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African Journal of Pure and Applied Chemistry

Full Length Research Paper

Copper-paracetamol complexes: Promising lead antibacterial drug candidates

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Antibiotic resistance by microorganisms has triggered the need to discover new antibiotics to replace the old ones. The study was designed to prepare copper-paracetamol complexes which will serve as lead compounds towards the discovery of novel antibiotics. Copper sulphate was reacted with paracetamol in the presence of sodium nitrate in borate buffer to give products which were separated into three layers when extracted with a set of organic solvents. The topmost, third layer was separated and further washed with 1:1:1 petroleum ether, ethanol and benzene to obtain a yellow extract. The first and second layers were also air dried to obtain products 1 and 2. The maximum wavelength of absorption of products 1, 2, yellow extract and paracetamol were 250, 350, 280 and 300 nm respectively. The infrared absorption peaks suggested that, the metal coordination products formed were different from the reactants (paracetamol and cupper). The atomic absorption spectra of the extracts further indicated the association of copper with paracetamol to form the coordination complexes. Products 1, 3 and the yellow extract inhibited *E. coli* and staphylococcus growth. In all four products were separated and their possible structures have been proposed in the text.

Key words: Copper, paracetamol, coordination complex, antibacterial and drug leads.

INTRODUCTION

Coordination complexes are of great biological importance in nature. They are mostly metal atoms (which are the central atoms) bonded to an array of molecules (usually organic compounds) to form the coordination complexes (Mukherjee, 2000). Metal coordination complexes serve as agents to mop out harmful metals in humans (Farrell, 1989). In oxygen circulation, hemoglobin, a metal coordination complex, transports oxygen from the lungs to tissues in mammals (Mairbäurl and Weber, 2012).

Metal coordinated compounds since 1980 has served as a means of synthesizing lead compounds in the field of drug discovery (Beckmann and Brooker, 2003; Kimura, 1986; Mewis and Archibald, 2010; Mewis and Archibald, 2010). In 2007, Lawal and Obaleye formed novel complexes of Co (II), Ni (II) and Fe (III) with aspirin and paracetamol. Their metal chelation complexes were found to be active against *Bacillus substilis, Serratia species and Escherichia coli* giving a new dimension to the search for antibiotics. Complexes of 2,2'-bipyridyl, formed from $CuSO_4$ were also found to be active against

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paracoccus denitrificans (Smit et al., 1980). In addition, synthetic coordination complex cis-$Pt(NH_3)_2Cl_2$ was identified as an anti-tumor drug to replace existing drugs that have lost their potency.

Antibiotics formed from chelation compound have some advantages over the existing drugs on the market. The 'slow' metal-ligand exchange rates of metal chelation compounds can be matched to the division rate of human pathogens offering an extended half-life to the coordination compounds. This enables the compounds to persist in the host to kill the pathogens hence abolishing the need for multiple dose of these drugs. In cancer research, this has been established, where cisplatin and carboplatin were found to have better anti-tumor activity based on their increased stability and half-life (Reedijk, 2008).

One of the most widely used over the counter analgesic is paracetamol (acetaminophen) (Hinz and Brune, 2012). It is used to relief pain, fever, headaches, and other minor aches. It is a major ingredient in numerous cold and flu remedies (Hinz and Brune, 2012). It is important to note that synthesis of paracetamol simple and can be done in the laboratory by nitrating phenol with sodium nitrate, separating the desired *p*-nitrophenol from the *ortho*-byproduct, and reducing the nitro group with sodium borohydride. The resultant *p*-aminophenol can be acetylated with acetic anhydride (in this reaction, phenol is strongly activating, thus the reaction only requires mild conditions) to form the final product (Bhattacharya et al., 2006). The increasing importance of metal coordination complexes in the design and discovery of novel of drugs led to the heightened interest of many scientist to study metal drug complexes as lead compounds (Farrell, 1989; Kimura, 1986; Mewis and Archibald, 2010). It was necessary therefore to synthesize novel compounds from the analgesics, which over the years demonstrated high efficacy and very low toxicity, through the formation of drug-metal complexes. This paper hereby reports the synthesis, characterization and antibacterial activity studies of novel transition metal complexes of paracetamol. Copper paracetamol complexes are promising lead drug candidates.

