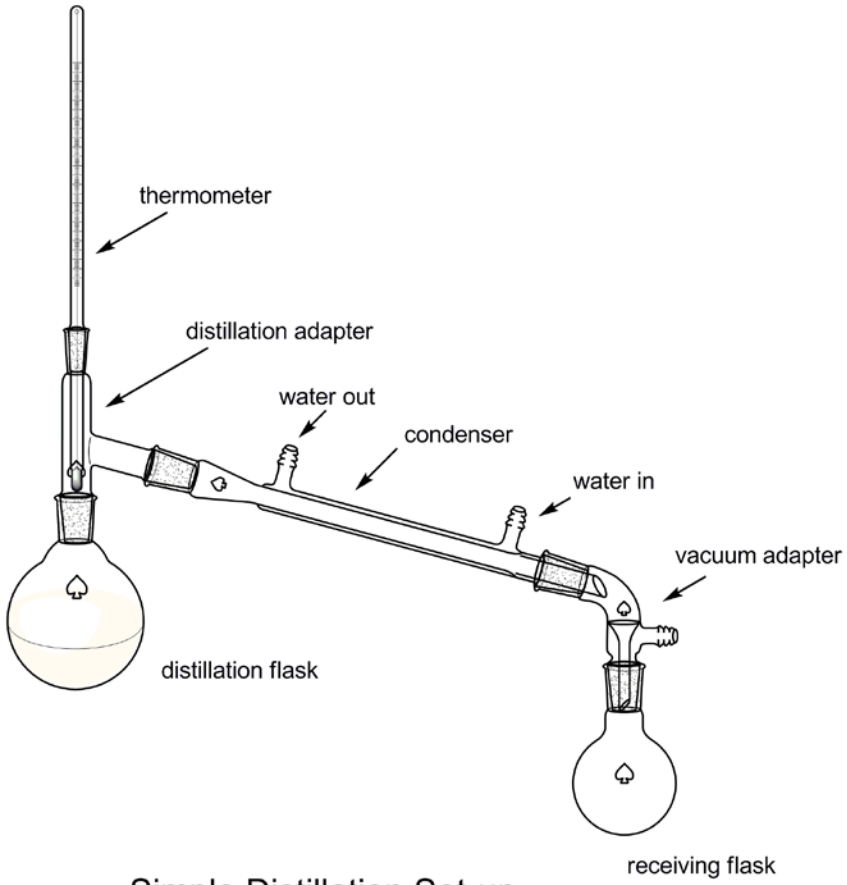
Isolation and Purification of Organic Compounds
Steam Distillation of Essential Oils

Distillation relies on the fact that the substance with the greatest vapor pressure will be enriched in the vapor phase in equilibrium with a boiling liquid phase containing a mixture of substances. The equations obtained from Rault's and Dalton's laws are provided in the Manual. Condensation of the vapor phase leads to a sample containing a greater proportion of the more volatile component.

