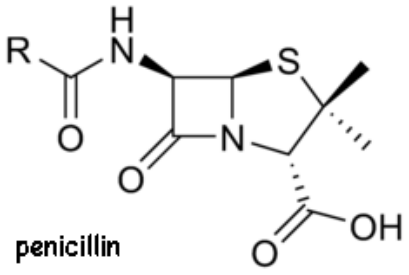
CHEM105: BIOCHEMISTRY AND SOCIETY

Experiment 7

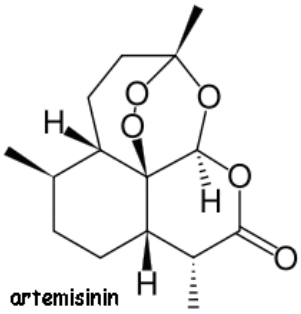
Traditional Chinese Medicine: Let's Find an Antibiotic

INTRODUCTION

Most of the commercially available medicinal drugs are derived from natural products - substances made by various organisms which they often use to protect themselves. Penicillin, derived from the fungus *Penicillium,* was discovered serendipitously in 1928 by bacteriologist Sir Alexander Fleming. Penicillin kills many bacteria by inhibiting their cell wall development. Some bacterial are resistant to penicillin, since they have an enzyme, penicillinase, which cleaves penicillin and makes it ineffective. Aspirin, acetylsalicylic acid, is made from salicylic acid, found in the bark of the willow tree. Natural salicylic acid derivatives have been used for thousands of years for pain and fever reduction, but it wasn't until a chemical derivative of it was made (acetylsalicylic acid - what we call now aspirin) which had superior properties that it became a standard medicine. Another drug, artemisin, derived from the shrub *Artemisia annua* which is been used in Traditional Chinese Medicine, is now the best drug to treat malaria. The structures of these drugs are shown below.



penicillin



artemisinin

In this lab, you will try to extract an antibacterial "natural product" from some traditional Chinese medicines. You will then test its efficacy by inhibiting the growth a common bacteria, *E. Coli* (not the dangerous kind). Antiseptics are agents that are used on living tissue, i.e. the skin of humans' before drawing a blood sample or as a preparation for surgery. Disinfectants are chemical agents used on inanimate surfaces, floors, walls, bedpans, etc. You will work in pairs for this lab (bring in 1 sample/pair).

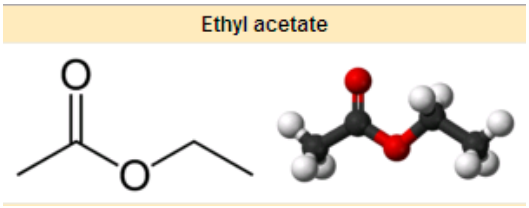
Preparation

The bacteria stock and nutrient agar plates were obtained from Professor Xie, Biology Department. The bacteria preparations need to be grown in a liquid, overnight culture (37^{o}C) and diluted 1/10 in sterile saline before the students prepare their plates

Procedure:

Preparation of material extract

As you remember from class, some substances, whose chemical structures have lots of oxygen atoms, dissolve in water (a polar solvent). Other substances, with few oxygens and many carbons and hydrogens, dissolve in organic solvents, like hexane (a nonpolar solvent), which itself has just carbon and hydrogen atoms. In this lab we will try to dissolve possible antibiotic substances found in traditional Chinese medicine in either water, or an organic solvent of intermediate polarity, ethylacetate, and test their antibacterial properties.



<u>If your solid is solid or semisolid in nature: (If it is a clear liquid, proceed to 7)</u>

1. Take a small sample of your solid and place it into a plastic mini-centrifuge tube (called a bullet tube). Add a small amount of water with a plastic pipette provided. See if it dissolves. If it does, skip steps 2-6.
2. Take a small rotary Teflon pestle attached to a drill and try to "homogenize" your sample as if it were in a blender. Do this for a few minutes.
3. Next centrifuge your bullet tube for two minutes. The use of the centrifuge will be explained in lab.
4. After centrifugation, remove the clear, (but probably colored, liquid layer from the tube and place it into a clean bullet tube. Label the tube to differentiate it from other tubes.
5. Add a small amount of ethyl acetate to the pelleted solid material from the first centrifugation. Use the small rotary Teflon pestle attached to a drill and try to "homogenize" the pellet as above.
6. Centrifuge again as above, and separate the clear, liquid layer from the tube and place it into a clean bullet tube.
7. Into each bullet tube with the removed liquid layer, add a small, paper disk using tweezers. Soak until the inoculated plates (next section) are ready for use.

Inoculation of Plates and addition of antibacterial agent:

1. Obtain a plate. Label each plate (on the bottom) with your name and number the sections on your plate from 1 -8.
2. Using the sterile swab and culture tube containing the bacteria (either non-toxic strains of *E. Coli* or), inoculate the <u>entire surface</u> of the plate by rolling the swab which you just saturated with the bacterial culture over the surface of the medium. You want to create a lawn of bacteria on the plate. Discard the swab as directed.

3. Once you have inoculated each plate with your bacterium, you are now ready to add the paper disks which have been soaking in the putative antibacterial solutions. Using tweezers, remove a paper disk from one of the solutions - first touch the disk to the side of the dish to remove excess liquid - and place it in the number 1 section of your bacteria plate. Continue until you have a disk from a different sample in each of the eight sections. Each plate should contain controls to ensure that the antibacterial properties observed arise from contact with something in your sample.
4. Incubate these plates with the cover up at 37 C for 24 - 48 hours.
5. Observe each plate (on Thursday) and note the zone of growth inhibition around each disk on the surface of the medium. Measure the **diameter** of the zone of inhibition in **mm** using the ruler provided and record this data in the table provided.

DATA: type of bacteria: _____

#	Possible solutions	diameter of ring (mm)
	Water	
	Your sample _____ in water	
	ethylacetate	
	Your sample _____ in ethylacetate	
SAMPLE PROVIDED IN THE LAB		
	10% bleach/water solution	
	liquid soap in water	
	antibacterial liquid soap in water	
	ampicillin	
	95% ethanol	

Disposal:

Place all disposal materials that came into contact with the bacteria into the red biohazzard bags provided.