The preparations that follow are very simple to carry out and illustrate typical examples of the formation of aromatic heterocyclic compounds, the chemistry of which will form a major part of your Organic Chemistry lectures at Year 3 level. A heterocyclic compound contains a ring system that is made up of carbon atoms and one or more heteroatoms, most commonly nitrogen, oxygen and/or sulfur. For further examples, see: Clayden, *Organic Chemistry*, Chapter 30.

Heterocycles with the maximum number of conjugated double bonds are aromatic and, just like benzene, show unusually high $^1\text{H}$ NMR chemical shifts in the 6 – 8 ppm range (remember that “normal” alkene protons have chemical shifts of 5 – 6 ppm).

Students on the Pharmaceutical Chemistry degree should carry out experiment 2B and 2D (synthesis of the pharmaceutical drug nifedipine).

The write-up for these experiments will ask you for a short Experimental Procedure and summary of characterisation data in a style typically used in a dissertation, a thesis or the supplementary material of a scientific paper. Reports should be submitted through VISION. The write-up for Experiment 2A or 2B is due by the stated deadline for your group, the write-up for Experiment 2C or 2D one week later — and you should take feedback for 2A/2B into account when submitting your report for 2C/2D.

**PRE-LAB EXERCISES**

a. Watch the following videos in preparation of your experiment: [Simple reflux](https://example.com)

b. Do the pre-lab webtest for Experiment 2 on VISION.

c. Prepare a table of reagents in your lab book.
2A The Fischer indole synthesis

Introduction

This preparation illustrates one of the most common ways of preparing indoles. The indole (1) ring system is a very important structure amongst heterocyclic compounds and is found in many natural products. These include ones with potent biological activity, which can be either of a useful type (e.g. the anticancer drug vinblastine from the periwinkle plant), or of the sort usually regarded as less desirable (e.g. the poison strychnine).

The Fischer synthesis allows you to make a substituted indole (4) from phenylhydrazine (2) and a ketone (3) in the presence of an acid catalyst. The mechanism is illustrated below and includes: (i) the formation of a phenylhydrazone; (ii) tautomerism of the imine to an enamine; (iii) a sigmatropic rearrangement involving the breaking of the N–N bond and the formation of a new C–C bond; and (iv) cyclisation to a five-membered ring followed by elimination of ammonia. It has been shown by isotopic labelling experiments that the nitrogen atom lost is the one further away from the phenyl ring in (2), as indicated by the asterisks in the following scheme.
Procedure

### Safety Notes

<table>
<thead>
<tr>
<th>Substance</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylhydrazine:</td>
<td>MAY CAUSE CANCER; toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed; irritating to skin, eyes and respiratory system; may cause sensitisation by skin contact; very toxic to aquatic life. This compound should only be handled in the fume cupboard and disposable gloves must be used. Do not inhale the vapours and avoid contact with your skin. If any phenylhydrazine gets spilled onto your hands, wash them with a little very dilute acetic acid and then thoroughly with soap and water. Ensure that no glassware that has been in contact with phenylhydrazine is taken into the open laboratory without first washing it (e.g. with acetone or methanol). Dispose of any phenylhydrazine residues into the non-halogenated solvent waste bottle.</td>
</tr>
<tr>
<td>Cyclohexanone:</td>
<td>Harmful if inhaled; flammable liquid and vapour.</td>
</tr>
<tr>
<td>Glacial acetic acid:</td>
<td>Flammable liquid and vapour; causes severe skin burns and eye damage.</td>
</tr>
<tr>
<td>Methanol:</td>
<td>Toxic by inhalation, in contact with skin and if swallowed can cause irreversible damage to the eyes; highly flammable liquid and vapour.</td>
</tr>
</tbody>
</table>

USE A FUME CUPBOARD FOR THIS EXPERIMENT.

To a mixture of cyclohexanone (2.9 g, 3.0 mL) and glacial acetic acid (15 mL) add phenylhydrazine (2.5 mL) with a graduated plastic pipette (see Safety Notes above) and reflux for 15 minutes. Cool the solution in ice/salt until it reaches 0 °C (check with a thermometer) and filter off the precipitate. Recrystallise the crude product from methanol–water. It is advisable to air-dry the product for at least 2 days (to attain constant weight and remove the smell of any residual acetic acid) before you analyse it.

