**University of Southampton – Chemistry Twilights**

**Extraction of a Natural Product**

**Background**

You might well be familiar with the spice called **nutmeg**. It is commonly used to add flavour to savoury dishes including potato dishes, curries and many puddings as well, for example in custard tarts (the brown dusting of powder on top). Nutmeg contains many chemicals and the ones that give flavour and smell are volatile (or have low boiling points).

In addition, the nut is also made up of compounds with much higher boiling-points, including complex organic polymers that give nutmeg its woody nature. One of these non-volatile compounds is **trimyristin**, a triglyceride, which is partly responsible for the taste and texture of nutmeg. As a saturated fat, it belongs to a class of compounds that have received much attention given their impact on human health. Diets high in saturated fat have been shown to lead to atherosclerosis (artery plaque build-up), high blood pressure and heart disease.

Trimyristin’s fatty characteristics also mean that the compound has potential as a lubricant additive in medicine tablet preparation, as an additive in cosmetics and in various medicines applied directly onto the skin (topical medicines).

**Practical objectives**

Many chemicals, which are now produced synthetically to meet a particular need in our daily lives (e.g. healthcare), were once isolated from nature. Isolation of new or known chemicals from nature remains an important part of chemistry. In some parts of the world where economies depend heavily on natural products, or areas with exceptional biodiversity (untapped natural resources), the natural product chemist role remains of key importance.

In general, the three key steps in natural product isolation are -

(i) **Extraction** of a mixture of compounds in to a particular solvent

(ii) **Separation** of the components of the mixture into separate chemical entities and

(iii) **Analysis** of the compound to determine its structure.

The techniques involved in each of these stages can vary from low- to high-tech. The techniques that you are going to use are **solvent extraction**, **gravity filtration** and **rotary evaporation** to isolate the mixture of compounds containing trimyristin.

You will follow this with **crystallisation** and **vacuum filtration**, which will be used during the purification to isolate trimyristin as a single compound. Finally, and subject to the time available, you will analyse your pure compound you have isolated to prove its purity and structure. You will look at **thin layer chromatography (TLC)**, **melting point** determination, and **infra-red (IR) spectroscopy**.

**Safety**

* You will be doing an experiment in an undergraduate laboratory where the chemicals and apparatus are potentially hazardous.
* We will provide disposable laboratory ‘pinafores’ and safety glasses, which must be worn at all times. Disposable gloves are available but are not recommended for most of the practical. We advise use of gloves when carrying out TLC analysis.
* You need to wear long trousers (or other suitable leg covering) and have shoes, which fully cover your feet.
* Music devices must be turned off whilst in the laboratory, and mobile phones should not be used, unless indicated.
* You will learn how to safely and effectively use a fume cupboard to ensure that you are not exposed to any of the chemicals that you are using.

**Help and support**

You will be working in pairs during the experiment and you should aim to share the work during the session. Several of our current undergraduate and postgraduate students, and teaching staff, will be around to help you during the experiment, and they will explain each of the techniques that you will be using.

**Experimental Guidance**

1. Transfer ground nutmeg (2.5 g, this will be pre-weighed for you and provided in a labelled glass vial) to a 100 mL (mL = millilitre) round-bottomed (RB) flask.

In the fume cupboard that you are working in, add a magnetic stirrer bar to the flask and clamp the RB flask over a magnetic stirrer plate, using a boss and clamp.

2. Using a measuring cylinder, add the extraction solvent, diethyl ether (25 mL) (sometimes labelled ‘Ether’).

3. Put a stopper in the flask gently and set the magnetic stirrer in motion, so that the nutmeg is thoroughly mixing with the solvent.

***Q***

4. Use the magnetic stirrer, to stir the mixture for 30 minutes at room temperature.

**At this point some more experimental techniques will be described, use any remaining time to prepare for upcoming steps.**



5. Separate the insoluble remains of the nutmeg through fluted filter paper (supported inside a clean dry filter funnel), collecting the extraction solvent (called the filtrate) that comes through, in a 100 mL RB flask.

6. Wash the brown nutmeg residue collected in the filter paper, with 10 mL of diethyl ether and collect this solvent in the same RB flask.

***Q***

7. Connect your RB flask to a rotary evaporator. A demonstrator will help you with this. Remove the solvent under vacuum. Carry out this process until you have a thick oil in the flask, which does not appear to change in volume, or until you see a solid spread over the inside surface of the flask.

***Q***

8. Check that the water bath in your fume hood has achieved a gentle boil. This will be turned on for you in advance.

**You have reached the point where you have a crude product. Now you have to isolate your target compound, trimyristin, by a process called crystallisation and then check whether you have a pure compound (by TLC) and what it is (using analytical techniques).**

9. Secure your crude product in your round-bottomed flask using a clamp, and add, using a Pasteur pipette, a small volume (half a pipette full) of propan-2-one, which is also known as acetone.

10. Hold the flask in the water bath using a clamp, to bring the acetone solvent to the boil. When the acetone boils, you should see that the solid in your flask starts to dissolve. Remove from the heat and if the solid is not completely dissolved, add some more acetone and return to hold in the water bath until the acetone boils again. Repeat until all the solid is fully dissolved in the minimum quantity of boiling acetone.

***Q***

11. Remove from the heat and allow to cool slowly to room temperature. You should see a solid reforming in the solution, which is the process of crystallisation occurring. This is a process that is best achieved with patience! If you do not see a crystalline solid forming, your demonstrators will be able to give you some tips about what you can do to ‘encourage’ the process.

