

Total Synthesis Project : Synthesis and study of Warfarin Derivatives (Analogues)

Coumadin (also called warfarin) is a blood thinner. Coumadin is a vitamin K antagonist to an enzyme called a carboxylase (it has a cofactor called vitamin K) together (the carboxylase and vitamin K) they are responsible for the carboxylation process of glutamic acid residues on Vitamin K dependent cofactors. This carboxylation is important for the formation of groups that can bind calcium and through this binding, anchor and localize the cofactors to the platelet membrane. This is part of the coagulation process of coagulation. Coumadin has a structure similar to that of vitamin K and essentially takes its place and shuts down the enzyme and associated redox needed to carboxylate these groups necessary for the coagulation process.

This document has been added on to and edited every week. I think it is important to reread it if you have not been following what is going on. There is a distinct possibility you will understand it better upon a second or third evaluation.

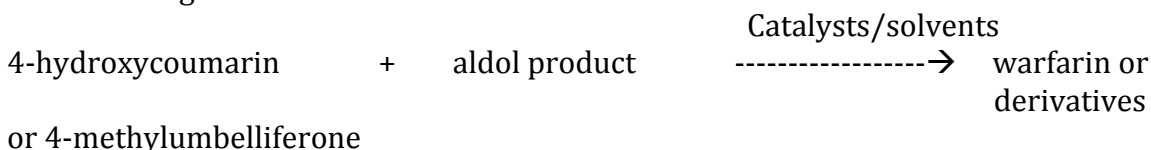
The goal of this project is to carry out a total synthesis of Warfarin (practice) and at least one derivative (a compound that has a small structural difference and might have a different biological activity). In this project, you will in groups of three. You will work more or less at your own pace, though we will cover certain topics in lecture or discussion each week. In addition to working out the total synthesis, you will look many of the references yourself, you will learn about the importance of the target molecules yourself, you will learn all about Thin Layer Chromatography and Column Chromatography (a method of analyzing the purity and content of product mixtures and a method to purify compounds based on TLC as a model), run more advanced NMR spectra to determine structure and possibly carry out some biological analysis of your products, such as clotting rates and/or protein binding of warfarin and your warfarin derivative. .

Each week you will be given some background material to explore and you will either have a lecture or a discussion session on that material. You may work ahead of this material, but it is important that you participate in the lectures and discussions. You will be responsible for the material on a quiz and when you write a journal style paper with your group at the close of the project. There will be a specific lecture on writing a journal style paper because the final goal of the project is to write a journal style paper. Though there will not be regular written assignments, it is expected that you will come into lab each week with a plan and with the assigned material prepared such that you as a group or individual could get up and discuss said material at the board. It is also important that once the lecture is given on how to write the journal style paper, that your group start writing up the parts of the paper that are ready to be written such as the introduction and the experimental section.

The overall plan for the synthesis will involve making an alpha, beta unsaturated ketone (different groups will make different compounds) using an aldol reaction and then purifying, characterizing that compound. The purified aldol product will be coupled to 4-hydroxycoumarin (possibly made in the lab) and in a separate combination, attempting an experimental coupling of an aldol product to 4-methyl-7-hydroxycoumarin (4-methyl umbelliferone) which can be made by the Pechman synthesis using resorcinol. The overall synthesis is a convergent synthesis utilizing aldol products, 4-hydroxycoumarin and/or 4-methylumbelliferone (4-methyl-7-hydroxycoumarin) which are coupled through conjugate addition using a rather sophisticated chiral catalysis to form warfarin and any derivatives. **The overall goal is to create compounds that are warfarin derivatives or analogues that might have different biological activity compared to the parent, warfarin.**

A large amount of this project will involve purifying and analyzing products. Please come in with an open mind, being psychologically prepared to have things fail. Come with a positive view that it is not about speed, but learning and that negative results are results. Scientists have to be very prepared, very thoughtful, very careful and yes, adventurous (in a safe way). They need to have the attitude that a negative result is a result and to try to come up with solutions and new ideas rather than having the "I want to start over." kind of view. Lab is not about performing a procedure perfectly. I would rather have students have some new reactions not work and learn how to trouble shoot or just understand chemistry better, than to simply execute a sequence with skill. This project is designed to be a more realistic chemistry experience, but at the same time it has some structure because of the very large number of students in the class.