METHODOLOGY

Pure, white paracetamol of melting point 170°C was obtained from Kinapharma Company Limited, Accra.

Preliminary absorbance scanning of $CuSO_4$ and paracetamol in aqueous solution

A solution of copper sulphate was prepared by dissolving 16 mg of $CuSO_4$ pellet in 100 mL distilled water; a solution of paracetamol was also prepared by dissolving the 15 mg of pure paracetamol sample in 100 mL of aqueous solution. Using distilled water as the blank, the absorption of copper sulphate and paracetamol were measured from 200 to 800 nm. The spectra was used to determine the purity of the two compounds.

Synthesis of copper paracetamol complex

A 0.2 M borate buffer of pH 8.2 was prepared and used to dissolve exactly 3.19, 1.38 and 3.02 g of $CuSO_4$, $NaNO_2$ and paracetamol in different beakers. All the mixtures were transferred into a dark conical flask and top up to 100 ml. The reaction was stirred at room temperature without heat for 2 h. The products from the copper-paracetamol reaction previously talked about were transferred into a separatory funnel. Exactly 100 ml of 1:1:1ethanol, petroleum ether, benzene were added to the separatory funnel. The setup was then left to sand for two hours and three different layers were formed. The first layer was fraction one (1), second layer (2) and third layer (3). There was a foamy layer on top which was named yellow fraction (Y).

Thin layer chromatographic identification of copper complexes

Exactly 50 ml of toluene, ethyl acetate and ethanol were measured into the chromatography tank and mix well to have 2:1:1 solvent front. Aqueous solution of paracetamol and copper sulphate were labeled as paracetamol and $CuSO_2$ respectively. The top fraction was labeled "3", the middle fraction was labeled "2" and the bottom fraction was labeled "1". The yellow crystal obtained from the extraction was labeled as yellow fraction.

The samples were spotted on a thin chromatographic plate. They were allowed to dry. The plate was place in a tank which was covered with a glass plate. The chromatographic plate was removed after the solvent was about 2/3rd of the length of the plate.

The plate was viewed under U.V short wave and iodine thank. The sports were located by and their retardation factor was calculated.

Spectroscopic scanning of the copper complexes

Exactly 0.1 g of the fractions extracted thus the first, second, third and yellow fractions respectively were measured in to separate test tubes and dissolved with distilled water. The solutions formed were filtered and their absorbance measured at wavelength range of 200 to 800 nm. Distilled water was use as the blank. The yellow fraction was dissolved in ethanol and with the ethanol as the blank its absorbance was measure from 200 to 800 nm. The respective spectra were taken. Exactly 0.1 g of the fractions were measured into separate test tubes and dissolved with DMSO. The solutions formed were filtered and their absorbance measured at wavelength range of 200 to 800 nm. DMSO was use as the blank. Absorbance was plotted against wavelength.

Infrared analysis of all the copper-paracetamol products

The first, second, third and yellow fractions were labeled 1, 2, 3, Y and paracetamol (P). Exactly 0.02g sample 1, 2, 3 were dissolved in 20 mL distilled water and filtered to have a clear solution while 0.02 g of the sample Y was dissolved in ethanol. Using water and ethanol as the blank for sample 1-3 and sample Y, absorbance of the samples were measure from 400 to 4000 cm^{-1}.

Atomic absorption spectra analysis of the copper-paracetamol products

Exactly 0.5 g of all the fractions (1, 2, 3 and Y) were measured and digested with 6mL of 30% nitric acid and hydrogen peroxide. The

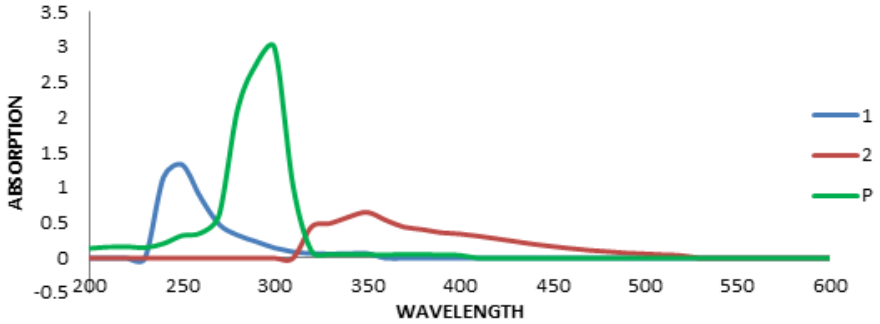

Figure 1. Absorption spectra of products 1, 2, and paracetamol.

