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SYNTHESIS, CHARACTERIZATION (FTIR) AND ANTIBACTERIAL TEST OF 3-(2-NITROETHYL) INDOLE.

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ABSTRACT: This study investigated the synthesis of 3- (2-Nitroethyl)indole with indole as the starting material. This reaction involved the formylation of the indole ring at position three using the Vilsmeier- Haacks reagent and then followed by coupling of the formylated indole ring with Nitromethane (proton donor) in the presence of sodium ethoxide which acted as the base. Characterization was done using Fourier Transform Infrared Spectroscopy (FTIR). The final synthesized compound was screened against four bacteria namely; *Klebsiella pneumoiae*, *Staphyloccus aureus*, *Salmonella typhi* and *Bacillus subtilis*.

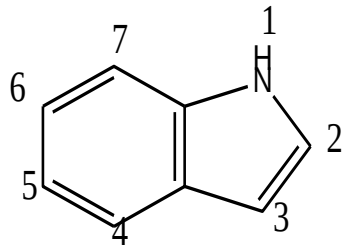
Keywords: formylation, bacteria, Vilsmeier- Haacks and coupling.

1.1 INTRODUCTION

Heterocyclic compounds are cyclic compounds with one or more atoms of other elements called a heteroatom. Some popular heteroatoms are Sulphur, Nitrogen and Oxygen. (Wang S., Yuan X. H., Wang S. Q., Zhao W., Chen X., & Yua, B., 2021). N-heterocyclic compounds have

biological activities such as antifungal, anti-inflammatory, antibacterial, antioxidant, antidiabetic, anticonvulsant, anticancer, insecticidal activity. (Mermer, A., Faiz, O., Demirbas, N., Demirbas, A., Alagumuthu, S., & Arumugam, 2019). Heterocyclic compounds provide one of the richest sources of novel compounds with diverse biological activity, as a result of the special properties of the resulting compounds to mimic the structure of peptides and to bind reversibly to proteins (Adreani, A., Burnelli, S., Grandaiola, M., Leoni, A., Locatelli, A., Morig, R., & Kunkel, M. W.,2008).

The indole ring system is common and an important heterocyclic compound in nature. (Nagendra, K. K., Neha, K., Pankaj, A., Naresh, K., Chang, H. K., & Akhiesh, K. V., 2013). It is found in a hugely diverse array of biologically significant natural compounds, from simple derivatives such as the neurotransmitter serotonin to complex alkaloids such as the clinically used anticancer agents like vinblastine and mitomycin, and the antihypertensive alkaloid reserpine, the importance of indoles to biological chemistry cannot be overstated. (Nagendra, *et al.,* 2013). Some important synthetic drug is made of indole motif, including sumatriptan, tadalafil, rizatriptan. The success of heterocyclic structures is the ability of a synthetic chemist to synthesize a compound and screen it against a variety of different receptors in order to yield several active compounds (Nagendra, *et al.,* 2013).



Indole

Discovery of new drug molecules involves exploration of several structural motifs and indole ring system represents one of the most important structural subunits in this field. (Kochanowska-Karamyan, & Hamann, 2010). Fluvastatin (a drug with an indole ring as one of the subunits), accounts for a total of $3.2bn in sales in 2010. The development of indole based drug has been the main theme in organic synthesis over the last century.

Based on the numerous importances of indole derivatives, their wide applications, and their potential for discovery of new drugs and compounds, it is highly desirable that massive research

in indole should be carried out resulting in the synthesis of wide variety of indole based compound. To the best of our knowledge no scientific report has been made about the synthesis, characterization and antibacterial test of 3-(2-Nitroethyl)indole. These were unveiled in this study.

2.1 MATERIALS AND METHODS

2.1.1 Equipment and Apparatus

The following were some of the apparatus used during the experiment; Measuring cylinder, retort stand, conical flask, oven, filter papers (whatman), distillation apparatus, Calcium chloride glass tube, 250ml Round bottom flask and flat bottom flask, Vacuum pump (VP280, 283l/min (10CFM), dual stage), autoclave (12 x 15 30 liter stainless steel autoclave), cotton wool, petri dish, swab sticks, inoculating loop, magnetic stirrer (scilogex ms-H280-Pro Temp Control Pack, 110v magnetic stirrer), capillary tubes, thin layer chromatography plates. The glass wares were pyrex grade.

