Lecture 3. Part 1

- Project based on a new understanding of an important biochemical process:

- **Understanding cholesterol biosynthesis**

Cholesterol: its importance to life .

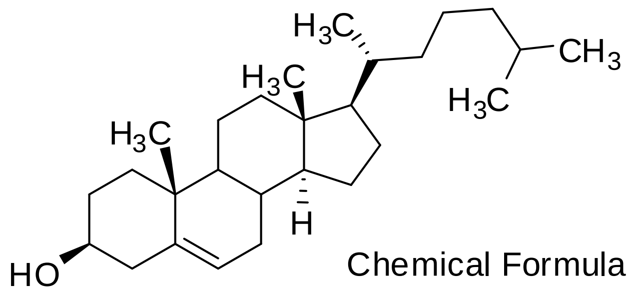
- Cholesterol
- a vital substituent of cell membranes
  - Brain and spinal chord High concentration
- Precursor to all steroid hormones including testosterone, estrone, cortisol, anxiety/stress monitoring steroid used to treat information
- Precursor to bile acids which are crucial to digestion
- (too much) Deposition in the arteries is associated with atherosclerosis and heart disease.

Very serious problem in today's society.

Sources of cholesterol

- Foods of animal origin
  - Meat, butter, lard
    - Commercial source spinal cord of cattle

Found in the body of all animals.



Chemical Formula: $C_{27}H_{46}O$

- Not in vegetables or plant derived food
- Biosynthesized in all animals

Can control cholesterol by controlling your diet, but there is a pharmaceutical alternative.

Pharmaceutics try to interfere with the biosynthesis of the compound.

Complicated molecule

Biosynthesis of cholesterol

Heavily studied in the 60s and 70s.

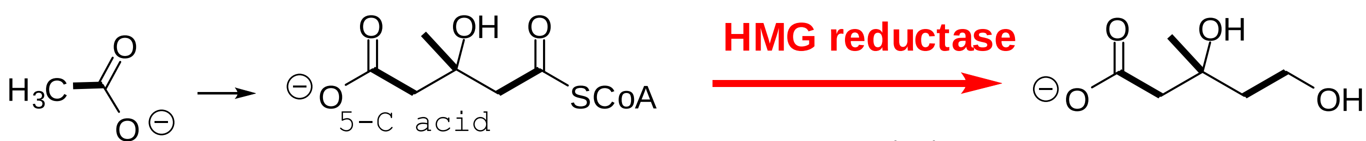
Many steps involved.

Many of the enzymes have been isolated and cloned.

- Well studied-multi-step involving many different enzyme
- Carbon source: Acetate [C2 –building block]
- First key intermediate –Mevalonic Acid

Some people produce less cholesterol than others.

- Our goal: to interfere with the production.



5-C acid

Rate determining step

Key Enzyme

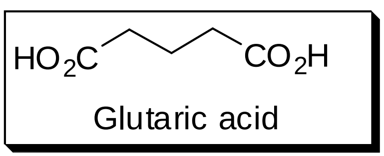
HMG-CoA

Mevalonate

3 units
of acetate

hdroxy-methylglutaryl- C

Acetic acid, etc.



Glutaric acid

If you can interfere with the HMG reductase enzyme, you can reduce the amount of cholesterol that the body synthesizes.

HMG reductase - mediates the redate determining step in cholesterol biosynthesis

CoA - stands for coenzyme A

Converts carbonyl group to an OH group.

