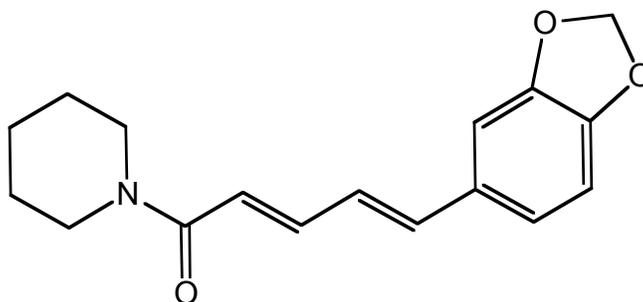


## Experiment # 2: Piperine – Synthesis and Isolation of a Natural Product

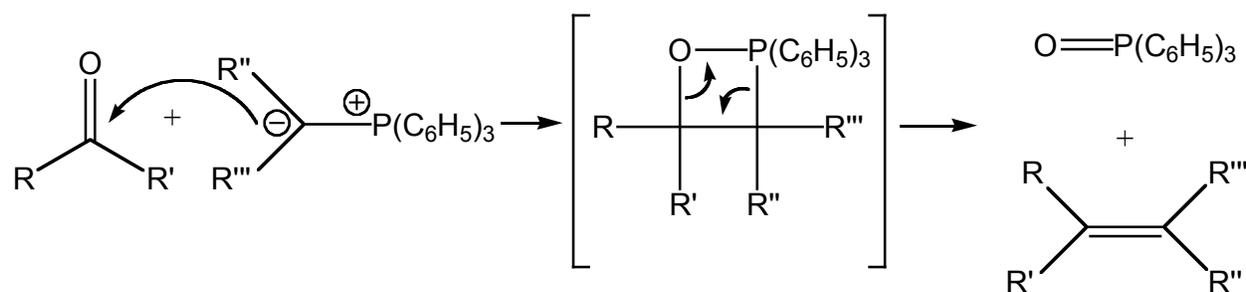
### INTRODUCTION

Piperine is the naturally occurring alkaloid that gives the spice black pepper its characteristic biting taste. The stereoselective synthesis of this natural product, in good yield from inexpensive and readily available starting materials, exemplifies the powerful synthetic utility afforded by the Wittig reaction and its variations such as the Horner-Wadsworth-Emmons reaction. The important impact of the Wittig reaction on modern synthetic organic chemistry was recognized by the 1979 Nobel Prize that was awarded in part to Georg Wittig for his discovery and subsequent development of this reaction.



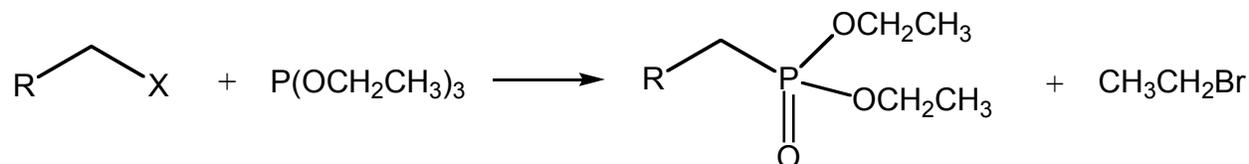
Piperine

The original Wittig reaction refers to the reaction of phosphonium ylides with aldehydes or ketones to form alkenes:

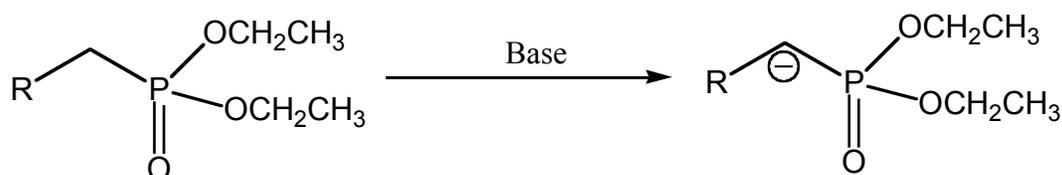


As shown, this reaction mechanism, which has been studied extensively, proceeds through a four membered oxaphosphetane ring intermediate. This intermediate breaks down to yield the corresponding alkene and phosphine oxide. Formation of the stable P=O bond of the phosphine oxide is a major driving force for the reaction. Although the original Wittig has proven to be a highly useful reaction in organic synthesis, there are

limitations with this reaction that have been largely overcome by the use of phosphonate ester carbanions (see below). Phosphonate esters can be easily made by reaction of an alkyl halide with a trialkylphosphite:

