

# 36B-BioOrganic Modifications for Technique Experiments

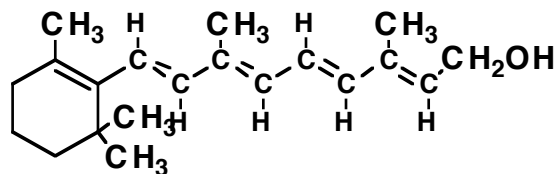
## Technique of Column Chromatography

### Experiment Title: Applying Column Chromatography As A Method to Isolate Plant Leaf Pigments.

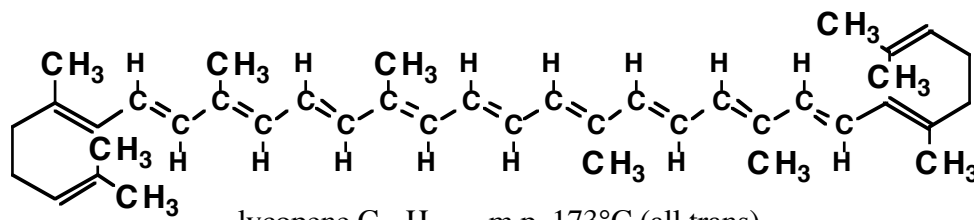
**Introduction:** Thus far you have utilized two techniques to isolate pure organic compounds: recrystallization and distillation. Another very valuable isolation technique that is often employed when very small quantities of sample are available is column chromatography. This separation method is very similar in principle to thin layer chromatography, and can be used for samples of many different sizes. TLC is used to determine an effective solvent system for the separation and then column chromatography is used to separate and isolate the desired compound(s). This laboratory assignment demonstrates the utility of column chromatography to separate natural products such as leaf pigments. **You will need to bring leaf samples with you to complete this lab.**

Background:

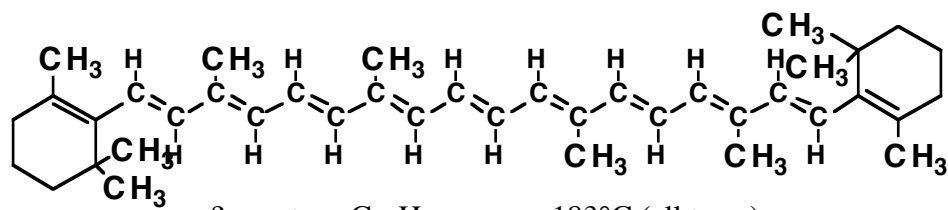
The orange and red pigments in fruits and vegetables such as tomatoes and carrots are hydrocarbons known as carotenoids. The two most common carotenoids, which incidentally are precursors to Vitamin A, are lycopene and b-carotene, the structures of which are shown below.



Vitamin A  $C_{20}H_{30}O$

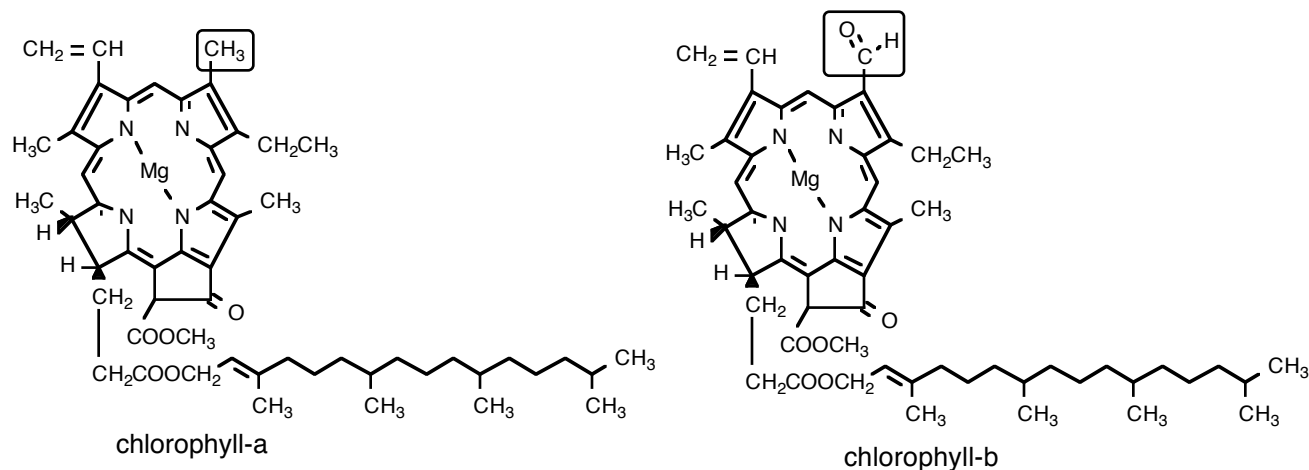


lycopene  $C_{40}H_{56}$  - m.p.  $173^{\circ}C$  (all trans)



The carotenoid pigments also occur in the leaves of plants but they are not obvious because of the presence of other pigments.

