Experiment 4 – Preparation of Fruity Fragrances

Learning Objectives

- Perform and understand Fischer esterification reactions
- Apply acid-base extraction in the reaction work-up
- Critical analysis of liquid-liquid extraction technique
- Observe and interpret hydroxamic acid tests for esters
- Interpret infrared (IR) spectra of starting materials and product to determine reaction success
- Predict and interpret ^{1}H NMR spectra of synthetic banana and apple oils

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* Please find "How to Prepare for Lab & Assignments" after the procedure in this doc.

Background: Esterification Reactions

Esters encompass a large family of organic compounds with broad applications in medicine, biology, and industry. Esters are represented by the structure R(C=O)OR', in which R and R' are alkyl or aryl groups. Esters are widespread in nature, occurring naturally in plants and animals. Small esters, in combination with other volatile compounds, produce the pleasant aroma of fruits. A symphony of chemicals is typically responsible for specific fruity fragrances, however, often one single compound plays the leading role. For example, artificial pineapple flavor contains more than twenty ingredients but ethyl butyrate is the major component. Examples of ester flavors and fragrances are shown in **Figure 1**. In contrast to previous experiments where students isolated compounds from plants, in this experiment, students will synthesize these compounds in the lab.

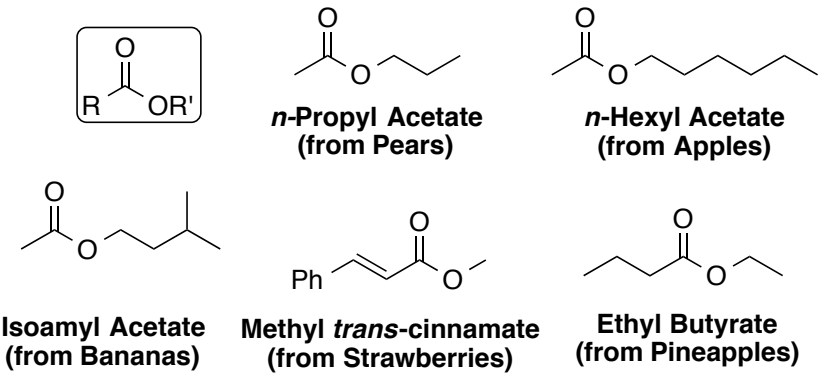


Figure 1. Examples of esters found in essential fruit oils.

Esters are carboxylic acid derivatives commonly synthesized by Fischer esterification (**Figure 2**). A carboxylic acid is reacted with an alcohol in the presence of catalytic amounts of mineral acids such as sulfuric or hydrochloric acids under refluxing conditions (heat to boiling). This reaction is reversible and thus is limited by its equilibrium constant and dictated by Le Chatelier's Principle. A large excess of either reactant pushes the equilibrium to favor products, thus increasing the yield. Constantly removing the product will also increase the yield, though this is not always possible or practical. A Fischer esterification is only recommended with primary and secondary alcohols and unhindered carboxylic acids. Steric hindrance near the reaction center slows down the esterification.

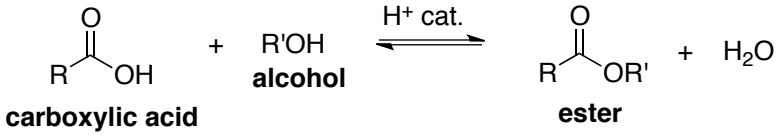


Figure 2. General scheme for a Fischer esterification reaction.

In this experiment, students prepare either banana oil from acetic acid and isoamyl alcohol or prepare sour apple oil from acetic acid and *n*-hexanol (**Figure 3**). Incidentally, isoamyl acetate is also the alarm pheromone of the honeybee and thus, it should be kept away from beehives! The reaction is performed at the microscale level using a Fischer esterification under refluxing conditions. A round-bottom flask is topped with a water-cooled condenser. The contents of the flask are heated to the boiling. Vapors travel up and inside the reflux condenser, where they condense back to a liquid and fall back into the round-bottom flask. This allows the system to remain open while heating, but without losing any reaction components. This particular reaction is run "neat" or without solvent. The acid and alcohol are both liquids and act as the solvent. It is important that the reaction flask does not run dry and that *cold water* be running through the condenser at all times.

