> The aim of science is to extend
> our thinking and reduce it to order.
> --Niels Bohr

Separation of Plant Pigments by Paper Chromatography

In a previous experiment you have seen how differences in the polarity of molecules can enable their separation through a process called *chromatography*. The name of the process indicates that originally it was a method having something to do with color. In gas or vapor phase chromatography this origin is obscured but in this experiment it is evident.

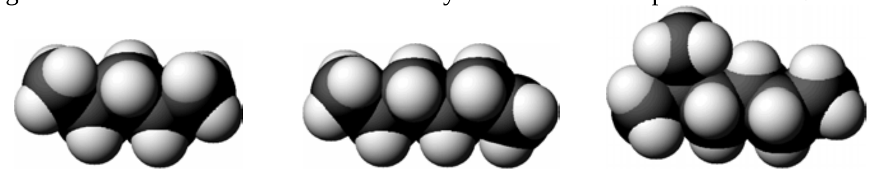
Anyone who has ever played on grass or had an unplanned encounter with a bush knows that plants contain pigments which adhere to varying degrees to fabric (and skin!). The fact that a small industry has risen up in aid of attempts to remove stains like these and many others shows that not all of the molecules can be treated in the same way if their removal is desired. Part of the reason behind that is different fabrics and the varying polarity of their molecules. The constituent fibers of cotton, for example, are very different from those of silk or synthetics like rayon. The plant pigments themselves also have varying polarities and so there are a number of possible combinations to address in stain removal.

The point of this experiment is not to develop a wash-day solution to all your grass stain problems but rather to look at the polarity of some of the common pigments in plant leaves (specifically common ivy) and how that polarity affects their interactions with the cellulose fibers in paper and a few solvents.

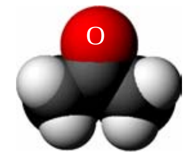
In paper chromatography substances are applied to a piece of absorbent paper. A solvent is then allowed to move through the applied substance. Sometimes the motion is radial, sometimes descending, sometimes (as in this experiment) ascending. In all cases the "wicking" action of the paper disperses the solvent in a particular direction and depending on the solubility of the applied substance, may move it along as well. This kind of effect can easily be seen with an ordinary water-based marking pen and a piece of paper toweling.

In many applications of paper chromatography the interaction of the paper, solvent and applied substance is very complex. The *stationary phase* would seem by simple logic to be the paper and the *mobile phase* which moves the applied substance along would seem to be the water. However, water can hydrogen bond with the cellulose in paper and in cases where the solvent is an aqueous solution, the water on the paper is often considered the stationary phase! The interpretation of such experiments in terms of the polarity of substances is often very difficult.

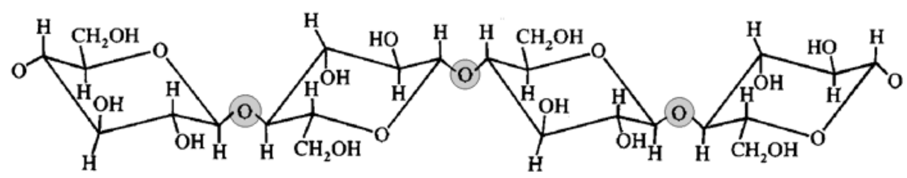
In order to avoid such problems, we have chosen a non-aqueous solvent system for this experiment. One of the solvents is *petroleum ether*, a mixture of non-polar compounds including pentane, C_5H_{12}, hexane, C_6H_{14}, and 2-methylhexane, C_7H_{16}. Because of the inherent symmetry at each carbon of the molecules, the only significant intermolecular forces in these hydrocarbons are dispersion forces:



In contrast, 2-propanone (acetone), C_3H_6O, has a structure in which an oxygen atom on the center carbon of the three-carbon chain creates a definite molecular dipole:

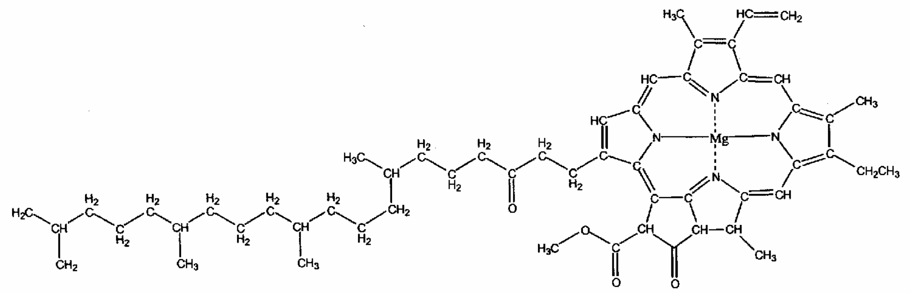


Unlike these small molecules, the cellulose in paper is a *polymer* (made up of many repeating unit structures connected as shown by the shaded oxygen atoms):

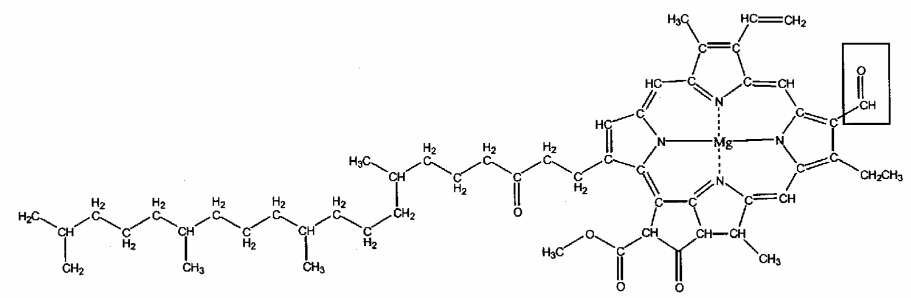


In the diagram above each vertex is occupied by a carbon atom which is not explicitly shown. The most important characteristic of this huge structure (which extends out at both "ends" shown here) is the presence of the -OH groups that permit hydrogen bonding and other strongly polar interactions.

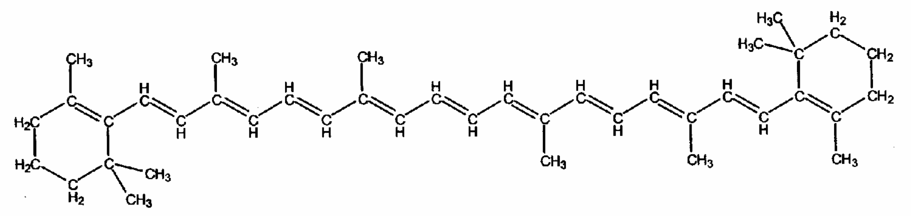
There are many different pigments in plant parts. Most are large molecules with hydrocarbon skeletons like that in cellulose. With such large molecules it is often difficult to make judgments about polarity. So for this experiment we have selected four compounds with distinct structural features. These compounds are present in relatively large amounts in ivy leaves and they also have characteristic colors which are easily seen in a simple chromatogram.



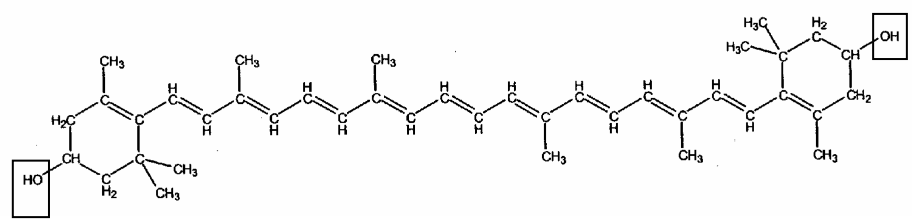
chlorophyll *a*



chlorophyll *b*



β-carotene



xanthophyll

The two forms of chlorophyll are identical except for the boxed part in chlorophyll *b*. Both compounds have a number of oxygen atoms on their hydrocarbon skeletons and that renders them somewhat polar. The additional structure on the *b* form is of interest in this experiment.

