Auxin: Indole-3-Acetic Acid (IAA), A Hormone with Diverse Effects: Synthesis and Applications

Developers:

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Grade Level:

9-12

Discipline:

Biochemistry, Chemistry, Biology, AP Chemistry and also
Science Fair Projects

Objectives:

Upon completion of the project, the students will be able to:

1. Identify auxins from their structural formulas.
2. Understand the process of refluxing in chemical synthesis.
3. Perform thin layer chromatography using silica gel plates to differentiate chemical substances.
4. Determine melting points of solids and use melting point to determine the purity of substances.
5. *Use ‘H-NMR spectroscopy to differentiate between different compounds.
6. *Use infra-red spectroscopy to differentiate between different compounds.
7. Prepare varying concentrations of solutions from a stock solution (of known concentration).
8. Determine the root initiation of lima beans with varying concentrations of IAA.
9. Graph IAA concentrations vs. number of rows of lateral roots and length of lateral roots.
10. Interpret the graphs in calculations.
11. Understand the functions of plant hormones.
12. Know the synthesis of indole acetic acid

(* for AP chemistry students only)

Specific Objectives:

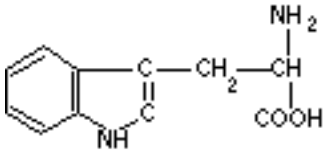
- To synthesize Indole-3-Acetic Acid (IAA).
- To determine the effect of different concentrations of IAA on root initiation of lima beans.
- To determine the effect of different concentrations of IAA with fertilizer solution on root initiation of lima beans.

Background:

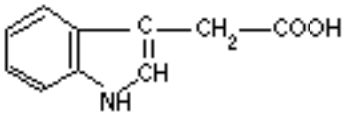
Plant hormones are chemical messengers which are produced in one part of an organism and transported to other parts, where they exert an effect out of all proportion to their very small concentrations. There are at least five major hormones in plants: Auxin, Cytokinins, Gibberellins, Ethylene and Abscisic acid.

The name auxin comes from the greek *Auxein*, meaning “to increase, or augment.” The most commonly known naturally occurring auxin is indole acetic acid or IAA. It is synthesized by plants from the amino acid tryptophan. IAA is produced in the apical meristems of shoots and diffuses downward, suppressing the growth of lateral buds. In young grass seedlings and other herbs, it plays a major role in stem elonga-

tion, migrating from the illuminated portion of the stem to the dark portions and thus causing the stems to grow toward the light.



Tryptophan



IAA

Auxin also increases the plasticity of the plant cell wall. A more plastic wall will stretch more during active cell growth, while its protoplast is swelling. Since very low concentrations of auxin promote cell wall plasticity, the hormone must be broken down rapidly to prevent its accumulation. Plants do this by means of the enzyme indole acetic acid oxidase. By controlling the level of both IAA and IAA oxidase, plants can regulate their growth very precisely.

Auxin also promotes the growth of vascular tissue in stems and the growth of the vascular cambium itself. It likewise increases fruit growth and also acts in other ways to prevent leaves, fruits or flowers from falling off prematurely. In high concentrations, auxins can cause uncontrolled growth and plant death.

The molecules of synthetic organic compounds that exhibit auxin activity have certain structural features in common. An active auxin usually has a ring system as a nucleus, with at least one double bond in the ring. To the nucleus is attached a side-chain that terminates in a carboxyl group. This group is separated from the nucleus by at least one carbon atom and bears a particular spatial relation to the ring system. Indole acetic acid is the perfect model for these structural requirements.

Materials and Equipment:

Part I

- 3-indolylacetonitrile
- potassium hydroxide
- ethanol
- water
- 2 beakers
- funnel (suction pump)
- filter papers
- thermometer
- hemispherical heating mantle (boiling bath)
- three-neck round bottom flask
- condenser (reflux)
- spatula

See Figure 1

Part II

- 8 jars (400-mL)
- aluminum foil (heavy duty)
- razor blade
- Indole acetic acid
- Peter's fertilizer solution
- measuring cylinders, 500-mL and 50-mL
- Pipette (25-mL)
- Lima bean seeds *(Phaseolus limensis)*
- ruler (centimer)
- 2 beakers
- potting soil
- fiber tray

Part I:

Synthesis of Indole-3-Acetic Acid (See Figure 1)

1. Mass 4.2g of potassium hydroxide (5g of 85% pure salt) and place in a dry three-necked flask.
2. Add 1 mL of water and dissolve the potassium hydroxide pellets (Be careful not to adhere any pellets to the sides of the flask).
3. Add 45 mL of ethanol and 5g of 3 indolylacetonitrile to the flask.

