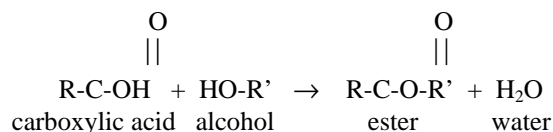


ESTERS: THE PREPARATION AND IDENTIFICATION OF AN ARTIFICIAL FOOD FLAVOR

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INTRODUCTION

The odors of flowers and the odors and flavors of fresh fruits are a result of a complex mixture of many chemical compounds, but one of the major constituents is a type of compound called an ester. (See Table 1) An ester is a compound formed from the reaction between a carboxylic acid and an alcohol. The generalized reaction is:



Carboxylic acids (or organic acids) commonly occur in nature. Most low molecular weight carboxylic acids have unpleasant odors, but higher molecular weight acids tend to be odorless. Fats, such as butter and margarine, contain carboxylic acids, with lactic acid, $\text{CH}_3\text{CHOHCOOH}$, being one that contributes to their characteristic odor and flavor. When butter becomes rancid, the odor is caused by the formation of butyric acid (butanoic acid), $\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$, the same compound that is responsible for the odor of sweat.

When a carboxylic acid reacts with an alcohol the product is often a sweet smelling compound called an ester. Esters find wide applications as flavors or fragrances used to enhance foods and beverages. A food technologist, may utilize a natural ester or a mixture of esters to produce characteristic flavors and fragrances in a food. In some cases, the mixture of esters used to approximate a flavor may not actually duplicate that in the natural food, but most people can be fooled. Only a professional taster, a trained person with a high degree of gustatory perception, can tell the difference between the natural and artificial flavors and fragrances.

Although esters find great applications in foods and beverages, they are not used in perfumes that are applied to the body. Esters, generally, are not stable to perspiration and tend to break down forming carboxylic acids which have unpleasant odors. Only the very inexpensive toilet waters use esters while the more expensive perfumes use essential oils that are usually extracted from natural sources.

SAFETY

Sulfuric acid is corrosive. Clean up any spills immediately. In the event of skin contact, wash the affected area well with cold water. If necessary, call your instructor for assistance.

Acetic acid and butyric acids are corrosive. Clean up any spills immediately. In the event of skin contact, wash the affected area well with cold water. If necessary, call your instructor for assistance.

Butyric acid and acetic acids have strong, unpleasant odors. Work with these compounds in the hood.

Organic liquids are flammable. Keep them away from flames or sparks.

DISPOSAL

Dispose of all waste materials in the proper containers, available in the laboratory.