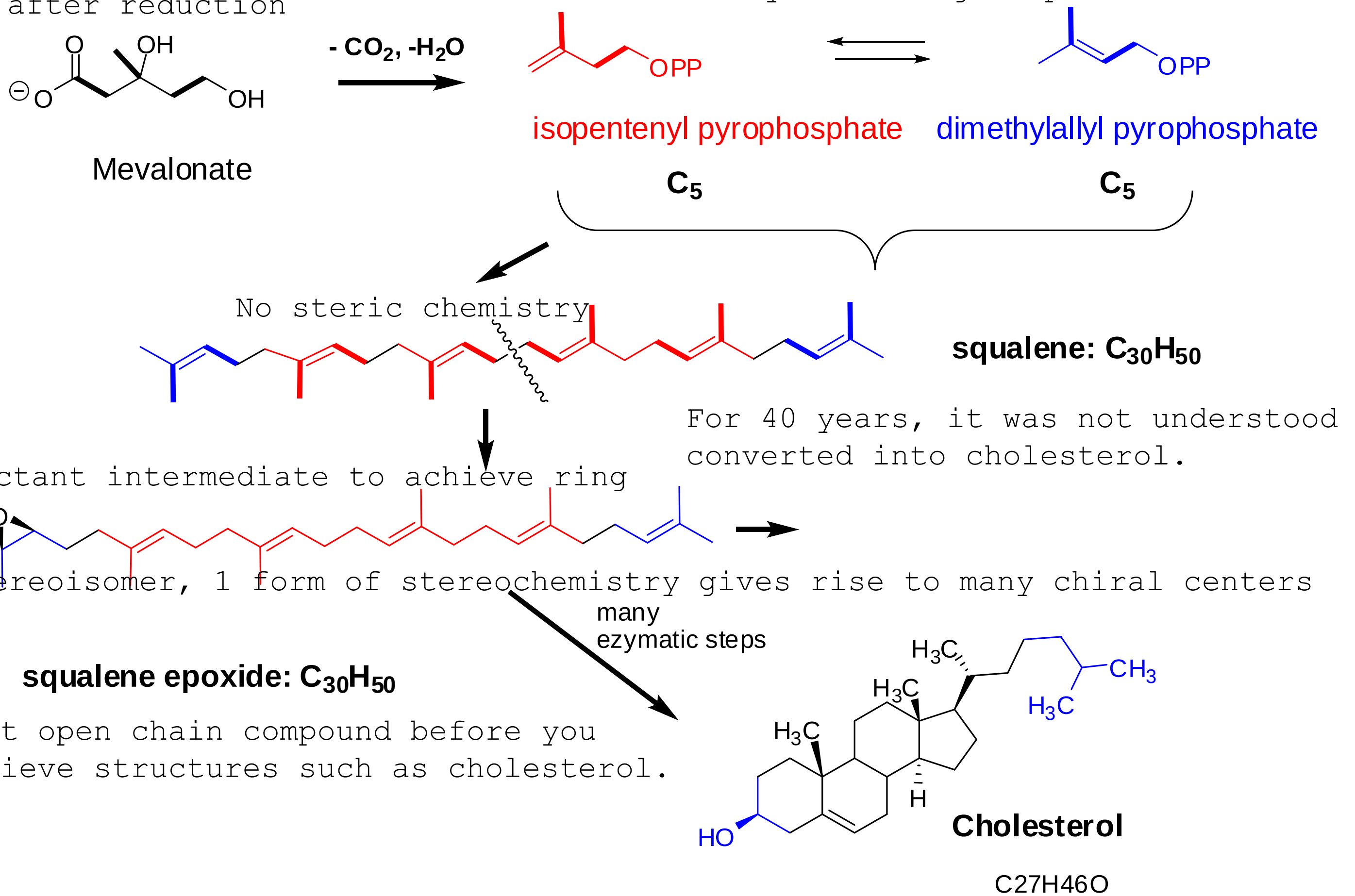
Biosynthesis of cholesterol

All the steroids are made from isoprenes.

- Mevalonate is converted into two C5 units {isoprene}

Isoprene unit is the basis of an incredible number of naturally occurring compounds.

Produced after reduction



No steric chemistry

For 40 years, it was not understood how squalene converted into cholesterol.

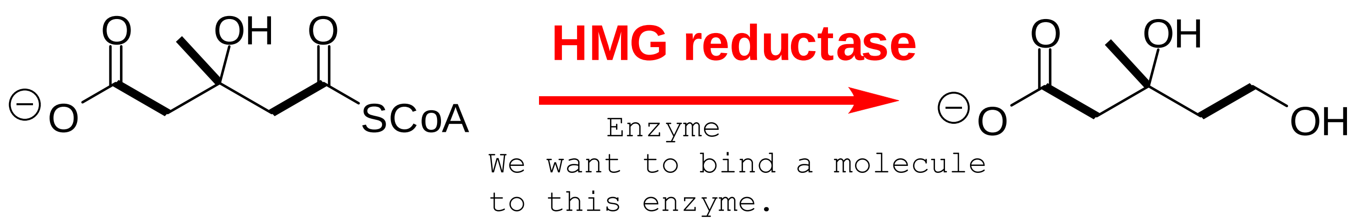
Epoxide reactant intermediate to achieve ring

One stereoisomer, 1 form of stereochemistry gives rise to many chiral centers

Last open chain compound before you achieve structures such as cholesterol.

Inhibiting Cholesterol Biosynthesis

- Interfere in the rate determining step by inhibiting the enzyme, HMG reductase, that carries out that step.
- How does one approach this?
  - The natural ligand for this enzyme is HMG-Co



HMG-CoA

Mevalonate

For interference:

  - A molecule with a similar structure would be expected to bind to HMG-reductase, sequester it, and thus prevent it from converting HMG-CoA into mevlonate and eventually cholesterol

Finding a lead compound

- 1971 –Japanese Biochemist – Akira Endo begins a search by focusing on micro-organisms

Must be able to understand a variety of things to produce drugs.

- Rational: Some micro-organisms may produce inhibitors of HMG-CoA reductase. Why?
- Compounds made bio-synthetically via the mevalonic acid pathway are required by micro-organisms to maintain their cell walls

Microorganisms need products developed from mevalonic acid.

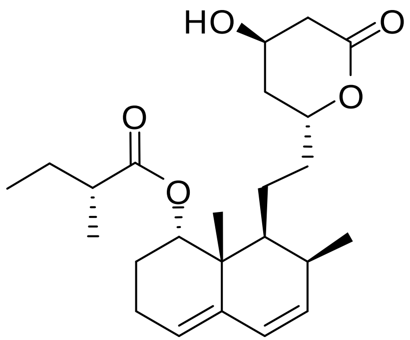
- Screening led to the first known inhibitor of HMG-CoA –reductase a compound called mevastatin

From screening microorganisms.