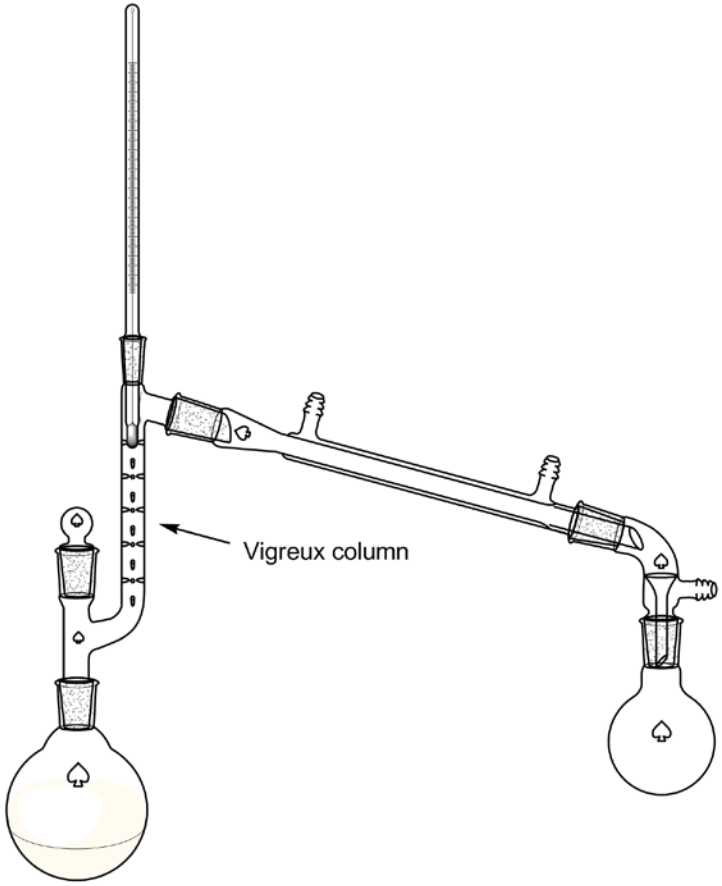
- **Simple distillation: a procedure in which the liquid-vapor equilibrium occurs once. This procedure will ordinarily lead to only partial separation of two volatile components unless one of them has much greater vapor pressure than the other. If one component of a two-component mixture has no measurable volatility this method will lead to a satisfactory separation of both components.**



Simple Distillation Set-up

Distillation methods continued

- **Fractional distillation: a technique that sets up a series of liquid-vapor equilibria, typically in a long vertical column packed with inert materials containing a large surface area. A Vigreux column with indentations along the length of the column is also commonly used. The multiple phase equilibria established in such a fractionating column may lead to efficient separation of mixtures of two volatile materials provided that there is a difference in their vapor pressures. The separation is achieved because the vapor component of each individual equilibrium becomes the liquid component for the next equilibrium established in the column. As each equilibrium is established, the vapor phase in equilibrium with the liquid phase becomes successively more enriched in the more volatile component. Fractional distillation is very important in the industrial separation of the components of crude oil.**



Fractional Distillation Set-up

Distillation methods continued

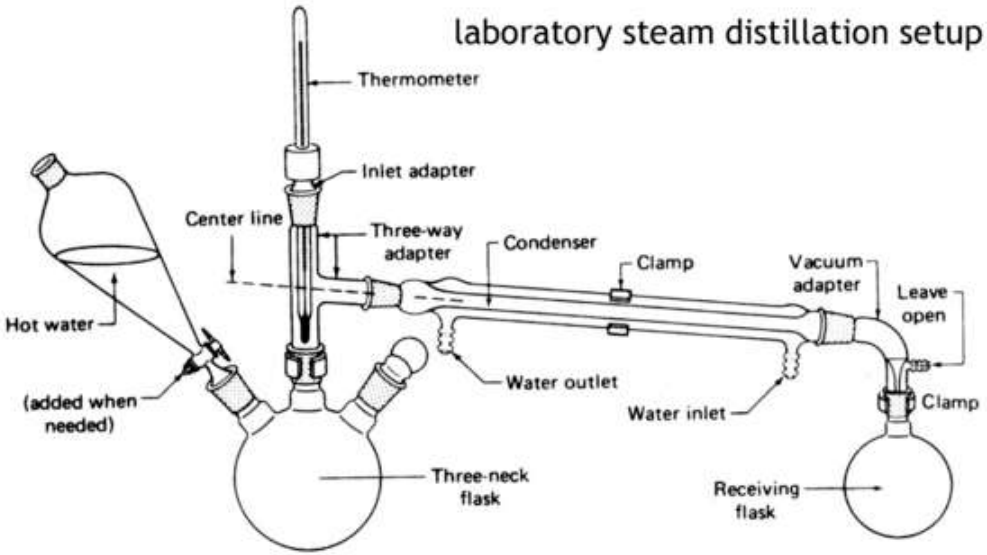
- **Vacuum distillation: If the desired compound decomposes at its atmospheric boiling point vacuum distillation can be used. The boiling point of a compound is reduced under vacuum because its vapor pressure reaches the lower pressure that occurs under vacuum at a lower temperature. Both the simple and fractional distillation set-up can be converted into a vacuum distillation by attaching a controlled vacuum source to the vacuum adapter outlet. You have already performed a version of a vacuum distillation during the rotary evaporation that isolated caffeine from EtOAc in Expt #2. In the rotary evaporation process the point is to isolate the less volatile component(s) dissolved in the volatile organic solvent. You will also make use of a rotary evaporation to isolate the essential oils in this experiment after the initial steam distillation that produces a two phase mixture of water and the oil.**