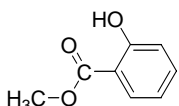
Formula	Common name	IUPAC name	Flavor/odor
$\begin{array}{c} \text{O} \\ \\ \text{HC-O-CH}_2\text{-CH}_3 \end{array}$	ethyl formate	ethyl methanoate	rum
$\begin{array}{c} \text{O} \\ \\ \text{H}_3\text{C-C-O-CH}_2\text{-(CH}_2\text{)}_3\text{-CH}_3 \end{array}$	n-amyl acetate	pentyl ethanoate	pears, bananas
$\begin{array}{c} \text{O} \\ \\ \text{H}_3\text{C-C-O-CH}_2\text{-CH}_2\text{-CH(CH}_3\text{)}_2 \end{array}$	isoamyl acetate	3-methylbutyl ethanoate	pears, bananas
$\begin{array}{c} \text{O} \\ \\ \text{H}_3\text{C-C-O-CH}_2\text{-(CH}_2\text{)}_6\text{-CH}_3 \end{array}$	n-octyl acetate	octyl ethanoate	oranges
$\begin{array}{c} \text{O} \\ \\ \text{H}_3\text{C-CH}_2\text{-C-O-CH}_2\text{-CH(CH}_3\text{)}_2 \end{array}$	isobutyl propionate	2-methylpropyl propanoate	rum
$\begin{array}{c} \text{O} \\ \\ \text{H}_3\text{C-CH}_2\text{-CH}_2\text{-C-O-CH}_3 \end{array}$	methyl butyrate	methyl butanoate	apples
$\begin{array}{c} \text{O} \\ \\ \text{H}_3\text{C-CH}_2\text{-CH}_2\text{-C-O-CH}_2\text{-CH}_3 \end{array}$	ethyl butyrate	ethyl butanoate	pineapples
$\begin{array}{c} \text{O} \\ \\ \text{H}_3\text{C-CH}_2\text{-CH}_2\text{-C-O-CH}_2\text{-(CH}_2\text{)}_2\text{-CH}_3 \end{array}$	n-butyl butyrate	butyl butanoate	pineapples
$\begin{array}{c} \text{O} \\ \\ \text{H}_3\text{C-CH}_2\text{-CH}_2\text{-C-O-CH}_2\text{-(CH}_2\text{)}_3\text{-CH}_3 \end{array}$	n-amyl butyrate	pentyl butanoate	apricots
$\begin{array}{c} \text{O} \\ \\ \text{H}_3\text{C-(CH}_2\text{)}_3\text{-C-O-CH}_2\text{-CH}_2\text{-CH(CH}_3\text{)}_2 \end{array}$	isoamyl valerate	3-methylbutyl butanoate	apples
	methyl salicylate	methyl 2-hydroxybenzoate	oil of wintergreen

Table 1. Some common esters used as flavoring agents.

Materials Needed

Carboxylic acid (See Table 2)
Alcohol (See Table 2)
Sulfuric acid, concentrated
Sodium sulfate, anhydrous
Acetone
100-mL ground glass neck round bottom distilling flask
distilling head to fit distilling flask
condenser
thermometer adapter for distillation apparatus
rubber tubing for condenser
125-mL Erlenmeyer flask
beakers, 250-mL, 400-mL, 600-mL
Separatory funnel, 125-mL or 250-mL
Funnel
Test tubes, 6 - 15 mm or 18 mm diameter
Test tube rack
Corks to fit test tubes
Thermometer, 250°C
Graduated cylinder 25-mL or 50-mL
Spatula
Glass wool
Ring support for separatory funnel
Ring stand
Bunsen burner or heating mantle
Clamps
Boiling chips
labels
Disposable IR card, PTFE substrate

Ester name	Acid needed	Amount of Acid needed	Alcohol needed	Amount of alcohol needed	Boiling point of ester
n-amyl acetate	acetic acid (glacial)	23 mL (0.4 mole)	n-amyl alcohol (n-pentyl alcohol)	21.6 mL (0.2 mole)	142°C
n-octyl acetate	acetic acid (glacial)	23 mL (0.4 mole)	n-octyl alcohol	31.5 mL (0.2 mole)	199°C
methyl butyrate	butyric acid	18.4 mL (0.2 mole)	methyl alcohol	16.2 mL (0.4 mole)	102°C
ethyl butyrate	butyric acid	18.4 mL (0.2 mole)	ethyl alcohol	23.3 mL (0.4 mole)	121.6°C
n-butyl butyrate	butyric acid	18.4 mL (0.2 mole)	n-butyl alcohol	36.6 mL (0.4 mole)	156.9°C
n-amyl butyrate	butyric acid	18.4 mL (0.2 mole)	n-amyl alcohol (n-pentyl alcohol)	43.2 mL (0.4 mole)	179°C
methyl salicylate	salicylic acid	6.9 g (0.05 mole)	methyl alcohol	30.0 mL (0.75 mole)	223°C

Table 2. Quantities of acids and alcohols needed for preparations with boiling points of esters.

PROCEDURE

Obtain a 100-mL ground-glass-neck round bottom flask, a condenser, and a ground-glass distilling head.

