Synthesis of NAA (2- (1-Naphthyl ) Ethanoic Acid) from Coal Tar and Application of Plant Hormone with Soy Bean and Cow Pea in Aqueous Medium

KhinMooh Theint¹, TinMyint Htwe²

Abstract— Plant growth regulators, which are commonly called as plant hormones, naturally produced non-nutrient chemical compounds involved in growth and development. Among the various kinds of plant growth regulators, 2-(1-Naphthyl)ethanoic acid especially encourages the root development of the plants. In this work, NAA was successfully synthesized from naphthalene which was extracted from coal tar. The purity of naphthalene, α-Chloromethyl naphthalene, α-Naphthyl acetonitrile, α-Naphthyl acetic acid or 2-(1-Naphthyl) ethanoic acid were also confirmed by Thin Layer Chromatography, and by spectroscopy methods. The yield percent of NAA based on naphthalene was found to be 2.1%. The yield percent of naphthalene from coal tar is found to be 4.09%. The effect of NAA on root development was also studied in different concentrations of soy bean (Glycine max) and cow pea (Vigna catjang walp)

Keywords— coal tar, cow pea (Vigna catjang walp), molecular spectroscopy, naphthalene, 2-(1-Naphthyl) ethanoic acid, plant growth regulator, root development, soy bean (Glycine max)

I. INTRODUCTION

PLANT growth regulators, usually called plant hormone, are substances which, when added in small amount, modify substantially the growth of plant, both stimulatory and inhibitory. They are often transported from one part of the plant to another [1]. Plant growth regulators (PGR) are small organic compounds that influence physiological responses to environmental stimuli at very low concentration (generally less than 10⁻⁷M). They are used extensively in agriculture, horticulture, and biotechnology to modify plant growth and development[2].They have great promise to increase the world food supply. Auxins were the first plant hormones discovered. Naphthalene acetic acid is one of the synthetic auxins. It is used to encourage root development in cuttings. So, it is also known as rooting hormone. Naphthalene acetic acid is widely used in vegetative propagation of plants from stem and leaf cutting[1].

NAA (1-naphthyl acetic acid; C₁₂H₁₀O₂) is a root inducing, auxin type compound that is sometime used in media, especially to promote callus growth. Rooting hormones are either mixed with talc and used as powder or mixed with liquid and used as a wet dip [3]. All rooting hormones should contain a fungicide. When propagating plants from cuttings, it is very important that they root as quickly as possible [4]. Synthetic auxins, Indole butyric acid and Naphthalene acetic acid are both widely used in vegetative propagation of plants from stem and leaf cutting. The application can be traced to the propensity for auxin to stimulate adventitious root formation [5]. The synthetic auxins are used in commercial applications largely because they are resistant to oxidation by enzymes that degrade IAA. In addition to their greater stability, the synthetic auxins are often more effective than IAA in specific applications. The synthetic auxins are favored in commercial applications because of their low cost and greater chemical stability [6]. Synthetic auxins such as Indole butyric acid (IBA) and naphthalene acetic acid (NAA) have proven most effective. IBA and / or NAA are more stable than indole acetic acid (IAA).

In the present work, NAA was synthesized from naphthalene. In this case, naphthalene was prepared from coal tar. The prepared NAA was checked by TLC and identified by UV and IR spectroscopic methods. The application of synthetic NAA was assayed on soy bean and cow pea.

II. EXPERIMENTAL PROCEDURE

In this work, coal tar was used as naphthalene source. There are four reaction steps involved in this synthesis. They are:

First step: Extraction of Naphthalene from coal tar
Second step: Preparation of α-Chloromethyl naphthalene from naphthalene
Third step: Preparation of α-Naphthyl acetonitrile from α-Chloromethyl naphthalene
Fourth step: Preparation of Naphthalene acetic acid (NAA) from α-naphthyl acetonitrile

Step (1)
Coal tar was purchased from Steel Mill, Pyin Oo Lwin, Myanmar.

The mined coal is usually converted coke, which is needed for the smelting of iron to steel. When coal is heated in the absence of air, volatile compounds evolved leaving the coke behind. The volatile materials consist of coal gas and liquid coal tar.

Coal tar is the dark brown to black oily to viscous product of characteristic odour obtained as a by product of the carbonization of coal [7]. It was distilled and four fractions were collected separately according to temperature. Fractional distillation of coal tar yielded many organic compounds according to the temperature applied.