A rotary evaporator

Distillation methods continued

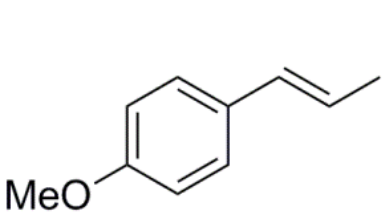
Steam Distillation: an alternative method for purifying compounds with high boiling points if they are water insoluble. The method relies on the fact that the vapor pressure above a mixture of two immiscible liquids is the sum of the vapor pressures of the two pure components. When such a mixture is heated so that the total vapor pressure equals atmospheric pressure the two liquids will co-distill. The mole fraction of each component in the vapor is governed by the vapor pressures of the pure liquids. The organic component is isolated from the water after distillation by extraction since it is water insoluble. In your experiment you will perform an extraction with CH_2Cl_2, followed by drying of the organic extract, and rotary evaporation to obtain the essential oil.

This is a common steam distillation set-up. It is designed to provide a continual supply of water from the separatory funnel for a distillation requiring more water than you will need.

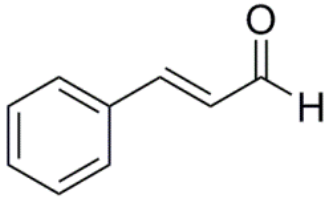


Essential oils

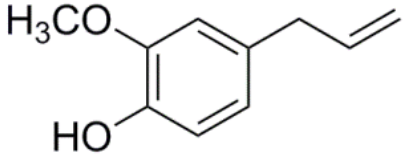
Mixtures of natural products traditionally obtained from plant sources by steam distillation methods are called essential oils. These oils have been known for centuries. They are used for food flavorings, perfumery, medicinal applications, and have even been used as insecticides. The oils are mixtures, but many of them contain 70-90% of a single component.



