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Synthesis, Characterization and Antibacterial Activities of Cu (II) Complex of Benzylpenicillin

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ABSTRACT

The sudden uprising of resistant human pathogens is a challenge in current antimicrobial therapy, therefore the need to encourage the development of novel metal-based drugs The efficacy of the various organic therapeutic agents can often be enhanced upon coordination with a suitable metal ion. Cu (II) benzylpenicillin,[Cu(Bpen)] complex was synthesized by the reaction of benzylpenicillin with $CuCl_2.2H_2O$. The complex was characterized by melting point, solubility, colour, conductivity, elemental analysis, infrared, UV/Visible, ^{1}HNMR and ^{13}CNMR spectroscopy. The spectral data showed that the ligand benzylpenicillin behaved as a pentadentate ligand coordinating to the metal ion through OH, C=O of amide, C-O of β-lactam, C=O of COOH and NH. A trigonal bypyramidal geometry have been proposed for [Cu(Bpen)]. The ligand and [Cu(Bpen) complex have been screened for their *in vitro* antibacterial activity against four gram positive strains (*Staphyloccocus aureus, Bacillus substilis, Bacillus cereus and Enterococus feacalis*) and four gram negative (*Escherichia coli, Enterobacter clocae, Pneumonia aeruginosa and Campylobacter felus*). It was deduced that [Cu(Bpen)] complex have improved antibacterial activity than benzylpencillin alone and it was evident that the overall potency of benzylpencillin was enhanced on coordination with copper ion.

Keywords: benzylpencillin, copper, trigonalbypyramidal, complex, antibacterial

INTRODUCTION

The emergence of resistant human pathogens is a major problem in current antibacterial therapy, therefore there's need to encourage efforts towards the development of novel drugs and transition metal complexes that have been found to be particularly useful in this matter (Witkop and Ramachandran, 1964). Coordination compounds are important classes in inorganic chemistry that have been widely studied nowadays and they have been known to play vital roles in the field of medicine since the discovery of cis-platin and carboplatin as anticancer agents (Shechter and Karlish, 1980; Thompson *et al*., 1999).Cisplatin,one of the first inorganic complexes discovered, is the most efficient drugs for the treatment of certain types of cancer; however, drug toxicity and resistance limit its utilization for a broader range of diseases. Throughout the years of scientific research copper (II) complexes have been found to possess various activities such as antiulcer (Bonham *et al*., 2002), antiamoebic (Rakel, 2007), antidiabetic (Davis, 2003), anticonvulsant (Rottkamp*et al*., 2000), anti-inflammatory (Adelstein and Vallee, 1961; Harris *et al*., 1998; Christen, 2000), antimicrobial (Strausak*et al*., 2001) and antitumor (Schaefer and Gitlin, 1999).Various copper (II) complexes have been reported with antimicrobial activities. For instance, Copper (II) complex of Kefzol was screened against two gram (+) and gram (-) bacterial strains. It exhibited a marked enhancement in Kefzol activity against all the tested bacterial strains (Chohan*et al.*, 2004).Six copper(II) complexes of 2-benzoylpyridine N(4)-cyclohexylthiosemicarbazone were tested against five types of bacteria and were found to have an elevation in their bacteria activity against *Bacillus Sp., Vibrio choleraol, Staphylococcus aureus and Salmonella paratyphi* (Joseph *et al*., 2004). Novel complex of Cu (II) was synthesized from cyclohexane-1,3-dione of the type $[Cu(L^2)_2].2NO_3.H_2O$ (where L^2 = 2-[2-(3-nitropheny)hydrazono]cyclohexane-1,-3-dione). It was screened against *Escherichia coli ATCC25922, Enterococcus feacalis ATCC29212, Staphylococcus aureus ATCC25923, and Salmonella typhimurium CCM583* respectively and was observed to show average level antibacterial activity against the bacteria compared to ampicillin (Turan *et al*., 2015). Pahontu *et al.* (2016) synthesized new Cu (II) complexes from 8-ethyl-2-hydroxytricyclotridecan-13-one-thiosemicarbazone.