2.1.2 Reagents

All the chemicals and reagents used in this research work were of analytical grade. Distilled water was also used. The table below shows the specific chemicals as well as their vendors.

TABLE 2.1: Reagent Used and Their Sources

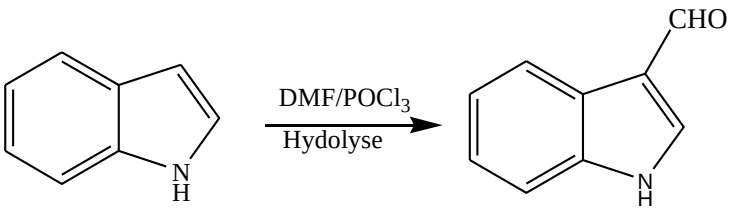
Reagent	**vendors**
Phosphorus trichloride ($POCl_3$)	LOBA
Dimethyl formamide (DMF)	LOBA
Nitromethane	LOBA
Sodium metal	KERMEL
Indole	ALDRICH
Hydrochloric acid	CDH

Sodium hydroxide (pellets)	KERMEL
Methanol	KERMEL
Ethanol	JDH

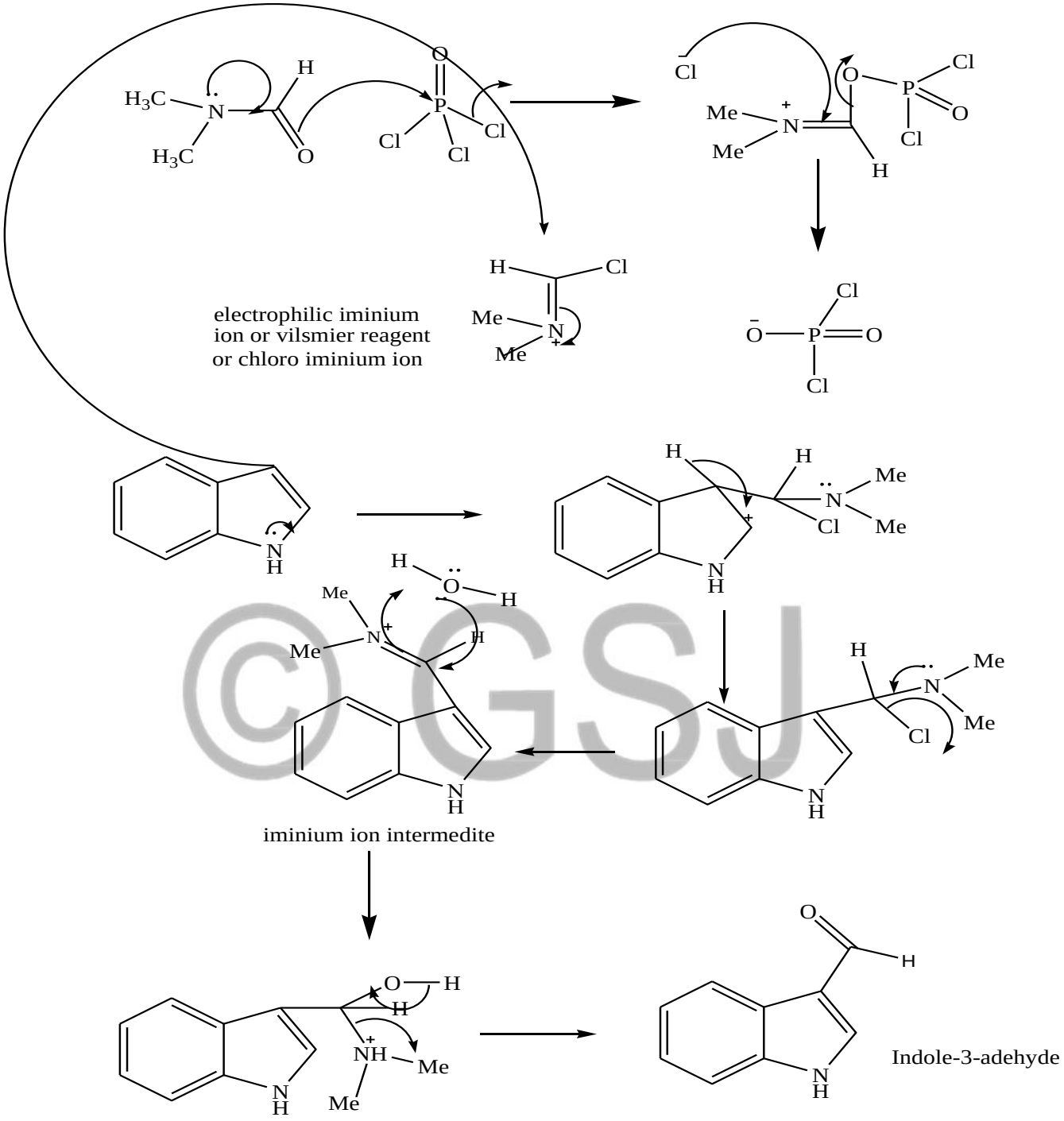
2.2.0 Methodology

2.2.1 Formylation of Indole Ring at Position Three

In order to synthesis 3-(2-Nitroethyl)indole, the indole compound was first formylated. This was done by adding 8.60ml of freshly distilled phosphorus oxychloride drop wise with stirring (scilogex ms-H280-Pro Temp Control Pack, 110v magnetic stirrer) to 28.80ml of Dimethylformamide (DMF) in a flask immersed in an ice bath for 30 minutes a pinkish colour of the formylation complex was observed during this step. (Masomeh A., 2019). 9.99g of indole was dissolved in 10ml of DMF and was added slowly with stirring to the flask containing DMF and Phosphorusoxychloride at a temperature below 10^{o}C. (Masomeh A., 2019). After the solution was completely added and thoroughly missed over a period of an hour, the temperature of the viscous solution was increased to 35^{o}C. The syrup was stirred efficiently for an hour at this temperature a canary yellow paste was formed. 