The following is the overall scheme:

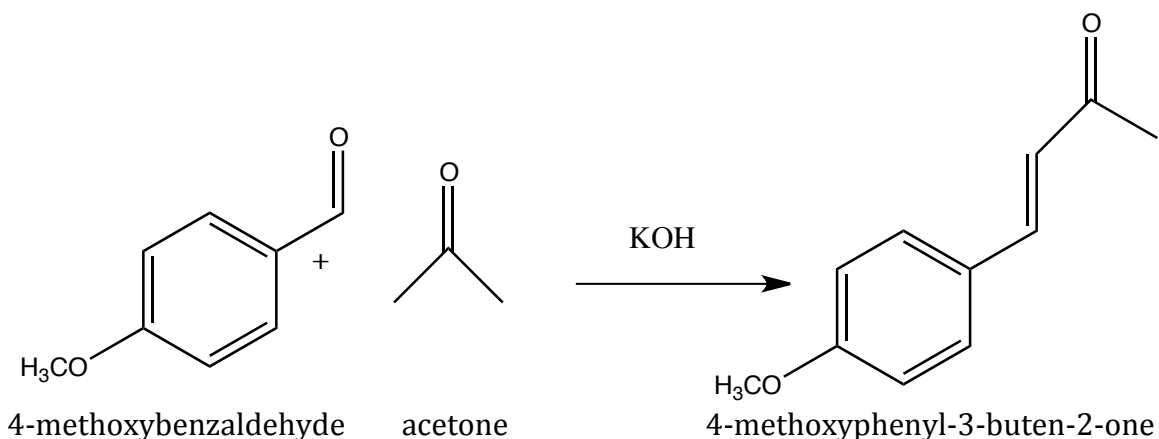
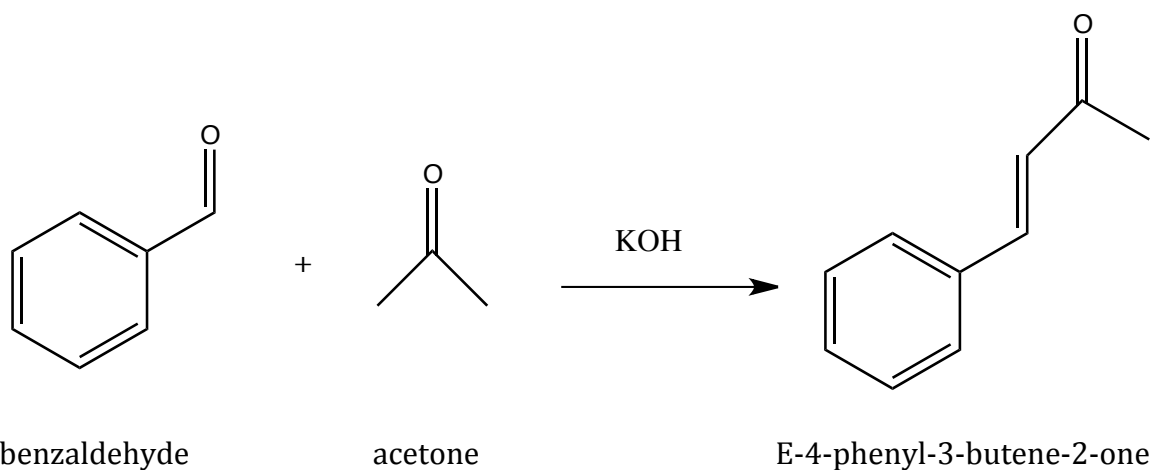


Week One (week of 3/18/2013)

During the first week, you and your group should be prepared to do the following three activities. This means you should have and procedures written in your lab notebook and you should have read up on the mechanism for any reactions and the theory behind any purification techniques.

A. Aldol Reaction

Aldol synthesis: The following is the compound that is needed for the synthesis of Warfarin, however, other aromatic aldehydes can be substituted in the procedure. It is expected you will try this synthesis and one derivative with a different aromatic aldehyde such as p-methoxybenzaldehyde as shown beneath the standard synthesis.



In the discussion session this week, it is expected that you will have gone over the aldol reaction and will be ready to discuss the procedure and mechanism at the board. The aldol reaction can be readily found in your text. If you haven't gotten to it in class, it is still your responsibility to look it up. When you come to lab you should have the scaled reaction procedure and the mechanism written in your lab book (this is the blow by blow description of the reaction showing intermediates and using arrow formalism).

General Procedure for Preparation of Aldols

Choose an aldehyde (several will be available) in lab for you group. Note, the procedure is written from the perspective of piperonaldehyde, but the quantities have to be recalculated for other aldehydes. It is expected that in any class all aldehydes will be used at least by one group and that the experience will not be discussed with other groups.

You can do this procedure at 5-10 times the given scale.

1. Place 0.15 g of piperonaldehyde in a 25 mL Erlenmeyer flask.
2. Add 1.00 mL of acetone and a magnetic stirrer. Stir the reaction on magnetic stir plate.
3. While the reaction is stirring, add 0.1 mL of the NaOH solution which is prepared in advance by you by dissolving 0.6 mL of NaOH in 1 mL of water. (What is the molarity of this solution?)
4. Cap the Erlenmeyer and allow it to stir for 45 minutes.
5. Pour the reaction into 20 mL of water.
6. At this point, you should have a cloudy solution with an oil. The oil should be centrifuged out. The oil should settle to the bottom of the centrifuge tube.
7. If any crystalline compound precipitates out, it should be isolated by vacuum filtration. The filtrate from this vacuum filtration and/or the supernatant from the centrifugation should be stored in the refrigerator until next week when more compound may be isolated by vacuum filtration. This week the oil and any isolated compound will be studied by Thin Layer Chromatography for purity. You can start with pure dichloromethane as your eluting solvent and add methanol as needed.

