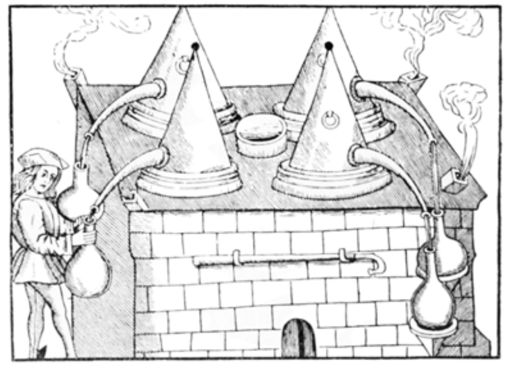
Fig. 33-1



Ancient still for extraction of essential oils and perfumes.

Experiment 33

STEAM DISTILLATION: SEPARATION OF BIOACTIVE MOLECULES

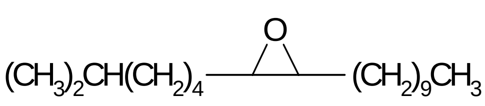
Text Topics and New Techniques

Pheromones, steam distillation, plant testing. The suggestions by Thomas Tworkoski for the procedure for plant testing are gratefully acknowledged.

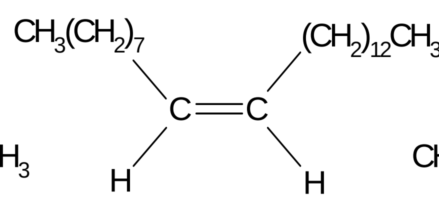
Discussion

As a result of the field of synthetic organic chemistry, many chemicals have been developed that we use to make our lives easier and more comfortable. Medicines and pesticides help to prevent and cure many diseases. Unfortunately, most chemicals do more than address their intended purpose. It is very important that very careful analysis of benefits versus risks be considered before the use of a chemical is initiated. Medicines almost always have undesirable side effects. The FDA very carefully evaluates the benefits and the side effects before approving a drug for use. Most pesticides are toxic at some level to all living things and the dose and ecological impact must be carefully assessed before the pesticide is applied. For more information on this topic, you should read the introduction to ***Exercise 12.***

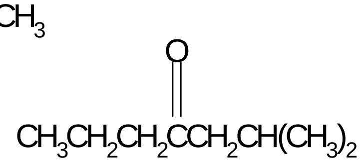
Synthetic organic chemists are continually searching for medicines and pesticides that are more specific in their actions. It has been known for some time that insects produce and emit chemicals called pheromones that enable the insects to communicate warnings or sexual signals. Dr. Jerrold Meinwald at Cornell University has been one of the leading research chemists in this field. By extracting these chemicals from huge numbers of insects, organic chemists have been able to isolate and characterize the pheromones of many pests.



disparlure - sex attractant of gypsy moth



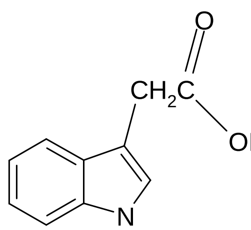
muscalure - sex attractant of common housefly



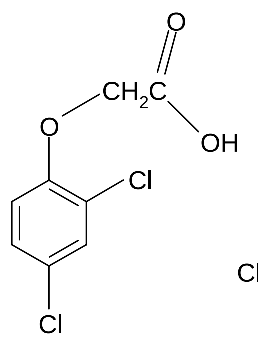
2-methyl-4-heptanone an ant alarm pheromone

Examples of pheromones that have been isolated are given above. The pheromones are usually relatively simple compounds that can be prepared in the laboratory. Once the pheromone has been isolated and characterized, synthetic organic chemists attempt to devise methods to synthesize the compound. The pheromone can then be used as bait to attract the insects to locations where pesticides can be locally administered without introducing the pesticide into the environment.