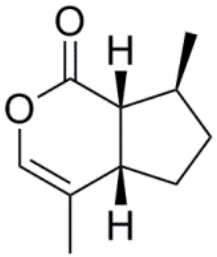
anethole
from anise seed



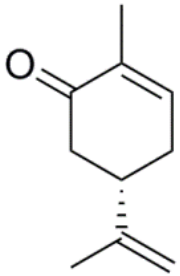
cinnamaldehyde
from cinnamon bark



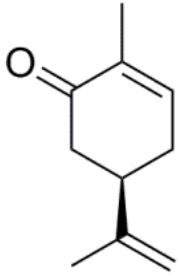
eugenol
from cloves



nepetalactone
(major diasteriomer)
from catnip



(+)-carvone
from carroway seed

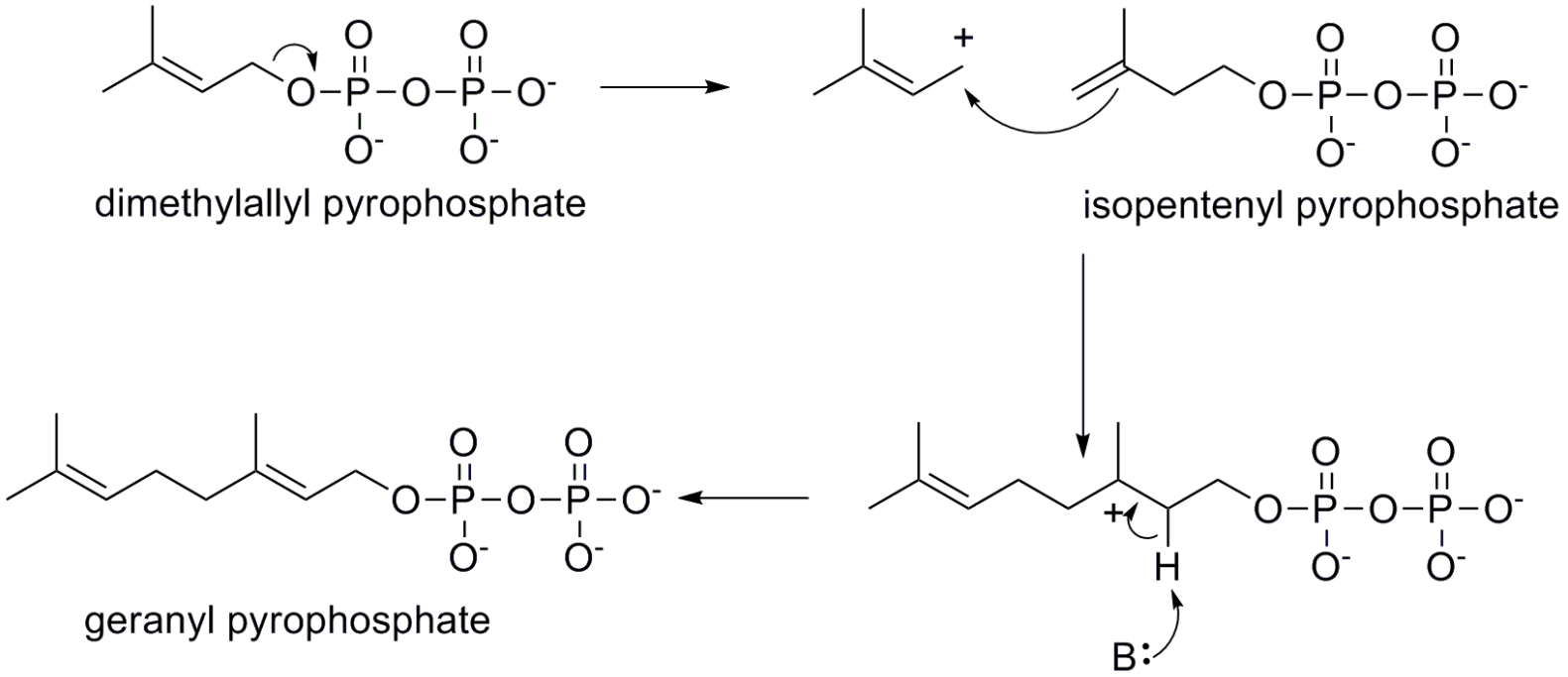


(-)-carvone
from spearmint

The major components of the six essential oils you will distill are listed above.

