Natural Products Chemistry. **The Isolation of Trimyristin from Nutmeg.**

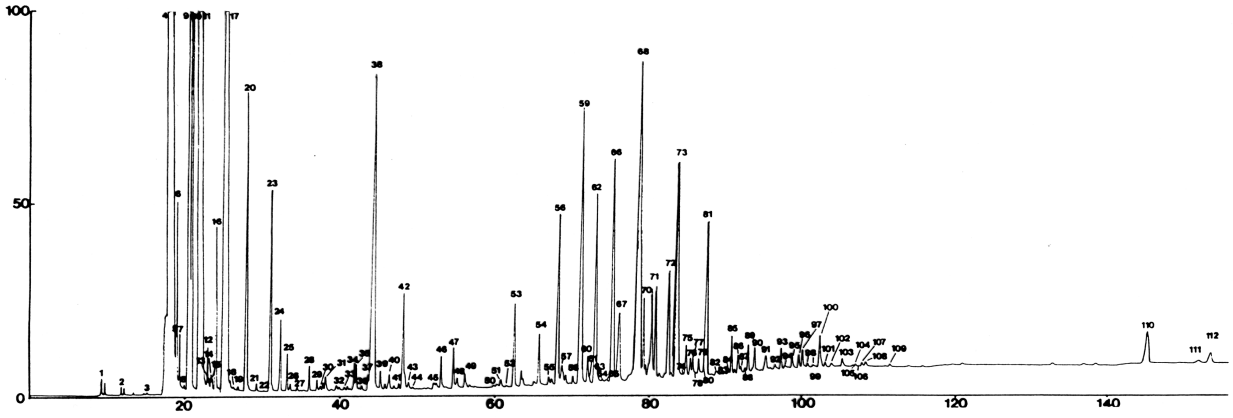
Over 40% of the medicinal chemicals used throughout the developed world today were originally isolated from natural sources. These sources include flowering plants, fungi, bacteria, and to a lesser extent, animals, especially marine animals. The subfield of organic chemistry that deals with isolating and studying chemicals found in nature is called natural products chemistry. The techniques of extraction, distillation, recrystallization, and chromatography are used to isolate and purify chemical compounds from natural sources. Methods such as infrared and nuclear magnetic resonance spectroscopy, mass spectrometry, and x-ray methods are used to identify the structures of the compounds. Laboratory synthesis of the compounds from simpler compounds provides confirmation of the structure as well as a laboratory source of the chemicals. In some cases, for further confirmation of structure, the isolated compound is chemically degraded to simpler compounds, which are more easily identified.

The biochemical machinery of living organisms produces vast numbers of organic chemicals. These can be broken down into primary metabolites and secondary metabolites. Primary metabolites are those chemicals needed by an organism to exist for even a short period of time and include proteins, carbohydrates, fats, and vitamins to mention some. Secondary metabolites are those chemicals that in a more indirect way have enabled a species to survive for the millions of years of its existence. Pheromones, which allow organisms to communicate with other members of its own species are one example of secondary metabolites. These include aggregation, alarm, sex, and trail pheromones to name a few. The ester, isopentyl acetate, is an alarm pheromone of the honeybee. If a honeybee is attacked, it releases this pheromone, which causes other honeybees to enter the fray. Allomones are another example. These are chemicals that allow organisms to interact in a way that is detrimental to other species. Examples of allomones include natural insecticides, herbicides, and antibiotics.

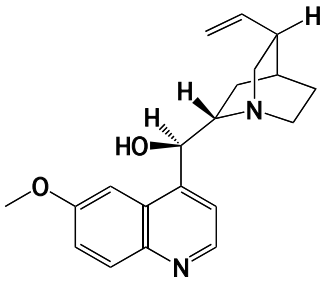
It is these secondary metabolites that are of interest to us as pharmaceutical (or medicinal) chemicals. Many have been found but a great many more await discovery. Antibiotics such as penicillin and erythromycin, chemicals such as paclitaxel (trademarked name, Taxol) used in cancer chemotherapy, painkillers such as morphine, and many more organic compounds, used to treat many different diseases, come from natural sources. It is estimated that there are between 10 million and 100 million living species on Earth and only 1.4 million have been formally identified and described. Of these 1.4 million only a small fraction have been studied in terms of their chemical constituents. Because each species may contain hundreds to thousands of organic chemicals, one can assume that there are very large numbers of as yet undiscovered medicinal chemicals existing in nature. Unfortunately, it has been predicted that by 2025, 25% of today's flowering plants will be lost to human endeavors, such as the burning off of rainforests for agriculture, meaning that a great many potentially useful natural chemicals will also be lost.

