

IJS GS**FEDERAL UNIVERSITY
GUSAU-NIGERIA****INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL SUSTAINABILITY****Evaluation of Fischer Esterification Method: Synthesis and Modification of Benzocaine; A Local Anaesthetic**Hamisu H. M. Anka^{1*}, A.M. Lawal², I.O. Alaekwe², K.O. Nwozor² and M.B. Danbatta²¹School of Pharmacy, Faculty of Science and Engineering, University of Wolverhampton, Wulfruna Street, Wolverhampton, WV1 1LY, U.K.²Department of Chemistry, Federal University, PMB 1001, Gusau, Zamfara state, Nigeria.*Corresponding author: H.H.MohDAnka@wlv.ac.uk +44 (0) 1902 321 000

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Abstract

Local anesthetics, or "painkillers," are a well-studied class of compounds. Chemists have shown their ability to study the essential features of a naturally-occurring drug and to improve on them by substituting totally new, synthetic surrogates. Often such substitutes are superior in desired medical effects and also in lack of unwanted side effects or hazards. This article highlight the synthesis and acetylation as well as characterization of benzocaine; a local anesthetic, using (Fischer esterification method); the resulting products were evaluated using Infrared spectroscopy, Melting point and thin layer chromatography; the %yield of the products(78% Benzocaine and 96% N-acetylbenzocaine) were determined to be highly acceptable and the analytical data from melting point(89-91 for benzocaine and 103 – 105 for N- acetylbenzocaine), Fourier transform infrared spectroscopy and thin layer chromatography all confirmed the presence of the products thus ascertaining fischer esterification as an efficient method for synthesis of benzocaine and its acetylation.

Keywords: Fischer esterification; anaesthetic; benzocaine; synthesis; painkillers.**1.0 INTRODUCTION**

Local anesthetics works by blocking nerve transmissions through state dependent binding of voltage gated Na⁺ channels leading to loss of sensation by certain areas of the body, they have

the potential to produce severe harm if use without caution; high doses may cause cardiovascular collapse and owing to its surfactant nature character, may cause

irreversible nerve injury (Chakrabarti and Patra, 2014); Local anesthetics, or "painkillers," are a well-studied class of compounds. Chemists have shown their ability to study the essential features of a naturally-occurring drug and to improve on them by substituting totally new, synthetic surrogates; often such substitutes are superior in desired medical effects and also in lack of unwanted side effects or hazards. Interest in cocaine lies in its anesthetic properties, the pure alkaloid was isolated in 1862 by Niemann; soon after the structure of cocaine was established, chemists began to search for a substitute as cocaine has several drawbacks for wide medical use as an anesthetic in eye surgery, it also produces mydriasis, It can also become a drug of

addiction, finally, it has a dangerous effect on the central nervous system. The first totally synthetic substitute was eucaïne; this was synthesized by Harries in 1918 and retains many of the essential skeletal features of the cocaine molecule. The development of this new anesthetic partly confirmed the portion of the cocaine structure essential for local anesthetic action. The advantage of eucaïne over cocaine is that it does not produce mydriasis and is not habit-forming. Unfortunately, it is highly toxic. A further attempt at simplification led to piperocaine. The molecular portion common to cocaine and eucaïne is outlined by dotted lines in the structure shown. Piperocaine is only a third as toxic as cocaine itself (Minard 2006).