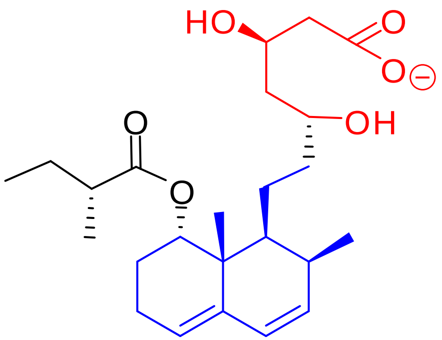
If one microorganism can prevent another microorganism from producing mevalonic acid, it has an evolutionary advantage over the other.

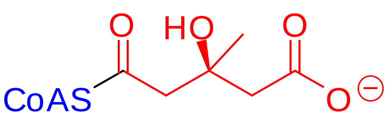
Structure of Mevastatin

- Mevastatin structure –comparison to HMG-CoA reductase



Carboyxlic acid end





HMG-CoA

mevastatin
- a potent inhibitor of HMG reductase,

Based upon the name of the microorganism.

Nature provided a molecule with a similiar structure to bind with the enzyme.

Did not become a drug: showed toxicity.

What is the correlation? From the chemist's point of view: one is an ester molecule, can be converted into an alcohol and a carboxylic acid.

Japanese company actually gave up after this discovery.

CEO of Merck took a chance, realized that small changes in the molecule might reduce the toxicity. Realized nature might make other compounds that are similiar.

Kept screening fungi, etc... found the oyster mushroom which has extracts that give a significant amount of a material that's closely related to mevastatin (large amount ~%3 dry weight.

Other companies also began screening.

- # Other naturally occurring HMG-CoA reductase inhibitors

Nature has specific ways of introducing methyl & hydroxyl grps.

Only difference is methyl grp.

Break the bond and create an acid out of it.

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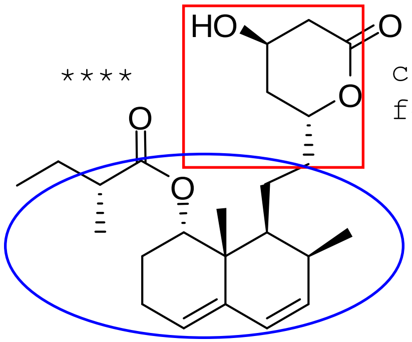


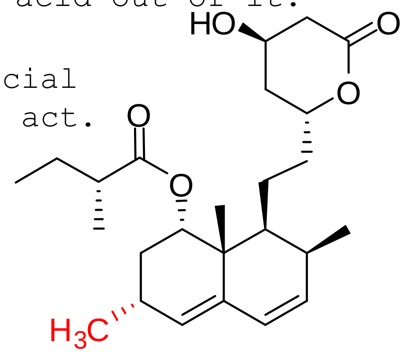
crucial for act.

Not particularly polar.

mevastatin

Replace with a similiar molecule.

serious side effects



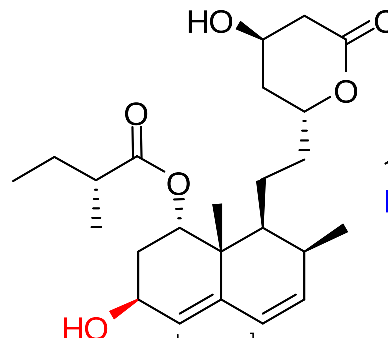
lovastatin

2 major compounds.

Oyster mushroom

Mevacor (R)

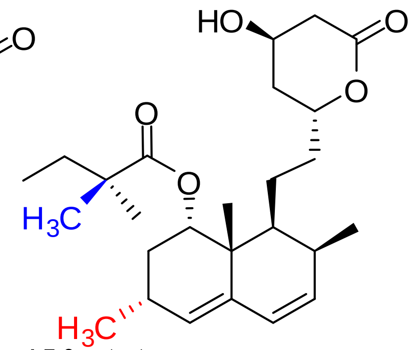
1st important cholesterol lowering drug.



cytochrome p450 (?)

pravastatin

Extra oxygen.

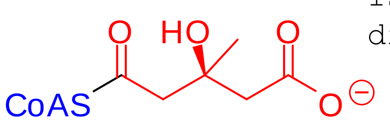


simvastatin

Zocor (R)

semi-synthetic

Another company wanted "me too" drug by introducing some substituent elsewhere in the molecule, synthetically (organic chemist's work).



HMG-CoA

Recognize ester, can use a methyl iodide, to create the molecule with similar properties.

Zocor is a well selling and well tolerated cholesterol lowering drug.

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First approach was to keep the chemistry simple.
Kept aromatic ring structure. Easy to synth. Planar.
Many types of variations are patentable.
Can make it polar/non polar by adding subst. (-CH3, -Cl, -F, etc...)
Create it so it is stable (won't be broken down by an enzyme. -F helps with stability, so does -SO2.

Proposals for making a synthetic variation of mevastation?
- what part of the mevstatin is crucial? (pharmacore)
Synthetic versions of the molecule would be patentable (lots of money).

This would be patentable b/c no one has ever made it.

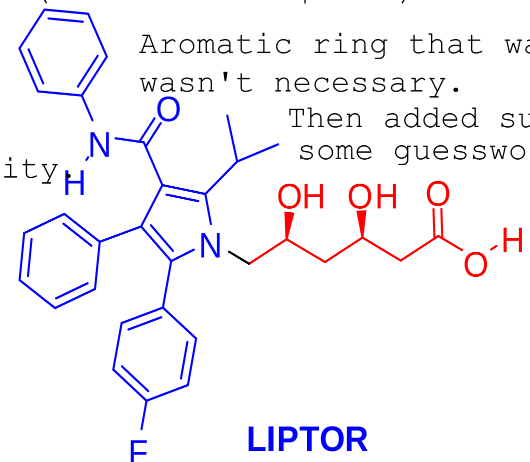
- Synthetic statins

Most successful drug ever made (outside of aspirin).