30g of crushed ice was added to the paste with careful stirring producing a clear, cherry red aqueous solution results (Masomeh A., 2019). 10ml of water and 20g of crushed ice was added to the above. A solution of 37.5ml of concentrated sodium hydroxide in 100ml of water was placed in a separating funnel and fitted on the flask. The sodium hydroxide solution was added dropwise with stirring until one third of the solution was added. The remaining two third was added rapidly with efficient stirring, heat was applied to the resulting suspension until it boiled. The solution was then cooled at room temperature and kept in refrigerator overnight (12 hours). The product was collected on a filter paper using a vacuum pump for the filtration and washed with 30ml of water. The compound was air dried. The product formed was recrystallized with methanol. (Masomeh A., 2019)



Scheme 2.2.1: eequation for the reaction



Scheme 2.2.2: mechanism for indole formylation

2.2.2 Preparation of sodium ethoxide

The sodium ethoxide used in this reaction was prepared by measuring 20ml of ethanol into a 250ml quick fit flat bottom flask. 1g of sodium metal was weighed and then cut into smaller

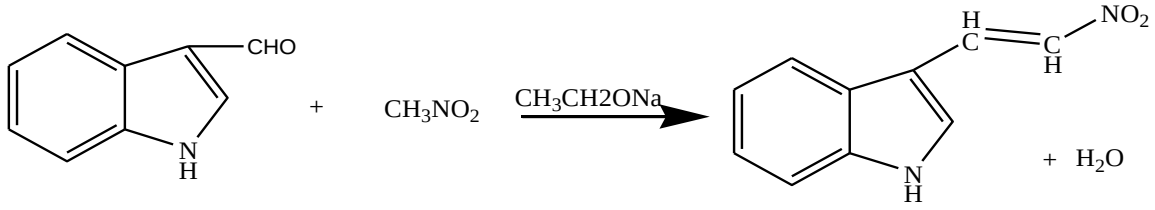
pieces. The sodium metal was then placed one after the other into the quick fit flat bottom flask containing the 20ml ethanol. The mouth of the flask was covered with calcium chloride glass tube to avoid oxidation. This content was stirred until the sodium dissolved. The sodium ethoxide is the medium through which the coupling of Nitromethane on the Indole-3-aldehhyde can be carried out.

