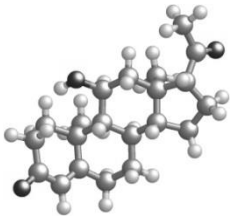


Chem!stry

Name: …… **( )**

Class: ……

Date: …… **/** …… **/** ……

Making and Testing a Penicillin

Apparatus:

100 cm^3 stoppered bottle or conical flask

2 × 25 cm^3 measuring cylinders

10 cm^3 measuring cylinder

Teat pipettes or droppers

Test tubes

2 × 100 cm^3 beakers

Glass rod or magnetic stirrer

Universal indicator paper or pH meter

50 cm^3 separating funnel

5 – 7 mm cork borer

Adhesive tape

Protective gloves

Protective goggles

Reagents:

6-aminopenicillanic acid

5.0 cm^3 of 1.0 mol dm^{-3} aqueous sodium hydroxide

0.5 cm^3 benzoyl chloride

5.0 cm^3 propanone

15.0 cm^3 ethyl ethanoate

10 cm^3 of 1.0 mol dm^{-3} hydrochloric acid

25 cm^3 saturated sodium hydrogencarbonate solution

4 agar plates impregnated with *Bacillus subtilis*

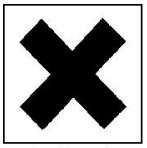
Ethanol (for sterilising apparatus)

Beaker of disinfectant (for sterilising apparatus and work surfaces)

Control solution of 6-aminopenicillanic acid

Control solution of sodium benzoate

Care: 6-aminopenicillanic acid can act as a sensitiser by inhalation or skin contact. Wear protective gloves and do not inhale the dust.


Irritant

Care: You should not perform this activity if you are allergic to penicillin.

Care: Benzoyl chloride is corrosive and a lachrymatory agent (it is a severe eye irritant) and must be used in a fume cupboard. It produces fumes of hydrogen chloride gas when exposed to moist air. Wear gloves and eye protection when handling this reagent.


Corrosive

Care: Propanone, ethyl ethanoate and ethanol are highly flammable liquids. Keep bottles stoppered when not in use and keep these reagents well away from naked flames.


Highly Flammable

Care: Consult your teacher before handling the bacterial culture. Wear gloves at all times. Cover any skin cuts with an effective waterproof dressing and wash your hands thoroughly at the end of the session. Report any spillages immediately. Any material that has come into contact with the bacterial culture must be sterilised before disposal.


Biohazard

The Reaction Scheme:

The starting material for this synthesis is 6-aminopenicillanic acid (6-APA). This is obtained from penicillin G which is produced naturally by the mould *Penicillium notatum*. 6-aminopenicillanic acid can be reacted with different acyl chlorides to produce a variety of new penicillins with different properties and a wide spectrum of antibacterial activity.

Your task is to convert the 6-aminopenicillanic acid into phenylpenicillin. You will not be able to isolate the phenylpenicillin in pure form, but you will be able to test its activity against the bacteria *Bacillus subtilis.* The reaction scheme that you will use is shown below in **Figure 1**.

