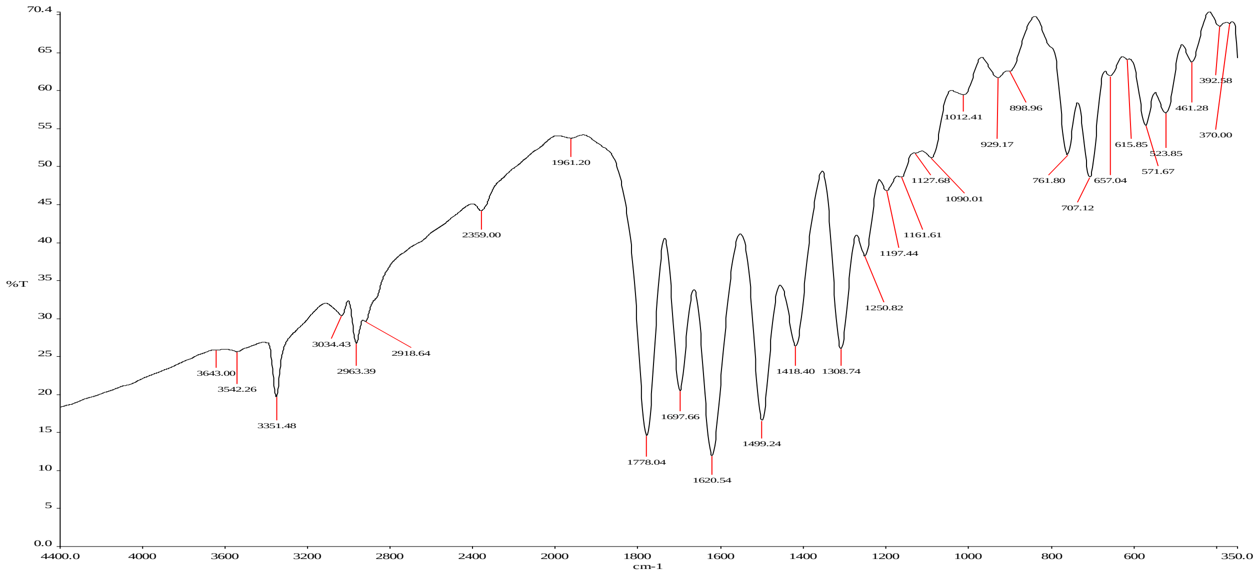


Figure 1: Infrared spectrum of benzylpenicillin

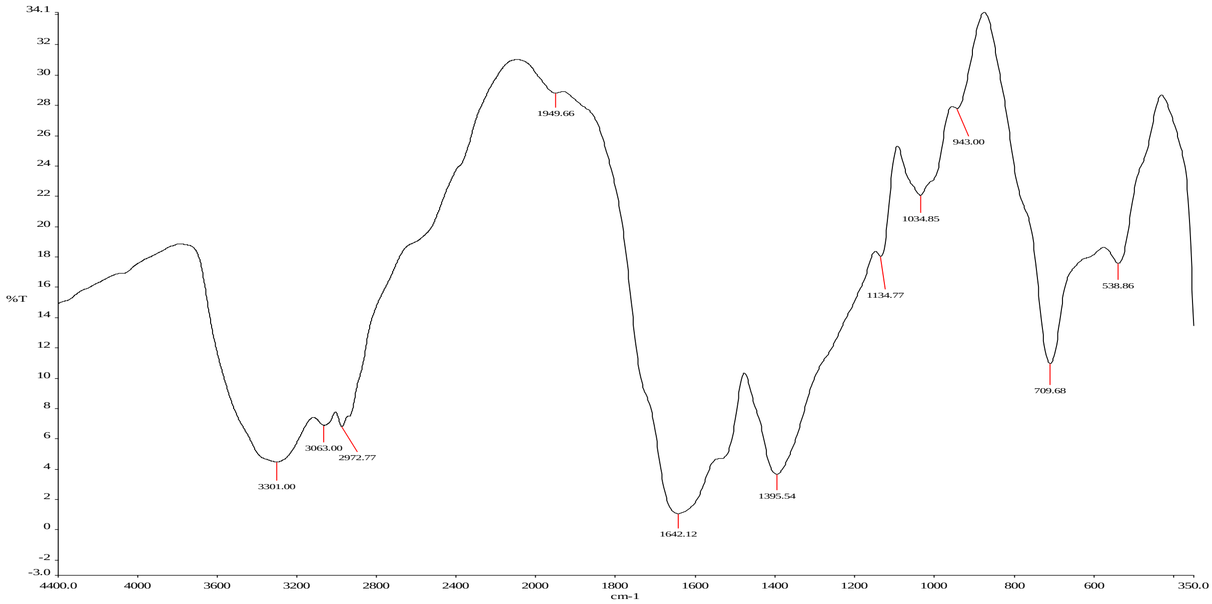


Figure 2: Infrared spectrum of Co(II) benzylpenicillin complex

The electronic spectral data of benzylpenicillin and its Co(II) complex are shown in Table 4. The electronic spectra of benzylpenicillin and its Co(II) complex are shown in Figures 3 and 4 respectively. The electronic spectrum of benzylpenicillin absorbed maximally at λ = 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 absorptions are as a result of the chromophores present in benzylpenicillin. These transitions have been assigned $\pi - \pi^*$ and $n - \pi^*$ and are known as Intra-ligand charge transfer

(ILCT). The electronic spectrum of [Co(Bpen)] absorbed maximally at λ = 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 absorptions are as a result of the chromophores present in [Co(Bpen)]. Ligand to metal charge transfer (LMCT) was observed at λ = 561.00 nm. This absorption band suggested that complexation occurred.