$$2C_2H_5OH + 2Na \longrightarrow 2C_2H_5ONa + H_2$$

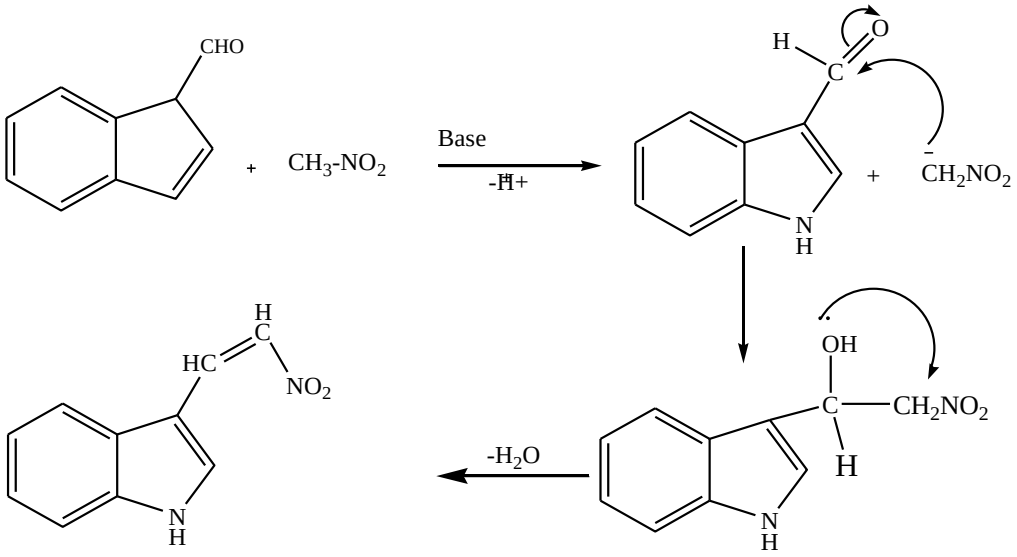
scheme 2.2.3: eqation for sodiumethoxide preparation

2.2.3 Coupling with Nitromethane

About 0.42ml of distilled Nitromethane was added to the prepared sodium ethoxide in drops with stirring.1g of Indole-3-aldehhyde was added to the content also and a magnetic stirrer was used to stir the content for three hours. This was left over night. Concentrated Hydrochloric acid was used to neutralize the reaction. Colour change was observed (from light orange colouration to fine yellow) and the solution formed a suspension. The suspension formed was then filtered with whatman filter paper. And washed with ethanol.



Scheme 2.2.4: equation for the coupling of Indole-3-aldehhyde with nitromethane.



3-Nitroetheneindole

Scheme 2.2.4: mechanism for the formation of 3-(2-Nitroethyl)indole

2.2.3 Purification of 3-(2-Nitroethyl)indole

The purification of 3-(2-Nitroethyl)indole was carried out using column chromatography. Column chromatography is a popular purification technique in organic chemistry. (Meyer, 2000). The silica gel was used for the packing of the column while the mobile phase was methanol and the 3-(2-Nitroethyl)indole was placed in a vertical glass column. The stopcock was opened in order to allow some of the eluent (methanol) to flow out and the purified sample was collected in a beaker. (Meyer, 2000).