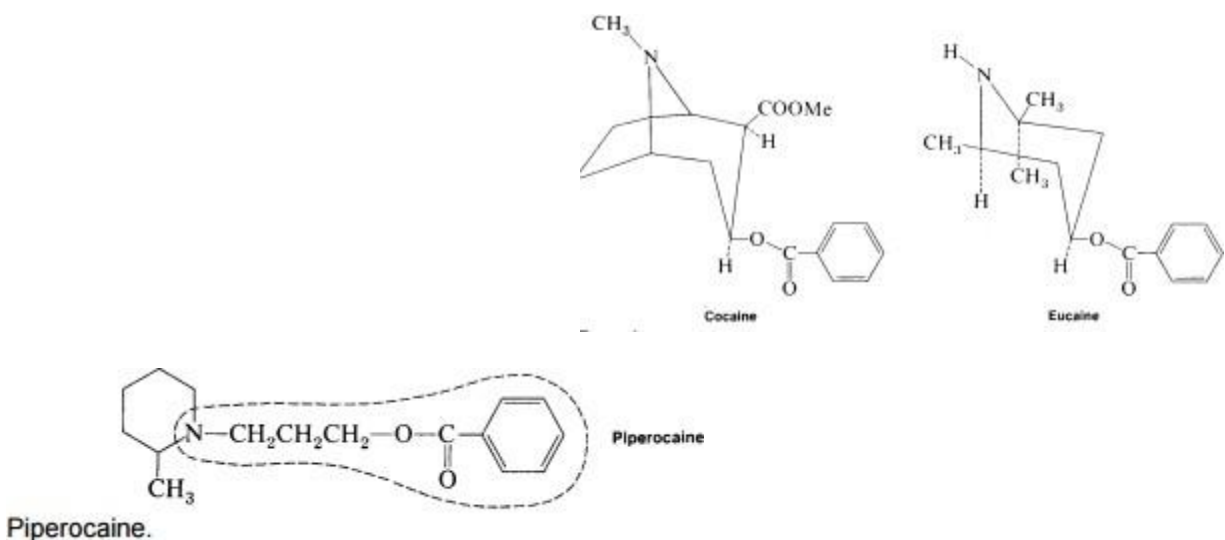


Figure 1: Structures of Cocaine, Eucaïne and procaine (Minard, 2006).

Local anesthetics arise as byproducts of synthetic analgesic industry when carboxylic acid ($R\text{-CO}_2\text{H}$) and alcohol ($R'\text{-OH}$) in an water eliminating reaction produces carboxylic esters ($R\text{-CO}_2\text{-R}$); an example is phenacitin an ethoxy compound by Oskar Hinsberg in 1888, Benzocaine in 1890 by Eduard Ritsert, procaine

by Alfred Einhorn's. Benzocaine is not the only ethyl ester introduce in to anesthesiology, meperidine in 1939 was the first synthetic opioid to be use in humans by Otto Eisleb, and etomidate by paul jessen in 1976 (Fragoza and Alston, 2008).