To demonstrate the large number of organic compounds that can be present in a typical natural source, consider the gas chromatograph (GC) of the extract of needles from the California juniper tree. The chromatogram shows 112 peaks, meaning that there are at least that many different compounds in that extract. Note also that GC allows only compounds which are able to be vaporized to pass through and reach the detector. Higher molecular weight compounds such as most of those of medicinal interest will not be seen on GC, so the actual number of organic

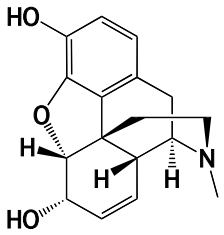
compounds in this extract is much greater than 112.



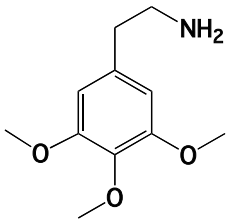
Some examples of naturally occurring organic compounds.



QUININE
(AN ANTIMALARIAL FROM THE BARK OF THE S. AMERICAN CINCHONA TREE)



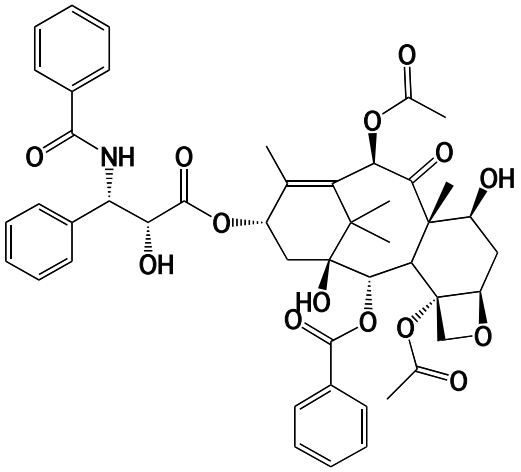
MORPHINE
(A NARCOTIC ANALGESICFROM THE OPIUM POPPY ORIGINALLY FROM ASIA)



MESCALINE
(A PSYCHOTOMIMETIC FROM THE PEYOTE CACTUS OF MEXICO AND THE U.S. SOUTHWEST)

Where does one start in a search for these organic chemicals? With such an immense number of organisms and their even greater number of chemical constituents, approaching the task in a completely systematic way is practically impossible. The time necessary to carry out a systematic study of the chemical constituents of all living organisms does not exist. Often, leads are found by anthropologists and ethnobotanists in their study of folk medicine. Over thousands of years, indigenous cultures from around the world have learned to use their native plants and animals for treating diseases endemic to them. If a particular plant for example shows promise, quantities of that plant would be collected and brought to a natural products chemistry laboratory, where the material would be extracted with solvents and tested for biological activity. If promising, the complex mixture of compounds would be separated by techniques such as further extractions, distillation, recrystallization, and chromatography. Those chemicals that show the most promise would be identified by spectroscopic and x-ray methods, physical properties such as melting points and boiling points, derivative formation, and independent synthesis to confirm the identity. Larger quantities could be obtained from the natural source or synthesized in the lab. Eventually, clinical trials, FDA approval, and commercial production and use would follow. The process from start to finish usually takes decades. The movie *Medicine Man* tells the story of the search for a natural pharmaceutical compound.

As an example of this process, consider the history of the drug paclitaxel or Taxol. Paclitaxel is extremely effective at treating a variety of cancers. Because of its effectiveness, a drug such as this is sometimes called a wonder drug or magic bullet drug. Paclitaxel was originally obtained by extraction from the bark of the Pacific yew tree, found in the Pacific northwest. The bark from each 100 year old tree yields about 0.3 g of paclitaxel which is enough for one dose for a cancer patient. If other sources had not been found, the Pacific yew tree would have been extirpated. Fortunately, laboratory syntheses were developed and other natural sources have been found. This drug today is one of the best examples for the importance of this type of research.



PACLITAXEL (TAXOL)
(CHEMOTHERAPEUTIC AGENTUSED TO TREAT VARIOUS FORMS OF CANCER - FROM THE PACIFIC YEW TREE OF THE AMERICAN NORTHWEST)

The chronology of the development of paclitaxel as a drug illustrates the challenges in this type of research and the long time period before such drugs come into regular use. As it was, the Pacific yew was almost lost to logging before paclitaxel was discovered in its bark.