2.2.4 Thin Layer Chromatography

Thin layer chromatography (TLC) was carried out to determine the number of components in the synthesized compound. The TLC plate was prepared in the laboratory. The R_f value obtained was calculated after purification of the synthesized compound. The formular below was used to calculate the retention factor values

$$\text{Retention factor } (R_f) = \frac{distance\ moved\ by\ compound}{distance\ moved\ by\ solvent}$$

2.2 5 Melting points

Melting points of the synthesized compounds were done by using a melting point apparatus (Cole-Parmer Stuart model SMP 20 digital melting point apparatus).

2.3 Antibacterial study of final product

The aim of antibacterial testing is to determine if 3-(2-Nitroethyl)indole will inhibits a visible growth of the bacterium being investigated under test condition. Since one of the biological activities of an indole based compound is its ability to act as an antibacterial.

2.3.1 Sample collection

The cultured microorganisms (*Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis,* and *Salmonella typhi)* were identified and collected from Microbiology department, University of Benin, Benin City.

2.3.2 Preparation of the Inoculum and Mueller Hinton Plates

The Broth and the agar were prepared according to the manufacture's specification. 1.35g of the Broth was dissolved in 500ml of water while the 27.50g of the nutrient agar was dissolved in 750ml of water. 2ml of the prepared broth solution was used to suspend each microorganisms which was left for 4hours that is the reproductive stage of the microorganism to awaken the microorganism. The agar solution was poured into the culture plates (after sterilizing it an autoclave) and left in a sterile environment to avoid contamination for 18hours at an ambient temperature. This was done to solidify the agar solution on the plates. Each plate was labelled according to the names of the organism that is to be analyzed. The plates were prepared in duplicates for each organism. (Jan Hudzicki, 2009)

2.3.4 Sterilization of Materials

The materials and chemical used for the antibacterial test like; reagent bottles, syringes, Mueller Hinton (MH) Agar solution and broth solution used were sterilized by heating the materials in an autoclave at high pressure for about 3 hours. While the inoculating loop was sterilized under a blue flame. (Jan Hudzicki, 2009).

2.3.4. Inoculation of the Plates

The dried surface of the MH agar plate was inoculated by streaking the swab sticks round the entire agar surface, the plates were rotated approximately 60 degrees each time to ensure an even distribution of the inoculum. (Jan Hudzicki, 2009).

2.3.5 Preparation of Sample Dilutions and Stock Solutions

50mg of the synthesized 3-(2-Nitroethyl)indole was dissolved in 1ml of distilled water. This represented the stock. Using serial dilution, 50mg, 40mg, 30mg 20mg and 10mg sample dilutions were prepared.

2.3.6 Placement of the antibiotic disks and the Sample Dilutions and Stock Solutions

The disk diffusion sensitivity test also known as Kirby Bauer disk method is a simple and practical method which uses antibiotic impregnated wafers (disk) to test whether a particular bacteria is susceptible to specific antibiotic or otherwise (Jorgensen J. & Turnidge, 2007; CLSI, 2009). A sterile cork borer 6mm in diameter was used to bore holes on the agar plates for the different concentration of 3-(2-Nitroethyl)indole solution, standard and control. After which the agar solution was placed on each well so that the diluted samples can set properly when they were added. The antimicrobial-impregnated disks (Septrin and Ciproflaxin were the standards used) were placed on the surface of the agar plate, each disk was touched on the plate to ensure complete contact with the agar surface, while the 3-(2-Nitroethyl)indole solutions were measured (about 0.12ml for each concentration) on each well base on their concentration which was indicated with a marker on the other side of the plate. The control which was water was measured (about 1ml each) into each plate. The plates were incubated for 18–24 hrs at 35-37^{0}C temperature. The antibiotics diffused from the disk into the agar. (Jan Hudzicki, 2009).

2.3.7 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration is the minimum concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibition concentrations are necessary in diagnostic laboratories to determine the resistance of microorganism to an antimicrobial agent and also to monitor the activity of a new antimicrobial agent. (Jan Hudzicki, 2009).

3.1 RESULT

3.1.1 Characterization and Identification of the Synthesized Compounds

From the table below, it is clear that the final synthesized compound 3-(2-Nitroethyl)indole has some properties which is different from the synthesized intermediate compound (3-Formylindole).