The green pigments in leaves are principally from chlorophyll-a and chlorophyll-b, which have the structures shown below



Chlorophyll-b differs from chlorophyll-a in that it has a formyl (-CHO) group in place of a methyl group ( $CH_3$ ), at the position shown in the above structure.

The leaves of plants contain not only the chlorophylls a and b, but also other pigments whose colors are masked by the chlorophylls in live, healthy leaves. The other pigments become visible in the fall when the leaf dies and the chlorophylls rapidly decompose. Among the other pigments are the carotenoids, the two commonest being lycopene and β-carotene, both of which are precursors to Vitamin A.

## Activities

Read the Introduction section of the Column Chromatography Experiment found in Chapter 8 of the Chem 36 Lab Guide (pgs 177-182) and also read an article by P. Keusch, Organic Chemistry Demonstration Experiments on Video Chemistry Visualized, [www.uniregensburg.de/Fakultaeten/nat\\_Fak\\_IV/Organische\\_Chemie/Didaktik/Keusch/D-TLC-e.htm](http://www.uniregensburg.de/Fakultaeten/nat_Fak_IV/Organische_Chemie/Didaktik/Keusch/D-TLC-e.htm). Also read for background information on plant pigments: The Chemical Pigments of Plants, J. Alkema, and S.L. Seager, J. Chem. Ed. 59, 1982, pgs. 183-186.

- ✓ In the first lab period you will extract the plant pigments from fresh leaves
- ✓ In the second lab period you will separate the pigments using column chromatography and characterize the plant pigments by UV/VIS

## PreLab:

Follow the prelab sheets when preparing your prelab and complete your Chemical Data Table with any reagents not found in the Common Shelf Data Table and include the structures of the plant pigments listed in Table 1 below. Highlight with colored pens or pencils the portion of the molecule that determines the class of the pigment. The classes are listed in Table 1 and the background article on plant pigments highlights the structures of these classes. Hand in a copy of the completed Common Shelf Chemical Date Table and a copy of your Chemical Data Table with your Prelab. When completing the procedure outline, make sure you have an explanation for every step in your procedure outline. You do not need to include a mortar and pestle in your diagrams but you do need to include a picture of your column set up.

### Prelab Exercises:

Answer questions a, b, and c of the Prelab Exercises on page 194 of your Lab Guide.

### Post Lab Questions:

Answer question:

Why is chlorophyll green?

Table 1. Plant Pigments

Class	Compound	Colors Produced
Carotenoid	Carotenes	Golden
Porphyrin	Pheophytin	Olive Green
Porphyrin	Chlorophyll a	Blue Green
Porphyrin	Chlorophyll b	Yellow Green
Carotenoid	Lutein	Yellow
Carotenoid	Xanthophylls	Yellow
Flavonoid	Anthocyanin	Red, Blue, or Purple (pH dependent)

# Column Chromatography Experiment: Separation of Plant Pigments

Taken from: P. Keusch, University of Regensburg, "Separation of Plant Pigments by Column Chromatography (CC), Organic Chemistry Demonstration Experiments on Video Chemistry Visualized

[www.uniregensburg.de/Fakultaeten/nat\\_Fak\\_IV/Organische\\_Chemie/Didaktik/Keusch/D-TLC-e.htm](http://www.uniregensburg.de/Fakultaeten/nat_Fak_IV/Organische_Chemie/Didaktik/Keusch/D-TLC-e.htm)

## Extraction of Plant Pigments

Working in a hood, rip two leaves into pieces and then add them to a mortar with 22 mL of acetone and 3 mL of petroleum ether and a spatula tip of  $\text{CaCO}_3$ . Grind the leaf pieces with a mortar until the solvent in the mixture becomes highly colored. Filter the pigment mixture into a separatory funnel and add 20 mL of petroleum ether and 20 mL of 10% aqueous NaCl solution. Remove the water layer and wash the organic layer 4 times with 5 mL of distilled water. Dry the organic layer with 4 spatula tips of  $\text{Na}_2\text{SO}_4$ . Gravity filter and concentrate the organic layer to 3 mL.

## Column Chromatography

Packing the Column. You will be carrying out a microscale column separation for the first step of this synthesis. The column you will need is found in your microscale lab kit, and it looks just like the one found in Figure 8.1 on page 196 of your Lab Guide. When using this column you will be separating no more than 50 mg of material at a time. Please keep this in mind because you do not want to overload your column. You will be using silica gel as your stationary phase. You will be using the slurry method to pack your column so make sure you review this procedure on page 197 of the your Lab Guide. The solvent system you will use for the separation is a 7:3 mixture of petroleum ether and acetone. Review the polarity of these solvents using Figure 7.8 on page 176 of your Lab Guide. Also review the polarity of silica gel as a stationary phase using Figure 7.5 on page 174 of your Lab Guide.