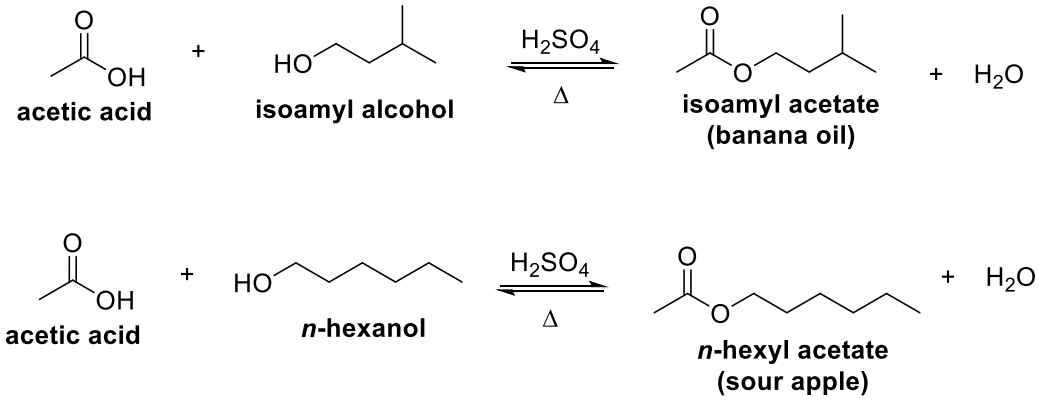


Figure 3. Reaction schemes for fruity fragrance synthesis *via* Fischer esterification

An acid-base extraction is performed with an aqueous bicarbonate (baking soda, $NaHCO_3$) solution to separate the ester from the unreacted acetic and sulfuric acid. This weakly basic solution also contains NaCl to improves phase separation in liquid-liquid extractions. This $NaHCO_3$-NaCl solution has a high ionic strength and draws residual water out of the organic layer. No additional organic solvent is necessary for the acid-base extraction because these esters are liquids and separate from the aqueous solution as an immiscible layer.

Time and quantity permitting, the ester product may be purified by microscale distillation using a Hickman still and a water-cooled condenser. Acid-base extraction is not an applicable method for separation of alcohol from ester, neither of which are acidic or basic! Column chromatography would be effective for separation since the alcohol and ester have very different polarities. The product is analyzed by IR and the hydroxamic acid test for esters and with comparison to alcohol starting materials. ^{1}H NMR spectra of banana and apple oil are provided for analysis.

PROCEDURE

Procedure Diagrams must be complete in your lab notebook before you can start the lab (see worksheet)

The statements in quotes are provided to give you guidance in writing the experimental methods section. One well-written sentence can explain an entire paragraph's worth of information!

1. Reaction Preparation and Set-Up

"To a 15-mL RBF was added…[chemical names (mmol, mL)]…and heated to reflux for 1 hour."

Pre-heat a sand bath on a hot plate at a medium setting. You may set this up as soon as you enter the lab, before the TA's pre-lab talk. Dispense 10 mmoles of the desired alcohol (isoamyl alcohol or *n*-hexanol) and 40 mmoles of glacial acetic acid into a 15-mL round-bottom flask (RBF) using a glass pipet and pluringe. Convert the *mmole quantities into volume (mL) before lab.* Add 3 drops of sulfuric acid and magnetic stir bar then attach a microscale water-jacketed condenser (figure on next page). Be sure the water is running through the condenser and reaction is stirring before heating. Heat to reflux with stirring in the sand bath and allow the reaction to reflux for one hour.

2. Reaction Work-up

"The reaction was quenched and washed with…"

Carefully lift the apparatus from the heat and allow the mixture to cool to ambient temperature. Disassemble the apparatus and turn off the water (clamp), but keep the hoses attached. Do not wash the condenser at this stage, as it may be used later. Transfer the liquid to a 16 x 125 screw-cap test tube with a pipet. Rinse the RBF with 2 mL of 5% $NaHCO_3$ in 15% NaCl solution. Slowly transfer the rinse to the screw-cap test tube. Stir the mixture with a microspatula until gas evolution (carbon dioxide) has subsided. Cap the tube and invert it several times to mix the layers. Frequently vent the system to release the pressure by momentarily unscrewing the cap. Let the system settle for about 10 minutes.