Aromatic ring that was 5-C. wasn't necessary.

Then added substituents. some guesswork, some not.

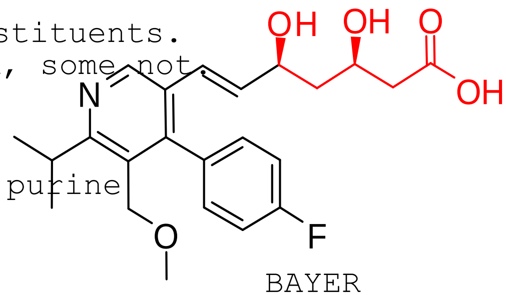
Adds polarity.



LIPTOR

prevents metabolizing.

PFIZER.



purine

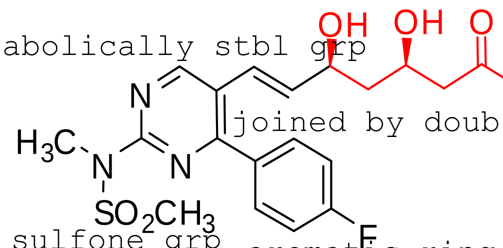
BAYER

6-C aromatic ring.

BAYCOL

strongest inhibitor. flaw? discovered when in gen public.

metabolically stbl grp



joined by double bond.

sulfone grp

aromatic ring

CRESTOR

same motif

All contain fluorines.

A trial and error process. Likely several hundreds, maybe a thousand compounds made.

- Structure relationship
  - To natural HMD-CoA reductase inhibitors
  - To each other

Lipitor vs Baycol

- Lipitor – a member of the “statin” family of drugs - the best selling drug in history.
  - Lowers blood cholesterol levels
  - Stabilizes plaque; prevents strokes
    - Total sales since 1996 > 125$ Billion
    - Patent ended ~2011
- Baycol Introduced by Bayer, about 1998, as a competitor to Lipitor
  - Many more serious side effects (deaths) including wasting of skeletal muscles and resulting kidney failure
  - Withdrawn in 2001.

For baycol, disease was 1 in 2500. very serious & bad.
huge variety of lawsuits involving baycol.

muscle wastes away, produces bad metbaolite by products, causing kidney failure.
this occurred in post marketing surveillance.
is this acceptable? left unchckd? death
1 person in 25,000 gets in this disease.

Other competitors: mevacor, crestor, zocor, pravachol, baycol.
zocor - semi synthetic.

With zocor, ~4500 patients with high cholersterol levels and heart disease, after 5 years 35% lowering of cholesterol and 42% reduction .

Epidemiology studies due to the widespread use of statins show a variety of "good and "bad" relationships.

"good" one study suggested a 50% reduction of colorectal cancer.

"bad" one study indicated high risk of diabeties.

pharmaceutical companies are saying the benefit of reduced heart disease that all of us above 50 should be taking the material. most doctors are saying we should not be going too far.
huge money maker.
how far do we take a drug? can it be given to the public without monitoring.

- SIDE EFFECT
- OTHER COMMENTS

Most are more or less tolerable, tingling etc...

One serious side effect rhadomylolysis [skeletal muscle wasting > kidney failure]

Lecture 3. The drug discovery process

- Choice of Projects

Natural product chemistry.

  - Ethnopharmacology

Traditional societies have used plants. Word was coined by professor at Harvard - Richard Schrotese (?) Friend of Timothy Leary (lsd dude). Worked with schotese, sent to amazon to work with cocoa plant and cocaine.

- Lead structures
  - Natural product, via ethnopharmacology

How to we use ethnopharmacology for getting to the lead structure of a drug?

  - Natural product libraries obtained via classical isolation

50-100-200 years ago. people didn't live as long as we do no. less cancer development because they didn't live as long as we do now. traditional societies don't have cancer treatments.

The drug discovery process. Via ethnapharmacology

- **Typical 'Common' health Problem**
  - **Infections** Every society had plant uses for infections.
  - **Stomach and intestinal ailments** Parasites, etc...
  - **Pain** Man & animals have always dealt with pain.

    Healers dealt with:
  - **Mental /nervous issues**
  - **Reproductive issues**

Traditional healers will have plants that they use for these areas.

What's worthwhile? We use the

- **Healer consensus** Ask a large amount of healers what plants they use for skin infections.

If there's quite a few that use the same plant, we have a general idea of what works. & vice versa. This is how we end up with potential new drugs for treating mental disorders & treating pain.

When we agree that certain plants might be useful for a particular purpose, we then:

- Lead structures via bio-assay guided isolation

Collect plants, tend to store them in alcohol (sterilizes it all, & prevents fermentation), sometime sundried.

Bio-assay guided isolation

A plant extract is shown to have the desired activity.

Activity can be in any particular area.

Extracts are typically prepared by grinding the plant sample, either fresh or dried with 95% ethanol

-other organic solvents used are ethyl acetate and dichloromethane

Why? Good solvents, dissolve many organic compounds, can therefore extract many of the constituents present in the plant.