B. You will run **Thin Layer Chromatography** of your isolated oil and any solid isolated, using the starting materials as standards.

It is expected that you will read up on Thin Layer Chromatography and Column Chromatography. The following is a reasonably good site for Thin Layer Chromatography.

<http://orgchem.colorado.edu/Technique/Procedures/TLC/TLC.html>

The following is a reasonably good site to get some background on Column Chromatography, which is a related topic and Column Chromatography may be carried out next week.

<http://www.youtube.com/watch?v=EytuRMS1154>

You should record the chromatography techniques in your lab note book as well as the synthetic procedure you have researched. Take good notes in lab lecture and when studying any references you use.

Thin Layer Chromatography – A Brief Description and General Instructions

1. Obtain a Thin Layer Chromatography (TLC) sheet from the desiccator. This sheet will be about the size of an iPhone, but much thinner. One side is a very thin coating of silica gel (a very polar stationary phase) that is in a binder. The silica gel is impregnated with a fluorescent compound that will glow green or orange depending on the compound impregnated. When exposed to UV light, the plates will look sort of like those glow sticks and necklaces kids

have in the summer. One side is white and sort of rough, the back side is plastic or aluminum. You will do your work on the rough side.

2. You should draw a line with a pencil very lightly about 1 cm up from the lower edge of the TLC plate. This is the origin. See the diagram below.
3. You need to prepare standards. Take a spatula tip of each of the starting materials in a vial and add about 1 mL of dichloromethane. This is sort of an estimate of the solid and solvent. You should also make a small solution of your reaction mixture/crude product. If it is liquid, you should take a drop or two and dissolve it in about 1 mL of dichloromethane. Again, the solutions are just approximate. The goal is to study the components of the reaction vs. the starting materials. New spots observed after elution and visualization indicate product, whereas spots seen at the same location as the starting material standards indicate incomplete reaction.
4. The standards and the reaction mixture should be spotted on the TLC plate as indicated on the diagram below. The idea is to use a different capillary pipet (these will be available in the lab) for each compound. You dip the capillary into the solution being spotted and it will enter the capillary via capillary action (no pun intended) and then it is a good idea to blot it a bit on a paper towel so you don't get a big blob of liquid on the end of the capillary. Then, lightly spot the solution on its mark on the TLC plate. The idea is to spot it and get the smallest spot possible. Then reapply it several times going for a small concentrated spot. You need to let the dichloromethane evaporate between each application. You will do this for the standards and the reaction as diagramed. It may take you a couple plates to get the hang of it. It is important for all members of your group to get the hang of this technique.
5. Realize it is really important to spot about a cm up from the bottom of the plate, to keep the spots about a cm apart and to spot in from the edge of the plate about a cm as diagramed.
6. **Preparing the Eluting chamber.** We will use a makeshift eluting chamber that will be either a 250 mL or 400 mL beaker with about a 0.5-1.0 cm of dichloromethane with about 1.0 mL of methanol added (eluting solvent). You need to use a piece of filter paper as diagramed below to saturate the beaker's atmosphere with the eluting solvent. Use a watch glass to cover the chamber.
7. **Eluting the TLC plate.** Dip your TLC plate in the solvent. It is important that the solvent does not immediately touch your spots. If it does, you need to adjust the level of the eluting solvent or spot a new plate. The former is preferable. The plate should be dipped, but also resting against the wall of the beaker. The back side toward the beaker, but on the opposite side of the

beaker from the filter paper. The solvent rises up the plate by capillary action. It will take about ten minutes. In this process, there is a competition for the compounds by the silica gel on the plate (very polar – the stationary phase) and the solvent system (varying degrees of polarity, but less polar than the stationary phase – this is the mobile phase). The compounds are in equilibrium between the stationary phase (the silica) and the mobile phase (the solvent). If the compound is extremely polar it will spend most of its time interacting with the silica gel and it won't move much. If it is of very low polarity (provided it is soluble in the eluting solvent), it will spend most its time in the solvent and will move way up the plate. Compounds move to varying degrees depending on their polarity. Compounds that are the same, will elute to the same point on the plate, so your standard lanes will help you identify compounds in the reaction mixture.