< 1962	Loggers burned Pacific yew as waste.
1962-7	Bark collected. Extracts found to kill leukemia cells.
1967	Taxol isolated as active compound.
1967-77	Supply problems, poor results limit study.
1978	Unique mechanism of action discovered.
early ‘80’s	Compound that can easily be converted to taxol discovered in European yew leaves and needles.
1984	Human trials, outstanding results.
1984-9	Toxicity, limited supply slow progress.
1989	Large-scale harvesting begins. Five year supply of trees remains.

1993 Harvest halted. Lab synthesis begun.

mid-‘90’s Regular clinical use begun.

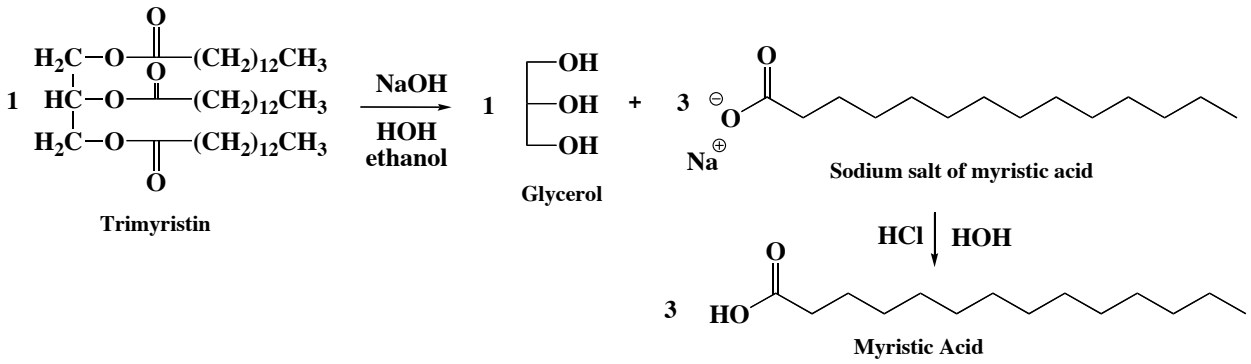
2000 Paclitaxel found in hazelnut leaves, twigs, and nuts and fungi endemic to hazelnuts.

It took 30 years for the drug to go from discovery to clinical use and if the right combination of fortunate events had not happened it might still be unknown. More detailed accounts of the Taxol story can be found on the course website.

Trimyristin from Nutmeg. This experiment illustrates the process of obtaining a pure organic compound from a natural source. Normally, because extracts from natural sources contain complex mixtures of compounds, isolation of a single pure compound requires long tedious separation and purification procedures. The extraction of nutmeg seed to isolate trimyristin is however an exception and can easily be done in one lab period. Ground nutmeg seeds are extracted with *tert* -butyl methyl ether and the resulting solid recrystallized from acetone to yield pure trimyristin. Most of the other numerous ether soluble components of nutmeg remain in the acetone solution. Basic hydrolysis of trimyristin, followed by acidification, yields glycerol and myristic acid. The myristic acid is isolated and identified by melting point.

Trimyristin belongs to the class of compounds known as fats or triglycerides. These are triesters of the trialcohol glycerol and unbranched long-chain (14 or more carbons) carboxylic acids called fatty acids. If the carboxylic acid portion of the fat contains no alkene groups, the fat is said to be saturated. If one alkene group is present the fat is monounsaturated. If more than one alkene is present the fat is polyunsaturated. In a monounsaturated fat, if the alkene is one carbon in from the far end of the long chain (away from the ester group), the fat is said to contain omega -1 (read as omega minus one - omega refers to the end of the chain so omega-1 refers to one carbon in from the end) fatty acids.

Because a fat is a triester of a trialcohol, hydrolysis yields one molecule of glycerol and three molecules of carboxylic acid for each molecule of fat. Thus, for example, hydrolysis of 0.012 mol of fat would yield 0.036 mol of carboxylic acid and 0.012 mol of glycerol.



Soap from fat. Sodium salts of long chain carboxylic acids are soaps. In fact soap is made by hydrolyzing fats. Greasy molecules, including fats themselves, cannot be washed away with

plain water because the greasy molecules are insoluble in water. Soaps however make it possible to wash away grease, using water as solvent. Soaps have a split personality. One end is the very polar ionic sodium salt of the carboxylic acid and the other end is the nonpolar hydrocarbon end. In water, soap molecules aggregate into a globular structure called a micelle. The nonpolar ends congregate on the inside of the globule and the polar ionic ends at the surface of the globule. Because the ionic ends are on the outside, the globule is soluble in water. The nonpolar inside of the globule dissolves the grease molecules and the ionic outside allows the globule to be washed away, grease and all.