4. Stir vigorously.
5. Reflux the mixture for six hours using a hemispherical heating mantle. The temperature of the boiling solution is about 85°C. The temperature of the boiling bath has to be 20°C higher than the boiling mixture. Refer Figure 1.
6. Take an extract from the refluxing mixture after every hour and perform a thin layer chromatography (using silica gel plates) of the extract, 3-indolylacetonitrile and indoleacetic acid. The solvent used is ethyl acetate/hexane 40/60 (by volume) prepared by mixing 4 mL of ethyl acetate with 6 mL of hexane.
7. Check the evolution of ammonia from the mixture using a moistened litmus paper.
8. You will see the formation of a crystalline solid inside the flask after 2-3 hours of reflux.
9. After refluxing the mixture for six hours, concentrate the solution to half by distillation.
10. Filter the solution using a suction pump and wash the solid with alcohol. The solid is the potassium salt of indoleacetic acid.
11. Dissolve the crystalline solid in 50 mL of water and neutralize it by adding concentrated hydrochloric acid drop by drop (The pH of the solution has to be 1). A precipitate forms. Filter it using a suction pump and wash the solid with water until the liquids are neutral (pH = 7).
12. Air dry the crystalline indole acetic acid.
13. Mix the filtrate from step 10 with 50 ml of dilute sulfuric acid and filter. The precipate is worked up as described in step 11.
14. Determine the melting point of the solid and compare your results with the value from literature.
15. Mix the prepared indole acetic acid with an authentic sample of IAA. Determine the melting point again to see whether there is a depression of the melting point.

Reaction

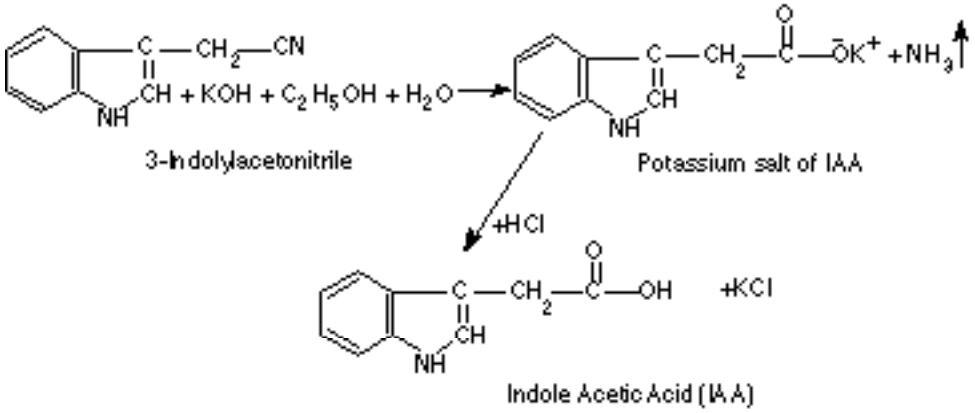
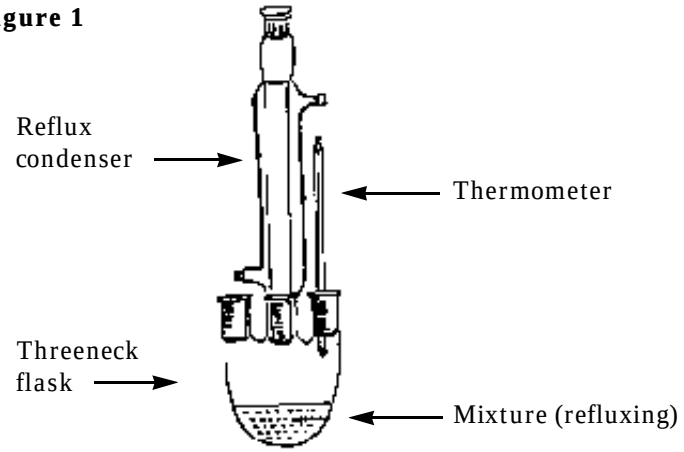
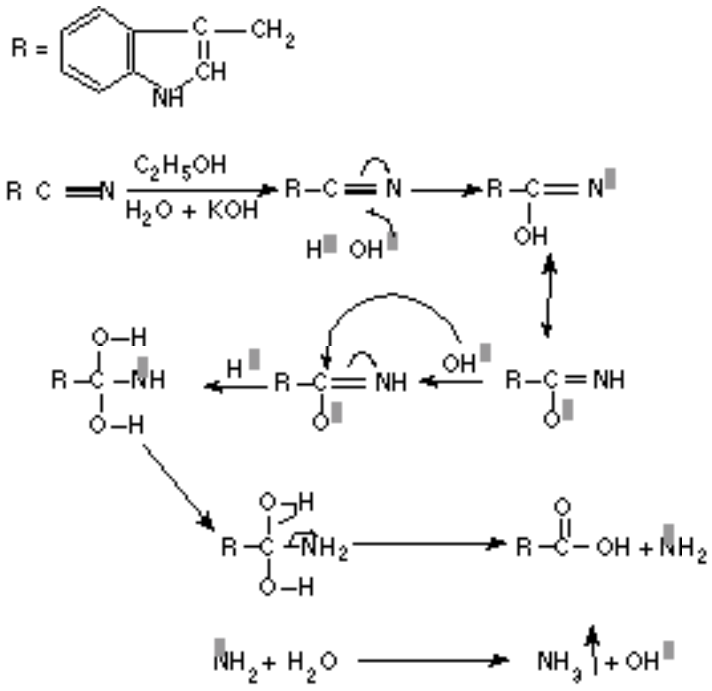