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The complexes were tested for their antimicrobial activity against *Escherichia coli, Salmonella enteritidis, Staphylococcus aureus, and Enterococcus feacalis.* The result showed that all the complexes showed stronger antibacterial activity when compared to the free ligand.. Common anti-bacterial agents have also been used as ligands to complex copper ions. It was noticed that the antimicrobial activity against *Mycobacterium smegmatis* of a metal ion complex in comparison to free ciprofloxacin, a bacterial gyrase inhibitor, increased three times (Patole *et al*., 2003). This may have resulted from the facilitated diffusion of the drug through the cell membranes, presumably by an increase in the lipophilicity of the drug (Jimenez-Garido*et al*., 2005). Complexes of cloxacillin with Co(II), Ni(II), Cu(II) and Zn(II) were synthesized and the coordination occurred through oxygen of carboxylate group and nitrogen of β-lactam group. These complexes were investigated and found to have an octahedral geometry with formula $[M(cloxa)(H_2O)_3Cl]$ and they are non-electrolytic in nature. It was also found that the complexes have improved antibacterial activity than cloxacillin alone and it was evident that overall potency of cloxacillin was enhanced on coordination with metal ions (Chohan and Supuran, 2006).Bamigboye*et al*. (2012) synthesized three complexes of mixed sulfamethoxazole-cloxacillin drugs. Both the sulfamethoxazole and cloxacillin acted as a bidentate ligands towards Mn(II), Cu(II), Zn(II) metal ions and all the three complexes were assigned octahedral geometry. The coordination of metal ion with cloxacillin was through carbonyl oxygen and lactam ring oxygen, while the coordination of sulfamethoxazole was through sulphone oxygen and amine nitrogen group and to complete the coordination of octahedral geometry two chloride ions were intruded. Investigations of antimicrobial activities of the complexes against the tested microorganisms were found to be more active than their parent ligands.Thousands of coordination compounds have been literally prepared based on well-conceived ideas of improving their efficacy and have been subsequently screened over a wide range of diseases but few of them have been successful in passing the clinical tests (Kostova, 2010; Moues *et al*., 2009; Rafique *et al*., 2010). It is for this purpose that this study sought to extend the landscape of drug design. We therefore present the synthesis, characterization and antibacterial activities of Cu (II) complex of benzylpenicillin

MATERIALS AND METHODS

All the chemicals used were of analytical grade. Benzylpenicillin was obtained from Shanxi Federal Pharmaceutical Company Limited, Shanxi, China. The melting points 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 and its Cu(II) complex (10^{-3} M solution) were recorded using Jenway Conductivity Meter 4510. DMSO was used as the solvent. The conductivity of DMSO which was used as solvent was 8.37 $Ohm^{-1}mol^{1}cm^{-1}$. The elemental analysis for C, N, H and S were obtained using a Perkin-Elmer 240B elemental analyzer. The UV-visible spectral measurement (190 – 900 nm) was obtained using UV-1800 series. The solvent used was Dimethylsulfoxide (DMSO). IR spectra were obtained on a Perkin Elmer Spectrum BX FT-IR spectrophotometer (4400-350 cm^{-1}) in KBr pellets. The NMR spectral measurement was recorded on nuclear magnetic resonance Bruker spectrophotometerat 400 MHz using tetramethylsilane as internal standard and DMSO-d6 as solvent.

Synthesis of benzylpenicillin - Cu(II) complex

The complex was prepared following reported procedure by Anacona and Figueroa, (1999). Exactly 2.216 g (0.013 mole) $CuCl_2.2H_2O$, in 10 ml of water was added to a solution of benzylpenicillin (4.35 g, 0.013 mole). The mixture was stirred for 1 hour and the solid complex which separated was removed by filtration and washed with water, ethanol and ether. The complex was dried under vacuum at room temperature for 48 hours. The complex was then stored in a neat andlabelled container after determining their percentage yield. The general synthesis for the benzylpenicillin - Cu(II) complex is proposed in Equation 1.

$$C_{16}H_{17}N_2O_4S + CuCl_2.2H_2O \longrightarrow [Cu(C_{16}H_{17}N_2O_4S)] + Cl_2 + 2H_2O \quad \text{-------Equation 1}$$

Antibacterial activity test

The organisms used were Gram-negative *Escherichia coli, Enterobacter clocae, Pneumonia aeruginosa,* and *Campylobacter felus*. The Gram-positive bacterial strains were *Staphylococcus aureus, Bacillus substilis, Bacillus cereus,* and *Enterococus faecalis*. These clinical isolateswere 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 mL of DMSO. Inoculation was done with the help of micropipette with sterilized tips and 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