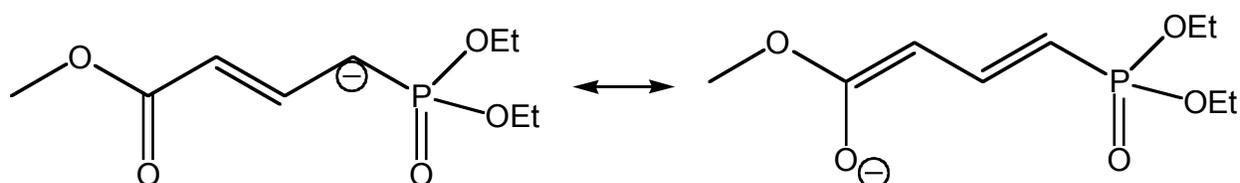


The carbanion is then generated by reaction of a base with the parent phosphonate ester:

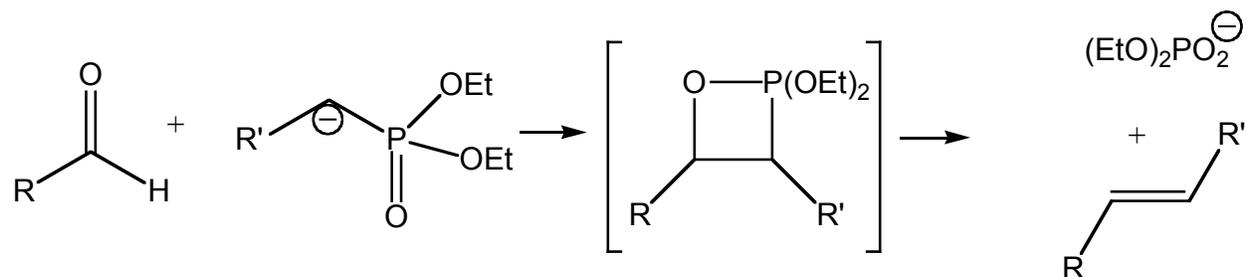


The phosphonate ester carbanions are significantly more nucleophilic than the phosphonium ylides used in the “classical” Wittig reaction and therefore react readily with a wider variety of aldehydes and ketones. Also, the phosphate side product produced from these reactions is highly water soluble and generally easy to separate from the alkene product in contrast with the phosphine oxide byproduct of the Wittig.

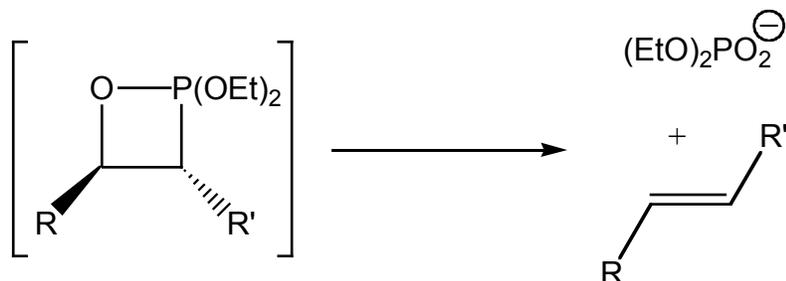
In order to undergo the Wittig type reaction with alkenes, the substituent on the  $\alpha$ -carbon of the phosphonate ester must be capable of stabilizing the carbanion by resonance. In the synthesis carried out here, the stabilizing group is methyl 2-butenate:



Reaction of these stabilized phosphonate ester carbanions with aldehydes and ketones to form the corresponding alkenes constitutes the variant of the Wittig reaction known as the Horner-Wadsworth-Emmons reaction shown at the top of the next page.



