

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

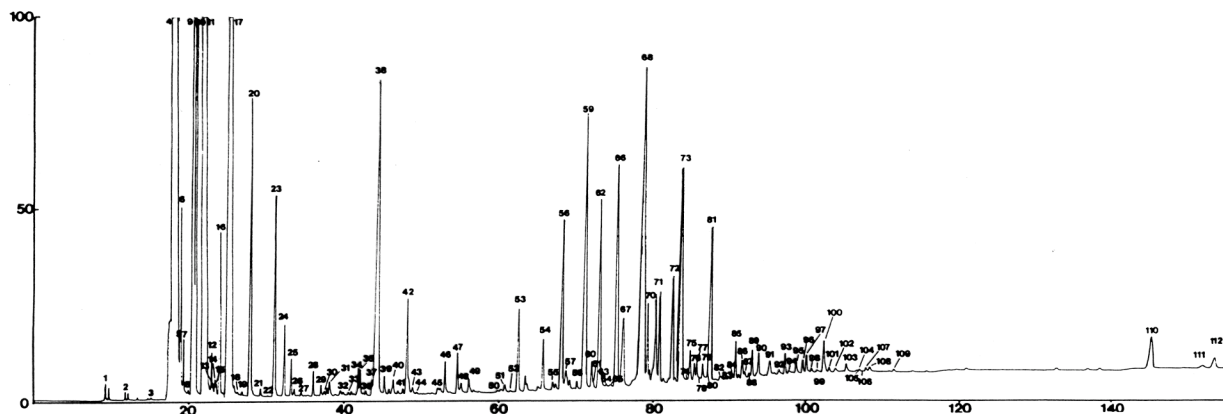
Over 40% of the medicinal chemicals used throughout the developed world today were originally isolated from natural sources. These sources include flowering plants, fungi, bacteria, and to a lesser extent, animals, especially marine animals. The subfield of organic chemistry that deals with isolating 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.

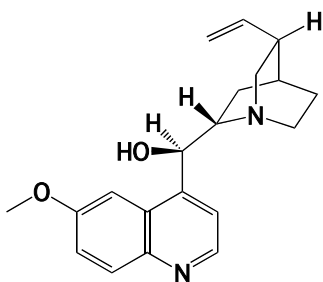
It is these secondary metabolites that are of interest to us as pharmaceutical (or medicinal) chemicals. Many have been found but a great many more await discovery. Antibiotics such as penicillin and erythromycin, chemicals such as paclitaxel (trademarked name, Taxol) used in cancer chemotherapy, painkillers 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

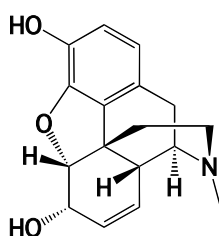
California juniper tree. The chromatogram shows 112 peaks, meaning that there are at least that many different compounds in that extract. Note also that GC allows only compounds which are able to be vaporized to pass through and reach the detector. Higher molecular weight compounds such as most of those of medicinal interest will not be seen on GC, so the actual number of organic compounds in this extract is much greater than 112.



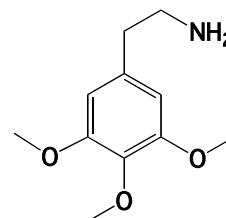
Some examples of naturally occurring organic compounds.



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



**MORPHINE**  
(A NARCOTIC ANALGESIC FROM  
THE OPIUM POPPY  
ORIGINALLY FROM ASIA)

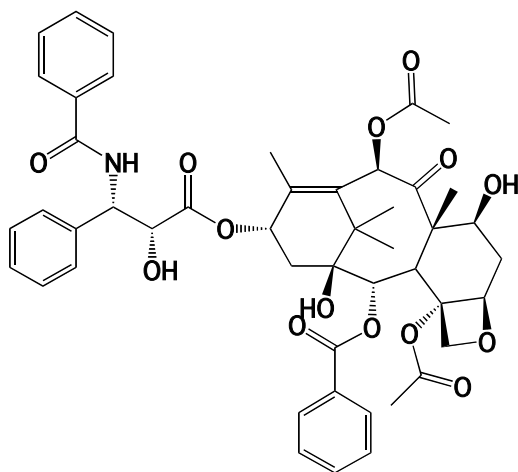


**MESCALINE**  
(A PSYCHOTOMIMETIC FROM  
THE PEYOTE CACTUS OF MEXICO  
AND THE U.S. SOUTHWEST)

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 AGENT USED 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.

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|---------|--|
| < 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.                 |

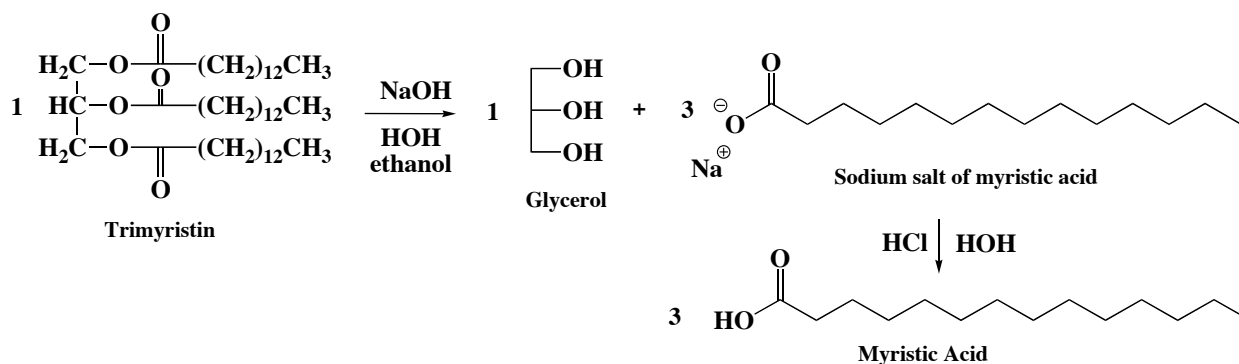
early '80's yew	Compound that can easily be converted to taxol discovered in European leaves and needles.
1984	Human trials, outstanding results.
1984-9	Toxicity, limited supply slow progress.
1989	Large-scale harvesting begins. Five year supply of trees remains.
1993	Harvest halted. Lab synthesis begun.
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.