Table 1: Some physicochemicalproperties of Benzylpenicillin and its copper complex

Ligand/complex	Colour	M.P(^{o}C)	Yield %	Conductance $Sm^2.mol^{-1}$	C (%) Found (Calc.)	H (%) Found (Calc.)	N (%) Found (Calc.)	S (%) Found (Calc.)
Bpen	White	209	-	236.0	53.92 (53.77)	4.81 (4.73)	7.87 (7.88)	8.98 (8.88)
[Cu(Bpen)]	Black	200	72	146.9	45.75 (48.54)	45.75 (48.54)	6.67 (7.08)	7.62 (8.10)

Bpen = Benzylpenicillin

Table 2: Solubility profile of benzylpenicillin and its copper complex in some selected solvents

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

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

Table 3: Infrared spectral data of benzylpenicillin and its copper complex

Ligand/complex	OH of COOH	N-H	C=O of lactam	C=O of amide	V_{assym} (COO)	V_{sym} (COO)	C-O
Bpen	3542.26	3351.48	1778.04	1697.66	1620.54	1418.40	1161.61
[Cu(Bpen)]	absent	3248.50	Absent	1640.37	1449.73	1362.26	1124.00

Bpen = Benzylpenicillin

Table 4: Summary of the UV/Vis peaks of benzylpenicillin and its copper 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
[Cu(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
			366.80
		LMCT	

Bpen = BenzylpenicillinLMCT = Ligand to metal charge transfer

Table 5: Summary of ^{1}H NMR peaksbenzylpenicillin and its copper complex

Compound	O-H (ppm)	C=OCH of β-lactam (ppm)	NH of amide (ppm)	Methyl protons (ppm)	Ar H (ppm)
[Bpen]	11	5.35	8.72	1.46 1.59	7.12-7.32
[Cu(Bpen)]	absent	absent	7.70	1.55 1.29	7.12-7.45

Bpen = Benzylpenicillin

Table 6: Summary of the ^{13}C NMR peaksbenzylpenicillin and its copper complex

Compounds	Phenyl carbons (ppm)	C=O of β-lactam (ppm)	C=O Amide (ppm)
[Bpen]	126.88-129.54	173.73	170.86
[Cu(Bpen)]	125.98-129.05	152.50	153.68

Bpen=Benzylpenicillin

Table 7: Percentage zone of inhibition (mm) of the benzylpenicillin and its coppe (II) complex against gram positive bacterial population

Ligand/complex	*Staphyloccocusaureus*	*Bacillus substilis*	*Bacillus cereus*	*Enterococusfeacalis*
Bpen	2.52±0.03^{a}	6.12±0.03^{a}	4.96±0.01^{a}	2.12±0.03^{a}
[Cu(Bpen)]	5.60±0.08^{b}	10.73±0.24^{b}	8.15±0.02^{b}	5.26±0.08^{b}

Means with different superscript are significantly different from each other ($P < 0.05$)

Table 8: Percentage zone of inhibition (mm) of the benzylpenicillin and its copper(II) complex against gram negative bacterial population

Ligand/complex	*Escherichia coli*	*Enterobacterclocae*	*Pneumonia aeruginosa*	*Campylobacter felus*
Bpen	10.43±0.03^{a}	1.32±0.03^{a}	6.82±0.03^{a}	7.32±0.02^{a}
[Cu(Bpen)]	16.84±0.02^{b}	8.11±0.01^{b}	14.82±0.05^{b}	10.44±0.01^{b}

Means with different superscript are significantly different from each other ($P < 0.05$)