8. When the solvent is about a cm from the top of the plate, you should remove the plate and lightly mark the level of the solvent. This is called the solvent front and its level is significant. Let the solvent completely evaporate off in the hood. The spots are visualized (most of the compounds we are working with are white and are not readily visible) with a UV lamp. Be careful not to expose your hands or your neighbors to the UV lamp. The background will fluoresce as previously described and your compounds should show up as blue or black spots in the background. Lightly circle them with a pencil. After this, you will more permanently visualize the compounds with the p-anisaldehyde stain. This entails dipping your plate into the stain with forceps and then, blotting the excess stain off with a towel. The plate then needs to be baked in an oven at about 100 °C for ten minutes or so. Be careful with the stain as it contains strong acids. Warfarin and derivatives stain purple, 4-phenyl-3-buten-2-one stains red and 4-hydroxycoumarin does not stain at all (white). This is very useful to tell what is going on. What would you expect on the first week? If it did not occur to you earlier, 4-hydroxycoumarin and 4-phenyl-3-buten-one are the starting materials that need to be spotted on the plate, flanking the reaction mixture. What would you expect next week?

You should record the chromatography techniques in your lab note book as well as the synthetic procedure you have researched. . Take good notes in lab lecture and when studying any references you use.

Summary of Week one:

- 1. Aldol net reaction in notebook with procedure.**
- 2. You will have attended lecture on overall project, aldol reaction and column chromatography.**

3. **You will be prepared to discuss the aldol mechanism in discussion with instructor/TAs.**
4. **Experimentally you will carry out the aldol for your derivative and for warfarin without purification.**
5. **You will study by TLC any materials isolated from your Aldol**
6. **You should run the NMR of your flutamide product.**

Week II

This week you should allow your aqueous filtrate or supernatant from your aldol reaction last week to come to room temperature and filter out any crystal that has formed. You should analyze this material and any material you isolated last week against your starting material standard from last week.

This week try running the TLC in pure dichloromethane with a couple of drops of methanol in the TLC chamber. In addition to establishing the purity of any new solid you isolate, it will also be able to move your product up the plate more which may be more suitable for your column. To establish the utility of adding methanol, please show your TLC plate to one of your instructors. This is important as the plate is a model for the column we will run this week.

Once you have establish the purity of your product mixtures, any that are not pure should be purified by column chromatography. It was observed last week that nearly every solid and oil initially isolated was at least contaminated with some starting material or by product.

Please watch the following video, but also make note of the following instructions that are more tailored to our lab.

The following is a reasonably good site to get some background on Column Chromatography, which is a related topic and Column Chromatography may be carried out this week. .

<http://www.youtube.com/watch?v=EytuRMS1154>

Please also watch the following youtubes I made for you in the lab. The first video is sort of botched, but it shows some stuff that can go wrong.

Basic Instructions for packing and eluting a Silica Gel Gravity column.

1. A small column will be provided to your group. Clamp the column using a mini-clamp to your monkey bars. Take the cap off the top and off the bottom of the column.
2. Place a 125 mL Ehrlenmeyer under your column.
3. Before working on the column, prepare a test tube rack with test tubes to collect fractions from your column. Smaller test tubes are better, but we

may have to use larger test tubes, due to limited equipment. Label the test tubes with numbers using the tape we have in the lab. Every test tube does not have to be labeled, but you might want to label every fifth tube.

4. Put a small amount of sand in your column (about a two or three centimeters).
5. Attach a syringe needle to the bottom of the column. Be careful.
6. Use a black rubber stopper to block the needle, by piercing the black stopper with the needle.
7. Pour ca. six centimeters of dichloromethane (or other solvent) into the column. This is the eluting solvent.
8. Weight out six or seven grams of silica gel into a 125 mL Erlenmeyer and add about 30 mL of dichloromethane. The silica gel will form a suspension in the solvent. Take the rubber stopper off the needle and after swirling the silica gel flask, start pouring the silica gel suspension into the dichloromethane in the column. It may flow rapidly in which case it is very important to control the flow. To do so, use the column cap. By screwing on the column cap, you can slow the flow. This is tricky and requires some finesse.
9. As the column flows, the silica suspension concentrates in the bottom of the column. There will be a layer of solvent on top of the silica concentrate as the silica settles. It is very important that the column not run dry. So, use the cap and the rubber stopper to control the flow.
10. You can put a small layer of sand on top of the column by sprinkling a few millimeters of sand through the layer of solvent. It will settle on top of the settled silica gel and serve as an important barrier to keep your sample from running dry.
11. Carefully allow the solvent to drain out to just above the sand layer. Use the rubber stopper to stop things if necessary!!!
12. The amount of silica gel can be used to separate .1 to 0.2 grams of material.
13. Weigh out about 0.2 grams of your crude material and dissolve it in a small amount of dichloromethane (the smallest amount possible – ten drops or so).
14. Using a pipet gently add this material to the top of the column. Normally, I run it down the side of the column so it does not perturb the column bed.
15. Carefully allow this to run into the column (do not allow column to run dry).
16. Use a small amount of dichloromethane to clean the compound off the sides of the column and allow this to run in (do not allow the column to run dry).
17. Do this several times until you are confident all the sample is loaded on the column.
18. After doing this, fill the column with the solvent and begin running the column at a slow rate (use cap to regulate flow) and collect the eluent (the solvent coming off with your compounds) in two mL fractions in your test tubes in the rack. This means every two mL or so, you change to the next test tube by moving the rack.
19. The column should parallel your work with your TLC plates. The material that is on the top of the TLC plate will be eluted first (will be found in the