Figure 1



Reaction Mechanism
(For Advanced Students)



Procedure:

Part II

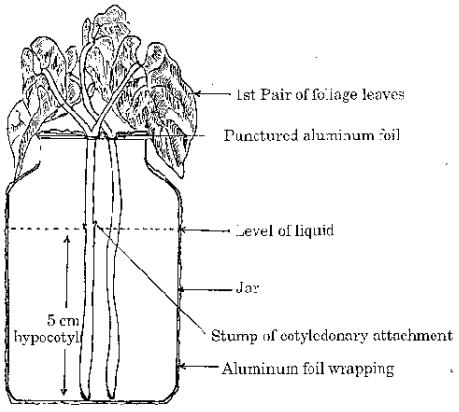
Root initiation by indole acetic acid:

1. Soak about 30 seeds of lima beans (*Phaseolus limensis)* for about one hour in tap water in a beaker.
2. Plant the seeds well apart and about 1/2" deep in a small, paper lined flat of potting soil.
3. Water the soil thoroughly; then germinate in the dark at 25°C for 5 days, until the hypocotyle begin to show.
4. Transfer the flat to the greenhouse, and grow for additional 7-10 days.
5. Completely cover the outside of eight 600-mL jars with heavy duty aluminum foil.
6. Prepare the IAA solutions as follows; 10 mg of IAA in 1 liter of water, 1 mg of IAA in 1 liter of water, 0.1 mg of IAA in 1 liter of water,10 mg of IAA in 1 liter of fertilizer solution, or prepare a stock solution of 10 mg of IAA in 1 liter of the solution and dilute accordingly.
7. Add 400 mL of each solution to the covered jars; also add 400 mL of distilled water and 400 mL of fertilizer solution not containing IAA to two separate jars to be used as controls.
8. Cover each jar with aluminum foil and fasten with rubber bands. Punch five holes in the foil using a pencil.
9. With a sharp razor blade, cut each bean plant at the level of the earth, remove both cotyledons, cut the hypocotyl 5 cm long, as measured from the point of cotyledon attachment to the cut base.
10. Put the hypocotyl through one of the holes in the lid and into the solution, with the pair of leaves projecting above the lid and cotyledonary stumps below the lid.
11. Repeat the procedure, one plant at a time, working rapidly to avoid drying of the

plants, until 5 plants have been placed in each jar. Refer to the diagram, Figure 2.

12. Place the jars in a row on the shelf above the laboratory bench.
13. Seven to ten days later, make the following measurements on each hypocotyl.
    a. Number of rows of lateral roots.
    b. Number of lateral roots larger than 1 mm in each row.
    c. Number of lateral root primordia shorter than 1 mm in each row.
    d Length of lateral roots in mm.
14. Determine the average for each treatment.
15. Graph the averages vs. IAA concentrations.

Figure 2



Sectional view of jar with plants in solution to be tested for its effect on root iniation.

Questions for Part I:

1. Write the structural formula of indole acetic acid.
2. How was the evolution of ammonia from the refluxing mixture tested?
3. What is the function of potassium hydroxide in the reaction?
4. How is the potassium salt of the acid converted to indole acetic acid?
5. What are the structural features of an auxin?
6.* How will you use NMR spectroscopy to distinguish between the hourly extracts and IAA.
7.* How will you use thin layer chromatography to distinguish between the products, reactants and hourly extracts from the refluxing mixture.

Part II:

(For AP chemistry students only)

1. Write the structural formula of indole acetic acid.
2. In the development of plant embryos, cells migrate from the suspensor to an area near the shoot apical meristem. Explain.
3. Hormones are chemicals that “work at a distance”. Plants have only one class of hormones, called gibberellins. True or False? Give reasons to your answer.
4. Many plants exhibit apical dominance, a phenomenon in which the shoot apex actively suppresses the formation of lateral branches. What is the reason for apical dominance?
5. State the inhibitor functions of plant hormones.
6. Design a project using plant hormones (IAA) for a science fair contest.