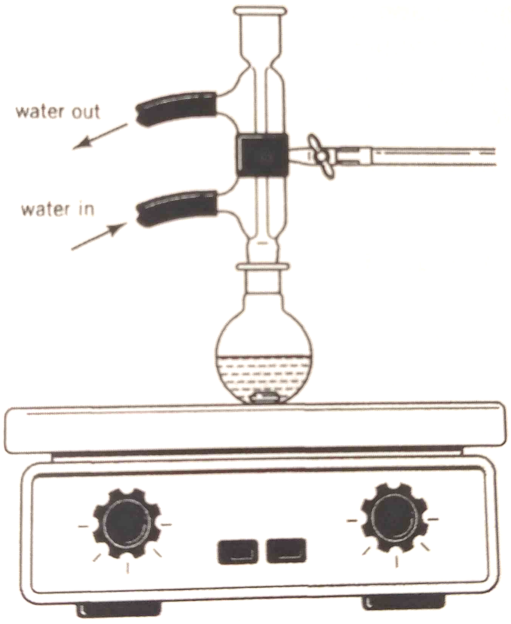
Use a pipet to transfer the lower aqueous layer to a labeled test tube. Keep this until the end of the experiment then discard it. Wash the organic layer remaining in the test tube twice with 1 mL of the $NaHCO_3$-NaCl solution. Invert and vent well in each wash. Collect the aqueous washes in the same labeled test tube as before.

"The combined organic layers were dried (Na_2SO_4) and filtered to afford..."

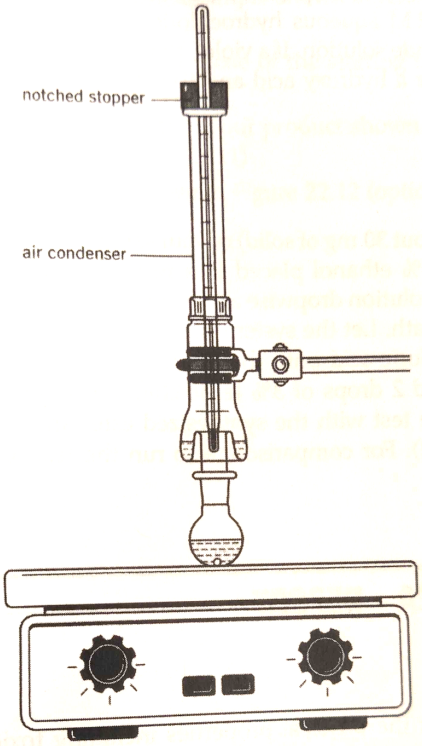
Remove any visible water from the product with a pipet. Dry the organic layer by adding a small microspatula-ful of anhydrous Na_2SO_4. Note that this drying agent is more granular than $MgSO_4$ and will create a similar but not identical snow-globe effect when sufficient drying agent is added. It may be necessary to add more, however, this may affect how much liquid can be obtained after filtration. Allow the product to dry for 5 minutes with occasional swirling.

<u>0.4 mL of crude product or more?</u> Filter using a pipet loosely packed with a small piece of cotton into a **5-mL round-bottom flask (RBF)**. Label and cap the RBF. Save in your drawer for next lab.

<u>Less than 0.4 mL of crude product?</u> Filter using a pipet loosely packed with a small piece of cotton into a **pre-weighed vial to obtain the mass of crude product**. Label the vial and save in your drawer for next lab.



Reflux apparatus with 15-mL round-bottom flask.



Microscale distillation apparatus.

3. Distillation – Day 2

"The product was purified via microscale distillation with a Hickman still to yield…"

Add a boiling chip to the RBF. Assemble a microscale distillation apparatus on top of the 5-mL RBF using a Hickman still, boiling chip, a thermometer, and an air condenser (figure above). Support the thermometer using a notched stopper resting on top of the condenser; this will make the removal of the thermometer very easy.

Distill by heating directly on a hot plate at a medium setting until two or three drops of liquid remain in the flask. Record the temperature during the distillation. If the Hickman still is full before the distillation is complete, carefully remove the thermometer and condenser and empty the still with a Pasteur pipet. Reassemble the apparatus to continue the distillation.

Transfer the distillate to a tared vial. Weigh the product. Please clean and return the shared glassware to the counter (5-mL RBF, Hickman still, and air condenser).