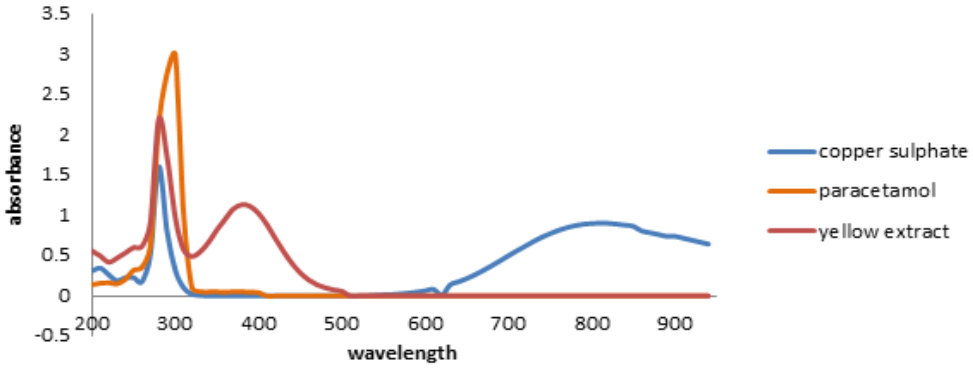

Figure 2. Absorption spectra of the yellow extract, paracetamol and copper sulphate.

digested samples were diluted with distilled water to a total volume of 10 ml. The absorbance of the samples was measured using the atomic absorption spectrometer. Reference concentrations of copper solution (2, 6 and 8 mg/kg) were used to plot standard curve. The concentrations of the samples were extrapolated from the standard curve.

Bioactivity assay

Exactly 0.05 g of all the fractions were dissolved in 1ml distilled water but the yellow fraction was dissolved in 1ml DMSO. Two microtiter titer plates were filled with varying concentrations (with dilution factor of 10 staring with 1M) of the samples labeled 1, 2, 3, Y, D, and para. Label D and para corresponds to dilute DMSO solution and paracetamol solution respectively. Each sample was applied to two rows on the microtitre plate. Exactly 100 µL of serum was added to each well of the two microtitre plates. The factions were inoculated with inoculated with E *coli* and *Staphylococcus aureus* for 24 h at 37°C. The content of the wells with no microbial growth were transferred to a fresh agar media plates. The plates were incubated for 24 h at 37°C. The plates were examined after the incubation for microbial growth.

RESULTS

To determine the purity of copper sulphate and paracetamol, their absorption spectra were recorded. In an aqueous solution, copper sulphate had two absorption peaks at 280 and 810nm (Figure 2) while paracetamol had a single absorption peak at 300 nm (Figure 1). These absorbance were compared to that of the products formed.

The extracting of the reaction products in 1:1:1 petroleum ether, ethanol and benzene gave four extracts; products 1, 2, 3, and the yellow extract. The wave lengths of maximum absorbance of the products were measured and compared to that of paracetamol and copper sulphate to establish whether new compounds were formed from the reaction (Figure 1 and 2). It was observed that the reaction products have maximum absorbance at wavelengths different from that of paracetamol and copper sulphate. The product labeled 3 did not absorb light within the wavelength of 200 to 800 nm. The maximum wavelength of absorption of samples 1, 2, and paracetamol are 250, 350, and 300 nm respectively (Figure 1). Therefore sample 1 and 2 were likely to be new products formed from paracetamol and copper sulphate. The wavelength of maximum absorption for the yellow compound was determined 280 nm. The yellow extract was probably a modified copper compound since it contains only one characteristic peak of copper (Figure 2).