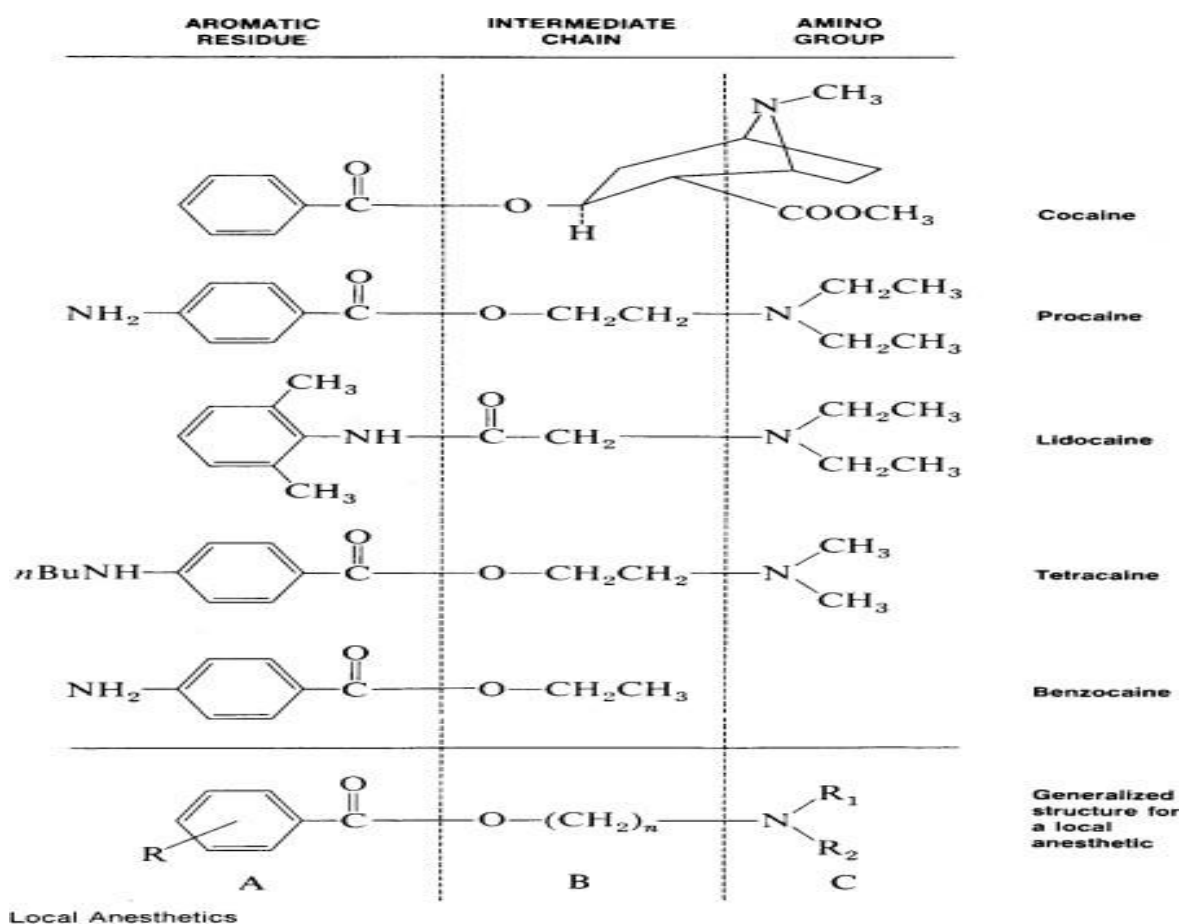


Figure 2: Structure for local anesthetics (Minard, 2006).

Benzocaine an anesthetic, used in dermal, topical and mucous formulations is a simple chemical compound that induces pain relief, its low solubility makes it not applicable for parental administration (Paun, *et al.*, 2013). This paper aimed at reporting the synthesis of benzocaine

2. MATERIALS AND METHODS

2.1 Materials

All the materials listed below that are used in the experiment were provided by chemistry laboratory, faculty of science and engineering, university of wolverhampton UK.

4-aminobenzoic acid, 100ml round bottom flask, absolute ethanol, concentrated sulphuric acid, anti-bumping granules, condenser, heating mantle, cork ring, NaOH solution, plastic teat pipette, universal indicator paper, 1M HCl,

by Fischer esterification of 4-aminobenzoic acid (PABA) and its modification, followed by subsequent evaluation of the products using Thin layer chromatographic and Fourier transformed infrared (FT IR) techniques.

500ml glass beaker, distilled water, watch glass, glass jar, melting point tester, Sense FT IR machine, ethanoic anhydride, micro Bunsen burner, solid sodium hydrogen carbonate.

2.2 Methods

2.2.1 Synthesis of benzocaine

3.0g of 4-amino benzoic acid was weighed in 100ml round bottom flask and 20ml of absolute ethanol was added and stirred (forming yellow color solution), 3ml of sulphuric acid was added

carefully while cooling in an ice bath(the reaction releases heat and turns milky color); 3 anti-bumping granules was put in the flask and was placed in a suitable heating mantle, after, a condenser was assembled and the mixture was then refluxed for 90minutes(the solution became a transparent brown color); the reaction was stopped by cooling in an fresh ice bath, after, the flask was removed from the condenser and was stood safely on a cork ring; about 25g of ice was added to the flask and 12.0ml Of 10% NaOH solution was subsequently added(the solution

turns white and frothy) until the PH was nearly 8 by testing with a small piece of universal indicator paper; the flask content was transfer to a 500ml glass beaker and was diluted to the mark with distilled water, then the content was stirred and filtrated under vacuum using Buchner funnel and vacuum pump; a white solid product with fine flakey texture was removed when no drips were coming through the filter paper and then was poured on a watch glass and dried in a drying cabinet at around 70°C for an hour.