4. Analysis: Hydroxamic Acid Test – Day 2

"Product formation was confirmed (or not) by the hydroxamic acid test for esters."

Perform this test with starting materials (alcohol and acetic acid), ethyl acetate (ester standard), and product in four separate test tubes. Add one drop of the sample to be tested to 1 mL of 0.5 M hydroxylamine hydrochloride (NH_2OH-HCl) in 95% ethanol in a test tube. Add 0.2 mL of a 6 M NaOH solution drop-wise and a boiling chip. Bring the mixture to a boil by heating in a water bath. Let the system cool and add 2 M HCl drop-wise until the pH is 2-3. If cloudiness develops, add 2 mL of 95% ethanol. Add 2 drops of 3% ferric chloride solution. A red-violet color is a positive test.

4. Analysis: IR spectroscopy – Day 2

"The [crude or purified] product was analyzed by IR."

Analyze the IR spectrum of the alcohol (provided in lab, posted on Canvas). Obtain the IR spectrum of your product using NaCl plates and identify ester peaks. *Is there an OH peak in the product?*

Interpret the ^{1}H NMR spectra of both ester products (may be provided in lab, also posted on Canvas). Assign every hydrogen on the structure to a signal on the spectrum (integration, splitting, and expected & observed chemical shift).

Table 1. Clean-up & Safety

Clean-up	**Safety**
Liquid waste: aqueous layers and solutions from chemical test	H_2SO_4, HCl, hydroxamic acid, NaOH, and acetic acid are *corrosive*
Solid waste: Na_2SO_4, pipets, filter pipets	Acetic acid, ethyl acetate, and ethanol are *flammable*
After analysis, dispose of your product in the liquid waste using a *very small amount* of ethanol from a wash bottle to aid the transfer.	Isoamyl alcohol is an *irritant*
Wash all glassware and wipe down counters; return shared glassware to reagent counter	Wear gloves & goggles throughout the experiment.
Clean IR plates with acetone saturated with NaCl. Return plates to the desiccator after use.	Be careful not to burn or melt the water hoses on the hotplates!

All used pipets & broken glass go in the glass waste box.
Please do not throw away glass in the trash as it creates an unexpected occupational hazard for our custodial staff.

Thank you for participating in community set up & clean up tasks ☺

References and Supplemental Reading

Palleros, D. R. "Preparation of Fruity Fragrances," *Experimental Organic Chemistry*, **2000**. Wiley: Hoboken. p. 473 – 485.

Mohrig 4th edition: Chapter 7.1 (Reflux), 22.7-11 (NMR)

Klein 2nd edition: Chapter 15.1-6 (NMR), 20.10 & 20.15 (Fischer esterification)

McMurry 8th edition: Chapter 13.11 (^{1}H NMR splitting), 21.3 (Fischer Esterification), & 21.10 (^{1}H NMR of esters)

How to Prepare for Lab + Assignments

Follow Canvas Exp 4 Module…

Before Lab

- Read this PDF – background, procedure, safety, pre-lab and in-lab questions
  - Option: listen to Caitlin read this document in the **8M Exp 4 Podcast**
- Attend and/or watch **lab lecture** – we go over everything you need for your assignments!
- Practice the lab online *via* **Slugs@home platform** - sites.google.com/ucsc.edu/slugshome/home
- Complete the **pre-lab questions** at the end of this doc - incorporated into Canvas quiz ☺
  - **Quiz** due before your enrolled section – check Canvas for due date
- Download the **Exp 4 worksheet** and prepare your **lab notebook**…

Lab Notebook Preparation – *worksheet = template / outline to copy by hand into lab notebook*

- **Purpose:** one-sentence summary of the main lab goals plus the reaction schemes
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical properties!
- **Procedure with Diagrams** – complete before starting lab; sample on Canvas
  - Use the procedure on the previous pages to create your hand-drawn experimental instructions
    - Simple sketches & labels for all **equipment, chemical names** with **amounts**, & **transfers**
  - Format: Break it up with flow charts, bullet-points, comic strip, and/or whatever works for you!
    - Avoid copying the procedure word-for-word.
    - Make it easy for anyone to follow your procedure without referring to this document.
  - **Slugs@home Exp 4 website** - Equipment & Safety pages; pictures & videos of the whole lab
  - The **class notes** include useful diagrams as well

During Lab

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- **Pre-lab talk:** tips for success and open Q&A
- Show your **lab notebook pages** to your TA
- Perform the experiment with a partner, fill out data & observations in **lab notebook**

After Lab – each partner submits separate, individual assignments

- Upload **Notebook Pages** to Canvas by midnight on lab day – graded on completeness / participation
- Complete & upload the **Lab Report** on GradeScope (GS) – due date on Canvas
  - In-lab questions & experimental methods – see last page of this document

Pre-lab Questions / Quiz – see your class notes!