Procedure.

As always, as part of your prelab outline, prepare a table of reactants, products, and solvents (same type of table as for the cyclohexene experiment). This lab will take most of four hours, so be well prepared and get started as soon as possible.

Extraction. Set your sand bath to about 20% (turn to 30, then down; make sure yellow power light is on). Weigh 1.00 g (± 0.05 g) of ground nutmeg onto weighing paper and transfer the nutmeg to the microscale round-bottomed (RB) flask, using the plastic funnel to assist in the transfer. Add 3 mL of tert-butyl methyl ether and two or three boiling chips to the flask and, using the black plastic connector, connect the distillation column/air condenser (not the longer chromatography column) to the flask. Be sure that the connector is not too frayed. Clamp the flask to a ring stand using the small three-pronged clamp and slowly lower the flask into a slight depression in the sand, without actually touching the flask to the sand. Carefully adjust the position of the flask so that the mixture boils very gently. The finely divided nutmeg causes the mixture to bump (boil violently). Excessive heating may cause some of the mixture to bump out of the flask; bumping is minimized by heating very gently. Heat just at the boiling point for 10 minutes, remove from the heat, and allow the suspended solids to settle for a few minutes.

In the following transfer procedure the idea is to leave behind as much solid as possible before adding the solution to the filter, because the finely divided solid tends to clog the filter. Without stirring up the solids too much, remove the condenser, carefully tip the RB flask slightly, and using a pipet, transfer as much of the liquid as possible to a centrifuge tube while leaving behind as much solid as possible in the RB flask. Allow the solids to settle in the centrifuge tube for a minute. Then again, while leaving behind as much solid as possible, transfer as much liquid as possible into a pressure filtration assembly and filter the liquid into a clean, dry, tared 25 mL Erlenmeyer flask (tared = weighed empty). Be sure that the filter assembly is resting in the flask prior to transferring the liquid, as some liquid will filter through immediately. The filtration apparatus is made by pushing the micro Buchner funnel (polyethylene frit in place - no filter paper) into the end of a plastic pipet which has had the tip cut off and which has had a small hole cut into the squeeze bulb). Allow most of the liquid to filter by gravity, then, while holding your thumb over the hole in the squeeze bulb, gently apply pressure to complete the filtration. Care must be taken when squeezing the pipet bulb on the filter pipet. Too much pressure might cause the filter to leak or fall off. Add about 2 mL of fresh tert-butyl methyl ether to the solid in the RB flask, warm briefly, let the solids settle for a minute, and pipet the liquid to the centrifuge tube as before. Again allow the solids to settle briefly in the centrifuge tube, then filter the liquid through the pressure filtration apparatus, into the same 25 mL Erlenmeyer flask. Doing a rinse

such as this helps to ensure that any trimyristin that was left behind in the RB flask and centrifuge tube is not lost, thereby helping to ensure that the yield of trimyristin is maximized. In the fume hood, carefully pass a gentle stream of air over the solution while warming the flask with your hand until all of the solvent has evaporated and a yellowish solid remains. Be careful to not lose material by spattering the solution with too fast an air flow. After most of the solvent has evaporated, a gummy yellowish solid will remain. This is the crude trimyristin. Residual solvent may take some time to completely evaporate, but the next step may be started even if the solid is not completely dry.

At this point raise the setting on the sand bath to about 30 so the bath will be hot enough for the hydrolysis below.

Recrystallization of trimyristin. After allowing the crude trimyristin to air dry for another 5 min, obtain its weight. Note that this is an approximate weight because the material is not yet completely dry. To the solid in the 25 mL Erlenmeyer flask, add 1 mL acetone per 50 mg crude material, warm on the sand bath to dissolve the solid (caution: acetone has a low BP so heat carefully or material will be lost), allow the solution to cool slowly to room temperature (about 5 min). If crystals do not appear after cooling, scratch the inside surface of the flask with a glass stirring rod at the air-liquid interface to induce crystallization. (If crystals do not form at this point continue anyway.) Cool the solution in an ice-water bath. If crystals have not yet formed, scratch the surface of the flask with a glass stirring rod at the air-liquid interface. Allow the solution to cool in the ice-water bath for 15 min. Collect the crystals by vacuum filtration on a small Hirsch funnel (always clamp the filter flask to prevent it from tipping over). Rinse the crystals in the following way: lift the funnel slightly to break the vacuum, cover the crystals with about 1 mL of ice-cold acetone, and immediately reapply the vacuum. Allow air to pass over the crystals for a few minutes then scrape the crystals onto a tared piece of 9 cm filter paper and allow the crystals to dry to constant weight. Save a small sample for a MP.