The fraction between (205-240°C) was found to rich in naphthalene together with phenol. The contaminant phenol was removed by acetylation according to the following reaction. Fig.1.

\[
\text{Phenol} + (\text{CH}_3\text{CO})_2\text{O} \xrightarrow{\text{NaOH}} \text{Phenyl acetate} + \text{CH}_3\text{COOH}
\]

The resulting solution was the naphthalene and collected [8]. It was confirmed by TLC, melting point, UV and IR spectra. The reactions taken place in preparation of Naphthalene acetic acid from naphthalene are as follows. Fig.2, Fig.3, Fig.4.

In each step, isolation, purification and characterization by molecular spectroscopy such as UV and FT-ir were carried out. The precursor naphthalene and the final product NAA were also checked by thin layer chromatography and confirmed by determination of their melting points. The solvent system used for TLC was carbon tetrachloride or n-hexane for naphthalene and Benzene, Acetone, Acetic acid (15:6:1) v/v for NAA. The FT-ir spectrum and Ultra-violet data of the isolated NAA were also recorded. The effect of NAA on root development was also studied in different concentrations of soy bean (Glycine max) and cow pea (Vigna catjang walp). Soy bean seeds were sterilized for (1 min) in 70% ethanol, soaked in 1% sodium hypochlorite plus a drop of a liquid detergent and then stirred gently (150 rpm) for 20 minutes, followed by three rinses in sterile distilled water.

Ordinary sand was put into tin tray (21cm × 28cm × 4cm) and sufficient water was applied until the sand was thoroughly wet. The soil medium was allowed to stand for at least ten hours to get saturated and stable condition. Thoroughly washed and sterilized seeds were grown in the prepared soil. Then the seed-containing tin trays were put in darkness and at 27°C. The sprouts appeared within 3 days. They were watered each day and after 7 days, they became about 5cm tall.

Hypocotyl segments (2.5 cm) in length were cut from the connection of two cotyledons of soybean plants after 7 days of growth in soil medium. The segments were placed in each test tubes containing the NAA solution of various concentrations (0.25 ppm, 0.5 ppm and 1ppm). For comparison, distilled water alone (ie.0 ppm NAA) was tested. The test tubes were
placed on the racks. The segments were incubated for 7 days at a room temperature, also in darkness.

After incubation period, the segments were then removed from the test tubes and the length of roots were accurately measured and recorded. Another experiment in soil medium, the segments were put into beakers containing various concentrations of NAA solution (0.25, 0.5 and 1.0 ppm) for 5 minutes. Then the segments were placed on the soil medium, the cotyledons being on top. The segments without NAA treatment were also planted for comparison. The segments are cultivated in soil medium for 7 days at room temperature, also in darkness.

After cultivation periods, the segments were then removed from the sand and the length of root, the number of roots, the length of hypocotyl length and the stem length were accurately measured and recorded [9].

II. RESULTS AND DISCUSSIONS

Naphthalene was prepared from coal tar. Table.1. shows the yield percents of naphthalene from it. The results are given as an average of six experimental determinations. In this work, naphthalene obtained from coal tar was used as precursor to synthesize naphthalene acetic acid (NAA). The synthesis of NAA from coal tar was also carried out because coal tar is more abundant. It is also a waste effluent of steel mill and can be purchased locally.

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>YIELD PERCENT OF NAPHTHALENE FROM COAL TAR</th>
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<tbody>
<tr>
<td>Experimental Yield (%)</td>
<td>Literature Value[10]</td>
</tr>
<tr>
<td>5.36%</td>
<td>8.00%</td>
</tr>
</tbody>
</table>

Table II shows the characteristic properties of naphthalene from coal tar. The melting point of naphthalene from coal tar is found to be nearly agree with the literature value. The other characteristic properties of naphthalene are also found to be nearly the same. Therefore, the naphthalene from coal tar was used as precursor in the synthesis of Naphthalene acetic acid (NAA).