β-carotene and xanthophyll are also identical except for the boxed structures on the xanthophyll. The β-carotene molecule is essentially non-polar, having only a hydrocarbon skeleton like hexane (although much more complex). The additional structures on the xanthophyll molecule should give it different properties.

In this experiment you will apply these plant pigments from ivy leaves to strips of chromatography paper. The strips will then be suspended in the two solvents already mentioned (petroleum ether and 2-propanone) as well as mixtures of the solvents. The pure solvents will readily distinguish between the polar and non-polar molecules. Petroleum ether should dissolve the non-polar compounds and move them along the paper.

2-propanone should dissolve the polar compounds and move them along the paper. Different degrees of polarity may be discerned by the gradual separation of compounds as they are carried along the paper. The more soluble (i.e., more polar) substances should move more closely with the solvent front, others will lag behind a little. Because cellulose itself is polar there may be some interaction with the polar pigment molecules but these should be considered minimal compared to the interaction with the 2-propanone.

The mixed solvents may suggest an "optimum" mixture in which it should be possible to achieve a nearly complete separation of all four compounds as well as further illuminate the differing polarities of the pigments.

Preparing to experiment

You will be provided with the following materials:

1. five strips of chromatography paper
2. five test tubes w/rack and rubber stoppers
3. petroleum ether, 2-propanone
4. fresh ivy leaves
5. forceps
6. ruler

Design an experiment to determine the mobility of plant pigments in various solvent mixtures using ascending paper chromatography. [see **Technique**]

Technique

1. Applying the pigments

You should make a pencil line about 1 cm from the end of each strip. This is where the pigments will be applied. The idea is to *not* have them sitting in the solvent when they are placed in the test tubes.

To apply the pigments, place an ivy leaf, top side down, directly on a strip so you can just see where the pencil line is and roll a large coin (a quarter is excellent) firmly across the leaf, pressing pigment onto the paper. Choose a fresh surface of the leaf and repeat this for the other strips. Then return to the first strip and apply more pigment in the same place. Do this about 5-10 times on each strip, allowing some time for the lines of pigment to dry in between applications. The pigment stain must be TOTALLY dry before running the chromatogram.

2. Preparing solvent mixtures

You only need a small amount of liquid in each test tube--enough to run up the paper strip but not so much as to have the applied pigment line sit in the liquid during the experiment. 20 drops total should be adequate. For the mixtures you can use drop ratios to approximate the following (listed as volume acetone : volume petroleum ether): 6:14, 4:16, 2:18.

3. Developing the chromatograms

This is the easy part. Draw a second pencil line about 1 cm from the end the strip opposite to where the pigments have been applied,. Label each strip for the solvent mixture. After placing the strips in the appropriate test tubes, stopper the tubes and wait until the solvent has reached the upper pencil line. Remove the strips from the test tubes and allow them to dry. In the meantime empty your test tubes into the beakers in the fume hood. The spots have a tendency to fade when exposed to light so you might want to circle them (pencil) and maybe make a few notes before leaving the lab.

Analysis

These questions should be answered in your laboratory notebook following your data and observations.

[In most chromatograms of this sort β-carotene is an intense yellow while xanthophyll is a very pale yellow. The chlorophylls will be green, with *b* being somewhat "dirty" or olive green). It might also be helpful to note here that when molecules have similar polarities but very different sizes, the larger molecule will typically move more slowly in chromatography.]

1. Examine the strip which was developed with pure petroleum ether. Which pigment(s) appear(s) to have traveled the farthest? Explain briefly.

2. Examine the strip which was developed with pure 2-propanone (acetone). Which pigment(s) appear(s) to have traveled the farthest? Explain briefly.

3. Select the strip developed in the mixture which shows the best separation of the four pigments. What is the solvent ratio? Considering the introductory remarks above and your answers to (1) and (2), explain briefly the order of the pigments.

4. Attach your five chromatograms to your lab report using scotch tape. DO NOT use staples!