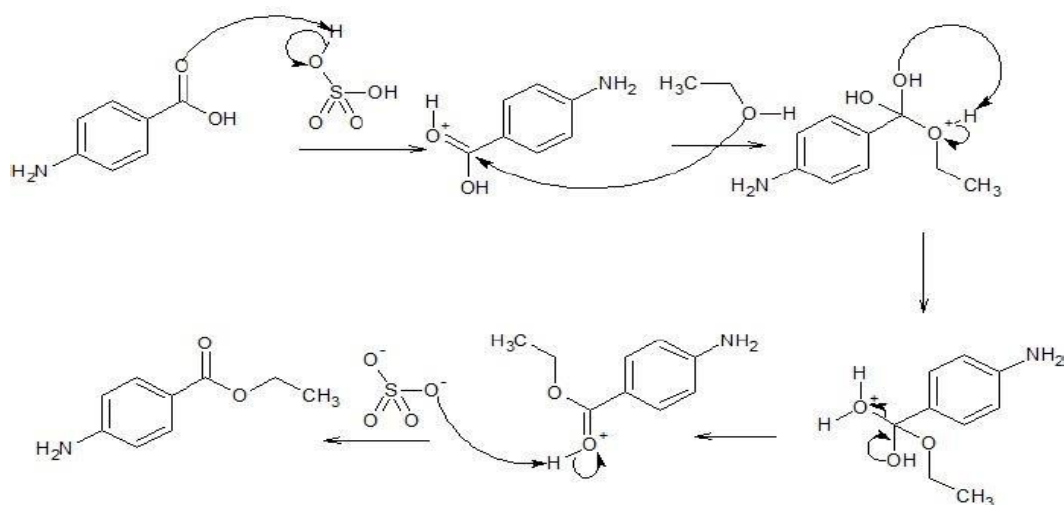
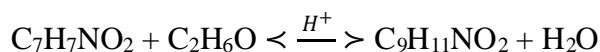


Figure 3: Reaction mechanism for synthesis of benzocaine (Xiang, 2012).

Reaction stoichiometry:



From the above equation 1mole of p-aminobenzoic acid (PABA) and 1mole of ethanol produces 1mole of Benzocaine and 1mole of water, therefore the stoichiometry is 1:1.

Thus, no of moles of PABA that will produce benzocaine is:

$$3\text{g} \times \frac{1\text{mole}}{137.14\text{g}} = 0.022\text{mole.}$$

20ml of ethanol will produce:

$$20 \times \frac{0.78945\text{g/cm}^3}{1\text{ml}} \times \frac{1\text{mole}}{46\text{g}} = 0.34\text{mole.}$$

The limiting reagent is (PABA), therefore if there are only 0.022moles of PABA, the maximum number of benzocaine that can be produce is 0.022moles. Thus, the theoretical yield is:

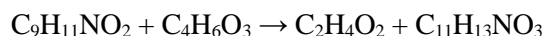
$$0.022\text{mole} \times \frac{165.189\text{g}}{1\text{mole}} = 3.6\text{g}$$

$$\text{Thus, \%yield} = 100 \times \frac{2.8\text{g}}{3.6\text{g}} = 77.7\%.$$

2.2.2. Acetylation of benzocaine

1.0g of benzocaine was measured in a test tube and 3ml of ethanoic anhydride was added using a disposable plastic teat pipette in a fume cupboard, the test tube was clamp in the cupboard in an upright position with sufficient clearance that allow a micro bunsen burner underneath; Using a very low flame with sufficient gap and careful control preventing boils out from the tube, the solution was refluxed to about 15minutes and then the flame was turn off and the mixture was allowed to cool to the touch; the tube content was poured into 25ml of cold water in 100ml conical flask and was swirled, followed by sequential addition of solid sodium hydrogen carbonate which disperses the excess acetic anhydride until no evolution of carbon dioxide was observed, the content was then diluted to the mark with distilled water and was stirred and filtrated under vacuum using Buchner funnel and vacuum pump; the product was removed when no drips were coming through the filter paper and then was poured on a watch glass and dried in a drying cabinet at around 70°C for an hour.