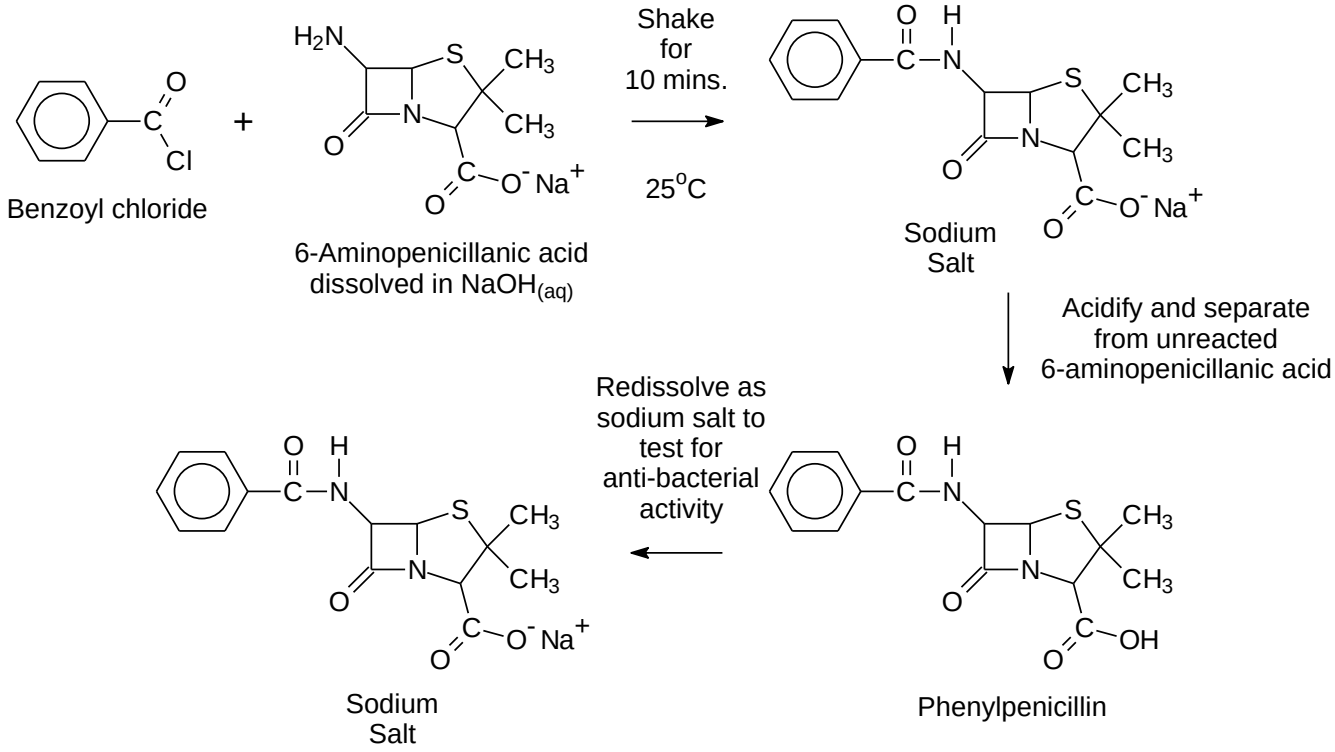


Figure 1.

The reaction scheme for the synthesis of phenylpenicillin.

Note: Benzoyl chloride is a relatively unreactive acyl chloride and can therefore be used in aqueous solution.

The 4-membered β-lactam ring is sensitive to extreme pH values and is consequently hydrolysed by strong acids and alkalis. To minimise destruction of the β-lactam ring, the pH of the solution is kept within the range pH 5 – 8 during the preparation. When you acidify the reaction mixture with hydrochloric acid during the purification procedure, the pH of the solution falls to pH 2, so you must work quickly at this stage.

As you go through the stages of the synthesis on page 3, use the column on the right-hand-side to keep track of the changes taking place. Where a reaction has taken place, draw the structure of the product and write a brief description about the change that has taken place at that stage.

When you carry out the extraction with ethyl ethanoate to purify the phenylpenicillin, make certain you know which chemicals are dissolved in each of the two solvent layers.

Method: **Part 1: Making and purifying phenylpenicillin**

1. Weigh out 1.0 g of 6-aminopenicillanic acid (**Care:** Wear protective gloves and do not inhale the dust) and mix it with 10 cm^3 of distilled water in a stoppered bottle (or conical flask).

2. Add 1.0 mol dm^{-3} of aqueous sodium hydroxide (**Care:** Irritant) drop-by-drop until the 6-aminopenicillanic acid just dissolves to give a clear solution. This should require approximately 5 cm^3 of the sodium hydroxide solution.

3. Working in the fume cupboard, dissolve 0.5 cm^3 of the benzoyl chloride (**Care:** Corrosive and a severe eye irritant) in 5 cm^3 of propanone (**Care:** Highly flammable) in a clean, dry test tube. Add this solution drop-by-drop, with continuous swirling, to the solution of 6-aminopenicillanic acid. Stopper the bottle and shake the contents gently for 10 minutes. (**Care:** you may need to occasionally release any build-up of pressure inside the bottle by carefully removing the stopper inside a fume cupboard.)

4. Transfer the reaction mixture to a 100 cm^3 beaker and add 10 cm^3 of ethyl ethanoate (**Care:** Highly flammable). Using a pH meter (or pH paper) adjust the pH of the solution to pH 2 by adding 1.0 mol dm^{-3} hydrochloric acid with continuous swirling. Any unreacted 6-aminopenicillanic acid forms a water soluble hydrochloride. Phenylpenicillin is more soluble in organic solvents than water.

5. Transfer both layers into a separating funnel and shake the mixture well. Once the two immiscible liquids have separated, transfer each into a separate 100 cm^3 beaker. Keep both layers. **Note:** The density of ethyl ethanoate is 0.90 g cm^{-3}. Ensure that you know which layer is which.

6. Return the aqueous layer to the separating funnel and add a further 5 cm^3 of ethyl ethanoate. Once again, shake the mixture and separate it into the two beakers. You can now discard the aqueous layer down the sink in the fume cupboard. **Caution:** do not discard the wrong layer!

Notes:

7. Now add 10 cm^3 of water to the remaining organic layer in the beaker. Adjust the pH to 6 – 7 by adding a solution of saturated sodium hydrogencarbonate. Transfer the mixture into the separating funnel and shake it well, taking care to release any build-up of pressure. Run the lower *aqueous* layer into a clean 25 cm^3 measuring cylinder. Add distilled water to adjust the volume of the solution in the measuring cylinder to 25 cm^3 and stir well. This solution contains the phenylpenicillin that you have synthesised.