Before assembling your column, check the small plug that fits into the bottom of the column to make sure that it has a small fritted disk inside. Next, make sure that the plug fits snugly into the glass column and is not easy to pull out. If it is loose, use a new bottom plug from your Organic Lab Equipment Kit. Finish assembling the chromatography column as depicted in Figure 8.1. Be sure to clamp the column securely and vertically to a ring stand. Then place an Erlenmeyer flask or small beaker under the stopcock of the column to catch any dripping solvent if you should spring a leak while packing the column. It is important to get into the habit of always having a beaker or flask under the column to catch any solvent that may accidentally drip out of the tip our your column.

Weigh out approximately 3 g of silica in a beaker and then add a small amount of the 7:3 petroleum ether/acetone solvent mixture to the stationary phase with stirring to create a consistent paste. This paste should be capable of flowing. Pour this homogeneous mixture into the column as carefully as possible using a spatula to scrape out the solid as you pour the liquid. The most important thing to remember when you are packing a column is to make sure you are creating an evenly distributed and packed stationary phase that is devoid of cracks, air bubbles and channeling. Add the petroleum ether/acetone (7:3) solvent mixture to the column to make sure that the entire column is below the level of the solvent. Once the column is packed, open the stopcock and allow the solvent level to drop to the top of the silica, but do not allow the solvent layer to go below this point. Allowing the solvent level to drop below the stationary phase, (known as letting the column to “run dry,”) should always be avoided since it allows air bubbles and channel formation to occur leading to poor separation. After the column is packed, carefully add a layer of sand (0.5- 1 cm) to the top of the column to minimize the disruption of the flat surface of the column when you need to add additional solvent.

**Preparing for fraction collection:**

Tare and label shorty vials, test tubes and small Erlenmeyer flasks for sample collection. You can use any combination of the three suggested fraction collection containers but be sure you have at least 10 labeled pieces of glassware before you begin running your column. You should also label one larger Erlenmeyer flask (25 mL) to collect the first fraction. Remember you will never leave your column unattended when it is draining solvent and you will always keep a collection flask under the stopcock to prevent the accidental loss of any sample.

**Adding the sample:** Add 10 drops of the concentrated plant pigment extract to the top of the sand using a pipette being careful not to disturb the top surface of the column. It is important to use a minimum amount of solvent to dissolve the pigments, if too much solvent is used the mixture will elute too rapidly and poor separation will result. A thin horizontal band of sample is best for an optimal separation. Drain some liquid from the column until the product is well within the silica layer. Add more solvent to the top of column being very careful not to disturb the surface.

**Sample Collection:**

Begin collecting solvent for your first fraction until you see the bands of color just above the stopcock of the column. When you reach this point you will begin collecting smaller fractions based on color making sure you have at least one small fraction between colors. If a yellow colored band remains on the column that is not eluting with the petroleum ether acetone solvent mixture, allow the solvent to run down to just the top of the column and then add a solvent mixture of 70:30 isopropanol/water. The compounds left behind maybe flavonoids that require a more polar solvent mixture to elute them from the column. Collect this polar mixture as one fraction. If a red or blue colored band still remains on the column you can elute it with a saturated solution of sodium bicarbonate.

**TLC of sample fractions:**

You will need to run a TLC on the first fraction (colorless fraction), and each colored fraction. See Table 1 for the color of each pigment fraction. Evaporate the solvent by blowing nitrogen over each sample. Remember you can spot at least three fractions on one TLC plate. If each colored fraction contains one component then you can begin UV/VIS analysis to confirm the identification of the plant pigment. If more than one spot exists in a fraction consult with your TA to determine a plan of action.

Cleaning up. When you are done with the column, pour the excess solvent into the proper waste container, pull out the bottom, and leave the “wet” column out in the beaker in your desk. The column will dry out by the next lab, and the dry used silica can then be easily emptied out into the waste bin.

### **Analysis of sample combined sample fractions using UV/VIS:**

Once the sample fractions of the various components have been evaporated, they can be prepared for UV/VIS analysis. Read pages 281-310 in your lab guide prior to beginning the analysis.

## **References:**

Lab Guide for Chemistry 36, 36B, 36H, Introductory Organic Laboratory, Robert Minard and Tracy Oriskovich Halmi, Kenneth Williamson, The Pennsylvania State University, 2003.

P. Keusch, University of Regensburg, “Separation of Plant Pigments by Column Chromatography (CC), Organic Chemistry Demonstration Experiments on Video Chemistry Visualized  
[www.uniregensburg.de/Fakultaeten/nat\\_Fak\\_IV/Organische\\_Chemie/Didaktik/Keusch/D-TLC-e.htm](http://www.uniregensburg.de/Fakultaeten/nat_Fak_IV/Organische_Chemie/Didaktik/Keusch/D-TLC-e.htm)

## **Final Report**

Include the UV/VIS spectra for all isolated pigment fractions. Attach all TLC plates and tabulate the  $R_f$ s for all spots. Discuss the order of elution of your pigments and whether or not they followed the order in Table 1 above. Create a table of plant pigments that you have isolated in this lab and include the name, class of compound, the color of the band, the  $R_f$  and UV max for each isolated compound. Discuss the success of the method in separating the pigments and whether or not you achieved the expected outcomes.