- water is used sometimes

very rarely. some focus on aqueous extracts.

Extracts have many, often 30 or more component compounds. Which is the active component?

conservative estimate. (30-100) incredibly complex extracts.

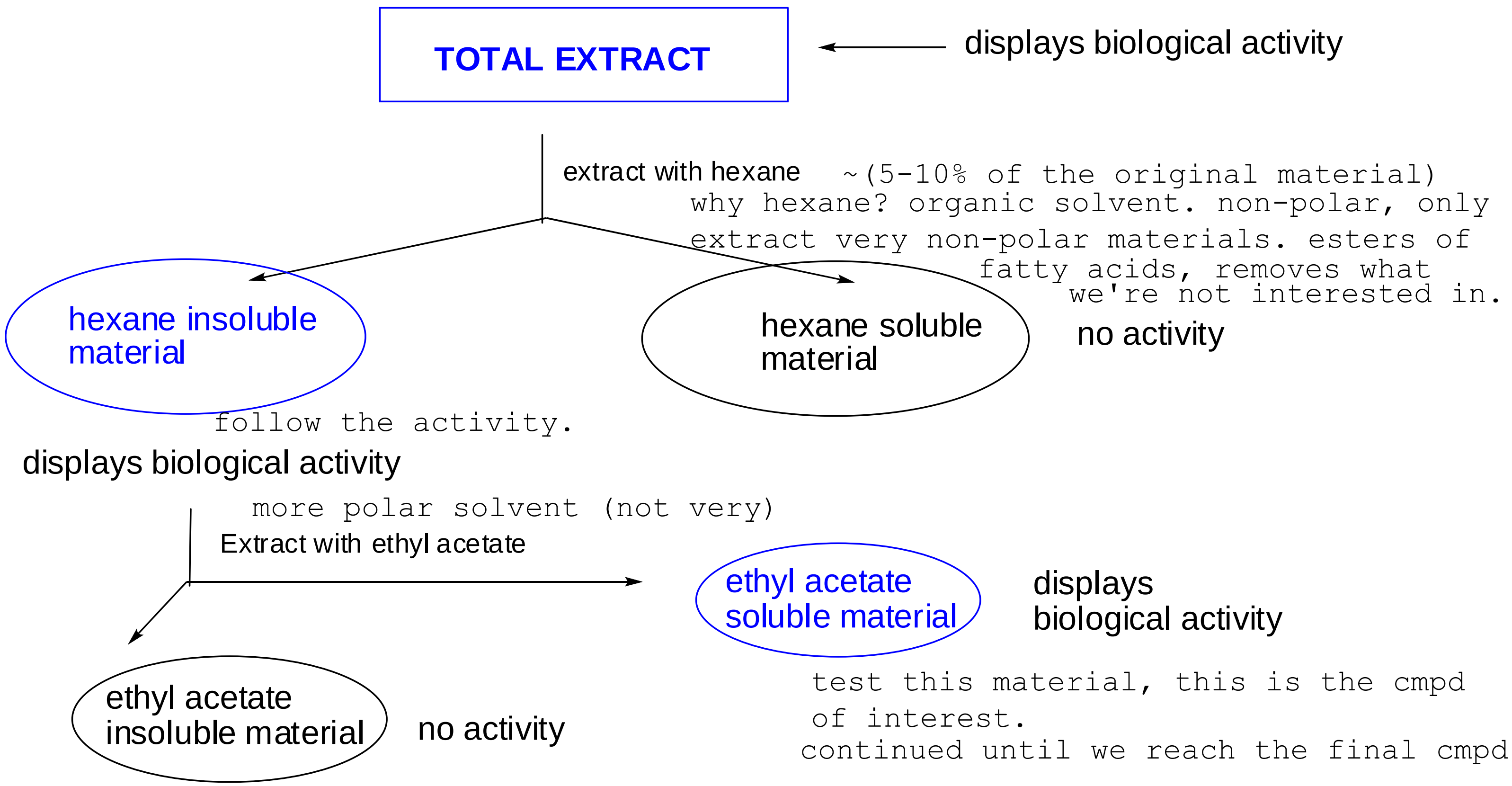
Standard method of deciding:

Use a standard operating procedure- Bioassay guided isolation to determine which is the active component.

Simple & effective concept.

Bio-assay guided isolation

- Procedure –de-convoluting the mixture-1



• Procedure: De-convoluting the mixture-2

Chromatographic separation of the components of the mixture in the fraction with activity

ethyl acetate soluble material — displays biological activity

through using different solvents to extract off the silica gel we get:

Silica gel chromatography yields many individual fractions with differen components
-some single pure compounds, others still as mixtures

e.g., 4 major fractions, which one has major activity? sometimes might even be 100.

Ckeck each fraction for activity

hopefully there is a pure fraction that shows all the activity. repeated this process many times over.

If the active fraction is a pure compound, then the process is complete
If it is still a mixture, then carry out a second, even third separation step until a pure active compound has been isolated

Structure determination, by various techniques:
Nuclear Magnetic resonance spectroscopy
Mass Spectrometry,
X-ray crystal structure determination.

requires experience and "brains"

lazy and get nice crystal material, you can send the information off to an expert and they will achieve a structure with every atom & ring is displayed and you're assured you have the correct structure.