Table 4: Electronic Spectral Data of Benzylpenicillin and its Co(II) Complex

Ligand/Complex	Chromophores	Transitions	λ_{max} nm
Bpen	C=C	π – π*	197.50
	C=O	n - π*	203.50, 209.50, 215.50, 226.50, 238.50, 255.50, 259.50, 270.50, 278.50, 284.50 and 317.50
[Co(Bpen)]	C=C	π – π*	197.50
	C=O	n - π*	203.50, 209.50, 215.50, 226.50, 238.50, 255.50, 259.50, 270.50, 278.50, 284.50 and 317.50
		LMCT	561.00

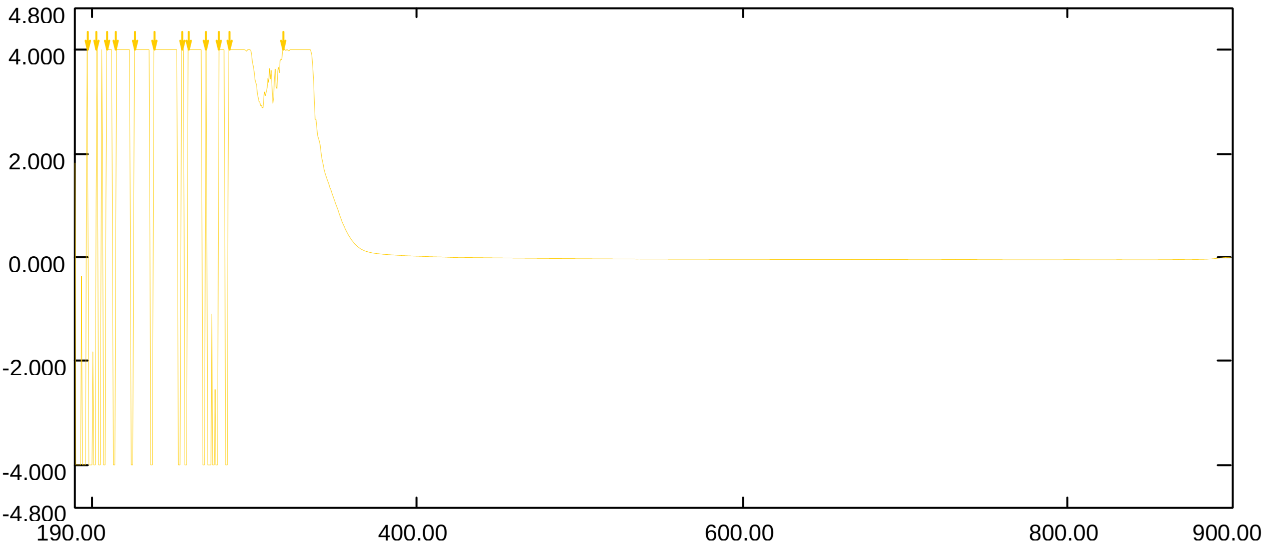


Figure 3: Uv/vis Spectrum of Benzylpenicillin

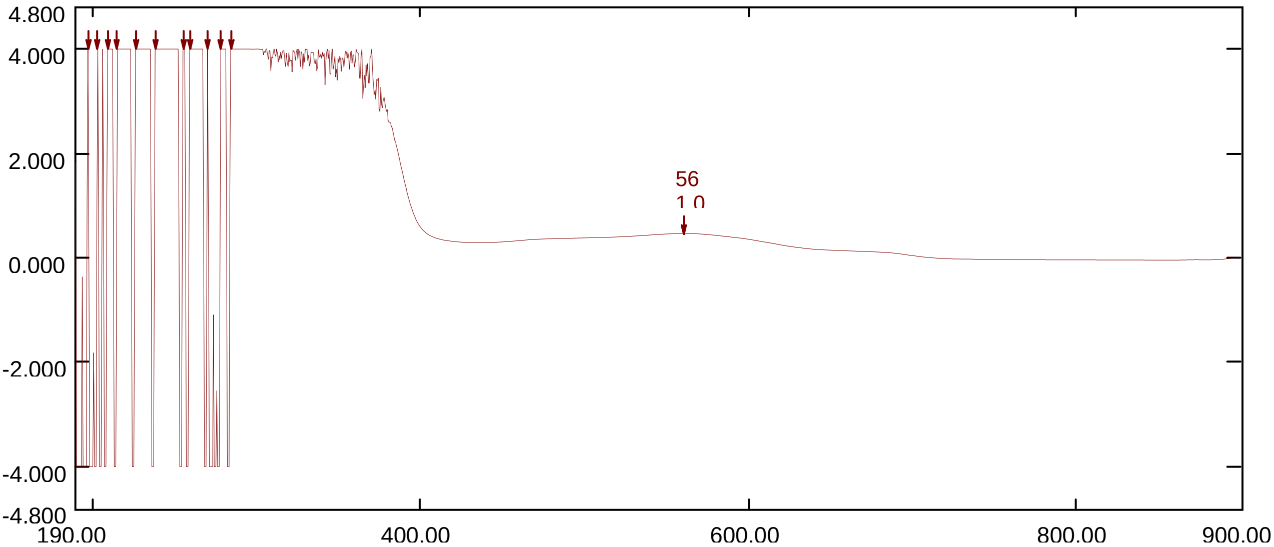


Figure 4: Uv/vis Spectrum of Co(II) Benzylpenicillin

The antibacterial activity of benzylpenicillin and its Co(II) complex against four gram-positive bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus,* and *Enterococcus faecalis*) is shown in Table 5. The antibacterial activity of benzylpenicillin and its Co(II) complex against four gram negative bacterial strains (*Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa,* and *Campylobacter fetus*) is shown in Table 6.

The result showed that the synthesized compound exhibited varying degree of inhibitory results on the growth of different bacterial strains. The complex showed significant decrease (p<0.05) in antibacterial activity against *Staphyloccocus aureus* as compared to the ligand. Significant increased activity was exhibited by the complex (p<0.05) as compared to the parent antibiotic against *Bacillus subtilis*, *Bacillus cereus, Enterococcus feacalis* and *Escherichia coli* bacterial strains. Moderate increased activity (p<0.05) was exhibited by the complex against *Enterobacter clocae* as compared to the ligand. The complex exhibited significant increased activity (p<0.05) against *Pseudomonas aeruginosa* and *Campylobacter fetus* bacterial strains as compared to the ligand. The increased antibacterial activity could be as a result of chelation which augmented the lipophilic character of the metal atom which in turn favours permeability through the lipid layers of the cell membrane (Angelo, 2020; Angelo *et al*., 2020).

