|  |  |  |
| --- | --- | --- |
| **Foundation Year** | **Extraction of a Natural Product** | **Semester 2** |

**Extraction of a Natural Product**

**Aim**

To extract a mixture of compounds into a particular solvent, to separate the components of the mixture into separate chemical entities, and to analyse one of the compounds to determine its structure.

**Introduction**

The spice nutmeg is commonly used to add flavour to savoury dishes including potato dishes, curries, and many puddings, for example in custard tarts (the brown dusting of powder on top). Nutmeg contains many chemicals, some volatile, which contribute but its flavour and smell. However, the nut itself is made up of much less volatile compounds, including complex organic polymers that give nutmeg its woody nature. One of the non-volatile compounds is trimyristin, a triglyceride, which is partly responsible for the smell, the taste, and the 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, high blood pressure, and heart disease.

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

**Skills associated with this practical**

|  |  |
| --- | --- |
| **Practical Skills**   * Solvent extraction * Gravity filtration * Rotary evaporation * Crystallisation * Vacuum filtration * Thin Layer Chromatography (TLC) * Melting point determination * Infra-red (IR) spectroscopy * Making observations | **Scientific Skills**   * Rf calculations * Analysis of melting point data * Interpreting IR spectra |

**Signposts**

Chemistry, Conoley & Hills, 3rd Edition, Chapter 11, Section 3 and Section 5.

**Understanding Hazard and Minimising Risk**

You must stand up throughout the practical, and safety glasses must be worn at ALL times in the lab. You must wear a labcoat whilst you are carrying out ALL practical work. Long hair must be tied back, and trousers (jeans are OK) must be worn. Open-toed shoes and clothing revealing bare skin are not permitted.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Substance | Amount | Hazards | Minimising Hazards | Disposal / Spillage |
| Acetone | <25 cm3 | Highly flammable, irritant | Wear gloves, **use in fumehood** | Pour into unchlorinated solvent waste |
| Diethyl ether | 25 cm3 | Highly flammable, irritant | Wear gloves, **use in fumehood** | Pour into unchlorinated solvent waste |
| Light petroleum ether | <10 cm3 | Highly flammable, irritant | Wear gloves, **use in fumehood** | Pour into unchlorinated solvent waste |
| Potassium permanganate solution (KMnO4(aq)) | <50 cm3 | Highly oxidising, irritant | Wear gloves | Clean up with tissue, seek advice from a demonstrator |

**Procedure**

Apparatus

PER PAIR: 100mL round-bottomed flask Hirsch funnel

Side-arm vacuum flask TLC tank and lid

Filter funnel Filter papers

Magnetic stirrer plate and bar Ice bucket

Measuring cylinder Pasteur pipette

Tweezers Plastic vial + lid

Stopper Water bath

Method

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 round-bottomed (RB) flask.

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

2. Add the extraction solvent, diethyl ether (25 mL) (sometimes labelled ‘ether’), using a measuring cylinder.

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 (**PHOTO**). 

***Q***

4. 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 (**PHOTO**). 

***Q***

8. Check your water bath (in fume hood) has achieved a gentle boil.

**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 round-bottomed flask containing your crude product using a clamp, and add, using a Pasteur pipette, a small volume (half a pipette full) of propan-2-one, 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, secure the clamp to a retort stand, 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 (**PHOTO SHOWING YOUR CRYSTALS**).

12. To prepare for the next step, you need to chill about 10-15 mL of acetone in your measuring cylinder, using ice (plastic ice bucket). When crystallisation is complete, you need to separate the solid material from the solution (yellow) 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 in to the funnel. If there is no solvent left, add a little cold acetone first, then swirl and pour (**PHOTO**).

***Q***

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. 

16. Leave air being drawn through your sample for 1 minute 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. Weigh the vial again to determine the mass of product isolated and vial.

**At this point, you should ask a 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 at step 27, and 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:** <http://youtu.be/jhWc5jFzNS0>

18. Collect equipment from the central TLC station. You will need two small sample vials, two TLC plates and two 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.

20. Dissolve a small amount of your pure sample in TLC eluent (20% light petroleum ether in diethyl ether; 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 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 height 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” (**PHOTO**).

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, use tissues to remove drips of excess solution, and then dry the plate using a 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 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 Rf vaue should be between 0 and 1 (**TAKE A PHOTO OF YOUR WORKINGS FOR THE Rf CALCULATION FOR YOUR SKILLS PORTFOLIO**).

**Melting Point Analysis**

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

**Infra Red (IR) Spectroscopy**

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**.

**Deadlines, Assessment and Feedback on Performance**

You are required to complete the *Skills Portfolio* document associated with this practical. This should be completed electronically with all photos inserted in the appropriate places and accompanying text typed in. The submission deadline for *Skills Portfolio*s will normally be midnight on the Sunday following the practical, although you will be given specific guidance during the practical session. Submission is via the e-submission system Turnitin which you will be able to access in the appropriate folder in the Laboratories and Coursework Blackboard course.

***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*

***Qq***

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