Table 3.1 Some Properties of the Final Product And Its Intermediate

Properties	**Compound 1**	**Compound 2**
Name	3-Formylindole	3-(2-Nitroethyl)indole
Chemical formular	C_9H_7NO	$C_{10}H_8N_2O_2$
Colour	Pale yellow but turned into cream white after recrystallization in methanol	Yellow but turns brown after some days.
Nature	Crystalline	Powdery
State	Solid	Solid
Solubility	Insoluble in methanol at room temperature but soluble when heat is applied	Insoluble in chloroform, petroleum ether, ethanol, partially soluble in methanol at room temperature and highly soluble in water.
Melting point (^{0}C)	196 – 198	Above 205
Mass obtained (g)	4.40	0.38
Yield (%)	35.20	29.30
Molar Mass (gmol^{-1})	145.16	188

Table 3.2: The table below shows the R_F values of 3-(2-Nitroethyl)indole using methanol and water as solvent.

Distance moved by the compound (cm)	**Distance moved by solvent (cm)**	**R_f**	**Solvent**
3.49	4.60	0.76	Methanol
3.86	4.60	0.84	water

Table 3.3: Antibacteria test of 3-(2-Nitroethyl)indole.

Bacterial used	**Standard drugs**	**Diameter**	**Zone of**	**Inhibition(mm)**	**(water as solvent)**		
	Cip ($30\mu g$)	S ($30\mu g$)	50mg/ml	40mg/ml	30mg/ml	20mg/ml	10mg/ml
k. *pnemoniae*	29.0	29.0	Nil	Nil	Nil	Nil	nil
S. *aureus*	28.5	28.5	Nil	Nil	Nil	Nil	Nil
S. *typlhi*	25.0	25.0	Nil	Nil	Nil	Nil	Nil
B. *subtilis*	25.6	25.6	Nil	Nil	Nil	Nil	Nil