The apparatus should be clean and dry. If not, take the time to clean the apparatus.

Assemble the apparatus for a *reflux* setup as shown in Figure 2 on the right side of this page. Start by clamping the flask in place, then attach the condenser and clamp it loosely to avoid a strain on the glass condenser-to-flask connection. Have your instructor check your set-up before proceeding.

Moisten the tubing connections on the condenser with some water and attach the rubber condenser tubing. Note that the water should flow in to the bottom of the condenser and out of the top into a sink.

Remove the condenser from the apparatus and lay it in a safe place as you obtain your reagents for your preparation.

Measure the amount of organic acid required for your preparation, as noted in Table 2. If the acid is a liquid, measure it in a clean, dry graduated cylinder. If it is a solid, weigh the necessary amount in a plastic weighing dish. *Carefully* note the odor of the acid. Describe the odor in your notebook or on your data page.

Using a clean, dry graduated cylinder, measure 5 mL of concentrated sulfuric acid. *Carefully*, pour the sulfuric acid into the dry 50 mL ground-glass round bottom flask. Take care to prevent the acid from coming in contact with the ground-glass surface of the neck. (The use of a funnel will prevent chemicals from coming in contact with the neck of the flask.) (See Figure 3) Do not rinse the graduated cylinder from the sulfuric acid.

Using the graduated cylinder from the sulfuric acid, measure the volume of alcohol as noted in Table 2. (NOTE: If the alcohol is a solid, weigh the necessary amount in a plastic weighing dish.) *Carefully* note the odor of the alcohol from its original container (not the graduated cylinder). Describe the odor in your notebook or on your data page.

Add the alcohol to the flask containing the acid.

Add two or three boiling chips to the flask.

Very lightly grease the “male” joint of the condenser with a thin film of silicone grease. (Your instructor will demonstrate how to do this.)

Secure the condenser in place with a clamp, but take care not to place any strain on the apparatus. Attach the tubing from the lower condenser tube to the water tap and the tubing from the upper condenser tube into a sink.

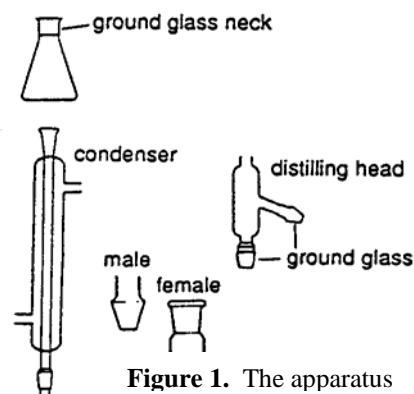


Figure 1. The apparatus

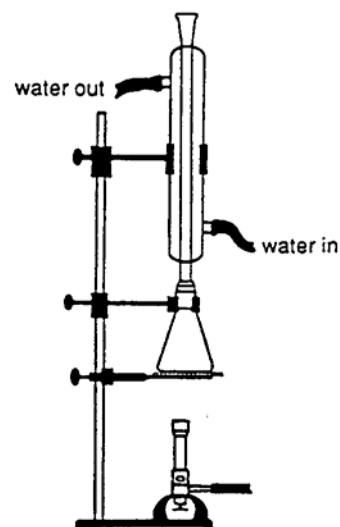


Figure 2. A reflux set-up

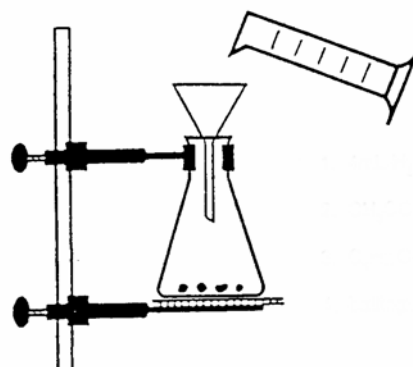


Figure 3. Pouring the sulfuric acid into the 50 mL flask

Have your instructor check your apparatus before proceeding.

