BPS 2110

- Room change:
  - **From the current room to CBY003 starting on Thursday !!!!**
- Questions
  - **A set of questions for part A of this course has been posted. These will be updated in the next two weeks.**
  - It is anticipated that at least 75 % of the October 2 test will have the same or quite similar questions

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September 14, 2012 2:11 PM

At the request of the International AIDS society, I'm forwarding you an announcement concerning a new program aimed at encouraging new initiatives in the field of HIV cure research. The society is very eager to encourage new thinking in an attempt to identify strategies to tackle this important but very challenging medical problem, and I therefore encourage you to consider making an application.

In addition, please feel free to forward this message to any of your colleagues who you think might be interested in making an application for support under this program.

International AIDS Society

Don't memorize structures of molecules.

Lecture -4. Natural Product as sources of new Drugs

Wheres the best source of new structures & new drugs? Synthetic chemists believe they can make the best molecules from nothing. Others believe nature is important in dev.

- 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

Natural product is not used as is, the natural product is an inspiration, i.e. Lipitor. Various organisms, in competition, try to kill other organisms so they create chemicals that are toxic to similar species. We use this to bioassay for antibiotics (relatively easy to do).

  - 50% of all new approved anti-cancer drugs have a natural product origin or are natural product derived

Also easy to test for anti cancer compounds. Been studying this disease for 60-70 years. All natural products have been tested for cyto toxicity.

If you understand the biochemistry, you can try to develop a drug based on the biochem of the diseas

  - Most Central Nervous system drugs have a synthetic chemistry origin but are based on the naturally occurring alkaloids with CNS activity.

Plant secondary metabolites - why do plants make this huge variety of structures?
1. just like in our body that we have hormones, plants have secondary metabolites that carry out functions.
They also ensure propagation of the species, they also provide protection/defence against insects & herbivores.

plants will make 8-10 different compounds with different structures, prevents immunity, ..
50% of the compounds were produced from 1983-2008, are synthetic based on molecular modeling & ideas on how to treat disease.

50% of the 983 drugs made in that time period have a natural product origin.

Hypotheses

- **Focused screening**
  - by learning from Healers (shamans) –healer consensus
  - via studying available literature describing the use of plants for medicinal purposes - Traditional Chinese Medicine

(most famous) they've been using plants for 1000s of years, even now the WHO say 80% of people in the world use natural products as their primary medicine.
even our society has people that prefer to take a natural drug instead of a pharmaceutical drug.

  - by focusing on plants belonging to families that are known to have certain types of bio-active compounds

pepper plants have been used for medicine, as insectidies, to season our food. these plants have active ingredients that are useful
900 members of this family world wide. costa rica has 5-600. not all have been investigated.

    - Rare plants in such families often give new and unusual compounds

unusual bioactivity. method to the madness to discovering bioactivity. 60-70 years ago techniques were less refined & focused.

About 10 years ago, the natural cancer society requested a site visit, scientists proposed a major study of plants from the islands along the equator in the pacific ocean. Have unique flora, collect every plant, test the extracts, to see if something can be found from this ecosystem. (huge project). NIH had a grp from san diego cali had developed a method for accessing the ocean floor

Random Screening

from different depths, they had not accessed whether there was any development of unique cmpnds.

- Screening of organisms that have previously not been investigated
  - Plants from areas not previously accessed
  - Rare plants
  - Micro-organisms growing in areas not previously accessible or growing under unusual conditions
    - Ocean floor at various depths
    - Near toxic dumps

Some are in phase 1,2 of clinical trials.

Something to resist toxic materials, you can find bacteria that help with the metabolism of cmpds that are toxic in origin.

Anti fungal cmpds, where would you go to collect these cmpds? First you find fungus in a rainforest, most are growing under wet-moist cond., maybe other plants that grow in the area create compounds that control/kill the fungus.

Random (rational) approach

- Suppose you are searching for an anti-fungal compound. Where would you collect plants that might generate anti-fungal compounds?
- New anti-biotic compounds would likely be synthesized by micro-organisms that compete against each other.

Look at micro-organisms in the soil. Likely to produce compounds that are toxic to other organisms.

- Insecticides are likely to be produced by plants that grow in areas with many insects

One of the plants haven't been consumed by insects, maybe that plant has a cmpd that protects against insects.

- Plants may produce compounds toxic to mammals to defend themselves against herbivors

Certain plants aren't eaten, those are the ones that produce the cmpd. Bitter tasting plants also accomplish this purpose.

This methods don't work all the time, but many times they do work.

More focus on plants because humans have more contact with plants. There is no ethnopharmacology with sea life in the same way that there is with plant products.

Natural Product as sources of new Drugs

- Terrestrial plants continue to be a key source of novel drugs
  - Amazing variety of structures continue to be discovered from plants
- Marine life, for ex. sponges, corals,
- Unusual marine life growing deep in the ocean under very high pressure.
- Different organism give different types of compounds

Biodiversity conservation, estimated 250k of plant species world wide 50% occur in tropical forests, which cover only 7% of land surface compared to 16% in 1950. (cutback of forests).

relatively modest portion of the plants have been investigated for their secondary metabolites which may have medicinal potential. there is a long way to go.
plants continue to be a source of potential medicine.

Natural Product as sources of new Drugs

- Plants,
  - Often sources for anti-cancer, anti-fungal, anti-oxidants
  - Also mood altering drugs
  - Most of the alkaloid derived drugs have a plant origin
- From Microorganisms
  - Often a source of antibiotics and anti-cancer compounds
- From the oceans
  - Typically many compounds have antibiotic and anti-cancer activity
  - Compounds which incorporate Cl and Br

The inherent advantages of natural products

- Natural products are produced by biological systems
  - **More likely to be bio-active. Why?**

the reason is obvious: the natural products are made in biological systems. the material is biological in origin, it is more likely you are able to absorb it and it will have biological activity. natural products are likely to be absorbed (this is the first step) and a key property of a drug. ADME - absorption, distribution, metabolism, excretion.

The inherent advantages of natural products

– Natural products are produced by biological systems

- **More likely to be bio-active. Why**

- **More likely to be bio-available. Why**

they are absorbed and have the right structure, right polartiy, etc... to fit into active sites and then be active.

The inherent advantages of natural products

– Natural products are produced by biological systems

- **More likely to be bio-active. Why**

- **More likely to be bio-available. Why**

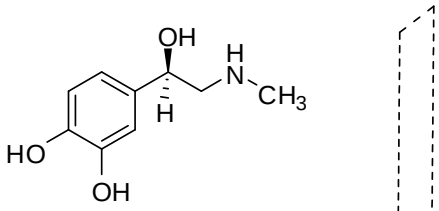
- **Are produced as single stereoisomers. Why is this important?**

VERY IMPORTANT:
right handed or left handed. one of those 2 isomers is likely to have the activity, the other doesn't. all kinds of discussions have been going on for 25 years, should company be allowed to sell racemic mixtures? what does the second isomer do, is it active? inactive? toxic? nature essentially always produces always one isomer. this is a tremendous advantage of making a drug.

Single isomers are desirable

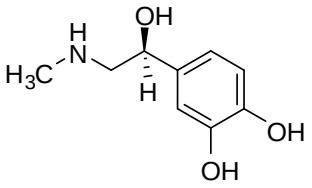
if you were making a drug, you'd have to have a good reason for making a 1:1 mixture.

- Enantiomers [non superimposable mirror images have different biological properties found in all bodies, produce in the (R) isomer. can produce both in the lab.
  - Example(s)



adrenaline- (R)-isomer

mirror plane



adrenaline- (S)-isomer

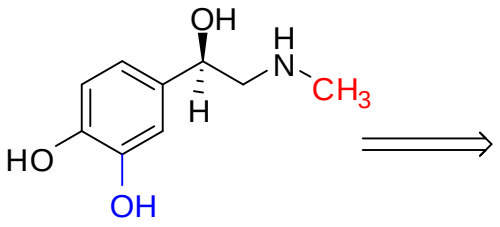
  - R isomer is ~ 20 times more active
  - Non-selective agonist of of all adrenergic receptors, including the major subtypes α_1, α_2, β_1, β_2, in the lung & the heart, basically speeds up things so you can fight, run, etc...
  - Physiologic responses to epinephrine by organ specific results, versus general results.
  - Heart Increases heart rate: activates α and $\beta 1$-receptors
  - Lungs Increases respiratory rate activates $\beta 2$-receptors open air ways, etc.
  - Systemic Vasoconstriction or vasodilation
  - Liver –raises blood levels of sugar and fatty acids

adrenaline - old british term. epenphrin is the new north american term.

Single isomers

1960s, golden standard for asthma compounds.