Reaction stoichiometry:



From the above equation 1mole of benzocaine and 1mole of acetic anhydride produces 1mole of N-acetylbenzocaine and 1mole of methanoic acid, therefore the stoichiometry is 1:1.

Thus, no of moles of benzocaine that will produce N-acetyl benzocaine is:

$$1\text{g} \times \frac{1\text{mole}}{165.189\text{g}} = 0.0061\text{mole.}$$

3ml of ethanoic anhydride will produce:

$$3 \times \frac{1.082\text{g/cm}^3}{1\text{liter}} \times \frac{1\text{mole}}{102\text{g}} = 0.032\text{mole}$$

The limiting reagent is benzocaine, therefore if there are only 0.032moles of benzocaine, the maximum number of N-acetylbenzocaine that can be produce is 0.032moles. The theoretical yield is:

$$0.0061\text{mole} \times \frac{207.2\text{g}}{1\text{mole}} = 1.26\text{g}$$

$$\text{Thus, \%yield} = 100 \times \frac{1.21\text{g}}{1.26\text{g}} = 96\%.$$

2.3 Evaluation of benzocaine and N-acetyl benzocaine

2.3.1 Melting point determination

The melting point of benzocaine and its modified form was determined using stuart melting point tester (Stuart SMP10) according to stuart instruction manual; small amount of the sample was inserted in to the sample tube, after setting the plateau temperature, the sample tubes were inserted in to block and the start button was pressed until the plateau light appeared, the start button was pressed again for ramping light to come on and the melting point was observed visually for all the samples

2.3.2 Infrared (IR) Spectroscopy

Infrared spectra of untreated benzocaine and its modified forms were obtained using (Bruka Alpha UK) FT IR equipped with a single bounce attenuated total reflectance (ATR) accessory; using micro spatula, a few milligrams of each samples benzocaine were placed on the middle of the sample stage after cleaning with acetone, the knob was then slowly rotated clockwise so that is above the sample and was then compressed by rotating the screw on the top of the arm to fixate the sample and then start button was pressed on the monitor until green bar appears; a scanning range between 400 to 4000 cm^{-1} with resolution of 4 cm^{-1} was employed and the spectra obtained were the result of averaging 16 scans. The same procedure was repeated for N-acetyl benzocaine.

2.3.3 Thin layer chromatography

Samples of PABA, benzocaine and crude N-acetylbenzocaine were spotted each in duplicate on silica(Kiesel gel 254) TLC plate(stationary phase) and were allow to dry before run in diethyl ether mobile phase(solvent) and TLC plate was visualize under UV light and iodine vapor.

$$R_f = \frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}} \text{ or}$$

$$R_f = \frac{\text{spot front}}{\text{solvent front}}$$

The retardation factor for p-amino benzoic acid (PABA) is:

$$R_f = \frac{4.4}{4.9} = 0.89$$

The retardation factor for benzocaine is:

$$R_f = \frac{4.6}{4.9} = 0.93$$

The retardation factor for N-acetylbenzocaine is:

$$R_f = \frac{3.4}{4.9} = 0.69$$

3.0 RESULTS AND DISCUSSION

Table 1: Yields from the synthesis of benzocaine and N-acetylbenzocaine.

	Percentage yield,	Melting point	R _f value
p-aminobenzoic acid(PABA)			0.89
Benzocaine	78%	89-91 ⁰ C	0.93
N-acetylbenzocaine	96%	103-105 ⁰ C	0.69

Benzocaine was synthesized using Fischer esterification reaction; the reaction is a reversible reaction that was why excess anhydrous ethanol was used to ensure the reaction proceeds towards the formation of an ester (product); the significance of the used of acid (H₂SO₄) was to catalyze the reaction when it protonates the carboxyl oxygen, this makes the carbonyl carbon more electrophilic so that the nucleophile (R-OH) can attack and eventually kick off water to produce an ester, as under basic conditions the -OH on the carboxylic acid and the base undergo proton transfer and the reaction does not progress. The importance of using sodium hydroxide to keep the pH(8) was that NaOH neutralizes H₂SO₄(H₂SO₄+ NaOH → NaSO₄+ H₂O); at pH of 8, the solution is weakly basic so there are no free floating protons to re protonate the carbonyl and make the reaction proceed again in the reverse direction.