To determine the effect of solvent on the absorption properties of copper paracetamol complex, the absorption spectra of all the products in dimethylsulphoxide (DMSO) were compared their corresponding spectra in aqueous

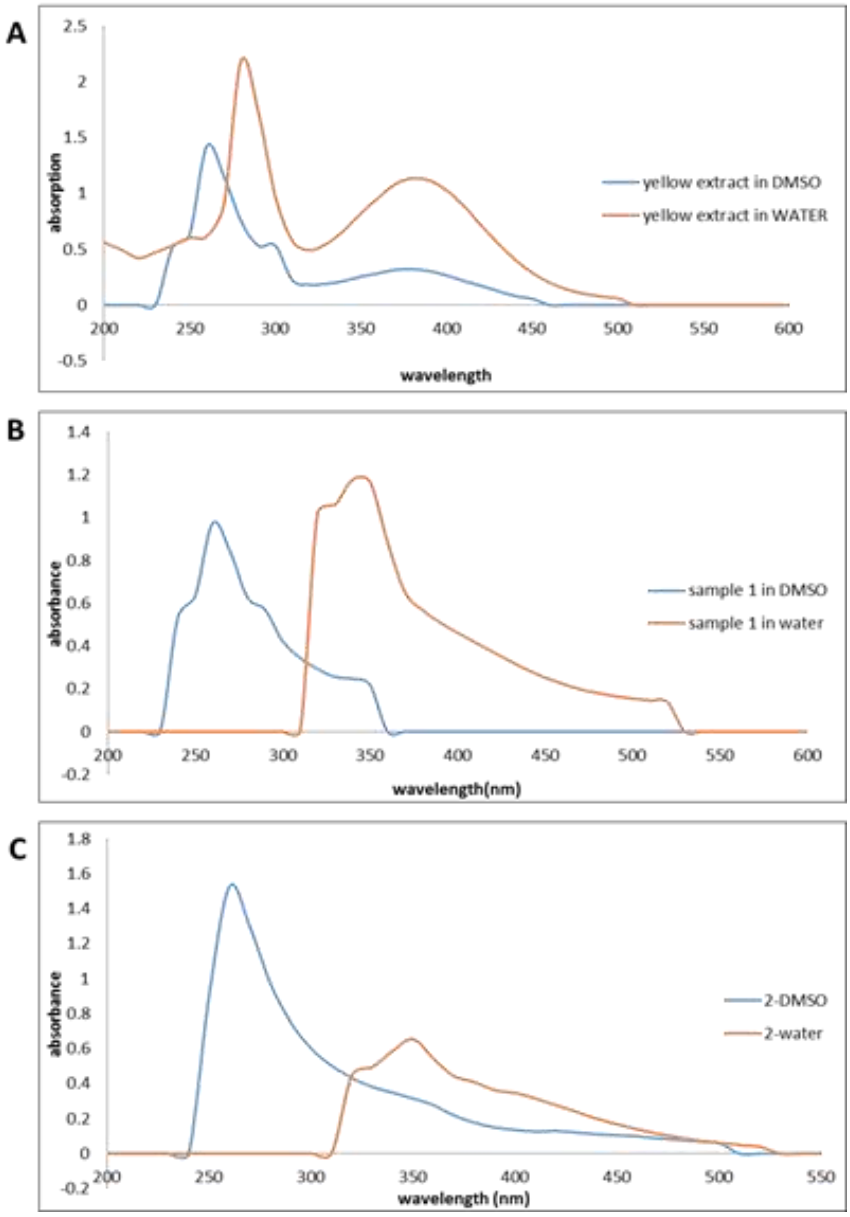

Figure 3. Solvent effect on the spectra of the coper complexes. (A) Absorption spectra of the yellow extract in water and DMSO. (B) Absorption spectra of product-1 in DMSO and water. (C) Absorption spectra of product-2 in water and DMSO.

solution. All the extracts in DMSO absorb at 260 nm except product-3 which did not show any absorption band. The change in wavelength for the reaction product/extracts 1, 2 and yellow corresponds to a blue shift (from a higher wavelength to a lower energy) (Figure 3).

To confirm that different compounds were formed, the samples were separated according to their polarity and their interaction with the solvent; the products formed have different RF value other than that of paracetamol. The chromatogram gave one spot for the yellow extract and two spots each (a and b) of the other reaction product (1, 2 and 3). Paracetamol gave a single spot while copper and sodium nitrite did migrate (Table 1). The yellow extract has the same RF value as that of sample 3a, similar values to sample 1a and 2a. The yellow extract is therefore likely to be present in all the three products (1, 2 and 3).