- **Salbutamol**- causes dilation of the bronchial smooth muscle,
- Unlike adrenaline -does not affect heart tissue



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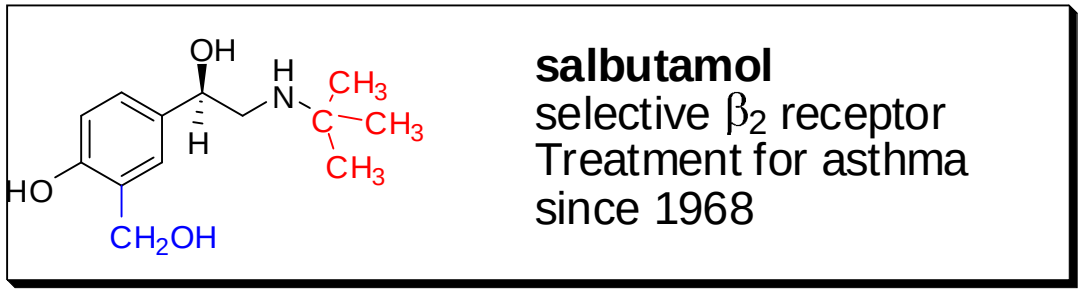


simple relationship. stereochemistry is exactly the same.

single isomers, if the stereochem was changed the other isomer is not nearly as potent.

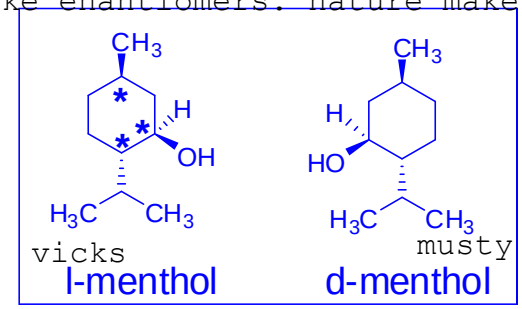
change OH to CH2 grp, because adrenaline having 2 OH grps, the body know it needs an enzyme to eliminate this. changes the OH grp into a CH2 grp rapidly, to make it an inactive. changing the methyl grp for a tertiary grp gave it the selectivity for one of those receptors over the other. a large grp might not fit into the other receptor but does fit into the B (beta) receptor.

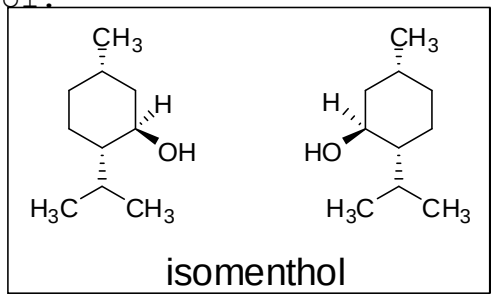
Single isomers are desirable

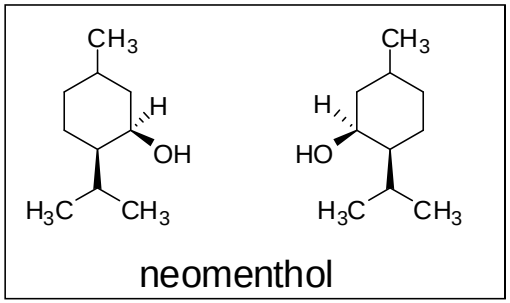
more stereocenters, more isomers.

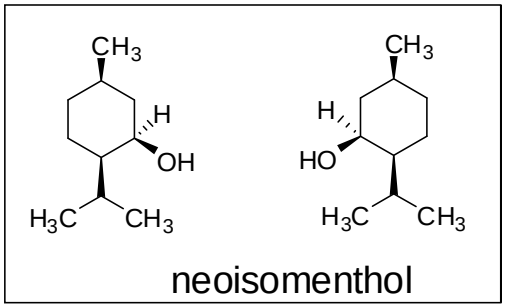
- Diastereomers are different compounds
  - Not surprisingly, they have different biological properties

this structure can have 8 different isomers, 4 sets of diastereomers. not similar like enantiomers. nature makes l-menthol.









  - Number of isomers 2^n where n is represents the number of chiral centers in the molecule
  - 8 isomers: 4 pairs of diastereomers

Racemic Drugs

simplifies the work.

- In a racemic mixture there are equal amounts of each enantiomer.

one of the things that has always been proved
is mixtures of racemic drugs are easier to synthesize
than one isomer, unless you already start with a
natural material or one that is biologically active.

- Racemic mixtures of drugs are easier to synthesize
- Usually one enantiomer is more active than the other.

Racemic Drugs

- Two enantiomers: One is the biologically active, the other is not.
  or more active than the other.
- Possibilities for the second isomer
  - No biological activity
  - Other biological activity
  - Toxic

need to be careful when pharmaceutical companies sell the racemic drug that the other enantiomer is harmless & does not have any effects.

up until 1990, that all the drugs were racemic. the assumption was made that one of them was not active. this was to simplify work. consequence? you need to take twice as much of the drug to have the same effect. the best scenario that you have is the other half of the drug is harmless, but this is not always true. sometimes the other isomer has unexpected biological effects or it has toxicity.

Chiral vs racemic drugs

- Up to about 1990 most drugs which had chiral centers were marketed as racemates
  - 50/50 mixtures of the isomer that carried the activity and that which is essentially inactive.
- Reason: Cost !! Theres no technical reason why you can't have single enantiomers.
- Consequence: - Need to take 2 X amount of drug.
- Best scenario:- The “other” isomer is harmless
- Other possibilities: The “other” isomer has
  - different biological effects
  - Has toxicity

Racemic vs Chiral Drugs

- Current regulatory practice:

Health Canada, FDA, European/Japanese health angecies, if you want to produce a drug:

- Ideally all chiral drugs should be sold as a single enantiomer.

you HAVE to try producing it as a single isomer.

sometimes:

- Allowance is made if it is shown conclusively that the second isomer is harmless OR

very important drug

- The drug cannot be prepared only as a racemate using currently available technology

(too difficult, too expensive)

- That enzymes in the patient convert one into the other – racemize the pure isomer

sometimes it can be shown that racemic versus single enantiomer
it doesn't matter, body converts one into the other: why bother worrying?

Biological racemization of drugs.

- The most “famous” example –thalidomide

simple chemical structure, one chiral center, causes birth defects. afterwards, people looked at the compound again, showed that one of the isomers was the isomer that caused the problem. if the other isomer is given, the birth defect problem would not occur. but: in the body, whether you give the left handed or right handed isomer, very quickly it is converted into a racemic mixture so you cant escape the terogenicity of the compound.

racemic mixture.

- Ibuprofen (Motrin, Advil)

considered by the WHO as a critical drug in central medicine. every hospital and every station in the countryside should have because it is a necessary drug to take care of simple problems. it is known as the (s) isomer is the active one, and yet the compound is still sold as a racemate because in the body there are enzymes that are capable of converting the (r) into the (s) and vice versa, so there is no point in making an isomer.

in many causes though, it is necessary to produce a single isomer.

MASS SPECTRA - some are sensitive enough to give the molecular weights of each compound, based on that you can come up with the formula of a compound. NMR (nuclear magnetic resonance) spectroscopy, gives a signal for each of the hydrogens that are unique in the molecule. carbon-13 NMR the isotope that is present in 1% of all the carbon atoms, gives a unique signal for each of the carbon in the molecule. signals are complicated but interpreted & simplified by the spectrometers. there are other ways of doing stereochemistry & connectivity, etc ...

HPLC (high performance liquid chromatography) illustrates how complex a natural extract can be.
each peak represents a different compound, there are some that are 30-50, maybe even more,
present in larger/smaller amounts, some you are aware of, others you aren't, etc...
newer machines an actually give molec. wgt. at the same time.

ADME

Lipinski Rule of 5

Bio-assay guided isolation

A plant extract is shown to have the desired activity.