Mechanism of benzocaine begins with an attack on one of the proton's of sulphuric acid by the carbonyl of (PABA); the oxygen then becomes protonated resulting in double bond breakage; the oxygen of the ethanol then attacks the carbon previously associated with the carbonyl group,

the lone pairs of the sulphur then deprotonate the ethanol's oxygen leading to the formation of an ester group; one of the hydroxyl group then binds to the proton of the of the sulphuric acid leading to its removal from central molecule; the negative charge of the carbon results in the formation of a double bond to the hydroxyl group, due to the newly found positive charge of the hydroxyl group, lone pairs of the Sulphur group deprotonate it forming the final product ester(benzocaine) and water (Figure 3).

The yield of both benzocaine and N-acetylbenzocaine is an acceptable yield; the major loss of yield in the case of benzocaine is due to fact that the reaction is an equilibrium reaction. The melting point data obtained had completely matched the observed range in Chemspider (2015).

Which shows the absent of any significant impurities in the product.

The IR data of the product (benzocaine) (Figure 6) is not very much different from that of starting material p-aminobenzoic acid(PABA) (Figure 4) beside for one peak in the (PABA) IR data at about 2400-3300cm⁻¹ which is as a result

carboxylic OH stretch; Very broad and complex with curve like shape due to strong hydrogen bonding.

The IR data for the product (Figure 6) is a good reference for the presence of the product (benzocaine) which is very similar to the reference data (figure 5) in the literature by Nist (2009); all of the significant peaks are present; the peak at 3419.39 represent -NH stretch, the strong peak at 3339.83cm^{-1} and the weaker at 3221.13 are -CH stretch for the benzene ring and could be seen clearly, as the -OH group in reference to starting material (PABA)(Figure 4) has been changed to $\text{-OCH}_2\text{CH}_3$, the weak peak at 2983.93 is -CH stretch of a methyl group, strong peak at 1678.98 is -C=O stretch of a carbonyl group, at 1632.36 is an aromatic C=C stretch, peak at 1593.50 is an NH bend, strong absorption in this case but this is not always so, peak at 1512.98 is an aromatic C=C stretch, the strong peak at 1271.34 is a C-O this peak account for the main difference in the product (Figure 6) from the starting material (PABA) (Figure 4) the band is strengthen by being

conjugated to a C=O group, the peak at 770.13 is a C-H bend possibly aromatic C-H bend but the finger print region is too complex to be completely confident of the assignment, the last peak at 501.50 is C-C skeletal bend of ethyl group.

The IR data of N-acetylbenzocaine(Figure 8) obtained is very similar to Casale and Ngeyen(2010)(Figure 7) in the literature;the band at 3331.06 is N-H amide stretch this band can be seen clearly but with weak absorption typical of a primary amines, at 2985.47 is C-H stretch, broadening of the base of this two bands might me due to impurities possibly the product was not completely dried; 1708.63 is an unconjugated ester C=O stretch the band at 1686.86 is an unconjugated C=O amide stretch, medium band at 1604.39 can be C=C aromatic stretch or N-H bend, 1523.30 is an aromatic C=C stretch, evidence of a doublet due to interaction with the ring substituent, at 1354.39 is aromatic C=C stretch, the strong band at 1240.01 is C-O stretch, 703.50 is a C-H bend.