1. Why is the reaction mixture extracted with **sodium bicarbonate ($NaHCO_3$)** and **sodium chloride (NaCl)** solution? What role does each salt play?

2. Calculate the **mass (mg)** and **volume (mL)** of alcohols and acetic acid that will be mixed from the mmol given in the procedure. Include these values in the reagent table in your notebook.

3. Determine the **limiting reagent** in the reactions. Calculate the **theoretical yield** of both syntheses in mg. Recall that catalysts cannot be the limiting reagent.

4. How is **Le Chatelier's Principle** on equilibrium used to increase the success of the esterification reaction?

5. Based on the techniques you have learned thus far in the organic chemistry lab, what are two methods that could be used to **separate unreacted alcohol from the ester**? Briefly explain **why** each would be expected to work. *Hint: you learned one of the techniques earlier this quarter; the other you learned in 8L.*

Take the **Exp 4 pre-lab quiz** *before* your enrolled section – check Canvas for due date

- The quiz incorporates the questions below - the questions may be reworded.
- **Be prepared with your responses to the pre-lab questions *before* starting the quiz.**
- There is a 20-minute time limit on the quiz and you get two attempts.
    - Make sure you have enough time to complete the quiz - you can't save and come back later.
    - If you choose to re-take the quiz, your grade will be the highest of the two attempts.

Though we encourage collaboration in this class, this is an individual quiz.

- The responses should be a product of your original work so that you are assessed on *your* understanding of the material.

Sharing your quiz or your responses in any format (screenshots, email, CHEGG, social media, text, carrier pigeon, etc.) is in violation of the UCSC academic integrity policy.

LAB REPORT

Canvas > **Experiment 4 Report** for submission details

Upload to GradeScope (GS) after both parts of the lab – see due date on Canvas

- **Select Pages** to correlate your responses to the GS outline ☺

A. In-Lab Questions – see your lecture notes!

1. Show the full arrow-pushing **mechanism** for the assigned ester (apple or banana), including charged reaction intermediates to account for all bonds broken and formed.

2. Draw the chemical reactions that **sodium bicarbonate** facilitates in the reaction workup. The equations should include the gas formed. Note: sodium bicarbonate participates in two different (though similar) reactions.

3. Report the **yield (mg)** and calculate the **percent yield** of the assigned synthesis. State whether or not the product was purified via **distillation**. Discuss 2-3 suspected sources of **product loss** (exact parts of the procedure, such as transfers between containers, when you most likely lost product).

4. Interpret the **IR of the assigned alcohol and product**. Include the functional group, bond, expected stretch, and observed stretch. Briefly discuss how you know the reaction went to completion (or not).

5. Report and interpret the **hydroxamic acid test** results. Draw the **chemical reaction** that occurred with your product – no abbreviations. What do the results suggest about the **success** of the experiment?

6. Interpret the **^{1}H NMR spectra** provided in lecture for *both* **(a)** banana oil and **(b)** sour apple. Spectra provided in lecture notes and posted online. Caitlin made supplemental videos on NMR interpretation of these esters - linked in the Canvas assignment for this report.

- Use the table format in the worksheet and add as many rows as needed to analyze each signal.
- Create separate typed **tables** for each ester and be sure to draw the **structures with each set of H's labeled** (A, B, C, etc.).

B. Experimental Methods

Writing guidelines and **sample experimental methods** are available on Canvas. Remember the sample experimental contains way more information than is pertinent to CHEM 8M students!

Use the **bold headings within the procedure** to get an idea of the level of detail to include in the experimental methods section (you may use those exact words!). You will need to **fill in your own data and descriptions** in place of "…"

Simply report whether the "presence of an ester was confirmed by the **hydroxamic acid test**" (no procedural details). **IR** is the only form of characterization to report, as you are not directly analyzing your sample by NMR.