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

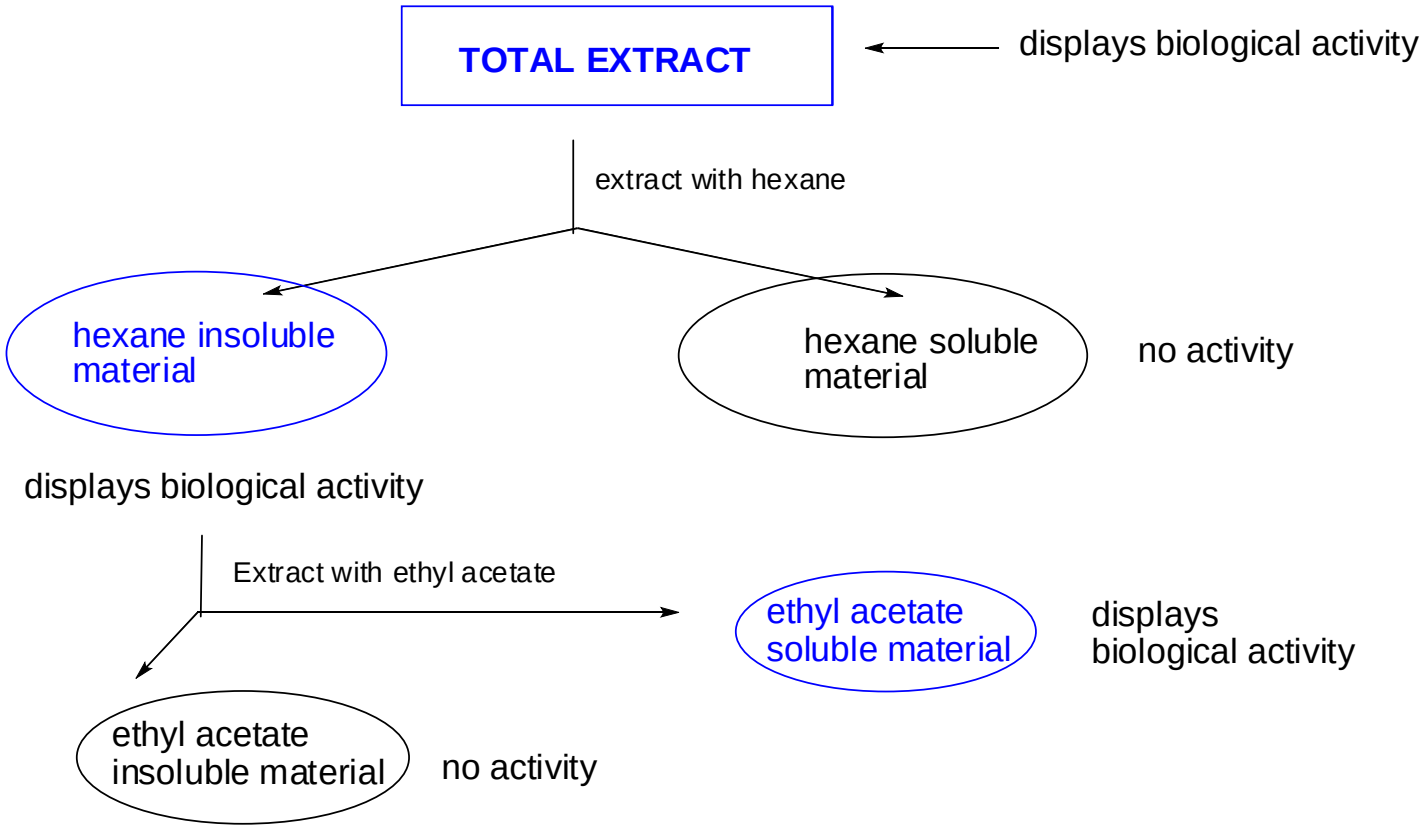
- water is used sometimes

Extracts have many, often 30- 100 or more component compounds. Which is the active component?

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

Bio-assay guided isolation

- Procedure –de-convoluting the mixture-1



De-convoluting the mixture-2

- Procedure:

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

ethyl acetate soluble material — displays biological activity

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

Check each fraction for activity

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.

Structure elucidation techniques

- High resolution mass spectrometry
  - Gives molecular weight of each substance to 4 decimal places > molecular fromula
- Nuclear Magnetic Resonance [NMR] spectroscopy
  - Proton NMR – gives signals for each unique hydrogen in a molecule
  - Carbon 13-NMR- gives a signal for each unique carbon in a molecule
  - Many different modern spin-spin correlations give connectivity between atoms in a molecule

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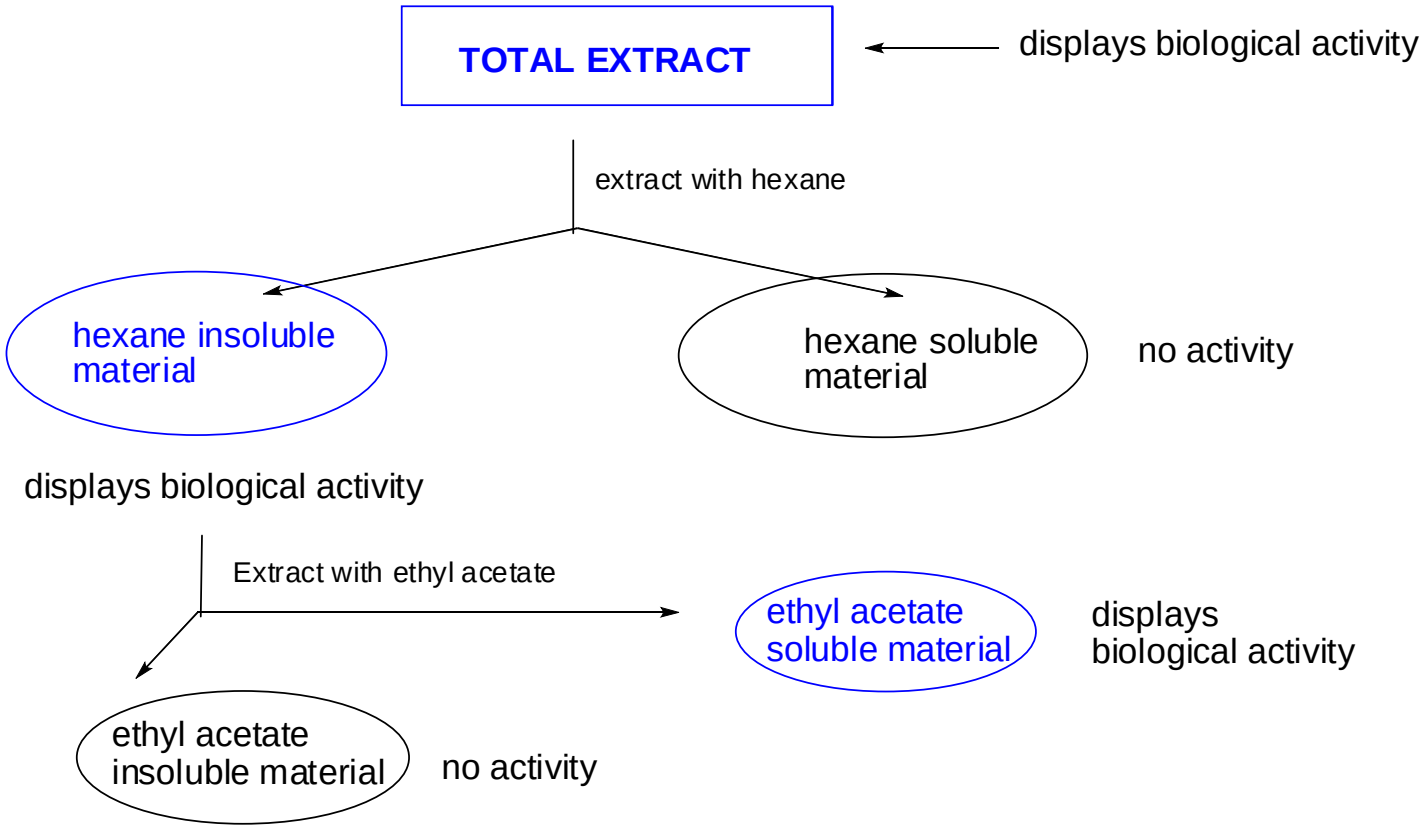

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Structure determination, by various techniques:
Nuclear Magnetic resonance spectroscopy
Mass Spectrometry,
X-ray crystal structure determination.