- earlier fractions off the column). Your product should come off later in the process.
20. The way you figure out what is in each fraction is by spotting four or five spots of every other fraction on a TLC plate vs. your starting material standard. Each plate could have as many as four fractions and you should put the starting material in the middle as a reference. You should elute the plates as you did last week and visualize using the UV lamp. If you wish you can also use the iodine chamber.
 21. Your goal is to ascertain which fractions contain your product in a pure form. Once these fractions are determined, they should be combined in a tared flask and rotavapped to dryness. If need be you can run another column to isolate more material.

At the end of the day, any material that is pure by TLC can be allowed to dry until next week and then studied by NMR, IR, and melting point.

Regardless of your situation (meaning if the bulk of your material appears pure by TLC), you should take some of your crudest material and run a column. It is very important to get the hang of chromatography for future work.

There are four possible coupling reactions to make warfarin-like compounds that can be attempted and we will start one or two of these next week, if we have pure material.

1. E-4-phenyl-3-buten-2-one (out of bottle) + 4-methylumbelliferone (made by you – see below) -----→ warfarin-like derivative 1
2. Your synthesized aldol project + 4-methylumbelliferone -----→ warfarin-like derivative 2
3. Your synthesized aldol project + 4--hydroxycoumarin-----→ Warfarin like derivative 3
- 4. E-4-phenyl-3-buten-2-one + 4-hydroxycoumarin -----→ Warfarin**

Reaction 4 is the basis, the model for all the other reactions.

You will try all of these on a small scale, probably starting with no. 4 to practice – starting next week.

Though it is recommended that you really take your time purifying your compounds, if you have excessive time, you can start making 4-methyl umbelliferone (4-methyl-7-hydroxy coumarin), which is roughly scheduled for next week.

Regardless, you should prepare to make 4-methylumbelliferone (4-methyl-7-hydroxy coumarin) by looking up the synthesis on the internet. You need to find a procedure in the Journal of Chemical Education that involves resorcinol reacting with ethyl acetoacetate using dowex beads covalently bound to sulfonic acid groups. When you find it, write the reaction and procedure in your notebook. You need to find the journal article. The term green chemistry is in the title. It is not adequate to just have some procedure you found on some college website.

Start trying to figure out the mechanism.

Additionally, you should begin reading about how anticoagulents such as coumadin (warfarin) work in the human body. Save the references for you paper.

This week in lab lecture, Dr. Lalonde will be giving a talk on how to write a journal style paper.

Our discussion will be a demonstration of the procedure given above by your instructor.

Summary of Week II

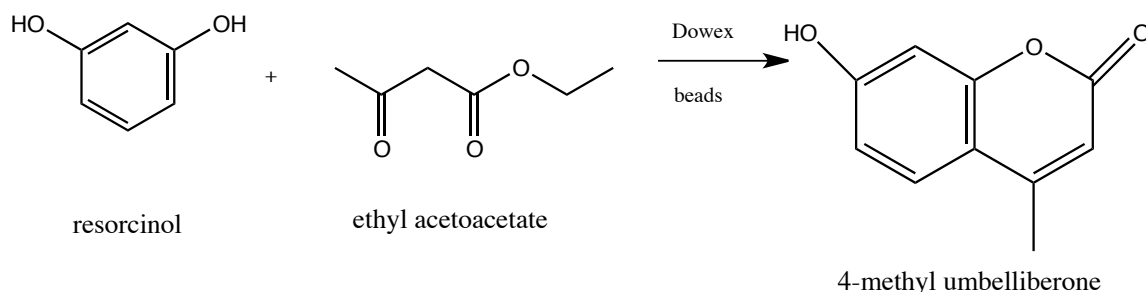
- 1. Attend lecture on how to write a journal style paper and watch and discuss demo given by instructor in lab.**
- 2. Isolate any crystals from your aqueous filtrate or supernatant from last week.**
- 3. Establish purity of these crystals.**
- 4. Work out the solvent system for your column by adding a bit of methanol to your TLC system and studying crude aldol product material vs. the starting material aldehyde.**
- 5. Run your column to purify your aldol product. Combine fractions containing your product and rotavap it to dryness.**
- 6. Look up Pechman Reaction and write it in your notebook. You need at least a reference from the Journal of Chemical Education.**
- 7. Start working out the Pechmann mechanism.**
- 8. Start reading about how Coumadin Derivatives work. Write down your references.**
- 9. Leave compounds to dry for future melting point, IR, NMR analysis.**

Week III

1. By now you should have looked up the synthesis of 4-methylumbelliferone (4-methyl-7-hydroxycoumarin) using Dowex beads (this is a source of acid), ethyl acetoacetate (when have you used this reagent before?) and resorcinol. You can run your reference by me if you are worried. As long as we have the reagents we

can try your synthesis. The following is the overall reaction for the Pechman condensation of 4-methylumbelliferone (7-hydroxy-4-methylcoumarin). We will be going over the mechanism in lab lecture. Try to work it out on your own if you can. You will also be doing the recrystallization of this product. I will also be going over recrystallization and melting points because I feel they are still not understood totally. In the short prelab discussion we will write out the first part of the 1, 2-diamino-1,2-diphenylethane catalyzed reaction used in the formation of Warfarin and derivatives.