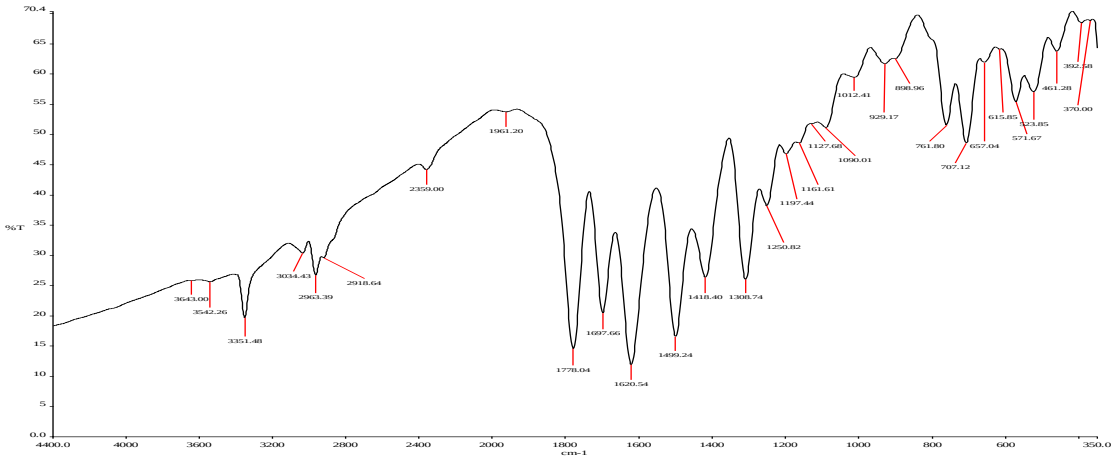

Figure 1: IR spectrum of benzylpenicillin

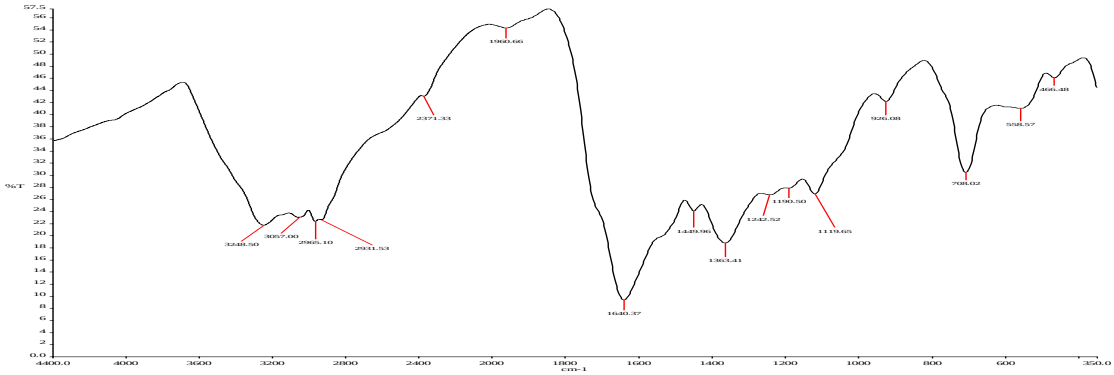

Figure 2: IR spectrum of benzylpenicillin-Cu(II) complex

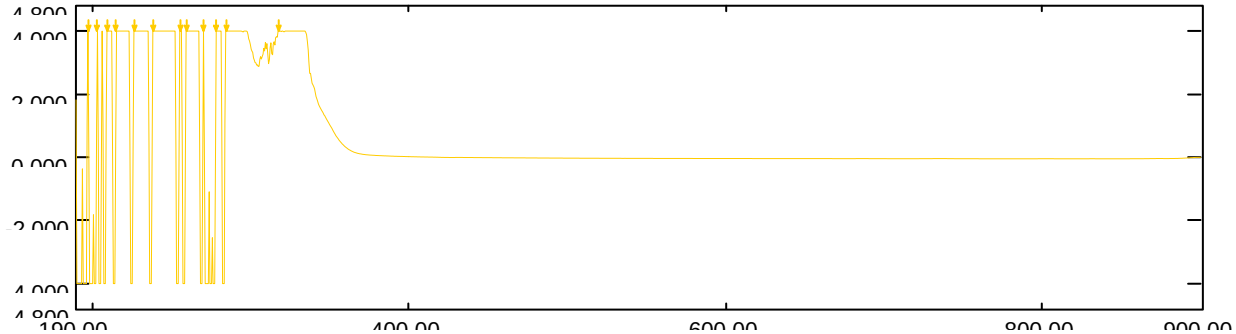

Figure 3: UV/Vis spectrum of benzylpenicillin

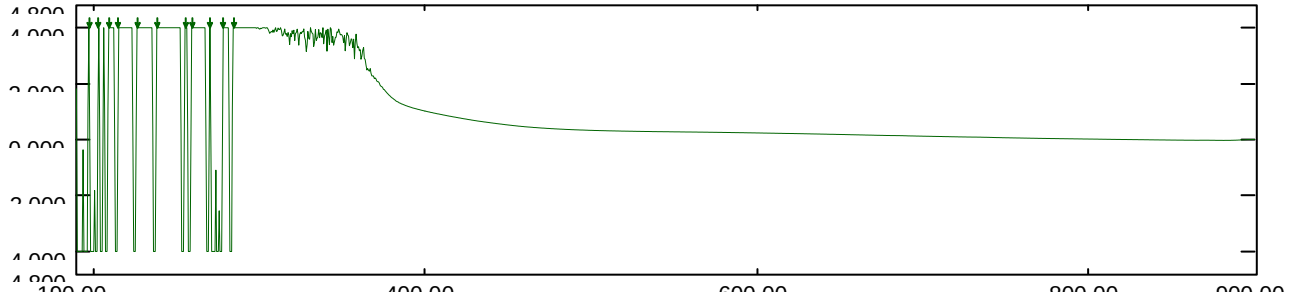

Figure 4: UV/Vis spectrum of benzylpenicillin-Cu(II) complex

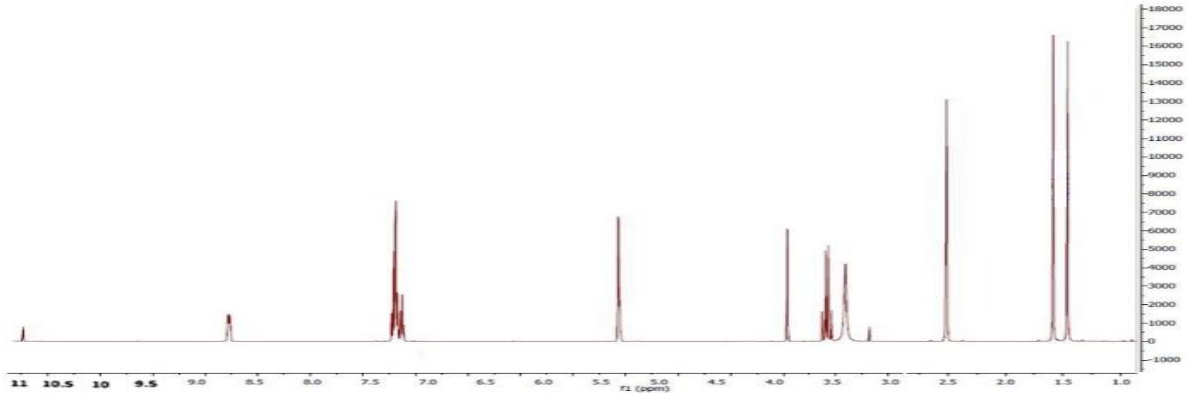

Figure 5: ^{1}H NMR Spectrum of Benzylpenicillin

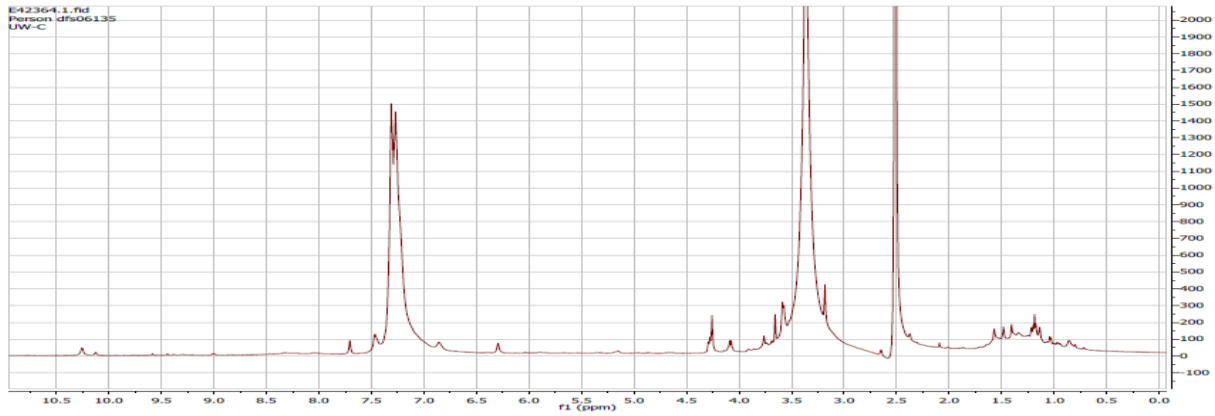

Figure 6: ^{1}H NMR Spectrum of benzylpenicillin-Cu(II) complex

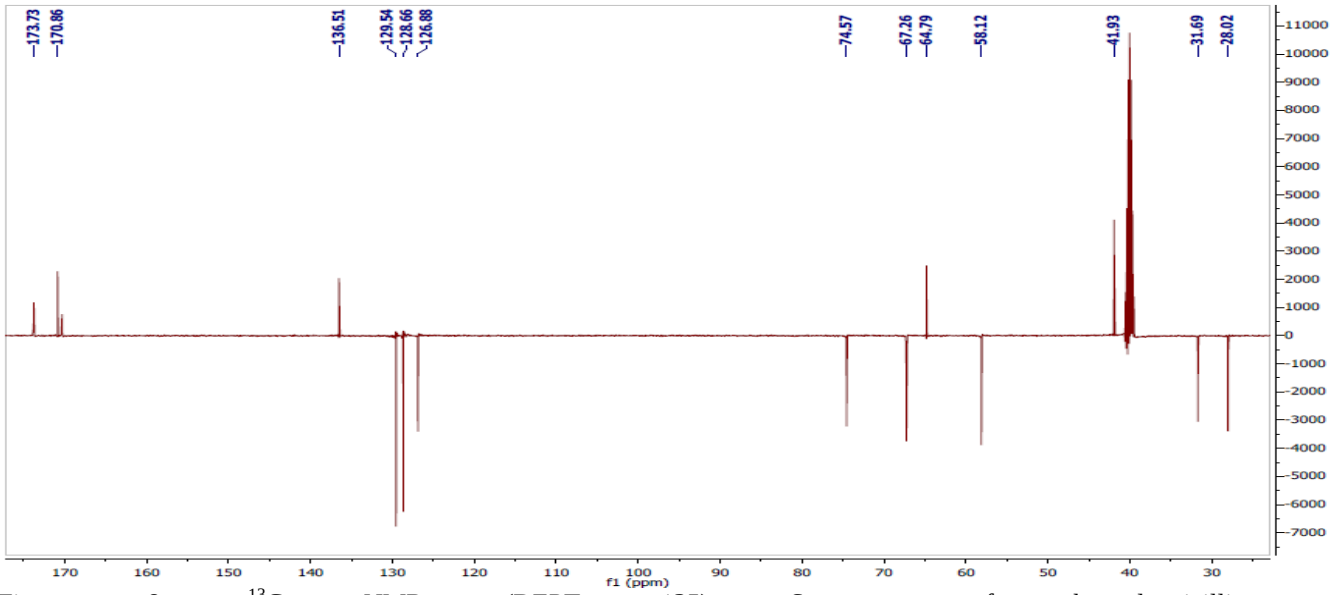

Figure 8: ^{13}C NMR (DEPT 135) Spectrum of benzylpenicillin

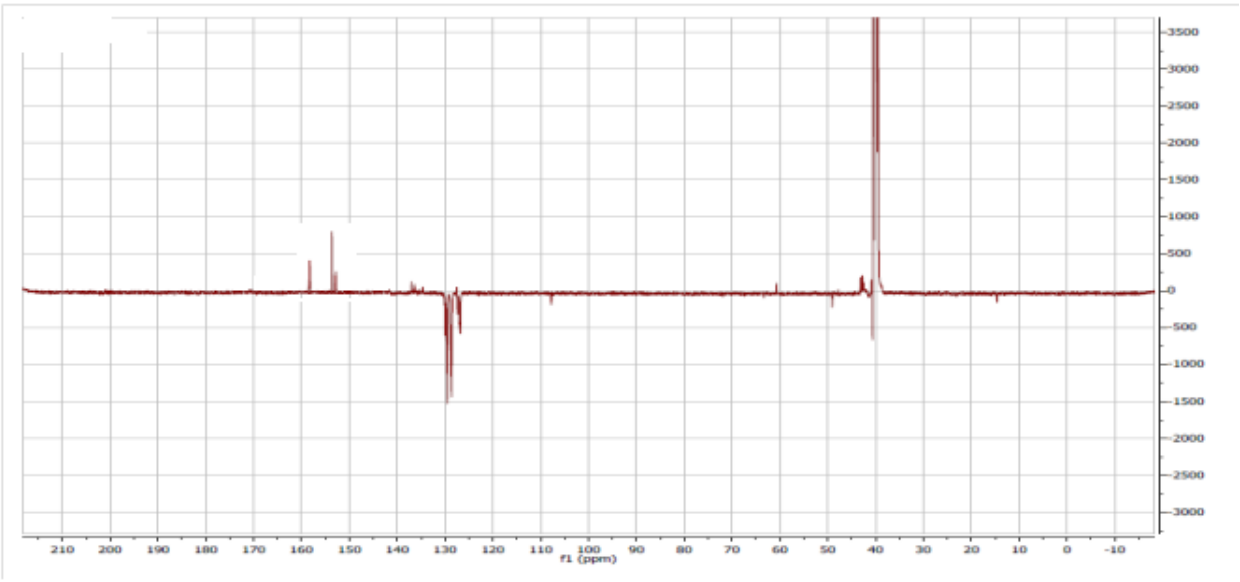

Figure 9: ^{13}C NMR (DEPT 135) Spectrum of benzylpenicillin-Cu (II) complex