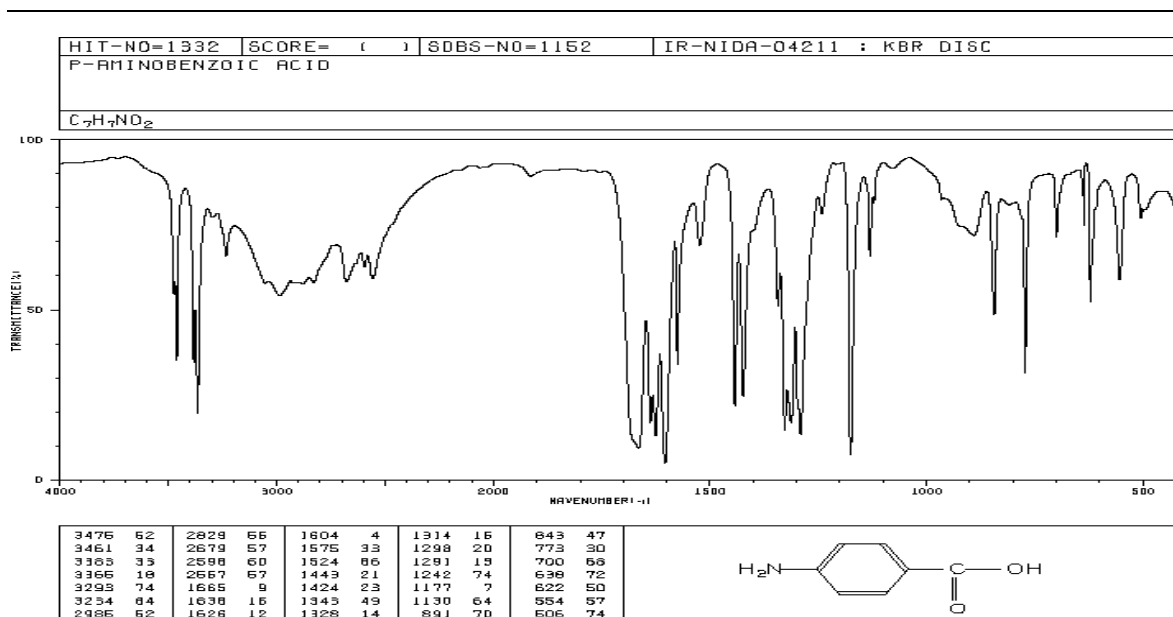


Figure 4: PABA reference spectrum (Liang, 2008).

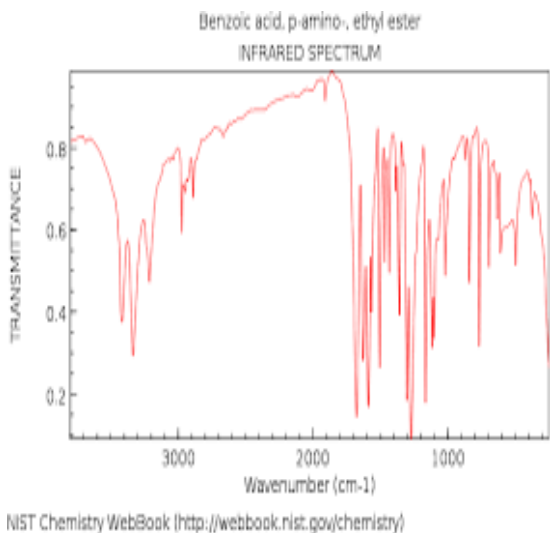


Figure 5: Benzocaine (Nist, 2009).

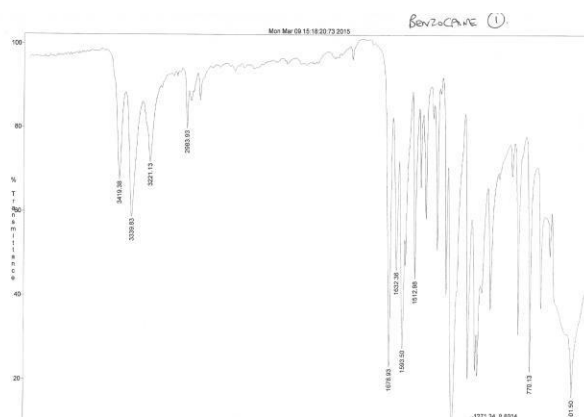


Figure 6: Synthesized benzocaine.