KEY: S= Septrin, cip=Ciproflaxin, nil= no activity

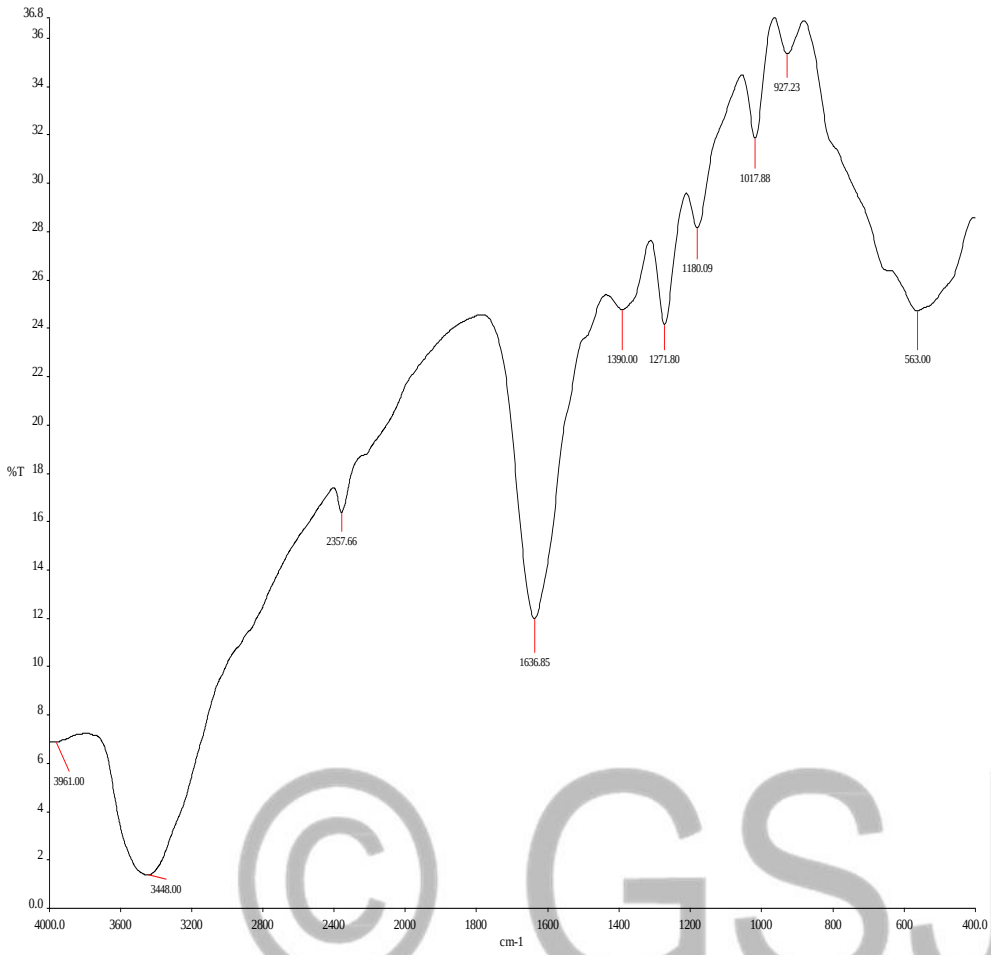


Plate 3.1: Fourier Transform Infrared (FTIR) of 3-(2-Nitroethyl)indole

Table 3.4 FTIR analysis of 3-(2-Nitroethyl)indole

Frequency cm^{-1}	**Appearance**	**Bond**	**Compound**
3448.00	A single band	N-H stretch	Secondary Amines
2357.66	strong	N-H bending vibration	Shoulder band of secondary amine
1636.85	Strong	C=C stretch	alkene
1390.00 and 1290	sharp	N-O stretch	Nitro group
1180,1017 and 927.23	Sharp	=C-H bend	Monosubstituted arene
750	weak	N-H wag	Aromatic amine

4.1 Discussion

4.1 Properties of the Final Product and its Intermediate.

Table 3.1 shows the properties of the intermediate compound and the final compound that were synthesized. These properties such as Melting points, colour and so on were found to be different in the two compounds that were synthesized.

4.1.2 Thin layer chromatography:

From the table 3.2 the development solvent used were water and methanol. It was observed that 3-(2-Nitroethyl)indole had a higher retention value in water (a retention factor of 0.84) at the same distance travelled by the development solvent (4.6) than in the methanol whose retention factor was 0.76. Although, both water and methanol are polar solvents but the synthesized compound seems to be eluted more in water than in methanol.

Since water being a more polar solvent gave a higher R_f value than methanol, it would be concluded that 3-(2-Nitroethyl)indole is a highly polar compound, because for compounds having very low polarity however, a lower-polarity solvent may be more effective in moving the solute up the plate and for compounds having high polarity, a high polarity solvent may be more effective in moving the solute up the plate. This is in agreement with the proposed structure of 3-(2-Nitroethyl)indole which have a highly electron withdrawing group (nitro group) present in the compound thereby bringing about unequal distribution of charges round the structure (polarization). The higher the distance moved by the sample in the mobile phase, the better the attraction or affinity it is to the mobile phase and lower the distance moved by the sample in the mobile phase, the lesser the attraction or affinity of the sample in the mobile. Since the methanol gave a lower distance that means the sample has a lower affinity for methanol than in water.

4.1.3 Antimicrobial Activity

Table 3.3 shows the activity of the 3-(2-Nitroethyl)indole against the bacteria used which were: *K. Pneumoniae, S. aureus, S. typhi* and *B. subtilis* was seen to be inactive from the highest concentration used that is at 50mg/ml to the lowest concentration that is at 10mg/ml. No zone of inhibition was observed after 24 hours of incubation as compared to the standard drugs (Ciproflaxin and septrin at 30µg) which had zones of inhibition towards the tested organisms (*S. aureus, S. typhi).* Since at the concentrations used no zone of inhibition was observed then that

means that at those concentrations and the kind of microorganism used, the synthesized compound will not be able to serve as antibiotics. This could either be that the synthesized compound 3-(2-Nitroethyl)indole is inactive towards the tested organisms used or that the concentration used was low to inhibit the growth of the bacteria tested.