Teaching Tips Part I:

1. One day before, assemble all the equipment for refluxing the mixture.
2. As the refluxing is done for six hours, advise the students to be early and also try to start the experiment at least one hour before the school day.
3. No sandals or open toe shoes are to be worn in the lab. Rubber gloves and goggles are to be worn at all times while conducting the experiment.
4. Label the hourly extractions from the refluxing mixture carefully, to be used for chromatography and spectroscopy. (This part of the activity can be done on another day.)
5. The volume of the extraction must be few milliliters as it will affect the yield.

Part II:

6. Select plants as similar as possible.
7. Do not place jars in the greenhouse, for there they would suffer excessive water loss.
8. The chemicals and equipment for the project can be purchased from:
   Fisher FMD
   4901, W. LeMoyne Street
   Chicago, IL 60651
   Telephone No. 1-800-955-1177

References:

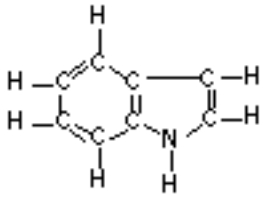
1. Arms, Karen and Camp, P.S. "Biology", 3rd Edition, Saunders College Publishing, Philadelphia
2. Bauer, Kane and Anderson, Ham. Process for Preparing Beta-Indolyl Acetic Acid. Patented November 19, 1940, United States, Patent Office
3. Bullock, M.W. and H. and J. J. *Journal of American Chemical Society*, 78, 5852 (1956)
4. Devlin, Robert M., "Plant Physiology", 3rd Edition, D. Van Nostral Company, NY
5. Hopson, Janet L. and Wessells, Norman K. "Essentials of Biology", 2nd edition, McGraw Hill Publishing Company
6. Johnson, Henbent E. and Crosby, Donald G., 3-Indole Acetic Acid, *Journal of Organic Chemistry*, 28, 1246 (1963)
7. Machlis, Leonard and Torrey, John G., Plants In Action, "Manual of Plant Physiology", W. H. Freeman Company
8. Postlethwait, J. and Hopson, Janet L. "The Nature of Life", Random House, N.Y.
9. Raven, Peter H. and Johnson, George B. "Biology", 2nd Edition, Times Mirror/Mosby College Publishing, St. Louis

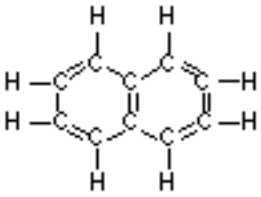
Extended (Further) Activities:

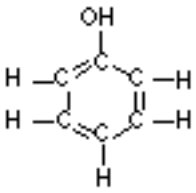
1. Students will be able to determine the effect of different concentrations of IAA on growth of apical meristems of shoots and roots of plants, in presence or absence of light.
2. They will be able to observe the prevention of abscission, the process by which a leaf or other organs fall from a plant, in the presence or absence of IAA.
3. Students will be able to see the effect of IAA as a herbicide on plants (IAA is applied in higher concentrations than those at which IAA normally occurring in plants).

Parent Compounds:

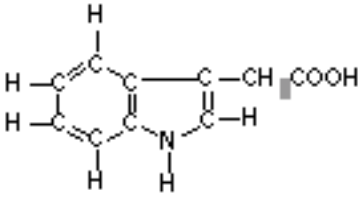
A. Parent Compounds
(all lack auxin activity)

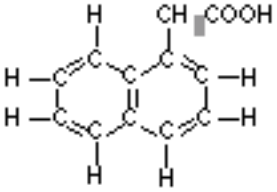

Indole

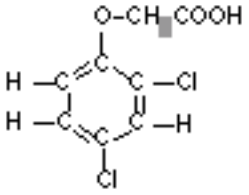

Naphthalene


Phenol

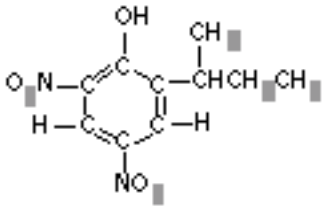
B. Auxin Plan Regulators

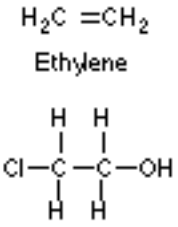

3-Indoleacetic acid
(IAA)


α- Naphthaleneacetic Acid
(NAA)

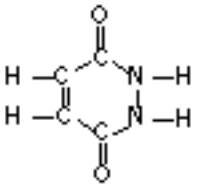

2,4-Dichlorophenoxyacetic
acid (2,4-D)

C. Nonauxin Plant Regulators


2,4-Dinitro-ortho-secondary
butylphenol (DNOSBP)


Ethylene

Ethylene chlorohydrin


Maleic Hydrazide
(MH)