Important *in vitro* Screens

- Antibiotic screens have been and continue to be done quite routinely. Need a variety of different pathogenic [harmful] bacteria growing in a culture and then applying solutions of different compounds to detect inhibition of growth.
  - MIC

- Anti-viral screens are carried out to detect compounds which kill different pathogenic viruses

- Anti-cancer screens look for compounds that kill different types of cancer cells but are relatively benign to healthy tissue
  - LC50

Natural Product as sources of new Drugs

- Terrestrial plants and to a lesser extent sea organisms continue to be a key source of novel drugs
- Surveys have been carried out to show where drugs introduced since the 1960's have originated.
  - 60% of all new antibiotics have a natural product origin
  - 50% of all new approved anti-cancer drugs have a natural product origin or are natural product derived
  - Most Central Nervous system drugs have a synthetic chemistry origin but are based on the naturally occurring alkaloids with CNS activity.

Natural Product as sources of new Drugs

- Terrestrial plants continue to be a key source of novel drugs
  - Untapped traditional knowledge
  - Amazing variety of structures continue to be discovered from plants
  - Often sources for anti-cancer, anti-fungal, anti-oxidants,
    - Also mood altering drugs
    - Most of the alkaloid derived drugs have a plant origin

Natural Product as sources of new Drugs

- From Microorganisms
  - Often a source of antibiotics and anti-cancer compounds
- From the oceans
  - Typically many compounds have antibiotic and anti-cancer activity

The inherent advantages of natural products

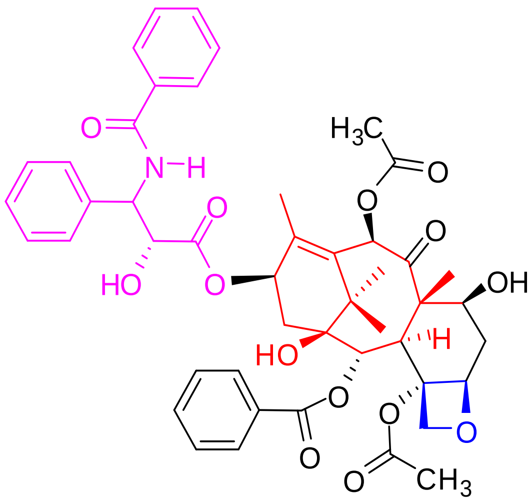
– Natural roducts are produced by biological systems
  - More likely to be bio-active. Why
  - More likely to be bio-available. Why
  - Single stereoisomer – why? – examples!

Single isomers are desirable

- Enantiomers often have different biological properties
  - Example(s)
- Diastereomers are different compounds
  - Not surprisingly, they have different biological properties
    - Example(s)

Structure variety in natural products-ovarian cancer

- Taxol



Orginally obtained from: Bark of pacific yew trees

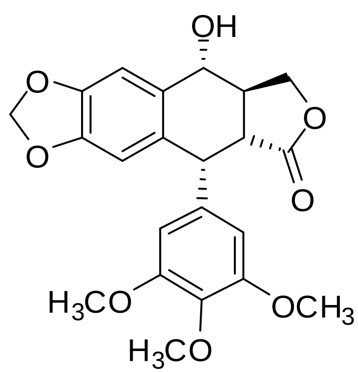
Clinical Uses: Chemotherapy

ovarian,, breat. lung

Discovered by screening for cytotoxic effects

- **

- Podophyllotoxin
  - Small cell lung cancer

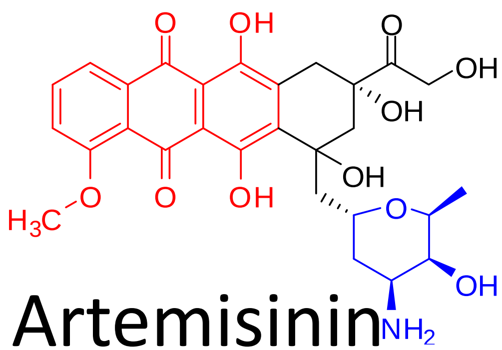


Isolated from the roots of the North American May Apple *[Podophyllum peltatum]*

Traditonal use natives: removal of warts

Structure variety in natural products

- Adriamycin



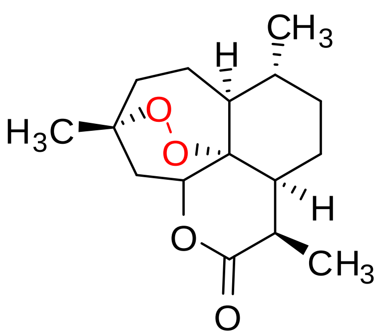
Discovered by screening for cytotoxixc activity

Clinical Uses, since the 1960s for a variety of cancers

including:

leukemia, , hidkins lymphoma, bladder, breast, stomach, lung...