[Cu(Bpen)] is a non-hygroscopic, air and photo stable crystalline powder. It is black in colour with a melting of 200 ^{o}C lower than the parent antibiotic (209 ^{o}C) (Table 1). The percentage yield of 72 % was very good. The micro analytical measurements and metal estimation data results suggested 1:1 metal ligand ratio and formation of the complex. The conductivity measurements in DMSO (Table 1) revealed that the ligand and complex were electrolyte with a value 236.0 and 146.9 Sm^2mol^{-1} (Geary, 1971). The conductivity of DMSO which was used as solvent was 8.37 $Sm^2.mol^{-1}$. The solubility of benzylpenicillin and [Cu(Bpen)] 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. 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. The solubility data suggested that the complex is

moderately polar.Infrared spectral data of benzylpenicillin and its copper complex are shown in Table 1 while infrared spectra are presented in Figure 1 and 2 respectively. The vibration frequency 1697. 66 cm^{-1} was assigned to C=O stretch of carboxylic acid. In the IR spectrum of [Cu(Bpen)] this wavenumber was shifted to 1640.37cm^{-1}. This suggests that coordination occurred through C=O of COOH. β-lactam carbonyl group which was observed at 1778.04 cm^{-1} in the spectrum of benzylpenicillinwas absent in the spectrum of [Cu(Bpen)]. This suggested the involvement of β-lactam carbonyl group in complex formation, hence, the formation of C-O→M bonding system. This also suggests that the β-lactam C=O was converted to C-O during complexation. The vibration frequency 3542.26 cm^{-1} was assigned OH of COOH in the IR spectrum of benzylpenicillin. This vibration frequency was absent in the IR spectrum of [Cu(Bpen)]. This suggests that OH was deprotonated during coordination. In the spectrum of the ligand, the wavenumber 3351.48 $cm^{-1}$was assigned N-H stretch. This wavenumber was shifted to a lower wavenumber (3248.50 cm^{-1}) in the complex. This suggests that complexation occurred throughN-H function group because increase in electron density, increases the N-H bond length and consequently slows down the vibration frequency.Theelectronic spectral data of benzylpenicillin and [Cu(Bpen)] is shown in Table 4. The spectra are presented in The multiplet signals observed in the ligand and complex in the range δ =7.12-7.45 ppm were assigned to aromatic protons. Finally, singletsobserved at δ= 1.46 and 1.59 ppm were attributed to the two methyl protons on the thiazolidine ring of benzylpenicillin were observed at δ= 1.29 and 1.55 ppm in the spectrum of the metal complex.