Turn on the water *carefully* and adjust the flow rate so that a slow, steady stream of water flows out the drain tubing.

Light the Bunsen burner (or turn on the hot plate or heating mantle) and heat the mixture in the flask to boiling. Adjust the burner flame (or heat) to achieve a slow rate of reflux (boiling liquid with condensed vapor dripping back into the flask) of about 2 drops of condensate per second. Reflux the mixture at this rate for 15 to 20 minutes.

While the mixture is refluxing, set a clean separatory funnel into a ring clamp support. Check that the stopcock is properly seated and in the closed position. Add 25 mL of distilled water to the funnel. (See Figure 4)

After the mixture has refluxed for 20 minutes, turn off the burner. Continue running water through the condenser until the liquid has stopped boiling. Then, turn off the water.

Carefully, remove the flask from the apparatus. (Make sure the condenser is securely clamped.) Cool the flask in a beaker of cold tap water.

When the flask has cooled to room temperature, pour the liquid from the flask into the separatory funnel. Rinse the empty flask with 20 mL of distilled water and add the rinse water to the separatory funnel.

Shake the mixture in the separatory funnel, venting every few shakes. Then set the separatory funnel in a ring support, on a ring stand and allow the mixture to separate. (See Figure 5)

While waiting for the mixture to separate, wash the 100-mL ground-glass-neck round bottom flask with detergent and water. Rinse well with distilled water. Rinse with a 5-mL portion of acetone. Allow the flask to dry, neck down, in the hood. (If necessary, an aspirator hose will be used to pull air through the flask to evaporate any residual acetone-water mixture.)

Assemble a distillation apparatus as shown in Figure 6, on the right. Lightly grease all ground glass joints. The apparatus should be clamped securely, but there should be no strain on the apparatus.

Have your instructor inspect your setup before continuing.

The liquids in the separatory should be separated. Drain the lower aqueous phase into a flask or beaker and discard it.

Drain the remaining organic layer into a 125-mL Erlenmeyer flask. The liquid will be cloudy due to small amounts of water mixed with it. Add two small spatula loads of anhydrous sodium sulfate, Na_2SO_4 , to the liquid in the

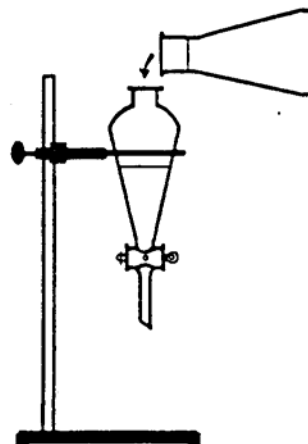


Figure 4. Adding water to the separatory funnel

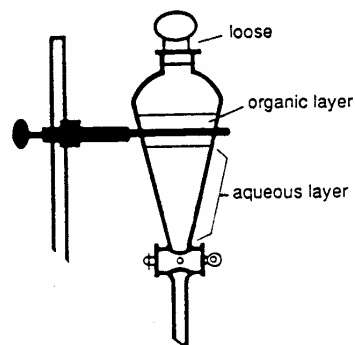


Figure 5. The separated mixture in the separatory funnel.

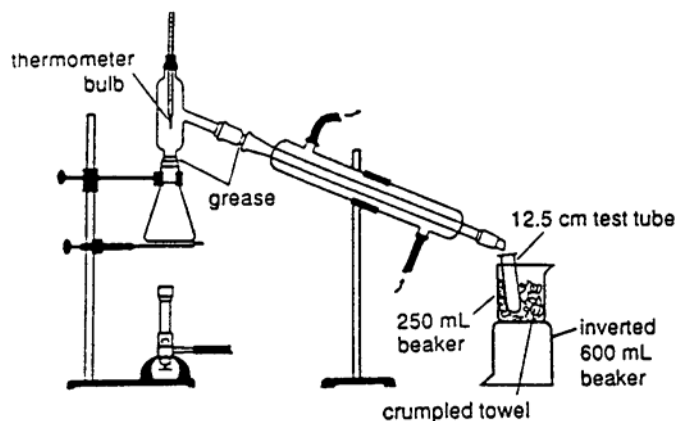


Figure 6. A distillation set-up

flask and swirl gently. The sodium sulfate will “cake up” as it absorbs moisture. Add additional sodium sulfate in two-spatula load increments, until the liquid is clear and the solid does not “cake up”.