- Artemisinin



Artimisinin -discovered in a screening program of tradional Chinese Medicines by Chinese Army Doctors

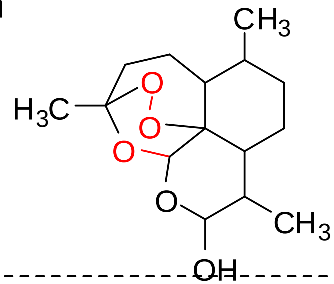
Currently, the most effective medicine for the treatment of malaria

Typically: Artimisinin Combination Therapy {ACT} to slow the development of resistance

Now being investigated as a potential anti-cancer compound based on studying the mechanism of action. Key: the peroxide bond

Structure variety in natural products- Antimalarials

Artemisinin

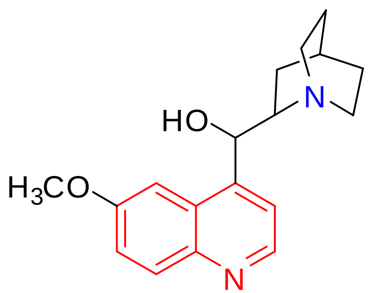


Based on Chinese Traditional Medicine

Best, most potent anti-malarial

Obtained from a shrub native to China:
Artemesia annua

Quinine

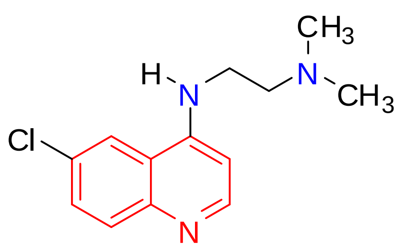


Based on traditional knowledge
(Peru)

Isolated from the Chincona
tree bark

First known potent antimalarial

Choroquine

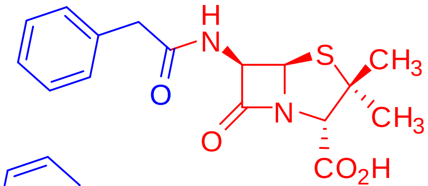


Synthetic
Excellent antimalarial
some resisant parasite

Structure variety in natural products

- **The Penicillins 1928->1942->**

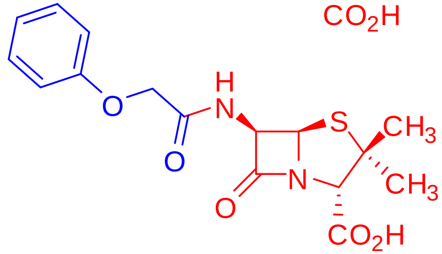
Penicillin V



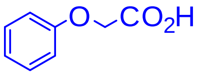
Original naturally produced penicillin
from the penicillium mold
Gram positive activity

Poor oral activity

Penicillin G

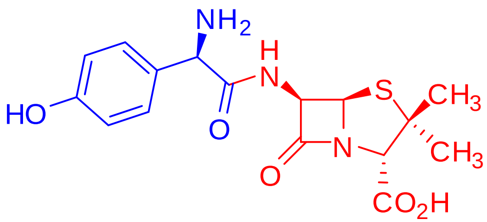


Also produced via fermenation by
adding



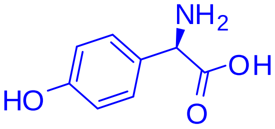
Gram positive activity
Good oral activity

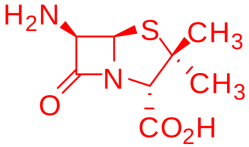
Amoxycillin



Broader spectrum activity;
includes Gram Positive and some Gram
negative bacterial

Orally active
Produced semi-synthetically



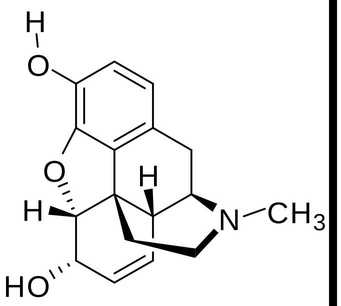


6-amino-penicillanic acid

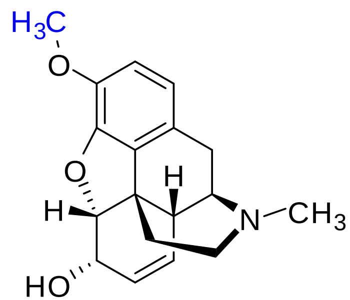
Structure variety in natural products

- Morphine –Alkaloids- natural or semi-synthetic

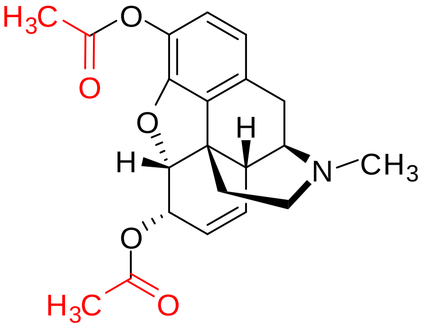
Morphine



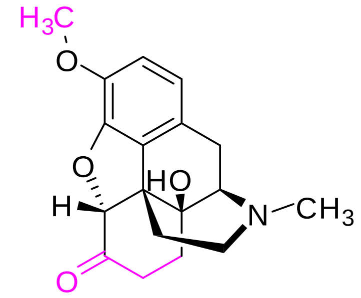
Codeine



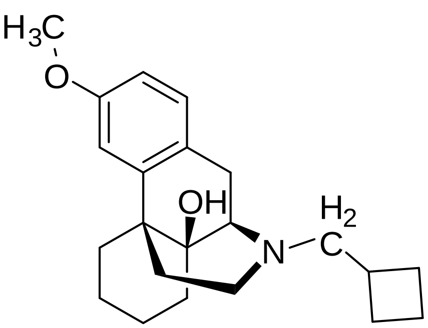
Heroin



Oxycodone



- Butorphanol
  - Synthetic

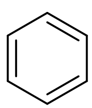


CNS active alkaloids

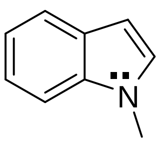
Common structure requirement for CNS active compounds:

Basic nitrogen atom joined to an aromatic ring via a satruated two carbon units

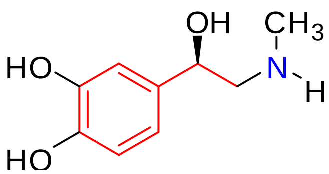
Aromatic rings are planar rings with all sp2 hybridized atoms with 6, 10, 14π electrons



benzene ring is a 6π eletron aromatic ring

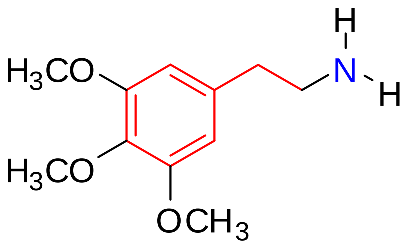


indole ring is a 10π eletron aromatic ring

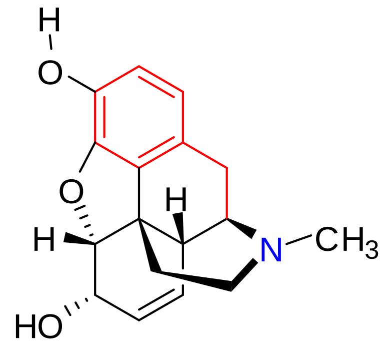


Adrenaline = epinephrine

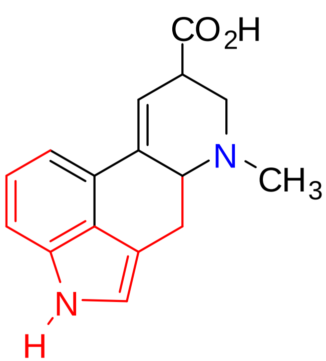
Natural CNS Active compounds



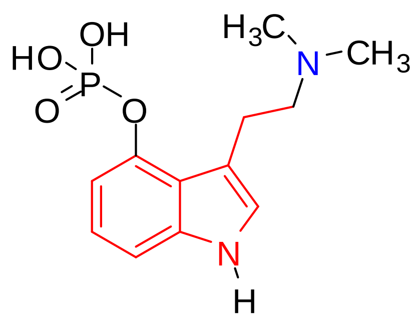
mescaline
peyote cactus



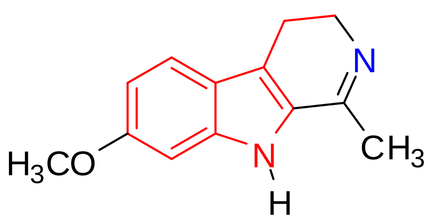
Morphine



Lysergic acid
Ergot fungus
growing on rye



Psilocybin
[sacred mushroom

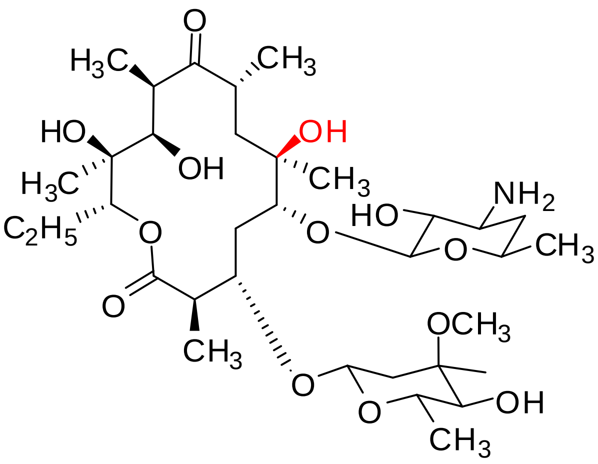


Harmaline
Halucinogenic drink
Amazon: Ayahusca

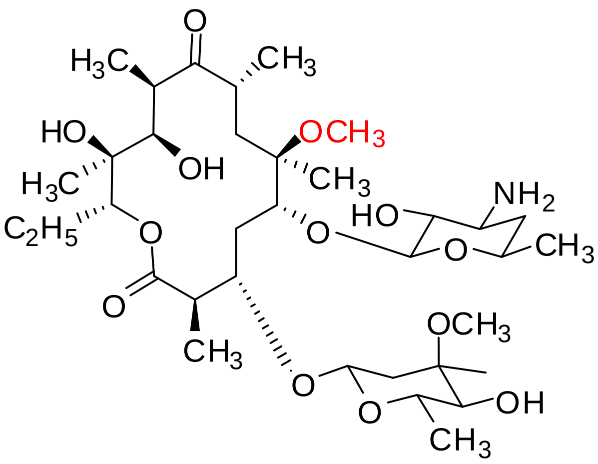
Structure variety in natural products

- Macrolide antibiotics from micro-organisms
  - Erithromycin and Clarithromycin

Erithromycin



Clarithromycin



Street drugs based on the Ar-$CH_2CH_2NR_2$ model

- Amphetamines, Dopamine , serotonin

Lecture 4.