Hydrolysis. Weigh 60 mg (± 5 mg but weigh exactly) of the trimyristin onto a tared piece of weighing paper and transfer it to a clean RB flask (use a funnel). Add 2 mL of 6 M NaOH, 2 mL of 95% ethanol, and a boiling chip and reflux the solution gently on the sand bath for 45 minutes. Note that more heat will be required to boil this higher-boiling mixture than was required for the extraction step. If less than 60 mg of trimyristin was obtained, scale the hydrolysis down by a factor of 2 (halve the amounts of all reactants – trimyristin, NaOH, and ethanol - but keep the reaction time the same). Overheating will cause ethanol to evaporate. Note the approximate level of liquid in the flask and if necessary, replace any ethanol that is lost to evaporation.

During the hydrolysis period, recrystallize the remaining trimyristin a second time, this time allowing the warm solution to cool to room temperature for 10 min before cooling in ice for another 10 min. In this second recrystallization dissolve the trimyristin in the minimum amount of acetone at the BP of the solution. Collect product by vacuum filtration and allow it to dry to constant weight. Compare the MPs of the once recrystallized and the twice recrystallized trimyristin.

After the hydrolysis has proceeded for 45 minutes, allow the flask to cool to RT and pour the contents into a 50 mL beaker containing 8 mL of water. Carefully, in the hood, add dropwise

with stirring, 2 mL of concentrated HCl (caution: corrosive liquid/noxious vapors). Myristic acid should precipitate. If a gelatinous solid is observed, there may not be enough HCl or the mixture may not have been stirred thoroughly. Cool the beaker in ice water for 10 min, with stirring, and collect the solid by vacuum filtration on a small Hirsch funnel (note: when getting the filter paper, be very careful that only one piece is selected. These tend to stick together. More than one piece will slow down the filtration considerably.) Rinse with three small portions of water, by breaking the vacuum, adding just enough water to cover the solid, and reapplying the vacuum as before. Filtration of the fine crystals is slow. Using too much rinse water will also slow the process considerably. Allow the solid to dry at least overnight, weigh it, take a MP, and calculate the % yield.

WASTE: Place all liquid wastes into the "Organic Liquid Waste" container.

BEFORE YOU LEAVE THE LAB: turn off the sand bath, Mel-Temp, air and vacuum, put away your equipment and lock your drawer, clean up your work areas, close the fume hood sash completely and ask your TA for her or his signature. If you see caps off of bottles, replace the caps. If you see spilled chemicals, clean them up or at least report it to your TA.

Summary of Results. Record the weight of trimyristin at all stages of the experiment. Report the following: (1), % recovery of crude and once recrystallized trimyristin from nutmeg (e.g., extracting 1.02 g of ground nutmeg seed resulted in a 70 % yield of crude trimyristin and, after the first recrystallization, a 50 % recovery was obtained, based on the original amount of nutmeg); (2), % recovery in the second recrystallization; (3), % yield of myristic acid obtained in the hydrolysis; (4), the MPs of once recystallized and twice recrystallized trimyristin, and myristic acid.

Postlab Questions

1.) What is the structural difference between saturated, monounsaturated, and polyunsaturated fats? Of which kind is trimyristin?

2.) In the first recrystallization, after the solid has been dissolved in warm acetone, the solution is allowed to cool slowly to room temperature. In an older procedure the warm solution was placed directly into an ice bath. How does this older procedure differ from a normal recrystallization and what might be the consequences?

3.) One triglyceride present in animal fats is tristearin (glycerol trioctadecanoate). What is the theoretical yield, in grams, of stearic acid upon hydrolysis of 1.8 g of tristearin? (See an Organic text, CRC Handbook, or ChemFinder.com for structure and MW.)

4.) In the hydrolysis of trimyristin the reaction mixture is heated at about 75° for 45 minutes. What is the purpose of heating a reaction mixture?

5.) If the mixture were heated at 40° for 45 minutes, what would be the result?

(revised, pws, 4/11)