The following is the net reaction for the Pechmann condensation of 4-methylumbelliferone (7-hydroxy-4-methylcoumarin).



The Dowex beads have sulfonic acid groups attached.

2. Analyze compounds isolated from last week.

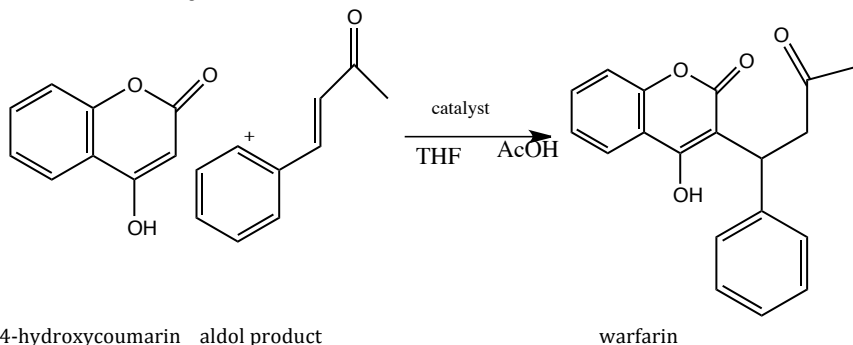
We will have NMR time. You probably want to make up two samples, one from your column and one from your vacuum filtration and we will establish by nmr which is most pure. It would be good if in advance you think about what the spectra will be like for the alpha-beta unsaturated ketones should look like. You will be running solids as films or pellets. If you would like to learn how to run a pellet, please see me or a TA. You also need to run IR of both and melting points. If you have impure compound. You may need to run another column. Running columns is an art and it does get better as you go along. We found in general that the larger columns were better because you can get more solvent in and run the columns faster. Does this make sense. It can save up to an hour. You may have to pump on your compounds to make them purer. You can talk to us about that.

3. Start first conjugate addition coupling to make Warfarin. **This will be your practice run and very important. You have to get the reaction to work before you start on the harder versions.** To prepare for this you need to find a green synthesis of warfarin. It was carried out using 4-hydroxycoumarin and 4-phenyl-3-buten-2-one with chiral 1,2-diamino-1,2-diphenyl ethane as the catalyst. We will be doing the small scale synthesis in THF and acetic acid as outlined below. This is a fairly recent [Journal of Chemical Education](#) article and as I recall, you have to download the procedure from supplemental materials. The reaction is done in a small vial and is very easy to set up. You must label your reactions. It is very

important. It is very important that you bring the journal article to lab and write the procedure in your notebook.

Note: I would really love to make some 4-hydroxycoumarin so that some students will have total synthesis of some of their molecules. The reaction is too dangerous to do in large groups though. If any of the students who wish to major would like to get together to make it, I would be delighted. I think you will enjoy it. We could do it one Saturday or just set up some time to do it. Please think about it. Julie Han who worked hard on parts of this synthesis would be there to help as well as me (MNS). Let me know so we can set it up.

The Green Synthesis of Warfarin.



4. Run TLC of starting materials above as a baseline. Develop your TLC in dichloromethane with a couple drops of methanol. Visualize with the UV lamp as well as using the p-anisaldehyde stain that will be provided. Record all observations in notebook. What colors are the spots with p-anisaldehyde? Make sure you are recording tlc data or saving the plates. At this juncture, you should start recording Rf values. These are the distance of from the origin to the middle of the spot divided by the distance from the origin to the solvent front. Keep good notebooks. Good notebooks lead to good papers.

5. If you are ahead of schedule, please start researching how warfarin-like drugs work. Read the chapter in Bruice on making medicinal compounds.

- 1. Attend lab lecture and prelab discussion**
- 2. Analyze material from last week.**
- 3. Do further purification?**
- 4. Carry out Pechman and purification.**
- 5. Do first Warfarin cross (no. 3 and get baseline TLC).**

6. Look up references for procedures, read about compounds you are making.

Realize you might not finish everything. At this point groups will start to not all be at the same points.

Week IV

At this point you have learned most of the techniques needed for the project. So the following will be simply a list of things you can be working on for the next couple weeks.