4.1.4 FTIR analysis

Plate 3.1 and table 3.4 shows the characteristic FTIR bands of 3-Nitroetheneindole. The FTIR spectrum displayed a single absorption band at 3550-3300cm^{-1} but centered at 3448.00cm^{-1} this indicates the presence of an amine or a hydroxyl in the compound since the region is known for N-H stretch and O-H stretch but the single nature of the band is an indication that it is a secondary amine since there is an absorption at 2357.66cm^{-1} which is the region for N-H bending vibration. It is a shoulder band, which is an overtone of the N-H bending vibration. A secondary amine usually displays a single spike due to the presence of only one hydrogen atom attached to the nitrogen atom. Therefore the absorption at 3448.00cm^{-1} is an amine group and not a hydroxyl group. The C-N stretching vibration of aromatic amines is found around 1335-1250cm^{-1} region, for the 3-nitroetheneindole spectrum it can be seen at 1271.80cm^{-1} .This confirms the presence of an amine at pyrrole ring of the 3-(2-Nitroethyl)Indole. The absorption at 1636.85 cm^{-1} is due to C=C stretching vibration of the ethene attached to the indole ring. This bond is conjugated because conjugated alkenes falls within the range of 1650-1600cm^{-1}. Also, this alkene is disubstituted because the region for a disubstituted alkene from sigma Aldrich IR spectrum table is around 1662 -1626cm^{-1}, this substitution might likely be in the cis – configuration due to the strong appearance of the band this might likely be the reason for the strong and sharp absorption. From the proposed compound, the indole ring and nitro group are the two substituent attached to the ethene The weak absorption band at 1390.00cm^{-1} and 1271.80cm^{-1} shows N-O symmetric stretch. This indicates the presence of a nitro group in the compound. The arene is seen around the finger print region (1225-950cm^{-1}) indicating the carbon – hydrogen (=C-H) bending vibration. The sharp absorption is as a result of the conjugated ethene with the ring which is a monosubstituent on the meta position of the indole ring.

4.2 Conclusion

Research in the field of medicinal chemistry pays attention to the development of new and better drugs and their successful introduction into clinical practice.

With the microorganisms and concentration used in this study, it could be seen that 3-(2-Nitroethyl)indole didn't give a positive result towards the bacteria that does not give the impression that the compound should be considered useless. As stated by Adreani*et al*., heterocyclic compounds have diverse biological importance which include antimicrobial, antiviral, antitubercular, anti-inflammatory, anticancer, antidiabetic, anticonvulsant, antioxidant, antidepressant and anticonvulsant activities (Adreani*et al*., 2008) of which only antibacterial study was carried out in this study that means that there could a likelihood that 3-Nitroethene could possess other biological importance. So, 3-(2-Nitroethyl)Indole should not be discarded or tagged as being useless.

4.3 Findings

The following were our findings during the research;

- A new compound has been added to organic chemistry
- 3-(2-Nitroethyl)Indole has no antibacterial activity at the following concentrations and bacteria 50mg/ml, 40mg/ml, 30mg/ml, 20mg/ml and10mg/ml. *K. Pneumoniae, S. aureus, S. typhi* and *B. subtilis*
- The synthesized compound is highly soluble in water even though the starting material was insoluble.
- 3-(2-Nitroethyl)Indole is a polar compound.
- The compound can easily change in colour from fine yellow to brown when exposed to atmospheric air.

4.4 Contribution to knowledge

A new compound, 3-(2-Nitroethyl)Indole can be synthesized and added to the library of organic compounds.

4.5 Suggestion for Further Study

- Antimicrobial studies should be carried out using other bacteria and fungi.
- Full characterization of the synthesized compounds should also be carried out.

- Toxicity test should be carried out.

4.6 Limitations

Full characterization of the synthesized compounds could not be carried out due to none availability of analytical instruments such as ^{1}HNMR and ^{13}CNMR. However, further work will focus on these areas.

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