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>CHARACTERISTIC PROPERTIES OF NAPHTHALENE</th>
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<tbody>
<tr>
<td>Naphthalene from Coal tar</td>
<td></td>
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<tr>
<td>Properties</td>
<td>Experimental Work</td>
</tr>
<tr>
<td>Melting Point</td>
<td>79.0°C</td>
</tr>
<tr>
<td>Colour</td>
<td>White</td>
</tr>
<tr>
<td>Odour</td>
<td>Strong smell</td>
</tr>
<tr>
<td>Solubility</td>
<td>Benzene, chloroform, cyclohexane, n-</td>
</tr>
</tbody>
</table>

According to UV data, the absorption peaks at 215.79 and 274.70 nm which are almost consisted with those of the literature value 218 and 275 nm [11]. The UV absorption spectrum of naphthalene from coal tar was depicted in Figure 5.

![Fig. 5 UV spectrum of naphthalene](image)

Naphthalene samples were identified by Fourier Transform infra-red spectroscopy. Fig.6. is FT-ir spectra of naphthalene samples from coal tar. IR spectrum revealed the presence of symmetric stretching C-H of ring, asymmetric and symmetric stretching vibration, over tone and combination bands of aromatic ring, C=C ring skeleton vibration (aromatic ring) and ring stretching at 3047.85, 2980.12, 1650, 1591.94, 1500 and C-H bending in plane, C-H out of plane bending and C=C bending(out of plane ring at 1387.68, 958.80 and 481.28. These absorptions were in agreement with literature values [12,13] thus, naphthalene from coal tar source is pure.

![Fig. 6. FT ir spectrum of naphthalene](image)
2-I( Naphthyl) ethanoic acid (C$_{12}$H$_{10}$O$_2$), melting point (128°C), UV spectrum revealed the presence of absorption peak at 215 and 274.70nm. IR spectrum revealed the presence of COOH and intense O–H and three adjacent hydrogen C-H out of plane bending vibration of a substituted naphthalene indicate at 1217.69, and at 931.34, 792.36 Figure.8.[4][13][14]. The effect of NAA on root development was determined in different concentrations of soy bean (Glycine max) and cow pea (Vigna catjangwalp) by bar graph and line graph.

Figure 7. UV spectrum of Naphthalene Acetic Acid

Figure 8. FT-ir spectrum of Naphthalene Acetic Acid

Figure 9. The graph of the length of soy bean cutting vs concentration of NAA (ppm) in aqueous medium

Figure 10. The graph of number of roots of soy bean Cutting vs concentration of NAA (ppm) in aqueous medium

Figure 11. The graph of the length of cow pea cutting vs concentration of NAA (ppm) in aqueous medium
The effect of NAA on root development and growth of plant was determined by bioassay method. The yield percent of naphthalene from coal tar is found to be 4.09%. The optimal concentration of NAA for root development is 0.25 ppm. When NAA was applied either in aqueous medium or soil medium, the hypocotyl length did not increase but the number of roots, root length and epicotyl length were all highest after applying 0.50 ppm NAA. In conclusion, for cow pea, hypocotyl cuttings should grow after treating with 0.25 ppm NAA. For cow pea, experiments were performed in both aqueous and soil medium. In aqueous medium, hypocotyl length did not increase but the number of roots, root length and epicotyl length were all highest after applying 0.50 ppm NAA. Also in soil medium, the hypocotyl length did not increase but other parameters (number of roots, root length, epicotyl length and hence stem length) were highest in higher concentration. In conclusion, for soy bean, hypocotyl cuttings should grow after treating with 0.50 ppm NAA.

For both species, NAA was applied to hypocotyl cuttings in aqueous medium and soil medium. Therefore, from this experiment the best condition for soy bean was to dip the hypocotyl cutting in NAA solution in concentration of 0.25ppm. Also in soil medium, the number of roots, root length, and hypocotyl length were highest in hypocotyl cuttings which was applied 0.25 ppm NAA. Only epicotyl and hence stem length were higher in higher concentration. In conclusion, for soy bean hypocotyl cuttings should grow after treating with 0.50 ppm NAA.

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**REFERENCES**


**III. CONCLUSION**

In this work, 2-(1-Naphthyl) ethanoic acid was successfully synthesized from coal tar. NAA was checked by TLC and identified by UV and IR absorption spectroscopy. The effect of NAA on root development and growth of plant was determined by bioassay method. The yield percent of naphthalene from coal tar is found to be 4.09%. The yield percent of NAA based on the precursor, naphthalene was found to be 21%. The optimal concentration of NAA for root development is 0.25 ppm. When NAA was applied either in aqueous medium or soil medium. For cow pea, the cuttings dipped in 0.50 ppm NAA gave the promising results.

**REFERENCES**