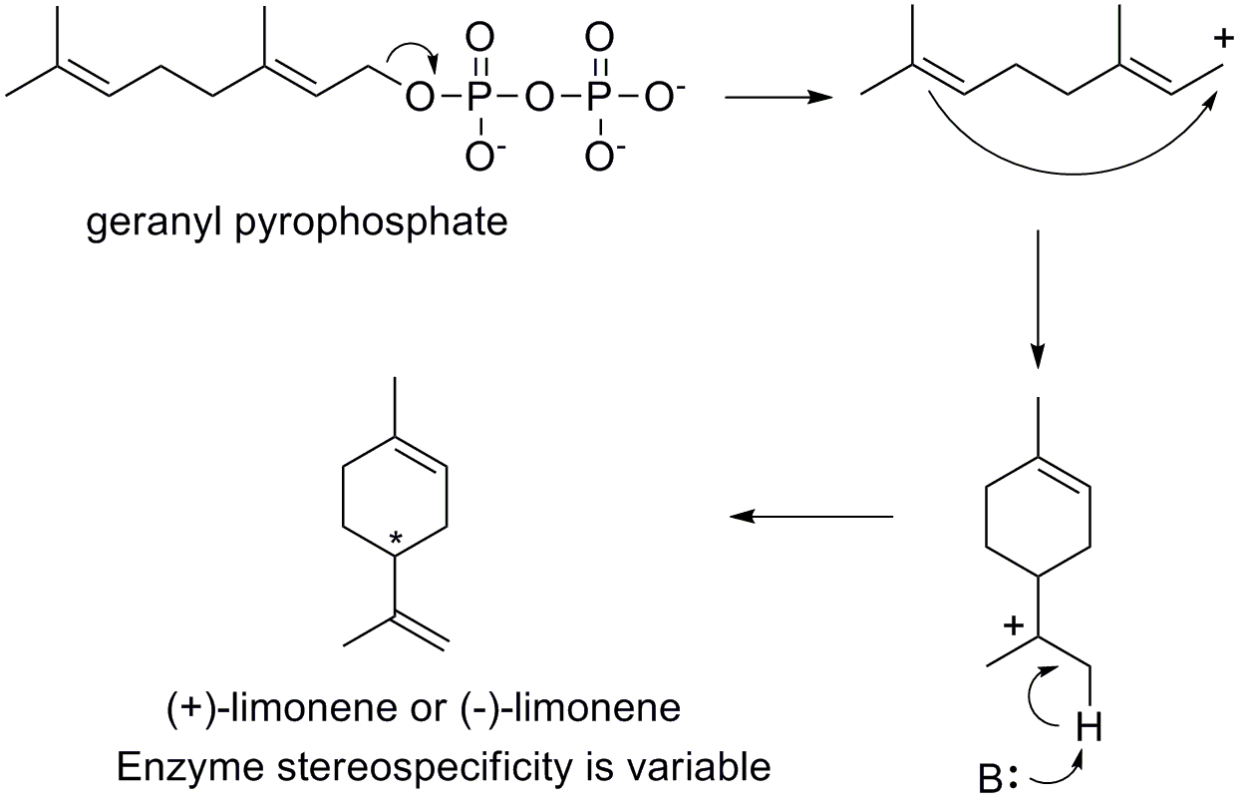
Natural Products Biosynthesis

In the Manual we mentioned that terpenes such as limonene and carvone are generated from isopentenyl pyrophosphate and dimethylallyl pyrophosphate. The biosynthesis of all monoterpenes starts with geranyl pyrophosphate that is produced by the action of the enzyme geranyl pyrophosphate synthase. The mechanism involves generation of an allylic cation from dimethylallyl pyrophosphate, nucleophilic attack of isopentenyl pyrophosphate on the cation, and β-elimination of a proton to form geranyl pyrophosphate. These are common reactions of carbocations.



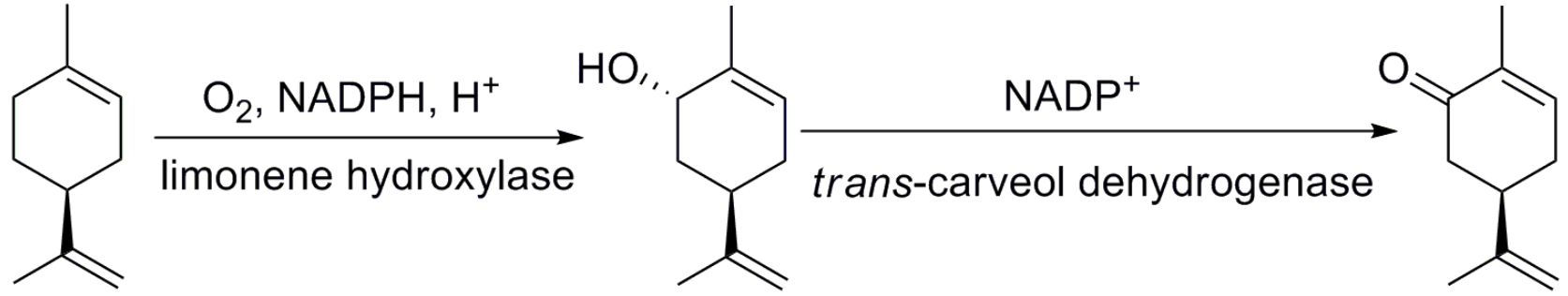
Natural Product Biosynthesis continued

Limonene biosynthesis starts with geranyl pyrophosphate. The enzymes involved are called limonene cyclases. Limonene cyclase in orange peel stereospecifically generates (+)-limonene, while the enzyme in spearmint leaves generates the (-)-enantiomer. The mechanism involves an intramolecular nucleophilic attack on the allylic cation formed by loss of pyrophosphate, followed by a β-elimination.