**Lab report:** For instructions, see page 30.
2B  Another synthesis of a heterocyclic compound

Introduction

Most heterocyclic syntheses involve fairly straightforward condensation reactions to form the heterocyclic ring, where a nucleophile attacks an electrophilic centre followed by the elimination of a small molecule such as water or ammonia. Such a process occurs in the final ring-forming step (iv) in the Fischer indole synthesis. Experiment 2B illustrates also how easy it is to form heterocycles by this sort of approach, and mechanistically this preparation is considerably simpler than the Fischer synthesis.

Procedure

Add a solution of hydroxylamine hydrochloride (0.695 g) in water (1.5 mL) to a solution of dibenzoylmethane (1.1 g) in methylated spirits (10 mL), then add 5 drops of “bench” aqueous sodium hydroxide solution. Heat the mixture under reflux for 1 hour, cool it to room temperature and collect the crude product by filtration. Recrystallise the crude product from methylated spirits. It is advisable to air-dry the product first for at least 2 days before you analyse it.

Lab report: For instructions, see page 30.
2C  The Hantzsch pyridine synthesis

Introduction

The German chemist Arthur Rudolph Hantzsch developed this multi-component synthesis in 1882. The reaction provides easy access to highly substituted symmetric 1,4-dihydropyridines which are readily oxidised to the corresponding pyridines with oxidants such as cerium(IV) salts, nitric acid or chlorine. The classical Hantzsch synthesis is a one-pot cyclocondensation between an aldehyde, ammonia and two equivalents of a β-ketoester such as ethyl acetoacetate.

Pyridine is an electron-deficient aromatic heterocycle that is not readily amenable to electrophilic aromatic substitution reactions. Highly substituted pyridines are generally best made by ring-closing reactions from acyclic precursors. Pyridine is also a weak base since the pyridine nitrogen can be protonated by strong acids.

The Hantzsch synthesis initially yields a 1,4-dihydropyridine which has two extra hydrogens at position 1 and 4 in the ring, with the N atom being defined as position “1”. Many 1,4-dihydropyridines are known to exhibit important bioactivity. Lately, the synthetically simple Hantzsch reaction has been used to make nifedipine and related pharmaceutical drugs that act as Ca\(^{2+}\) channel blockers for the treatment of high blood pressure.\(^\text{11}\)

In this experiment you will synthesise a structural analogue of nifedipine. The product of preparation 2C has also a number of uses in agriculture as a carotene stabiliser in grass meal, as a stabiliser of vitamin A in edible oils, and as a stimulator for the growth of pigs and cattle.

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Procedure

Safety Notes

Hexamethylenetetramine (urotropine): Flammable solid; may cause an allergic skin reaction.

Ethyl acetoacetate: Causes serious eye irritation.

Ammonium acetate: Causes skin and eye irritation; may cause respiratory irritation.

Methylated spirits (ethanol): Highly flammable liquid and vapour.

Methanol: Toxic by inhalation, in contact with skin and if swallowed can cause irreversible damage to the eyes; highly flammable liquid and vapour.

Sharps: Never point syringe needles in the direction of a person.

In a 50 mL round-bottom flask suspend ammonium acetate (2.0 g) and urotropine (0.80 g) in methylated spirits (22 mL). Add ethyl acetoacetate (7.8 mL) and anti-bumping granules, then reflux for 1 hour, gently swirling from time to time throughout the reaction. At the end of the reaction period, cool with an ice−water bath. Collect the precipitate by suction filtration and rinse the filter cake with well-chilled methylated spirits (2 mL), water (4 mL) and again well-chilled methylated spirits (2 mL). Recrystallise the crude product from methylated spirits. Check the purity of your product by HPLC.

HPLC analysis: Weigh out 2 mg (on the analytical balance) of your recrystallised sample and dissolve it in 2 mL of HPLC-grade methanol in a beaker. Make sure that everything has dissolved. Then follow the instructions on how to set up the HPLC and inject the sample solution. Paste a printout of the HPLC trace in your lab book. Estimate the % purity of your product.

Lab report: For instructions, see page 30.
**2D Synthesis of nifedipine (for students on the Pharm Chem degree)**

**Introduction**

For an introduction to the Hantzsch synthesis, see Experiment 2C.