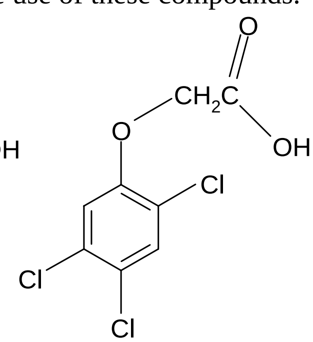
As a result of the evolutionary process, many plants have developed the ability to synthesize compounds that attract insects for functions such as pollination and protection from threatening insects. A major field in organic chemistry is the isolation, characterization and biological testing of chemicals from natural products. Many of our most useful medicines have been discovered this way. Plants also synthesize chemicals that affect and regulate their growth. Some synthetic herbicides include compounds that have been modeled after natural plant hormones but essentially cause the plant to grow itself to death. 2,4-D and 2,4,5-T illustrated below were modeled after the natural plant growth hormone IAA and used extensively as pesticides. Among the pesticides that included these compounds was *Agent Orange* which had widespread use as a defoliant in Viet Nam. Unfortunately, the synthesis of 2,4,5-T is accompanied by the synthesis of TCDD. TCDD is an extremely toxic compound that is also a potent mutagen and teratogen. Many people in Viet Nam suffered from the indiscriminate use of these compounds.



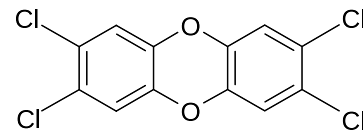
indole-3-acetic acid (IAA)
natural plant hormone or
auxin



2,4-dichlorophenoxyacetic acid
(2,4-D)

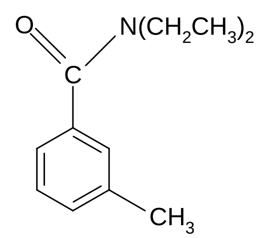


2,4,5-trichlorophenoxyacetic acid
(2,4,5-T)



2,3,7,8-tetrachlorodibenzo-p-dioxin
(dioxin, TCDD)

One of the goals of organic chemistry is to develop herbicides that do not have undesirable side effects such as those associated with 2,4,5-T. In this experiment, you will use steam distillation to obtain essential oils from cinnamon and clove samples and test their activity as herbicides on plants.



A recent literature report claimed that cinnamon leaf oil kills mosquito larvae and could have applications as an insect repellant [Chang]. You are probably familiar with the commercially available, *N,N*-diethyl-*m*-toluamide commonly called DEET and sold by one company as “OFF”. People liberally apply DEET products to exposed parts of their bodies to repel mosquitos. This has become especially important recently in the United States as the mosquito is now a vector almost everywhere in the country for the potentially dangerous West Nile Virus. Entry on the market of another useful repellant would be beneficial and probably a sales winner. Although you will steam distill cinammon and clove samples in this experiment, we will not be able to test the oils on humans as human testing appropriately must conform to stringent requirements and guidelines that cannot be satisfied in this course. However, another interesting report does afford a way that will enable you to test for some biological activity. According to Thomas Tworkoski, cinnamon leaf and clove oils can be used as herbicides. In fact, some commercially available herbicides are formulated from these oils. To determine their effectiveness, your steam distillation products will be tested on ragweed seedlings and compared to the action of other herbicides and some synthetic auxins and herbicides.

Techniques

Distillation of compounds with high boiling points is difficult, dangerous and potentially destructive as many compounds decompose at high temperatures. As you have experienced, one method for avoiding the high distillation temperature is vacuum distillation. Vacuum distillation lowers the boiling point on the order of 100 C° and usually prevents decomposition. Another commonly used technique for lowering the boiling point is called steam distillation. Boiling occurs when the vapor pressure in the system equals the confining (most commonly atmospheric) pressure. In the case of miscible liquids (such as cyclohexane and toluene in ***Experiment 8***), vapor pressure and the boiling point are non-linear functions of the mol fraction of the mixture. For immiscible liquids, the two liquids essentially behave independently and boiling occurs when the sum of the vapor pressure of the two liquids is equal to the confining pressure. For steam distillation, one of the liquids is water and the other an immiscible organic liquid. Usually the organic liquid has a much higher boiling point than water and therefore a much lower vapor pressure. Because of this, the vapor pressure of the mixture does not reach the confining pressure until the mixture is heated to close to the boiling point of water. The distillate is primarily water with a small amount of the organic substance. Because water is inexpensive and non-toxic, the use of large amounts of water to distill over small amounts of the organic is still a worthwhile procedure. In addition, since the organic substance is immiscible in water, it is relatively easy to separate either directly from the distillate or by extraction with a low boiling organic solvent such as ether or dichloromethane.