1. Lab lectures will include work shops where you write out the mechanism for the catalysis of the formation of Warfarin from coumarin compounds and aldol products by 1, 2-diamino-1,2-diphenyl ethane. You will receive a worksheet to work on with your instructor and TAs. There will only be two more weeks of lab lecture. The first week we will talk about carbon NMR, the proton nmr of warfarin and the first step in the catalysis.
2. It is important to check your Warfarin Reaction using TLC in dichloromethane with a few drops of methanol as your developing system and the UV lamp as a preliminary visualizing system and the p-anisaldehyde stain as a more permanent visualizing agent. It is up to you to decide if your reaction is complete or if it needs another week of work.
3. You need to check your 7-hydroxy-4-methylcoumarin (4-methyl umbelliferone) for purity. Measure the mass, melting point, IR and proton NMR.
4. At this point, you can decide which if any of the above microscale reactions outlined on page nine you should carry out. You have already done no. 3 (or have it in progress). If your traditional Warfarin reaction (no. 3) appears to be working you should definitely set up no. 4 – this is most likely to work well. If you can set it up on two or three times the microscale level given in the procedure (we used the small scale – do not use the large scale, it uses too much catalyst and the catalyst is expensive).

You are welcome to also do 1 and 2, but do these on the small scale since they are less likely to work well.

If you set up three microscale reactions, you should test them via TLC with UV lamp and staining against standards before you leave lab so you have a

baseline. Remember to make the solutions just take a drop or of your reaction mixture and put it in 10-20 drops of dichloromethane. Take a spatula tip of your starting materials and put them in 10-20 drops of dichloromethane.

Note: It is important in all TLC to let the plate run up to within a cm of the top of the plate, to make small concentrated spots (see me or one of the tas about making smaller spotting pipets), to not spot too close to the bottom or the edges, to let the plate dry before visualizing, especially before dipping in the stain. It is important to let the stain dry before baking it.

When keeping a record of your TLC plates, you need to record Rf values of all important bands, especially products. This is a ratio of measurement of the height of the center of the band in question from the origin to the height the solvent rose to the origin. The Rf values for products should be included in the data you include in your journal style paper – in the experimental section.

5. How is your no. 3 reaction coming (your test of the catalyst mediated coupling of 4-hydroxycoumarin with E-4-phenyl-3-buten-2-one. Is there evidence of warfarin being present (warfarin stains bright blue or purple with the anisaldehyde stain). Does the reaction appear to be finished? Do you believe it needs more time? More catalyst? You can base these decisions on the plate and you can discuss them with myself and the other instructors. Hopefully you can start isolating this warfarin by column chromatography by next week. If you think it is finished you could run the column this week! In any event, you should work out a solvent system using TLC and dichloromethane that move the warfarin product about halfway up the plate and separates it well from its contaminants such as the starting material. This could be accomplished by trying different combinations of methanol and dichloromethane with your product mixture vs. the standards. I hope the use and value of TLC is being understood and appreciated.

Brief Summary of Week IV (what you might be doing)

1. **Testing your Warfarin Reaction via TLC to see if it is proceeding.**
2. **Deciding whether to let your warfarin reaction to proceed or to separate it via column chromatography today. If so, working out the chromatography conditions as described above.**
3. **Setting up your other crosses, 1, 2, 3 – do three on two or three times the procedure scale. We are doing the small scale reactions. The catalyst is expensive. Get baseline TLC on all.**
4. **Characterize your Pechman product using NMR, IR and melting point.
If it is not clean, recrystallize it again. Without this, obviously, you can't do crosses 1 and 2.**
5. **Learn how to use a flame to make smaller pipets.**

Weeks V and VI

1. First a few notes sent via email.

I have been told that students do not understand what we are doing in lab. Of course, I feel bad about this.

I am going to go over the project again on Monday and Tuesday in lab lecture.

I am also going to go over the complete mechanism of the warfarin reaction.

Just briefly.....

Coumadin (also called warfarin) is a blood thinner. Coumadin is a vitamin K antagonist to an enzyme called a carboxylase (it has a cofactor called vitamin K) together (the carboxylase and vitamin K) they are responsible for the carboxylation process of glutamic acid residues on Vitamin K dependent cofactors. This carboxylation is important for the formation of groups that can bind calcium and through this binding, anchor and localize the cofactors to the platelet membrane. This is part of the coagulation process of coagulation. Coumadin has a structure similar to that of vitamin K and essentially takes its place and shuts down the enzyme and associated redox needed to carboxylate these groups necessary for the coagulation process.

Please see readings outside my door.

You are making two to four different Warfarin analogs in the hope that they will exhibit different anticoagulant or blood thinning activity. Different structure may equal different function. It is totally possible that some of our compounds have not been made and are novel anticoagulents with unique activity. This is not just a repeat of some old organic lab.

The idea is that each of the four compounds you are potentially making and using have different activities in the carboxylase reaction (structure is related to activity). It could be that a given compound is more active or less active.