Lately, the Hantzsch dihydropyridine synthesis has been used to make nifedipine and related pharmaceutical drugs that act as calcium channel blockers and are used for the treatment of high blood pressure. Second-generation drugs of the nifedipine type are no longer symmetric and possess different ester groups. The scheme underneath shows how nitrendipine is made by a variant of the Hantzsch synthesis using equimolar amounts of an aldehyde, methyl acetoacetate and ethyl 2-aminobutenoate. It also requires no further source of ammonia. Ethyl 2-aminobutenoate is a stable enamine — you should be able to identify from which starting materials it is made.

![Chemical structure of nitrendipine and reaction scheme](image)

**Procedure**

**Safety Notes**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Safety Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitrobenzaldehyde</td>
<td>Harmful if swallowed; causes skin irritation; causes serious eye irritation; may cause respiratory irritation.</td>
</tr>
<tr>
<td>Methyl acetoacetate</td>
<td>Causes serious eye irritation.</td>
</tr>
<tr>
<td>Aqueous ammonia</td>
<td>Causes severe skin burns and eye damage; very toxic to aquatic life.</td>
</tr>
<tr>
<td>Methanol</td>
<td>Toxic by inhalation, in contact with skin and if swallowed can cause irreversible damage to the eyes; highly flammable liquid and vapour.</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>This drug has to be treated like any other highly toxic compound due to its pharmacological potency and ability to lower blood pressure. Beware that a typical daily pharmaceutical dose is only 15 – 30 mg, and even a small dose can lead to collapse and increased heart rate in people with normal blood pressure.</td>
</tr>
</tbody>
</table>
USE A FUME-CUPBOARD FOR THIS EXPERIMENT.

In a 50 mL round-bottom flask heat a mixture of 2-nitrobenzaldehyde (2.27 g), methyl acetoacetate (4.0 mL), methanol (4 mL) and concentrated ammonia (1.6 mL) to reflux for about 4 hours. At the end of the reaction period, allow the reaction mixture to cool. If necessary, use an ice–water bath until a precipitate forms.

Continue with the work-up and bottling of the sample in the fume cupboard. Collect the precipitate by suction filtration and wash the filter cake with water (10 mL) and methanol (5 mL). Recrystallise the crude product from methanol.

Check the purity of your product by HPLC.

HPLC analysis: Weigh out 2 mg (on the analytical balance) of your recrystallised sample and dissolve it in 2 mL of HPLC-grade methanol in a beaker. Make sure that your sample is completely dissolved. Ask the lab technician for the best conditions for the HPLC analysis of nifedipine, then follow the instructions on how to set-up the HPLC. Ensure that your HPLC run is not contaminated by a peak from the product of experiment 2C which can be dragged over if the syringe or the HPLC inlet have not been thoroughly cleaned prior to use. Paste a printout of the HPLC trace in your lab book.

Lab report: For instructions, see page 30.
Lab report

Your write up should consist of:

- a suitable title (“Synthesis of …”),
- a ChemDraw reaction scheme showing the starting materials, reagents and product (no mechanism!), and
- a journal-style experimental procedure followed by
- a complete listing of m.p., HPLC purity, NMR, IR and MS data.

This is the style typically used in a dissertation, a thesis or the supplementary material of a scientific paper. For details on the style, have a look at the examples on VISION. The procedure and characterisation data should be a single, concise paragraph giving all the information a trained chemist would need to repeat your experiment and/or check the spectroscopic evidence, but leaving out unnecessary details (e.g. type of round bottom flask, glassware). Usually, this will be no more than half a page in length.

Your report should follow RSC guidelines for the presentation of experimental data, see http://www.rsc.org/images/Author_guidelines_tcm18-186308.pdf (Part 4.0 Characterisation of new compounds).

Analyse the spectroscopic data of your product. NMR spectra are available online on VISION.

Submit your report through the TurnItIn link on VISION at ORGANIC REACTIONS 1 (B19OC) > LABORATORY > EXPERIMENT 2. Your submission has to be your own work which you certify by using TurnItIn. The write-up for 2C/2D is due one week after your submission of 2A/B (and make sure that you check the feedback on your previous work).
MS data from SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (National Institute of Advanced Industrial Science and Technology)