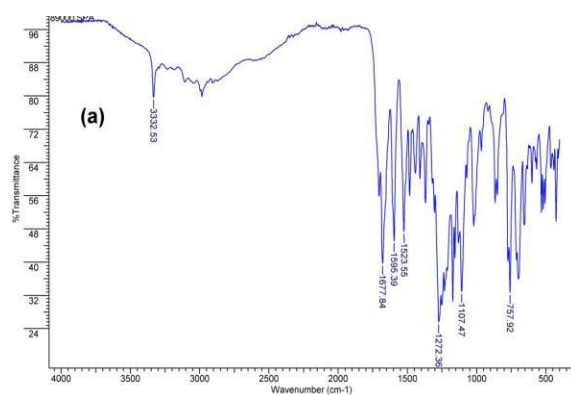


Figure 7: N-acetylbenzocaine (Casale and Nguyen, 2010).

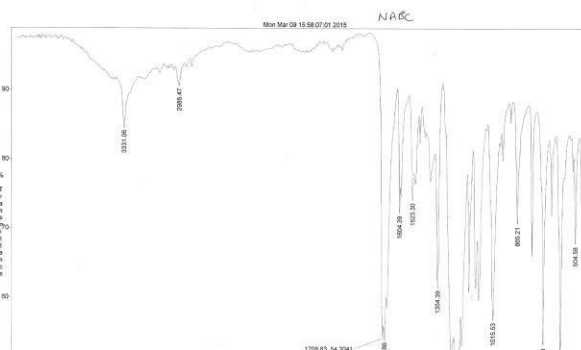


Figure 8: Synthesized N-acetylbenzocaine

Figure 9 shows the TLC plate visualized under UV light, the first 2 spots at the left side are spots of PABA, in the middle are of benzocaine, and N-acetyl benzocaine at the right side of the plate; N-acetylbenzocaine have the lowest retardation factor value (R_f) (Table 1) showing more polarity as it interact more with polar stationary phase (Silica gel) followed by PABA slightly more polar than benzocaine (Figure 9). On top of N-acetyl benzocaine near solvent front (Figure 9) are traces of benzocaine, which

means it haven't quiet fully reacted; the pale blob in line with PABA and on top of N-acetyl benzocaine might be PABA but it hasn't move as fast as PABA because it took long time to break free of N-acetyl benzocaine.

Iodine stained TLC plate (Figure 10) clearly shows PABA and trace of benzocaine but no sign of N-acetyl benzocaine; this is because the interaction of amines with iodine is via the nitrogen lone pair to form molecular complexes (Charge-transfer complexes) and the

reaction is dependent upon the availability of hydrogen in the amine molecule, PABA and benzocaine are primary amines having more hydrogen thus more chance to be oxidized by iodine than N-acetyl benzocaine; a secondary amine having less number of hydrogen resulting



Figure 9: UV visualized TLC plate.

in weak charge transfer complexes; the absent of unreacted traces of benzocaine in the iodine stained TLC plate is because is negligible, it only shows in the UV visualized TLC plate (Figure 9) because is highly UV sensitive.

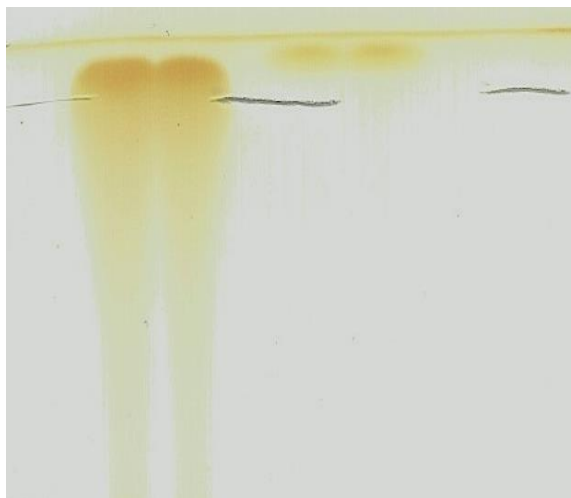


Figure 10: iodine stained TLC plate.

4.0 CONCLUSION

In general the percentage yield and evaluation results of both products showed that the products were successfully acquired, this signifies the efficiency of the experimental procedures used, though the drying time could have been increased to remove the water that is suspected

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to cause the broadening of bases of the IR bands especially in the N-acetyl benzocaine IR spectrum; further studies are recommended to employ more sophisticated evaluation techniques like HNMR, MS, HPLC, GC, GC MS.

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