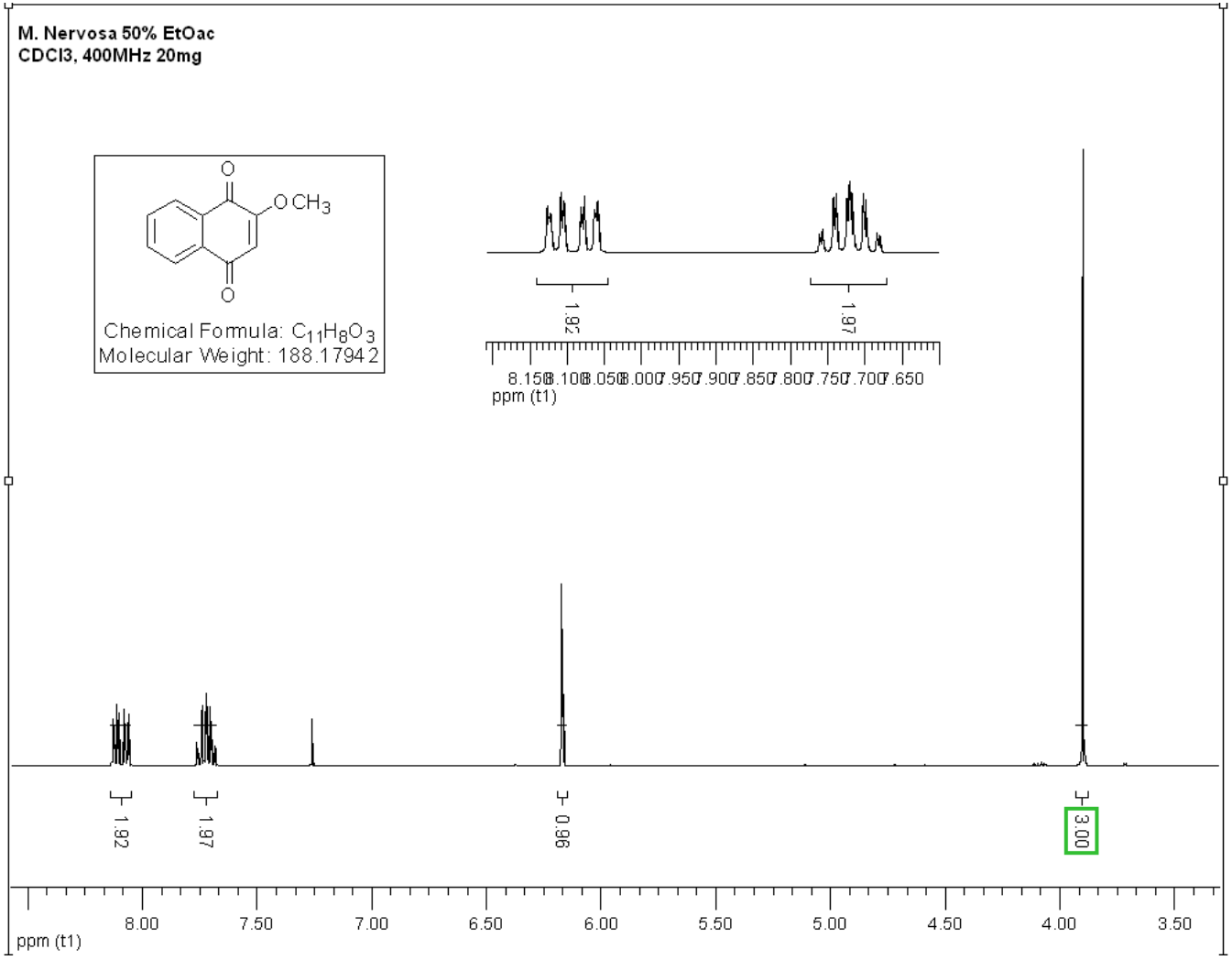
Chromatographic Separations

- High performance Liquid Chromatography [HPLC]
- HPLC –mass spectrometry [HPLC/MS]
- Gas Chromatography – for volatile compounds
- GC-MS
- Silica gel chromatography -Flash Silica Gel Chromatography
  - routine method
  - Suitable for large scale work.
- Combination: Flash silica followed by HPLC.

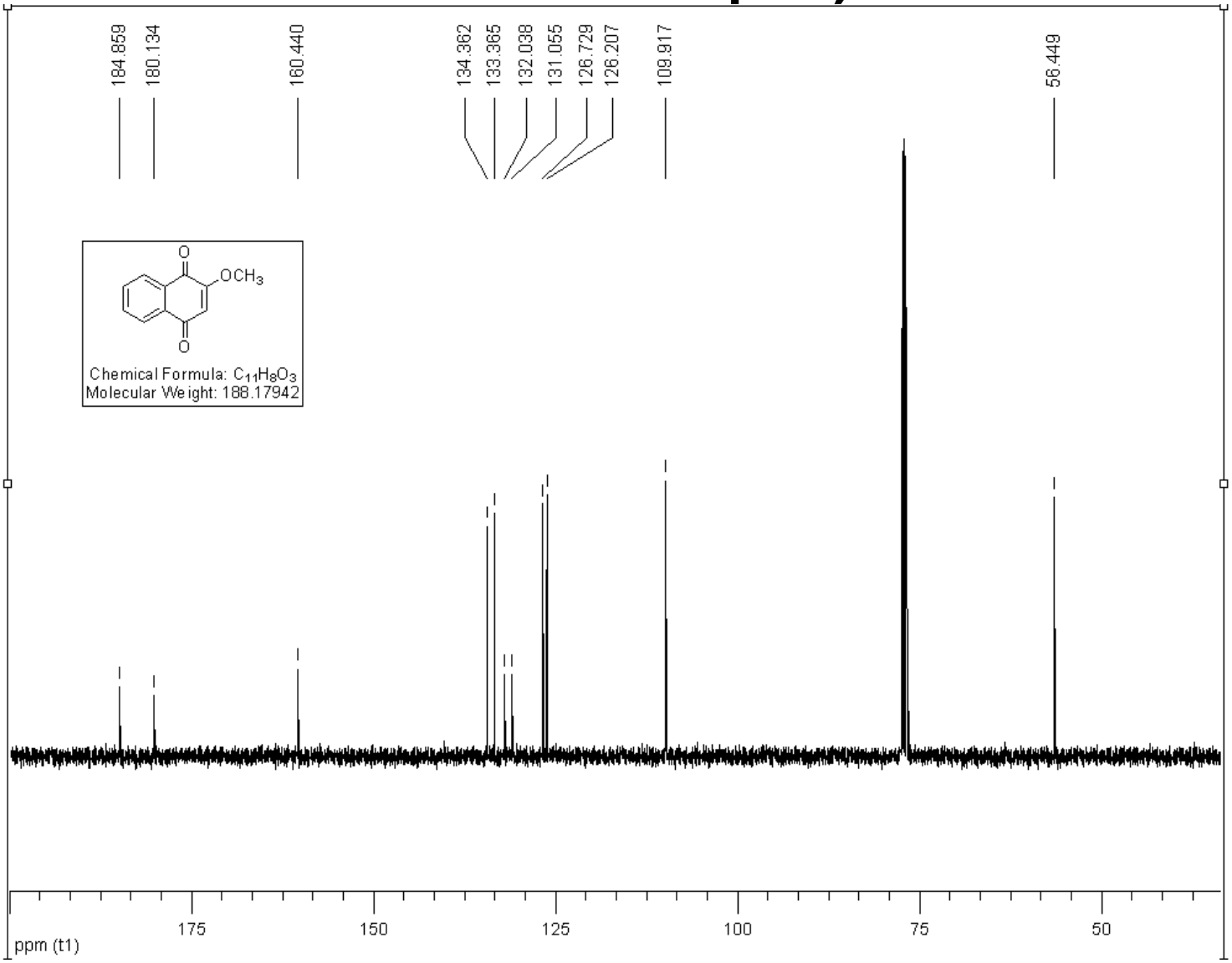
Chromatographic Separations

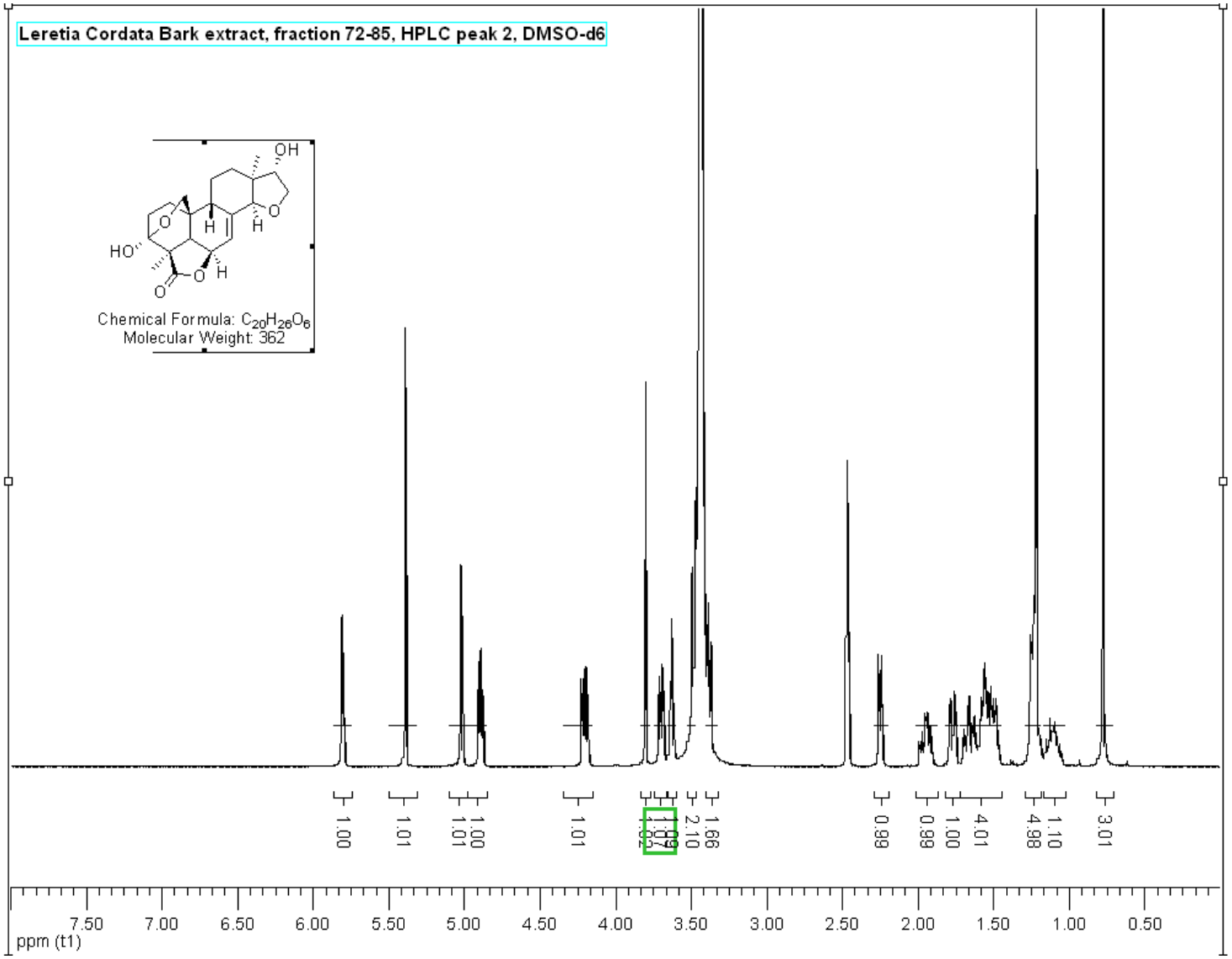
- High performance Liquid Chromatography [HPLC]