Atomic absorption spectroscopy was used to determine the presence of copper in the reaction products, copper was present in all the reaction products extracted at different concentrations (Table 2). The yellow extract however had the lowest concentration of copper.

To evaluate the antibacterial activity of the reaction

Table 1. Distance move by the samples spots from the origin and retardation factor of all the extracts.

Sample	Distance moved from origin (cm)	RF
$CuSO_4$	0.00	0.00
$NaNO_2$	0.00	0.00
Paracetamol	3.50	0.58
Yellow fraction	5.10	0.84
1a	4.80	0.79
1b	4.30	0.71
2a	4.90	0.80
2b	4.20	0.61
3a	5.10	0.84
3b	4.40	0.74

Table 2. A table of copper atomic absorbance and their corresponding concentration.

Sample	Concentration (mg/kg)
Paracetamol	0.00
Yellow extract	15.29
Sample 1	450.94
Sample 2	427.82
Sample 3	442.10

Table 3. The minimum inhibition concentration of the reaction products/ complexes.

Sample	Minimum Inhibition Concentration (MIC) in mg/ml	
	E. coli	***S. aureus***
paracetamol	No visible inhibition	No visible inhibition
Complex 1	6.25	12.50
Complex 2	No visible inhibition	No visible inhibition
Complex 3	1.56	1.56
Yellow fraction	6.25	6.25

products, the products were assayed against *s. aureus* and *E. coli.* The growth of *Staphylococcus aureus* and *E. coli* were inhibited by complex 1, 3, and the yellow fraction (Table 3).

DISCUSSION

Copper has a high oxidizing power and reacted rapidly reacted with paracetamol in the presence of sodium nitrate in borate buffer to give compounds which separated into three layers when extracted with 1:1 petroleum, ether and benzene. This reaction was possible because of the reactive groups of paracetamol (phenol oxygen, amine nitrogen and the acetal oxygen) and high complexing power of copper. The three layers were formed as a result differences in the densities of the compounds formed. Similar results were obtained by Nour El-Dien et al. (2005) when they complexed copper with L-dopa. L-dopa has reactive group attached to a benzene ring as seen in paracetamol. They observed that the L-dopa-copper complex gave a colored chelate.

Based on the RF values, the thin layer chromatography suggested that different compounds formed during the reaction. The differences in the RF values implied that the reaction products have different polarities and sizes compared to paracetamol. The yellow extract has the same RF value as that of reaction product/extract 3a and

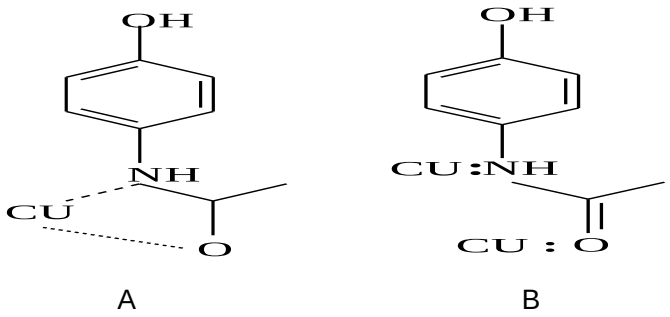

Figure 4. The possible constituents of copper paracetamol products in the yellow extract.

similar values to extract 1a and 2a. The compound in the yellow extract is therefore likely to be present in all the three products (1, 2 and 3). This was confirmed when a colorless 1:1 petroleum-ether and benzene changed color to yellow upon its addition to the other reaction products. The yellow extract may consist of intermediate compounds which formed the compounds in the other products. This may account for its presence in all the products (1, 2, and 3).