Table 5: Percentage zone of inhibition (mm) of benzylpenicillin and its Co (II) complex on gram positive bacterial population

Compound	**Bacteria (gram positive)**			
	Staphyloccocus aureus	*Bacillus substilis*	*Bacillus cereus*	*Enterococus feacalis*
Bpen	2.52 ± 0.03^{b}	6.12 ± 0.03^{a}	4.96 ± 0.01^{a}	2.12 ± 0.03^{a}
[Co(Bpen)]	2.32 ± 0.03^{a}	14.63 ± 0.04^{b}	6.74 ± 0.01^{b}	7.12 ± 0.03^{b}

Bpen = benzylpenicillin Means with different superscript are significantly different (P< 0.05)

Table 6: Percentage zone of inhibition (mm) of benzylpenicillin and its Co (II) complex on gram negative bacterial population

Compound	**Bacteria (gram negative)**			
	Escherichia coli	*Enterobacter clocae*	*Pseudomonas aeruginosa*	*Campylobacter felus*
Bpen	10.43 ± 0.03^{a}	1.32 ± 0.03^{a}	6.82 ± 0.03^{a}	7.32 ± 0.02^{a}
[Co(Bpen)]	14.71 ± 0.01^{b}	3.11 ± 0.01^{b}	16.43 ± 0.03^{b}	11.08 ± 0.01^{b}

Bpen = benzylpenicillin Means with different superscript are significantly different (P< 0.05)

Based on continuous variation method, elemental analysis, electronic and FTIR spectral data, a tentative structure (Figure 5) have been proposed for the Co(II) complex of benzylpenicillin.

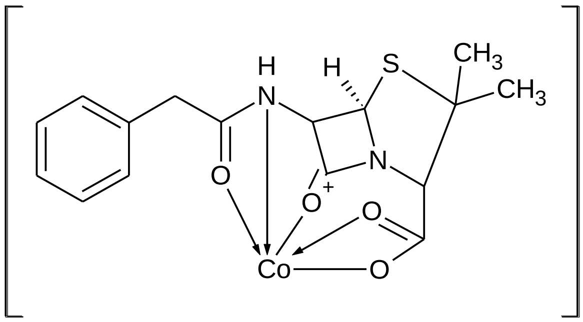


Figure 5: Proposed structure of Co(II) complex of benzylpenicillin

CONCLUSION

Co(II) complex of benzylpenicillin was synthesized. Benzylpenicillin and its Co(II) complex showed significant antibacterial activity. The benzylpenicillin ligand was found to behave as a pentadentate ligand and trigonal bipyramid geometry was suggested for the metal complex. The Co(II) complex showed enhanced antibacterial activity when compared with pure benzylpenicillin. It was deduced that Co(II) benzylpenicillin complex have improved antibacterial activity than benzylpencillin alone and it was evident that the overall potency of benzylpencillin was enhanced on coordination with cobalt ion.

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