Structure elucidation techniques. Proton NMR



Structure elucidation techniques, Carbon 13 NMR

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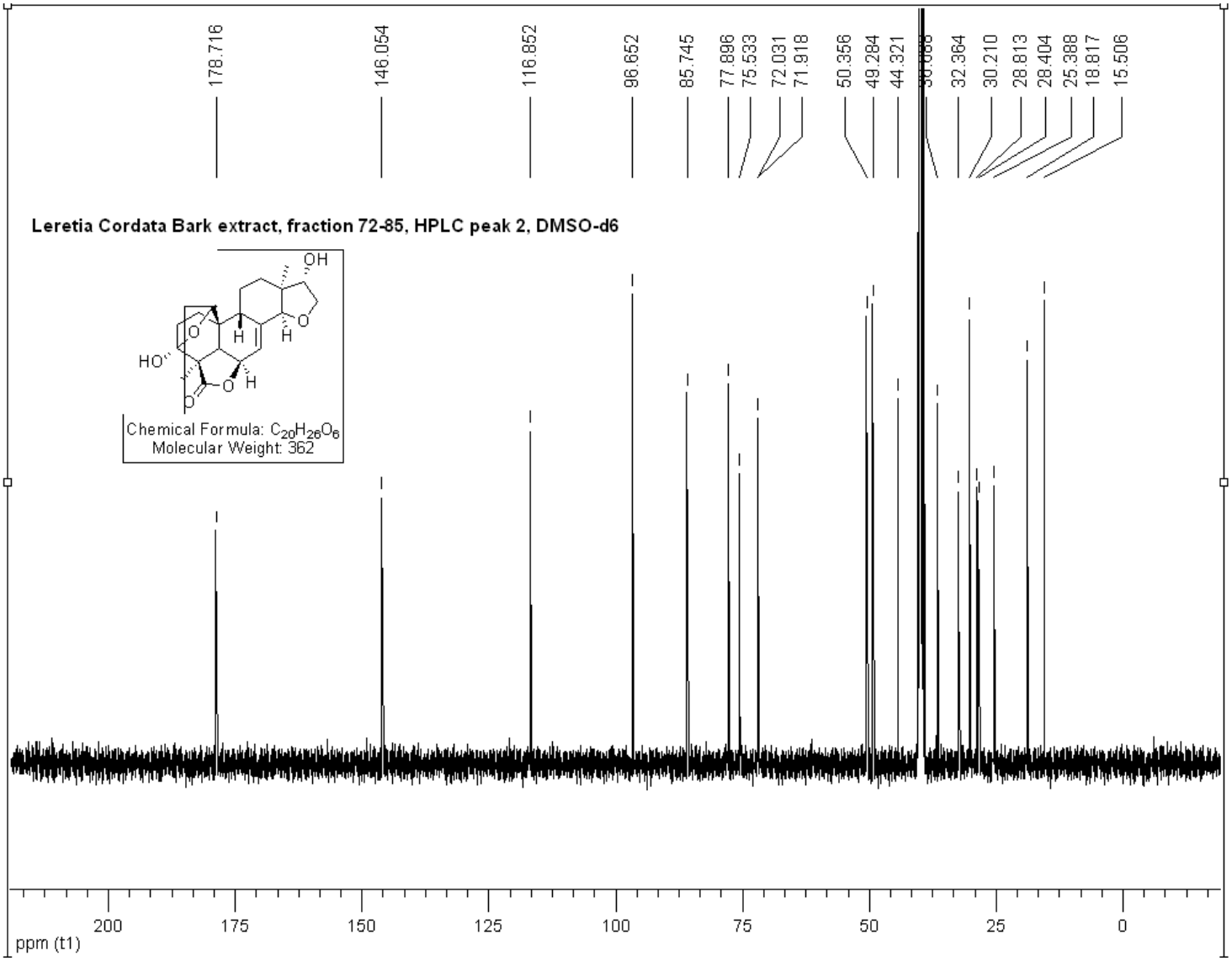
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44.321
32.364
30.210
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Leretia Cordata Bark extract, fraction 72-85, HPLC peak 2, DMSO-d6
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Chemical Formula: C20H26O6
Molecular Weight: 362
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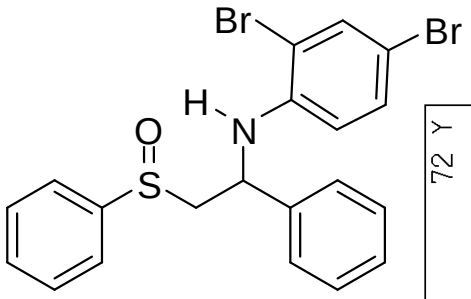
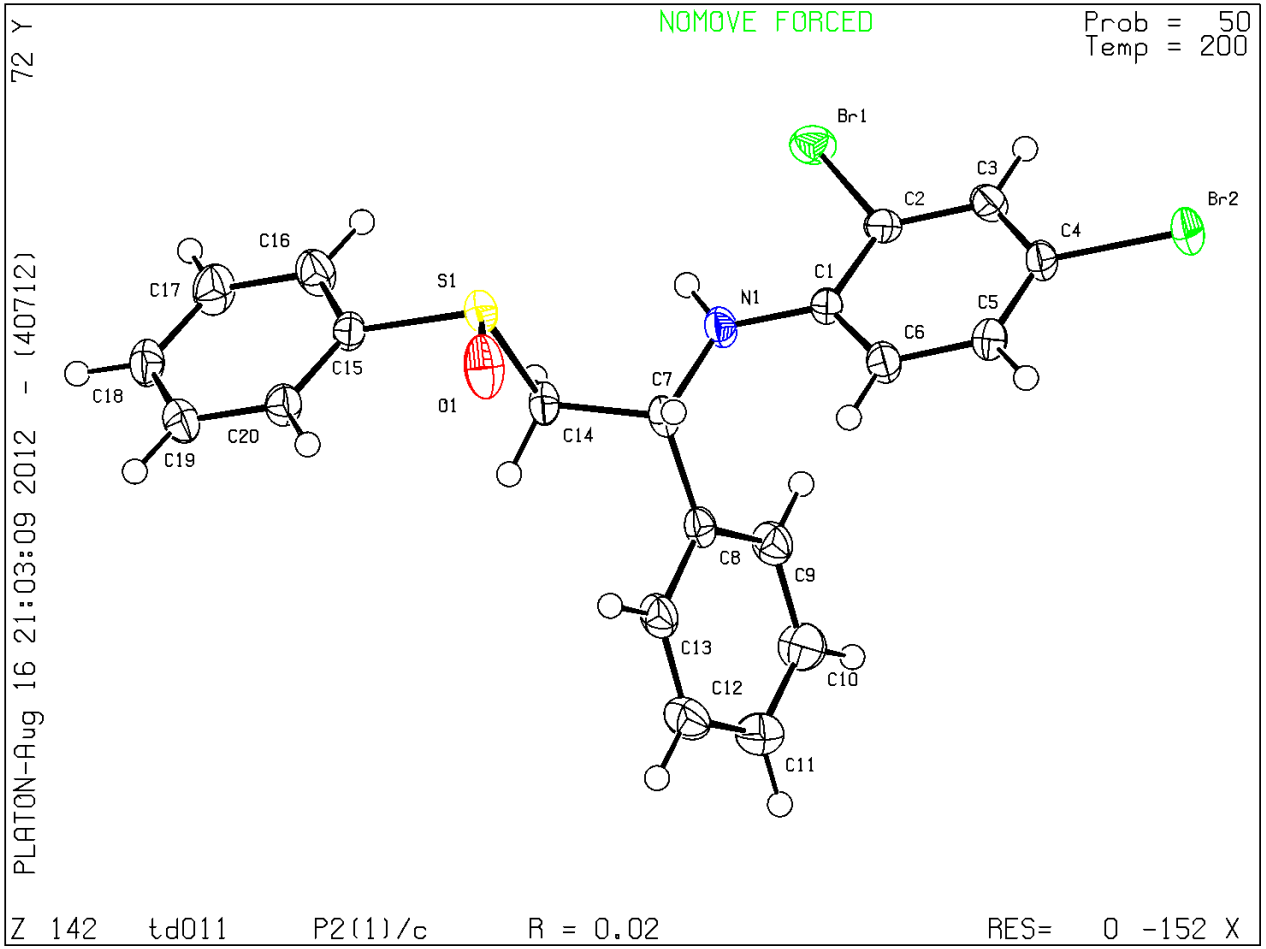
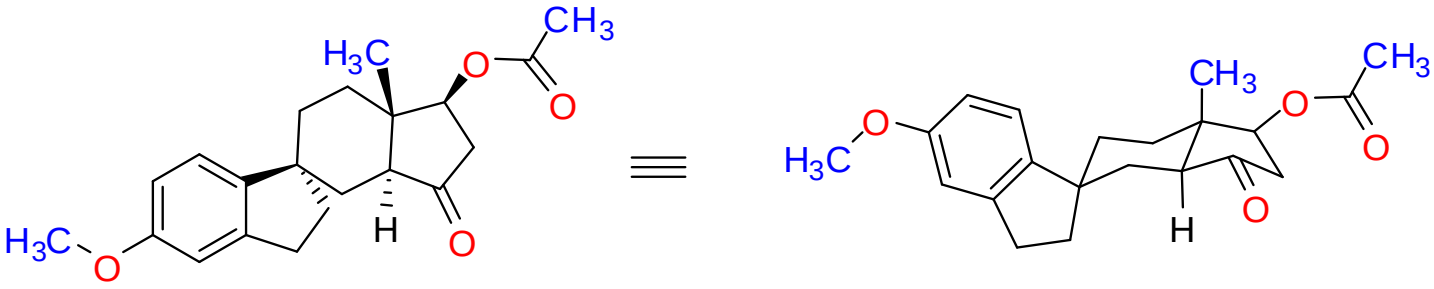
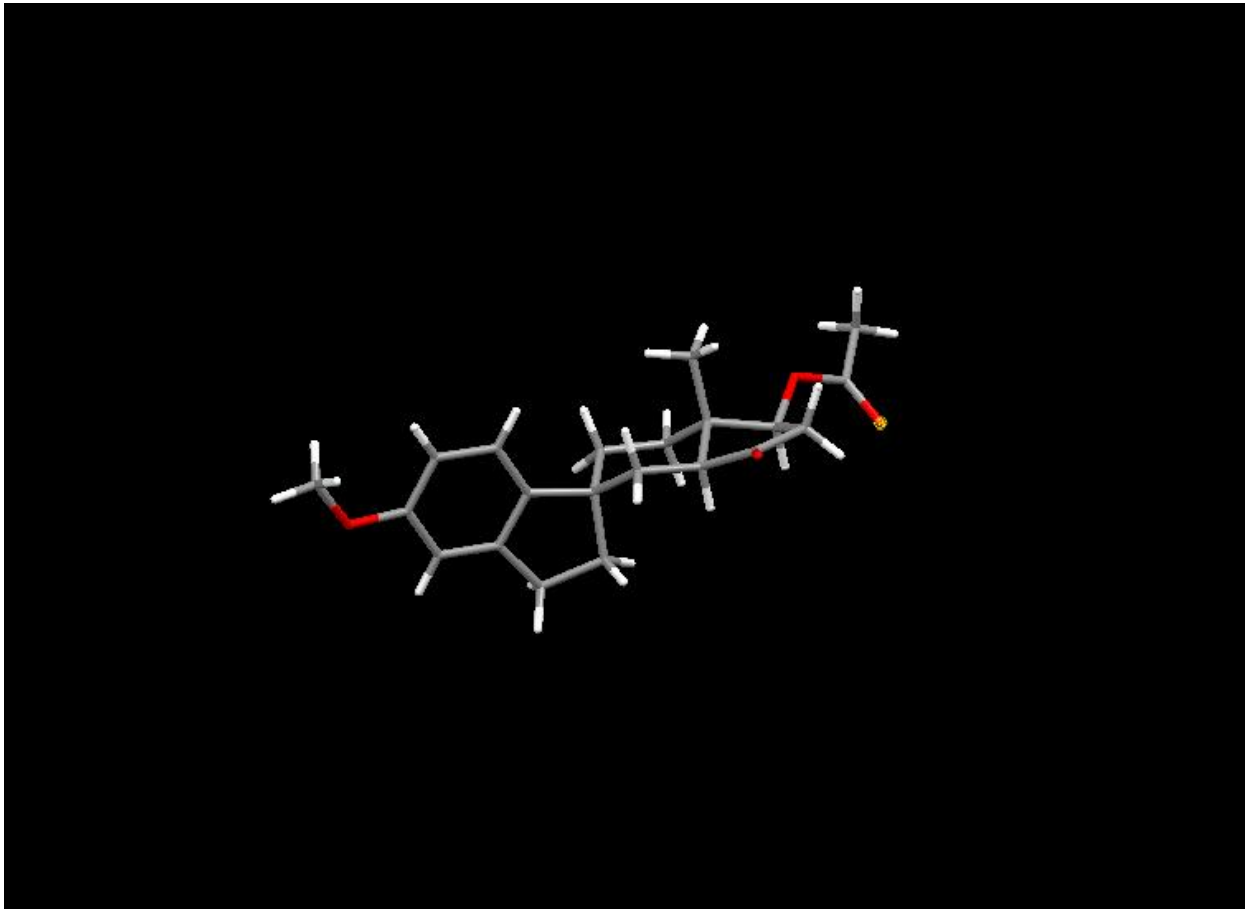
X-ray crystal structure determination