12. To prepare for the next step, you need to chill about 10-15 mL of acetone in your measuring cylinder, using ice in a plastic pot. When crystallisation is complete, you need to separate the white solid from the yellow-coloured solution using vacuum filtration.

13. Secure the vacuum filtration apparatus (Hirsch funnel and flask) using a retort stand, boss and clamp. Add a small filter paper to the funnel and connect the orange vacuum tubing to your flask (from the grey tap). Check your apparatus with a demonstrator before turning on the vacuum.

14. Swirl your mixture around in the flask, and pour it out carefully into the funnel. If there is no solvent left, add a little cold acetone to your mixture first, then swirl and pour.

15. You will notice that some solid remains in the RB flask, and that the solid might be yellow-coloured. You need to transfer all of this solid on to your filter paper using cold acetone, and wash the solid you have collected with a little more cold acetone.

***Q***

16. Leave air being drawn through your sample for 1-2 minutes to dry it, then disconnect the tubing and turn off the vacuum supply. Keep the filtrate for TLC later.

17. Weigh a small plastic screw cap vial (WITHOUT the lid) and then transfer the solid into this weighed vial very carefully – you do not want to lose any of your compound.

**At this point, you should ask your demonstrator if you should perform the TLC, mass calculation, IR or melting point next.**

**Instructions for TLC start at step 18, instructions for melting point start at step 27, and instructions for IR start at step 28.**

**TLC is a complicated procedure, so to make it easier, we have made a video, which can be accessed at the following link or by scanning the QR code below:** <http://youtu.be/jhWc5jFzNS0>

18. Collect equipment from the central TLC station. You will need two small glass sample vials, two TLC plates and two glass capillary tubes. Take care to handle the TLC plates by the edges only, to avoid damaging or contaminating the plates. A demonstrator will be there to help.

19. Draw a line gently using a pencil on the silica side of the plate. Do this on the narrow side of the plate about 1 cm from the bottom. Lightly draw two crosses on the plate labelling one P for pure and the other F for filtrate.

***Q***

20. Dissolve a small amount of your pure sample in TLC eluent (two or three drops will do). This is your pure sample for TLC. Your filtrate TLC sample is made by taking some of your filtrate (see diagram above) and diluting it with an equal amount of eluent.

21. Using a capillary tube, draw up some of your sample solutions and gently 'spot' them onto your pre-drawn cross on the TLC plate, waiting for it to dry in between each spotting. You can prepare both plates simultaneously in this way.

22. Fill your TLC jar with TLC eluent up to a depth less than the depth of the line on your TLC plate (<1 cm)

23. Carefully place your TLC plate into the jar using tweezers, and screw the cap on. Observe the solvent running up the plate and remove your plate before the solvent front reaches the top. When you remove the plate, immediately mark where the solvent reached on the TLC plate with a pencil line (this is called the “solvent front”).

24. Take your TLC plate to the central bench. First, visualise your plates under UV light (circling any spots which appear). Secondly, dip one plate in one of the KMnO4 dye jars and dry using the hot air gun. Return your plates to your bench.

25. On your worksheet, sketch your TLC plates making sure you record the following: Appearance of spots under normal light, appearance of spots under UV, appearance of spots using KMnO4 dip, distance of spots from the origin, distance of solvent front from origin. TLC plates fade over time so this is essential to preserve their information.

26. To calculate RF (retention factor) values you must divide the distance travelled by a sample spot, by the distance travelled by the solvent front.

You can do this by measuring the distance between the origin line and the respective parts. Your answer should be between 0 and 1.

27. Conduct a **melting point** experiment on your solid compound using the instructions provided **– pure trimyristin has a melting point of 56-57 °C.**

28. Collect an **infrared spectrum** of your compound by taking it to the spectrometer and following the demonstrator’s instructions. Compare it with data for the pure material.



When you have completed the above steps, you have completed the extraction and chemical analysis of the natural product, **trimyristin**.

We hope you have enjoyed the practical. Please be sure to ask any questions you may have of our demonstrators and staff before you leave, and feel free to take pens and any literature if you would like.

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**University of Southampton – Twilight Chemistry**

**Extraction of a Natural Product**

**Overall objectives:**

(i) **Extraction** of a mixture of compounds in to a particular solvent

(ii) **Separation** of the components of the mixture into separate chemical entities

(iii) **Analysis** of the compound to determine its structure.

Which techniques will you perform to complete each of these objectives? (You may want to check out the information sheet at the front of the lab script).

|  |  |  |
| --- | --- | --- |
| Extraction | Separation | Analysis |
|  |  |  |

Record below observations including length of time taken for each stage. Information like this is vital for results to be publishable.

Draw out your TLC plates below. What conclusions can you draw from this?

Calculate the yield of your product (% by mass of nutmeg)

How reliable do you think your results were?



Link to online script: <http://www.edshare.soton.ac.uk/13830/>

Link to TLC video: <http://youtu.be/jhWc5jFzNS0>

***Q*UESTIONS**

***Q***

Why is it necessary to thoroughly mix the nutmeg with the solvent, and stir for 30 minutes?

***Q***

What is the benefit of washing the brown nutmeg residue with extra diethyl ether?

***Q***

What effect does a vacuum have on evaporation of your solvent?

***Q***

Why do we need to dissolve the solid in the minimum amount of boiling acetone? *– CHALLENGING QUESTION*

***Q***

What is the purpose of washing your solid you collected with a little more cold acetone?

***Q***

What do you think the filtrate might contain, and what might TLC analysis show you?