The products have different wavelength for maximum absorption compared to the starting material (paracetamol) which explains the fact that they have different chromophores other than that of paracetamol. The additional chromophore might be due to the presence of copper attach to paracetamol. The yellow extract absorbs at a wavelength different from paracetamol but similar to copper sulphate solution. This suggests that the yellow extract is likely to be a modification of copper. According to Razeghi, (2009), copper has different colors in different compounds due to its electron configuration. It has one electron in the 4s subshell instead of two. The energy of a photon of blue or violet light is sufficient for a *d* band electron to absorb and transition to the half-full *s* band. There was a blue shift when DMSO quenched the absorption of the reaction products at other wavelengths except 260 nm. Absorption within the visible range is directly affected by DMSO and results in a red shift (a shift in frequency towards lower energy or longer wavelength) and a blue shift (shift in the frequency towards a higher energy or a shorter wavelength) (Diana et al., 2012; Miller et al., 2014). It is therefore indicative from the results that, there was a blue shift in the sample 1, sample 2 and yellow extract. The blue shift indicates that the copper might interact with the polar groups of paracetamol to cause its derivatives to have the same wavelength of maximum absorption in an aprotonic polar solvent (DMSO). The blue shift of the products (1, 2, and yellow extract) confirms the formation of new products from the copper paracetamol complex reaction.

The functional groups of compounds can give rise to none, one, or more than one infrared absorption band, depending on the nature of the group. These bands are unique to the groups and can be used to characterize the molecule. Comparing the absorption groups present in paracetamol and the reaction products, it observed that the yellow extract has all the functional groups as in paracetamol except that which corresponds to amide and ketone groups, and N-H deformation. The additional absorption peaks may be due to the presence of copper. This implies that copper may have formed complex with paracetamol at amide and acetyl sites (Figure 4). This conclusion was validated since the atomic absorption spectrum of the reaction products indicated the presence of copper. The product labeled 1 had all the bands in paracetamol except CH_2 scissoring, copper might complex with the paracetamol at the methyl group. This might alter the absorption of the CH_2 group in the product 1 (Figure 5). The reaction product 2 and 3 possess the band for paradisubstituted benzene only. This implies that the copper may have form complex with all the functional groups including the benzene ring. Lawal and Obaleye (2007) also synthesized novel complexes of Co (II), Ni (II) and Fe (III) with aspirin and paracetamol and characterized them using infrared. According to them, paracetamol complexes coordinate through the oxygen of the hydroxyl and the amide groups. This was similar to my results with indicated that the copper attached itself to the reactive groups of paracetamol to form coordination bonds.

From the atomic absorption spectra, the copper concentration of products 1, 2, 3 and the yellow extracts, were found to be 450.94, 427, 442.10 and 15.29 mg/kg respectively. The presence of copper in the products point to the fact that, copper has actually chelated to the paracetamol to give us copper-paracetamol complexes.

These are possible structures of complexes in the yellow extract. A) One copper atom chelating to the amine group and the acetyl group. B) Two different copper atoms chelating one each to the amine group and the acetyl group.

The antibacterial susceptibility test for the reaction products showed that, complex 1, 3 and yellow extract were able inhibit the growth *E. coli* and *S. aureus.*

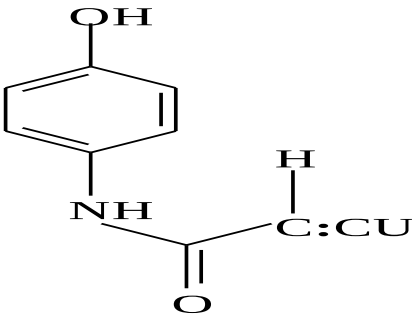

Figure 5. Copper paracetamol product that may be in reaction product 1. This is a possible structure of copper-paracetamol complex in sample 1. The copper was able to chelate to the methyl group on the paracetamol.

Sample 2 however did not inhibit any of the micro-organisms. This result was similar to the results obtained by Lawal and Obaleye (2007), where they investigated antibacterial activity of the metal aspirin/ paracetamol complexes against *Bacillus substilis, Serratia species and E. coli*.

Conclusion

Copper-paracetamol coordination complexes were formed and their possible structures predicted. Mass spectrometry and Nuclear Magnetic Radiation (NMR) analyses would have given sufficient data for complete structural elucidation of products.

The compounds formed were active against *E. coli* and *S. aureus,* pointing to the fact that coordination complexes are promising lead compounds to combat the increasing rate of resistance to antibiotics.

Conflict of Interests

The authors have not declared any conflict of interests.

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