The water for the steam distillation can be supplied either externally or internally. In today's experiment, you will mix clove samples with water and steam distill. In other cases, steam is continually produced externally to the distillation flask and transferred via tubing to the flask. For plant testing, the following recommendation by Thomas Tworkoski of the USDA is included:

> If I were composing a laboratory, I'd use common ragweed (Ambrosia artemisifolia L.) seedlings that were about 2 weeks old (Valley Seed Service, PO Box 9335, Fresno CA 559-435-2163 $15/quart of seed) grown in a commercial medium and small pots (2-3 inch diameter). Ragweed was very sensitive and a good bioassay. I'd apply the distillate at full, half, and 10% strength (aqueous) with an atomizer (a TLC plate sprayer would work fine) in a hood or outside (although pleasant at first, the aroma becomes overpowering). Lower concentrations might be needed if you want to illustrate a dose response. Obviously, I'd replicate each concentration at least 3 times. A critical problem is the immiscibility of essential oils with water and this can be overcome with adjuvants but vigorous shaking while spraying the plants will achieve reasonably uniform coverage.

Procedure

Transfer 20 g of cloves to a 200 mL round bottom flask and add about 100 mL of water. Distill until you have collected about 50 mL of solution. Add another 50 mL of water to the distillation flask and distill until you have collected an additional 50 mL of liquid. Combine the distillates and transfer them to a separatory funnel.

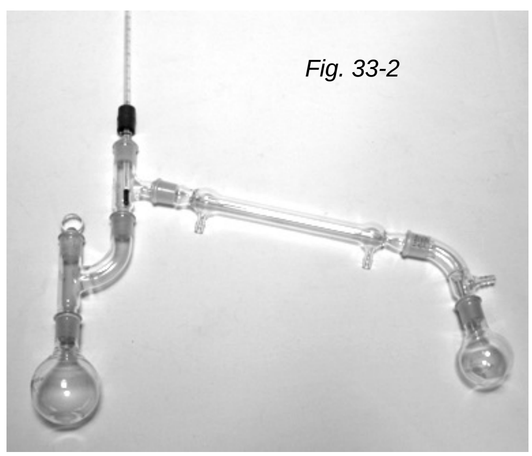


Fig. 33-2

Extract twice using moderate shaking with 15 mL portions of dichloromethane. Evaporate or distill off the CH_2Cl_2 and analyze the oil using ir and nmr.

Test ragweed plants as described above with the products from the clove oil. In addition, test plants with cinnamon leaf oil, Roundup, eugenol, cinnamaldehyde and synthetic or natural plant growth hormones.

References

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Prelaboratory Preparation - *Experiment 33*

First, be sure to list all the goals of the experiment. Prepare a table for insertion of useful and observed data. Do some research on cinnamon and cloves. Try the ***Merck Index*** under "oils of" and search online.

Observations

Report all relevant observations including boiling points, spectra and plant responses

Conclusions

This section should include the following:

1. Were the goals of the experiment achieved? Explain your answer.
2. What percentage of your spice were you able to obtain as an oil? Could this percentage be improved?
3. What was the identity of your product? Explain your answer.
4. Evaluate your product(s) as a potential herbicide. How did it compare to other compounds? Be thorough in your descriptions and explanations.