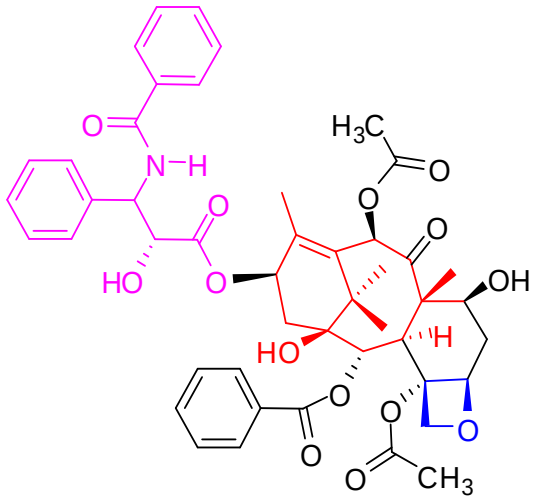
X-ray





Structure variety in natural products-ovarian cancer

- Taxol



Orginally obtained from: Bark of pacific yew trees

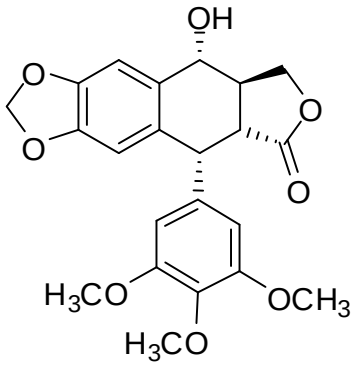
Clinical Uses: Chemotherapy

ovarian,, breat. lung

Discovered by screening for cytotoxic effects

- **********

- Podophyllotoxin
  - Small cell lung cancer

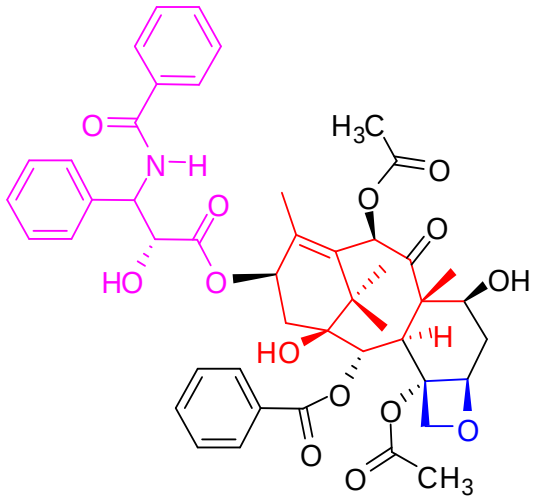


Isolated from the roots of the North American May Apple *[Podophyllum peltatum]*

Traditonal use natives: removal of warts

Structure variety in natural products-ovarian cancer

- Taxol



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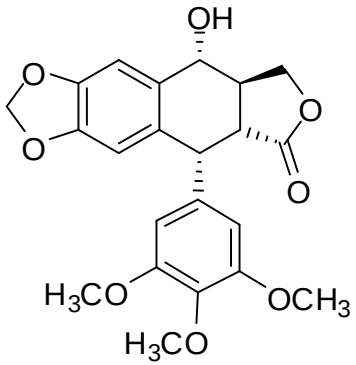

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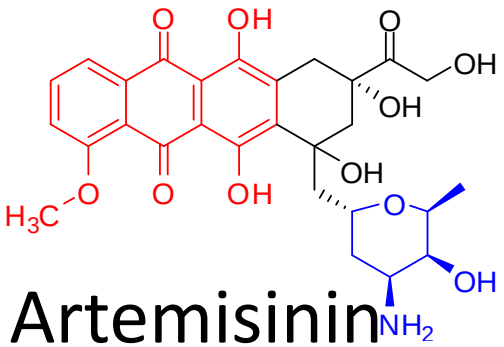


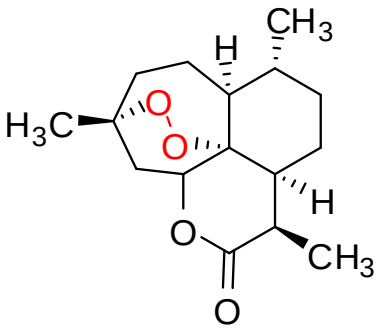
Discovered by screening for cytotoxixc activity