Does it make sense that if you vary the structure it could vary the activity of the drug. Does it make sense that if you make and purify different structures that are related to warfarin that they could be antagonists to vitamin K to different extents.

It is noteworthy that 4-methylumbelliferone is active in its own right. Yes, just making this compound is an accomplishment and can go in your paper.

Is it clear that we made warfarin itself as a model for everything? It is your TLC model, your practice for doing the chemistry to make other, possibly unique compounds, your model for column chromatography.

The idea is using the very well developed synthesis of warfarin from 4-hydroxycoumarin and 4-phenyl-3-buten-2-one as catalyzed by 2, 3-diamino-2,3-diphenylethane you are attempting that same chemistry with slightly different substrates.

By reacting 4-hydroxycoumarin with say, 4-(4-chlorophenyl)-3-butene-one in the presence of the same catalyst you might be able to make a different anticoagulant that would have exactly the same structure as warfarin except for the presence of a Cl. Some groups have nitro groups, some methoxy, etc. You are trying to generalize the reaction to make warfarin to make a slightly modified structure.

Then you carried out the Pechman - this produced 4-methylumbelliferone.

4-methylumbelliferone could be used in place of 4-hydroxycoumarin to make two more compounds .

So if adding A + B results in warfarin, where A is 4-hydroxycoumarin and B is 4-phenyl-3-buten-2-one.

Adding A+ C where C is slightly modified from B could result in a slightly modified compound that might have slightly different biological activity.

But what if you change A to 4- methyl umbelliferone? Then by reacting with compounds B and C, respectively you could create two more potential anticoagulants. Does this make any sense?

Realize it is my expectation that you probably will not complete all four compounds. You might complete A + B and A +C from beginning to end, including analysis.

The 4-methylumbelliferone reaction if started and just analyzed by tlc will probably be adequate. It is not expected or desired that you work outside your lab time.

Finally, what you are doing is new and some of it is unique.

I hope this makes some sense. I have copied some info on coumadin (warfarin) and it is outside my door. You can pick it up, it might help you with your paper and getting the relevance of what you are doing.

Finally, I am still working on this, but I am ordering anticoagulant kits and we may do some testing of our compounds just for fun for those who are interested. It does involve using a prick of blood. Surprised I did get permission from our head of safety. Cool

Regardless, it is purely voluntary.

- 2. Please pick up the supplementary readings outside my office – one packet per group please. If you have not been doing background reading as shown above, these should help you inform yourself and give you good models for style. Also, there is some good spectral data included for warfarin compounds.**
- 3. I will go over the big picture again during lab lecture and I will go over the mechanisms. Please take good notes. The lecture will also be available by tegrity.**
- 5. This week is the last week of lab lecture and it is the second to last week of organic chemistry lab. Lab will be over next week and you will end wherever you are.**
- 6. It is your responsibility to reread this entire document so you begin to understand what you are doing.**
- 7. This week I would imagine your Warfarin reaction – no. 4 – the original model reaction should be near finished. If it is you should have worked out a solvent system on TLC that will reasonable separate warfarin from its starting materials. This may require .5 to 1 percent methanol in the solvent. If this is the case a member of your group needs to pack a column with the correct amount of silica and solvent system and load the dried sample (see procedure, small scale) on the column. The amount of silica used is based on the mass of the sample after blowing of the solvent with air. Again, refer to the procedure. You should have everything in your notebook at this point. We will not be providing procedures. Elute the sample into test tubes using your solvent system. Visualize with the UV lamp and the stain.**
- 8. Some groups may need more of their original aldol product. This could be purified by column chromatography as done in the past by another member of the group.**

- 9. Some groups may have reaction 3 completed enough that they can isolate their warfarin analogue by chromatography. This would be done analogously to warfarin itself (do this first so you get the hang of it). Many though will not be able to do this until the last week as the reaction may not be done.**
- 10. Some will finally have 4-methylumbelliferone purified ready to react and can start reactions 1 and 2 shown above. It is expected that these reactions will not be isolated, but rather will simply be followed by TLC. Do not anticipate that you will be running columns of either of these next week unless you are ahead and have time.**
- 11. If your 4-methylumbelliferone is not pure, try either recrystallizing it from ethanol or soaking it in a few mL of dichloromethane and vacuum filtering it. Realize 4-methylumbelliferone itself is a biologically active molecule and may very well have anticoagulative properties.**

Thanks in general for working on what was almost an entirely new curriculum. We are still working out the bugs. Realize lab should be exploratory in nature and you should be thinking about and questioning what you are doing.

Your second quiz is as follows – done individually. 1. Why do you think your 1 and 2 reactions did or did not work. 2. This should be based on the TLC plate (or isolation if you have accomplished this and accompanied by full mechanism – the mechanism for the general reaction will be covered in lab lecture so take notes). Find in the literature some synthesis of a biologically active umbelliferone derivative, write out the reaction and give the reference. Due to me by the end of the course. This must be confined to one page and should take you an hour tops.