Natural Products Biosynthesis continued

The limonenes are converted into the corresponding carvones by the action of two enzymes: a limonene hydroxylase, and a *trans*-carveol dehydrogenase. The reactions that occur in spearmint leaves are shown below.

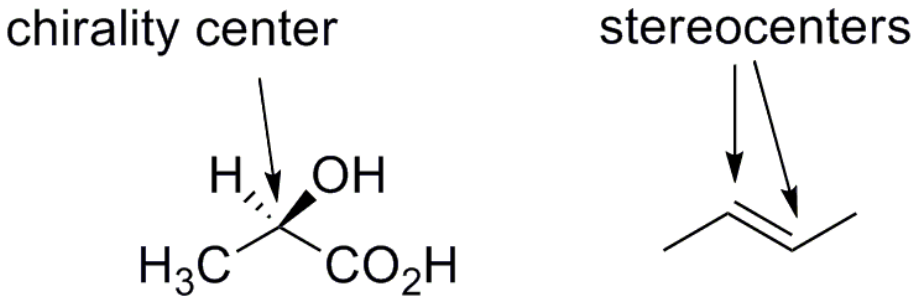


(-)-limonene (-)-*trans*-carveol (-)-carvone

The mechanism for the first reaction involves the combination of an allylic free radical and hydroxyl radical generated from the reaction of limonene with limonene hydroxylase, an enzyme that belongs to the family of cytochrome P_{450} oxygenases. The second reaction is an $NADP^+$ mediated oxidation of a secondary alcohol to generate a ketone. You have not discussed these types of reactions in CHM 241 yet, but you will get to them soon.

Stereoisomerism in natural products

Most naturally occurring organic materials have one or more stereoisomers. Stereoisomerism can arise as the result of chirality centers (chiral centers asymmetric centers), tetrahedral atoms bonded to four different groups, or stereocenters (stereogenic centers) an atom at which the exchange of two substituents leads to a stereoisomer. All chirality centers are stereocenters, but not all stereocenters are chirality centers. The trigonal (sp^2) carbons of (E)- and (Z)-alkenes are stereocenters, but not chirality centers. Stereoisomers can either be enantiomers, stereoisomers that are non-superimposable mirror images, or diastereomers, stereoisomers that are not mirror images. All but one of the major components of the essential oils you will isolate have a stereoisomer. In fact, both enantiomers of carvone are present in the list, and are isolated from different sources.



This Week's Experiment

In this experiment you and your partner will isolate an essential oil from one of six different sources by steam distillation. After the steam distillation is completed you will separate the oil from the water it co-distills with by extraction into CH_2Cl_2. After drying and evaporation, the essential oil will be isolated.

After you obtain an IR spectrum of your essential oil you will cooperate with five other pairs of students who isolated each of the other oils to analyze them by TLC. You will also perform TLC analyses on pure materials that are present in the oils. You will obtain R_f values for the major components of each oil and for the pure compounds. You will use the TLC and IR results to determine the identities of as many components as possible in each oil. You will also examine the structures of the components of essential oils to identify sources of stereoisomerism in these compounds.

Experimental Hints

1. **Heating at too high a temperature can lead to bumping, be careful.**
2. **The distillation should be done at a rate that allows you to collect about 100 mL of distillate in about 35-45 min once distillation commences.**
3. **Make sure that the CH_2Cl_2 extract is thoroughly dried and filtered into a dry round bottom flask before rotary evaporation. It will be difficult to remove water at the stage of rotary evaporation.**
4. **Work carefully with the other five pairs of students when you perform the TLC analysis. It is particularly important that all samples (including the standards) be analyzed with the same solvent composition since all the TLC results will have to be compared to each other.**
5. **Make sure to get all the TLC data in your notebook before you leave lab. You will need it all to complete the summary report.**