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

including:

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

- Artemisinin



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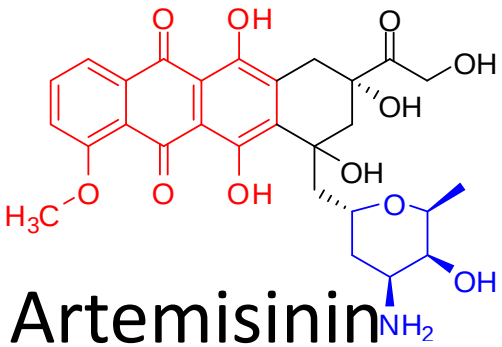
Now being investigated as a potential anti-cancer compound based on studying the mechanism of action. Key: the peroxide bond

Structure variety in natural products

- Adriamycin



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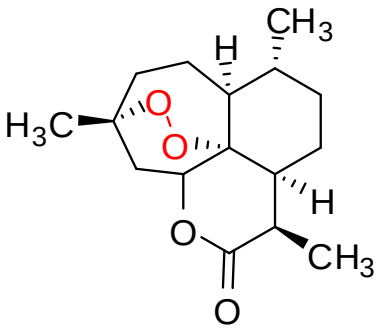

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Structure variety in natural products- Antimalarials

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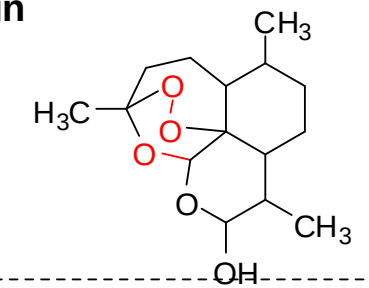
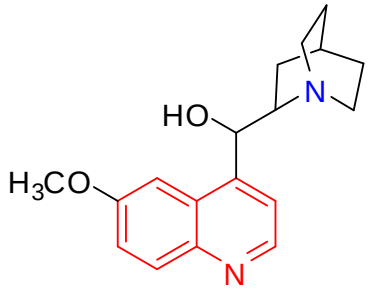


Based on Chinese Traditional Medicine

Best, most potent anti-malarial

Obtained from a shrub native to China: Artemesia annua

Quinine



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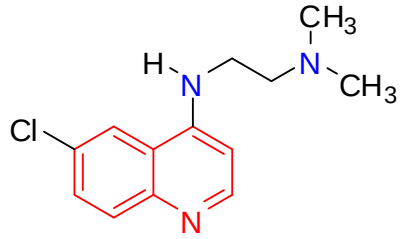
Isolated from the Chincona tree bark

First known potent antimalarial

Choroquine

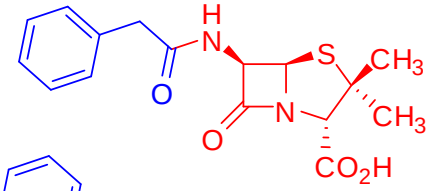


Synthetic
Excellent antimalarial
some resisant parasite

Structure variety in natural products

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

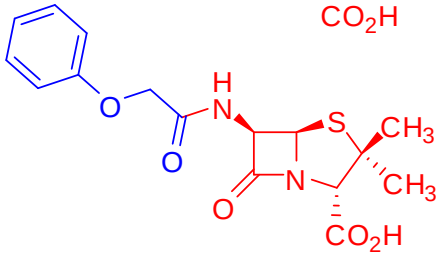
Penicillin V



Original naturally produced penicillin from the penicillium mold
Gram positive activity

Poor oral activity

Penicillin G



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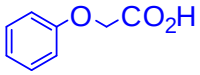
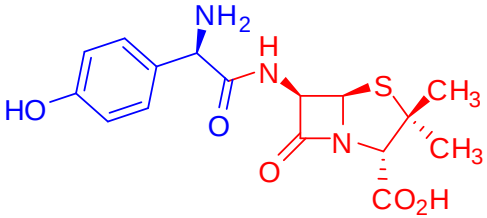


Gram positive activity
Good oral activity

Amoxycillin



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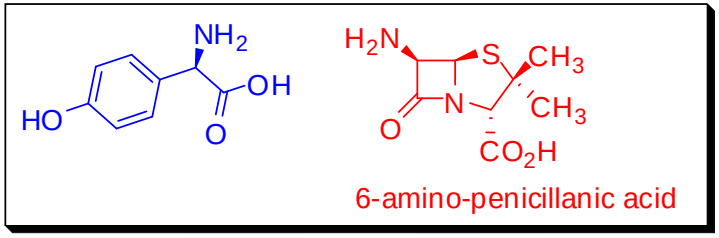
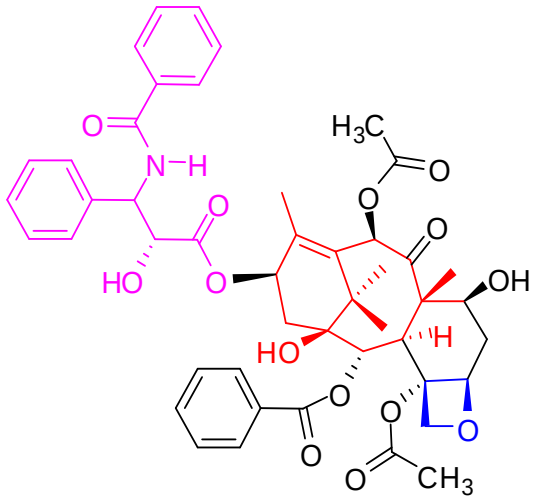
Orally active
Produced semi-synthetically



Structure variety in natural products-ovarian cancer

- Taxol



Orginally obtained from: Bark of pacific yew trees

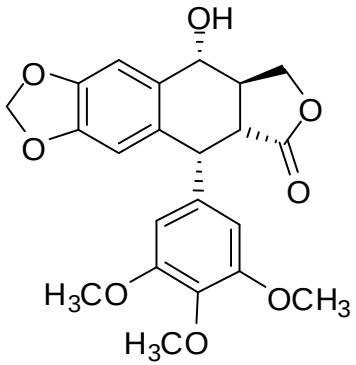
Clinical Uses: Chemotherapy

ovarian,, breat. lung

Discovered by screening for cytotoxic effects

- **********

- Podophyllotoxin
  - Small cell lung cancer

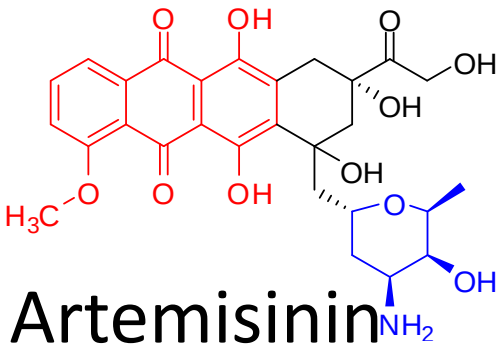


Isolated from the roots of the North American May Apple *[Podophyllum peltatum]*

Traditonal use natives: removal of warts

Structure variety in natural products

- Adriamycin



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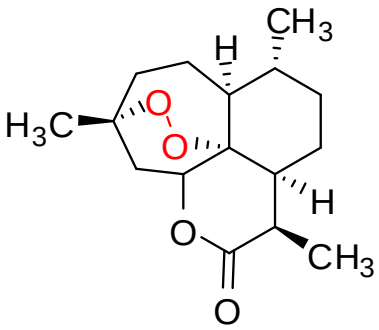

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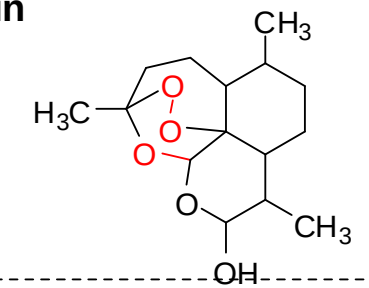

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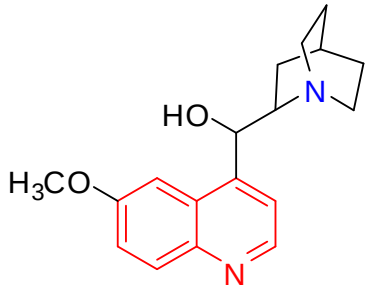

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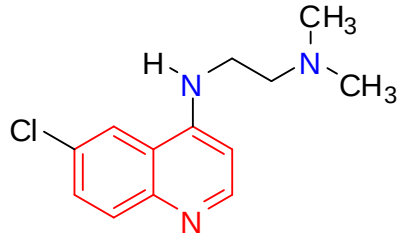

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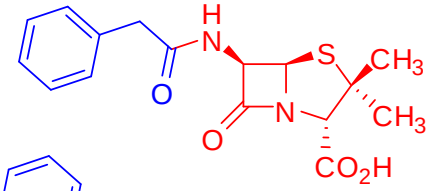

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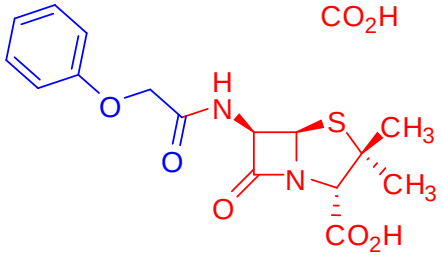