Carefully, loosen the condenser and distilling head on the distillation apparatus and remove the 100-mL round bottom flask. Place a piece of glass wool in a funnel and place the funnel on the distillation flask. Decant the organic liquid from the 125-mL Erlenmeyer flask containing the sodium sulfate into the funnel. Be careful not to pour any of the sodium sulfate into the funnel. (See Figure 7)

Add 2 or 3 boiling chips to the round bottom flask and replace it into the distillation apparatus.

Turn on the water and adjust the water flow through the condenser to a slow, steady stream.

Check that the test tube receiver is properly held by a piece of crumpled paper towel in a position to catch drops falling from the condenser.

Have your instructor check the apparatus before you start heating.

Light the burner or turn on the power to the heating mantle and begin to heat the distilling flask.

After boiling starts you will observe a film of condensate moving gradually up the interior of the distilling head. Watch the thermometer readings carefully as the vapors approach the thermometer bulb. Record the temperature reading when the thermometer is bathed with a thin film of condensate and liquid just begins to collect inside the condenser. Adjust the heating rate so that drops fall from the condenser into the test tube at the rate of one drop every 2 to 3 seconds. Continue this heating rate and check the thermometer regularly.

When the temperature remains constant during collection of 10 to 12 drops, carefully remove the first test tube and replace it with another clean test tube.

Continue the slow heating rate as long as the temperature remains within $\pm 3^{\circ}\text{C}$ of the “constant” reading. If the temperature changes by more than about $\pm 3^{\circ}\text{C}$ or if the receiver test tube is more than $\frac{3}{4}$ full, change receivers.

Keep a record of temperature changes for all fractions collected and label the test tubes in the order used.

Continue distillation until nearly all of the ester has distilled. Under no circumstances should you heat the flask to dryness.

When distillation is complete, turn off the burner or heating mantle and the water and let the apparatus cool to room temperature.

Carefully, note the odor of the ester in the test tubes.

Use the infrared spectrophotometer to confirm the identity of the ester you prepared. Your instructor will provide additional information on this procedure.

Optional: Use the gas chromatograph to determine the purity of your ester. Your instructor will provide additional information on this procedure.

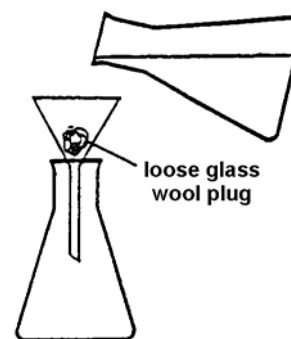


Figure 7. Filtering a solution through a glass wool plug

Report Form

ESTERS: THE PREPARATION OF ARTIFICIAL FOOD FLAVORS

Name _____

Date _____

Ester Prepared _____

Acid used _____

Quantity used _____

Describe the odor of the acid:

Alcohol used _____

Quantity used _____

Describe the odor of the alcohol:

Distillation Data:

Property	Fraction number from distillation				
	1	2	3	4	5
Boiling range °C					
Mass of fraction					
Odor					

On the basis of boiling range and odor, the fractions that appear to be pure ester are

The total mass of the fractions that are the pure ester is _____ g

How does the infrared spectrum of your ester compare with the standard spectrum?
(attach your infrared spectrograph to the report form)

Does the gas chromatograph indicate that the ester is pure?
(attach the gas chromatogram to the report form)