The ^{13}C NMR (DEPT 135) spectral data of benzylpenicillin and [Cu(Bpen)] is shown in Table 6. The^{13}C NMR (DEPT 135) spectra are presented in Figures 8 and 9.In the ^{13}C NMR spectrum benzylpenicillin, C=O of amide was observed at 170.86 ppm. The band for C=O of amide was shifted upfield 153.68 ppm in the ^{13}C NMR spectrum of [Cu(Bpen)]. This shift suggested that C=O of amide was involved in complexation to Cu(II) ion. C=O of carboxylic acid was observed at 173.73 ppm in the ^{13}C NMR spectrum of the ligand. This band was shifted upfield (152.50 ppm) in the ^{13}C NMR spectrum of the complex. This also suggested that C=O of carboxylic acid was involved in complexation to Cu(II) ion. The total numbers of carbons were equal to the peaks in the DEPT experiment.

Figures 3 and 4. The electronic spectrum of benzylpenicillin maximally absorbed 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 were as a result of the chromophores present in benzylpenicillin. These transitions have been assigned π – π* and n – π* and are known as Intra-ligand Charge Transfer (ILCT). The electronic spectrum of [Cu(Bpen)] maximally absorbed 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. These transitions have been assigned π – π* and n – π* and are known as Intra-ligand Charge Transfer (ILCT). The absorption band at λ = 561.00 nm in the spectrum of the complex have been assigned ligand to metal charge transfer (LMCT).The ^{1}H NMR spectral data of benzylpenicillin and [Cu(Bpen)] are shown in Table 5. The ^{1}H NMR spectra are presented in Figures 4 and 5. The singlet at 11.00 ppm corresponded to hydroxyl group of carboxylic acid. This chemical shift was absent in the ^{1}HNMR spectrum of [Cu(Bpen)]. This suggested that –OH was deprotonated during coordination. The doublet observed at δ = 8.72 ppm in ^{1}H NMR of benzylpenicillin was assigned to NH proton of amide. This chemical shift was shifted to 7.70 ppm in the ^{1}H NMR spectrum of [Cu(Bpen)]. This shift suggested that coordination occurred through N-H functional group

Based on elemental analysis, IR, UV-Visble, ^{1}HNMR and ^{13}CNMR, the structure in Figure 10 have been proposed for benzylpenicillin -copper (II) complex. The structure of benzylpenicillin is shown in Figure 11.

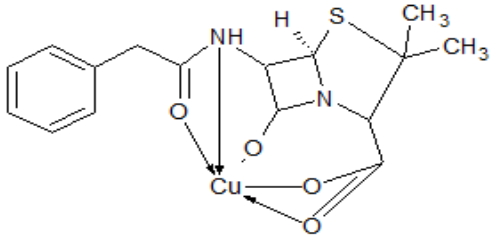

Figure 10: Suggested structure of benzylpenicillin - copper (II) complex

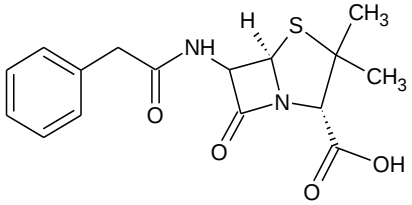

Figure 11: Structure of benzylpenicillin

The antibacterial activity of benzylpenicillin and its copper (II) complex against four gram positive bacterial strains (*Staphylococcus aureus, Bacillus substilis, Bacillus cereus,* and *Enterococus faecalis*) is presented in Table 7 while the antibacterial activity of benzylpenicillin and its copper (II) complex against four gram negative bacterial strains (*Escherichia coli, Enterobacter clocae, Pneumonia aeruginosa,* and *Campylobacter felus*) is presented in Table 8.The result showed that the synthesized compound exhibited varying degree of inhibitory results on the growth of different bacterial strains. The inhibitory activity of the complex [Cu(Bpen)] was significantly higher($p<0.05$) against *Staphyloccocusaureus, Bacillus substilis, Bacillus cereus, Enterococusfeacalis, Campylobacter felus, Enterobacter , Pneumonia aeruginosa*and *Escherichia coli*as compared to benzylpenicillin. 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 (Jimenez-Garido*et al*., 2005).

CONCLUSION

Copper(II) complex of benzylpenicillin was synthesized. Benzylpenicillin and its copper (II) complex were characterized and tested for their antibacterial activity. The benzylpenicillin ligand was found to behave as a pentadentate ligand and a trigonal bypyramidal geometry was suggested for the metal complex. The complex was also found to have improved antibacterial activity than the free ligand.

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