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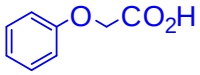

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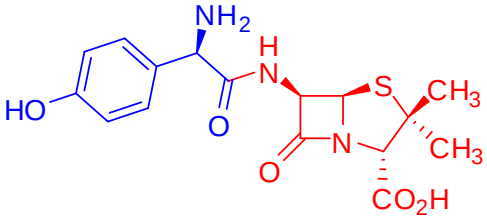

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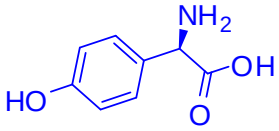
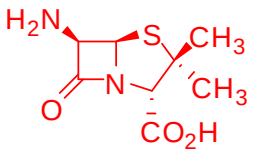


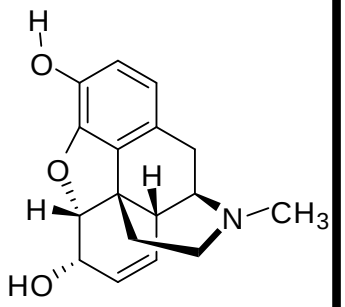


6-amino-penicillanic acid

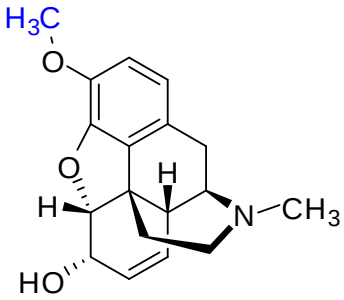
Structure variety in natural products

- Morphine –Alkaloids- natural or semi-synthetic

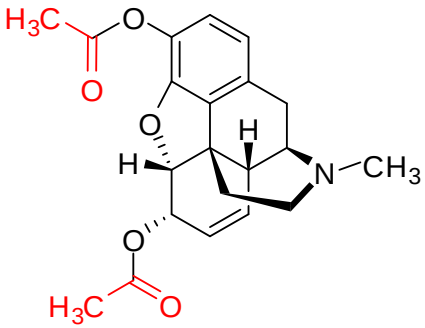
Morphine



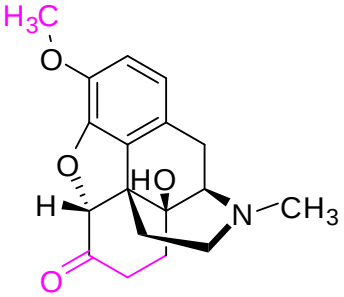
Codeine



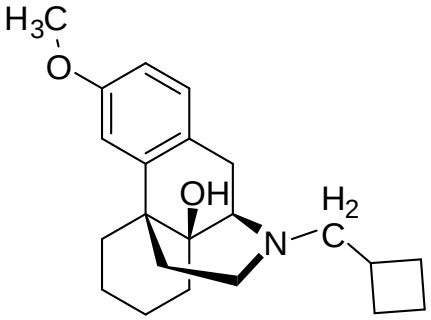
Heroin



Oxycodone



- Butorphanol
  - Synthetic

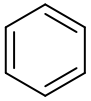


CNS active alkaloids

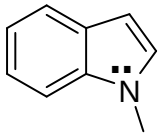
Common structure requirement for CNS active compounds:

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

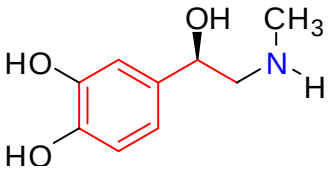
Aromatic rings are planar rings with all sp2 hybridized atoms with 6, 10, 14π electrons



benzene ring is a 6π eletron aromatic ring

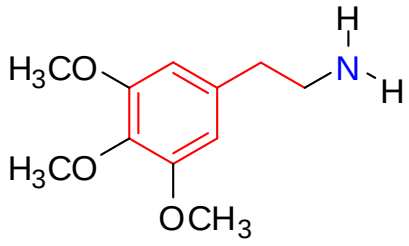


indole ring is a 10π eletron aromatic ring

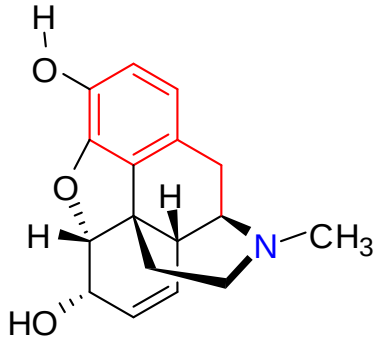


Adrenaline = epinephrine

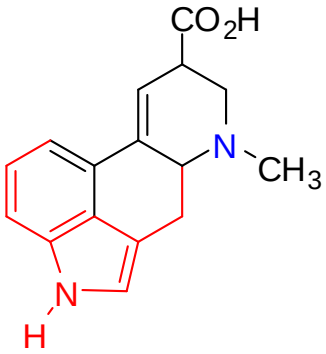
Natural CNS Active compounds



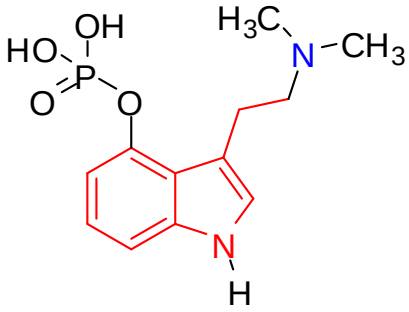
mescaline
peyote cactus



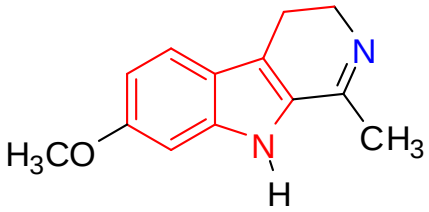
Morphine



Lysergic acid
Ergot fungus
growing on rye



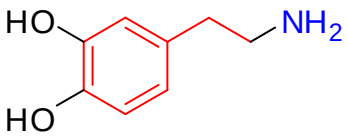
Psilocybin
[sacred mushroom



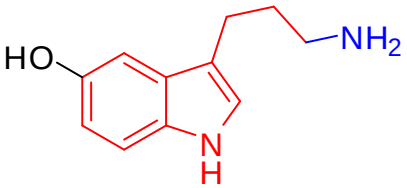
Harmaline
Halucinogenic drink
Amazon: Ayahusca

Neurotransmitters and “street drugs”

- Dopamine , serotonin and amphetamines

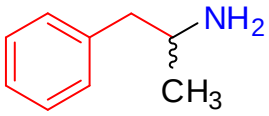


Dopamine
neurotransmitter

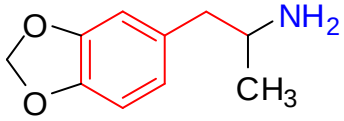


Serotin
neurotransmitter

contributes to feeling well



amphetamine
stimulant



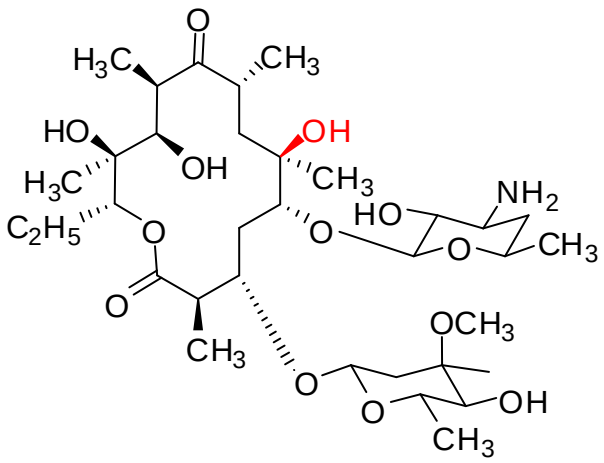
MDMA (3,4-Methylenedioxy-N-methylamphetamine)

Ecstasy,

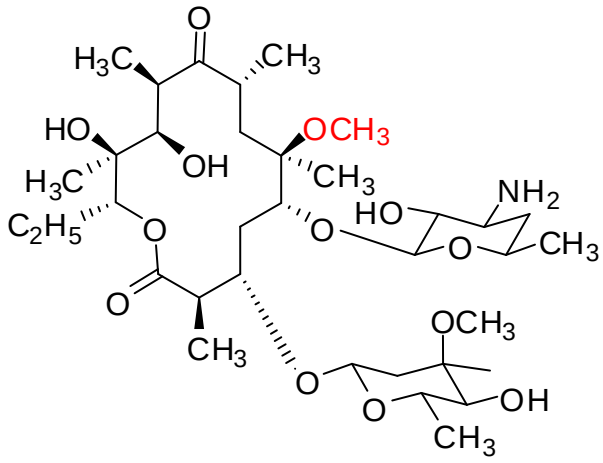
Structure variety in natural products

- Macrolide antibiotics from micro-organisms
  - Erithromycin and Clarithromycin

Erithromycin



Clarithromycin



Lecture 5.

- **Converting natural product leads into drugs**