Notes:

<u>Method:</u> Part 2: Testing for antibacterial activity

Note: Consult your teacher before handling the bacterial culture and **follow the safety instructions carefully**.

8. Take 1 cm^3 of the phenylpenicillin solution and dilute it to 10 cm^3 with distilled water. Stir well.

9. Dip the cork borer into a beaker of ethanol (**Care:** Highly flammable). **Hold the cork borer horizontally** so that the flames do not pass up the centre and burn your hand. Pass the cork borer through a Bunsen burner flame to ignite the ethanol. Hold the cork borer to one-side of the flame and allow the ethanol to burn off. This will heat the surface of the cork borer to approximately 60 °C so that it is sterilised. (**Care:** Make sure that the beaker containing the ethanol is placed away from the Bunsen burner. Allow the cork borer to cool before returning it to the beaker of ethanol.)

10. Use the sterilised cork borer to make a well in the centre of an agar plate that has been impregnated with *Bacillus subtilis*, by pressing the cork borer into the agar and then lifting out the cut plug of agar using a sterilised metal spatula. **Note:** The metal spatula should be sterilised in the same way as the cork borer. Place the agar plug directly into a beaker of disinfectant. Re-flame the cork borer and spatula after use. Fill the well in the agar with the dilute phenylpenicillin solution.

11. Replace the lid on the agar plate and seal using two pieces of adhesive tape, as shown in **Figure 2**. Do not completely seal around the rim of the agar plate as this may create anaerobic conditions and encourage the growth of harmful bacteria. Label the agar plate with your name, class and date. Write something further to indicate the treatment that has been given to the plate.

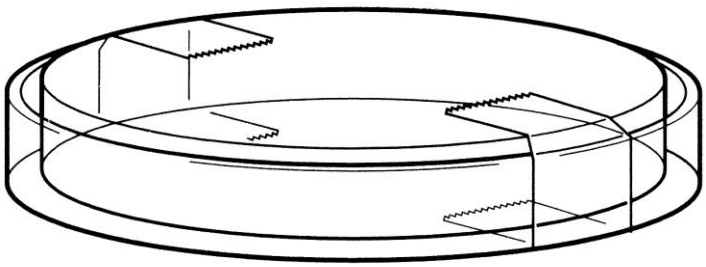


Figure 2.

12. Now set-up three control plates in a similar way to compare with the sample of penicillin. Fill the well in the first agar plate with the solution of 6-aminopenicillanic acid that has been provided. Fill the well in the second agar plate with the solution of sodium benzoate that has been provided. Fill the well in the third agar plate with distilled water. The control solutions of 6-aminopenicillanic acid and sodium benzoate have concentrations of 50 $\mu g\ cm^{-3}$, comparable to that of the phenylpenicillin solution. Cover and seal the plates as before.

13. Take care not to tip the plates. Leave them in a secure place at room temperature for 24 – 48 hours.

14. Make sketches of the four plates at the end of this time. (**Care:** do not open the plates once they have been sealed.) Regions of bacterial growth will cause the agar to appear cloudy. Regions where bacterial growth has been inhibited will cause the agar to appear clear. Use a ruler to measure the radius of the agar plate and the radius of any inhibited bacterial growth. Use these results to calculate the area of the agar plate and the area of any inhibited bacterial growth, hence calculate the percentage by which bacterial growth has been inhibited on each of the four agar plates.

Notes:

Care: Any material which has come into contact with the bacterial culture must be sterilised before disposal, or before returning to stock cupboards. The sealed agar plates must be sterilised in a pressure cooker or an autoclave before disposal.

Questions:

a) Explain why the phenylpenicillin that you made is called a *semi-synthetic* penicillin.

b) Why was an aqueous solution of sodium hydroxide added to the 6-aminopenicillanic acid in step **2**, before treatment with the benzoyl chloride?

c) **i)** What product, other than phenylpenicillin, is formed when 6-aminopenicillanic acid reacts with benzoyl chloride?

ii) Name / identify the type of chemical reaction that has taken place.

iii) Name the functional group that is formed by this chemical reaction.

iv) What name is given to this functional group in biology?

d) In addition to benzoyl chloride, give the full structural formulae and names of *two* other compounds that could be used to synthesise phenylpenicillin from 6-aminopenicillanic acid.

e) Explain the chemistry behind the method that was used to purify the phenylpenicillin.

f) Explain why it is necessary to have three control plates when testing the phenylpenicillin for antibacterial activity.

- Scan the QR code below for the answers to this practical.

http://www.nygh.sg/miscellaneous/nanyang_discovers/medicinal_chemistry/synthesis_of_penicillin_ans.pdf