**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 preform to complete each of these objectives? (you may want to check out the information sheet at the back)

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

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

Depict your TLC plates below, what conclusions can you draw from this?

Calculate the yield of your product

How reliable do you think your results were?

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**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
* You need to wear long trousers and have shoes which fully cover your feet.
* Mobile phones will be turned off whilst in the laboratory.
* You will learn how to use a fume cupboard to ensure that you are not exposed to any risk associated with 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 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 (2.5g, this will be pre-weighed for you and provided in a labelled bottle) to a 100 mL (mL = millilitre) round bottomed (RB) flask. Add a stirrer bar to the flask and clamp the RB flask over a magnetic stirrer using boss and clamp in the fume cupboard that you are working in.

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| 2. Add the extraction solvent, diethyl ether  (25 mL) (sometimes labelled ‘Ether’) using a measuring cylinder.  3. Stopper the flask and the set the magnetic stirrer in motion such that the nutmeg is being thoroughly mixed with the solvent.  4. Stir the extraction 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** |  |

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| 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. |  |

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| 7. Connect your round bottomed flask to a rotary evaporator and 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.    8. Check your water bath (in fume hood) has achieved a gentle boil | Rotovap10 |

**You have reached the point where you have 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 in the jaws of 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 over 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 over the water bath until the acetone boils again. Repeat until all the solid is just dissolved in the minimum quantity of boiling acetone.

11. Remove from the heat and allow to cool slowly to room temperature.

12. You might see a solid reforming in the solution which is the process of crystallisation occurring which is a process that is best achieved with patience. If you do not see a crystalline solid forming your advisors will be able to give you some tips about what you can do to ‘encourage’ the process.

13. When crystallisation is complete you need to separate the white solid from the yellow coloured impurities using vacuum filtration. To prepare you need to use a Pasteur pipette with a wide opening and you also need to chill around 10 mL of acetone on ice in your measuring cylinder.

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| 14. 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 vacuum tubing to your flask (from the grey tap). |  |

15. Check your apparatus before turning on the vacuum. Using your adapted pipette suck both solid and liquid and transfer them drop-wise direct onto the filter paper. Repeat until all the solid has been collected in the funnel.

16. You will notice that some solid remains in the RB flask and also that the solid might be coloured. You need to transfer all this solid and wash the solid you have collected with a small volume of cold acetone.

17. Add 2 mL of cold acetone to the RB flask and transfer using your pipette to the funnel which should still be under vacuum. If necessary repeat the process once more.

18. Leave air being drawn through your sample for 1 minute and then disconnect tubing and turn off the vacuum supply.

19. Remove a small sample of the solid and put in a small sample vial before dissolving in two or three drops of acetone (you must make a concentrated solution here).

20. Dilute the filtrate with acetone (approximately 5 mL)

21. Check the thin layer chromatography of the solutions from 19 and 20 above. You need to prepare two plates both with 2 samples (19, 20). Each one needs to be visualised under UV light and then one plate dipped in potassium permanganate (purple) and one in phosphomolybdic acid (green). Ask a demonstrator for help analysing your results

22. Weigh a 7 mL screw cap vial (WITHOUT the lid) and then transfer the solid into this weighed flask very carefully – you do not want to lose any of your compound.

23. Weigh flask and contents together. Work out the weight of trimyristin that you have isolated. Bearing in mind that you started with 2.50g of nutmeg you will be able to work out the % of nutmeg that is made up of trimyristin.

24. Conduct a melting point experiment on your compound **– pure trimyristin has a melting point of 56-57ºC**

25. Collect an infrared spectrum on your compound and compare it with data for the real material

**Information sheet**

**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 the volatile (or low boiling) ones. In addition, the nut is made up of much less volatile compounds inclusive of complex organic polymers that give nutmeg its woody nature. One of the non-volatiles is trimyristin, a triglyceride which is partly responsible for the smell, 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 given that diets high in saturated fat have been shown to lead to atherosclerosis, high blood pressure and heart disease. Its fatty characteristics also mean that the compound has potential as lubricant additive in tablet preparation and as additives 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 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 representing an untapped natural resource, the role of a natural product chemist 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 lo- to high-tech. The techniques that you are going to use are solvent extraction, filtration and rotary evaporation to isolate the mixture of compounds containing trimyristin. You will follow this by crystallisation and vacuum filtration and vacuum desiccation that will be used during the purification to isolate trimyristin as a single compound. Finally, and subject to the time available, you will analyse the structure of pure compound you have isolated to prove its purity and structure. You will look at thin layer chromatography (TLC), melting point determination, and infrared IR spectroscopy. Although you will not have time to collect your own data in full we will try and show you the mass spectrometer (MS) and also the nuclear magnetic resonance (NMR) spectrometer.