A remarkable aspect of this reaction is its generally high degree of stereoselectivity for the *trans* alkene when both *cis* and *trans* possibilities exist. The preference for the *trans* product is understandable based on steric effects in the cyclic intermediate. The *trans* alkene results from the less sterically strained four membered ring in which the largest groups are on opposite sides of the ring:

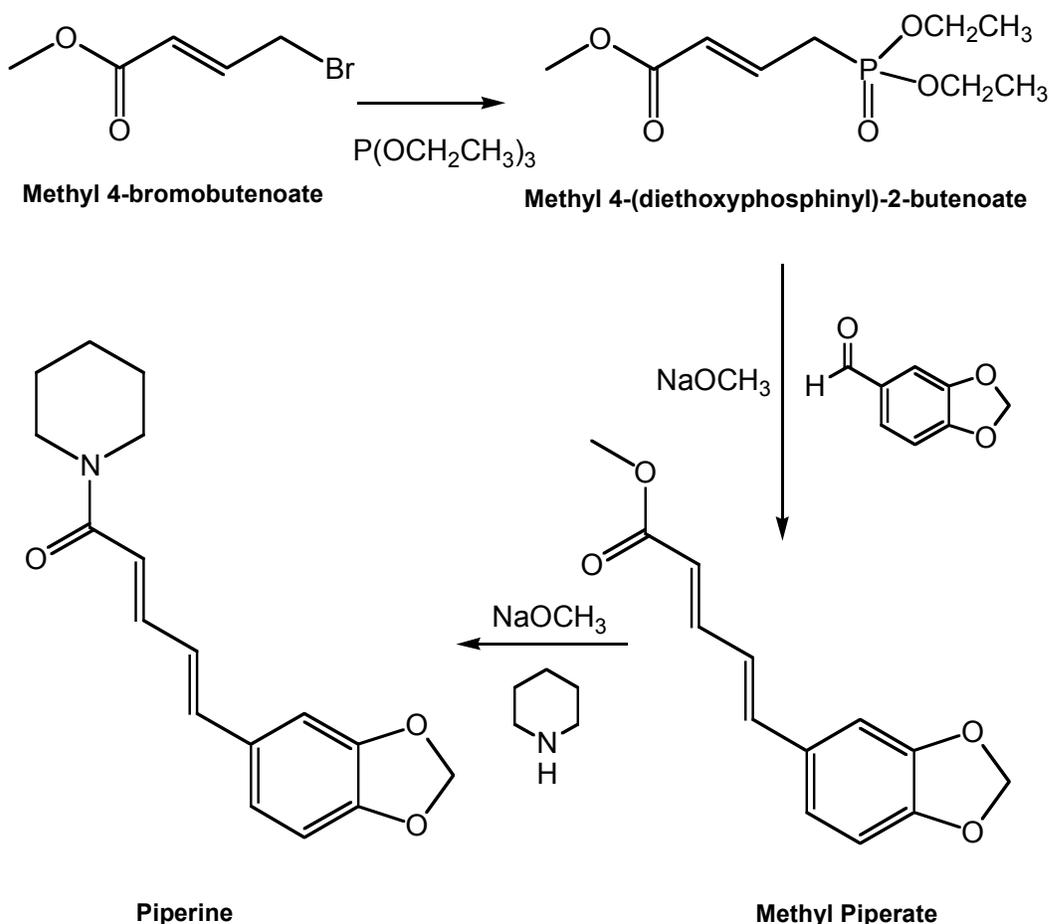


Such stereoselectivity is very important in natural product syntheses where the biologically important properties of a compound are usually specific to one stereoisomer. In the case of piperine, the unique property of its desirable peppery taste is specific to the (*E,E*) stereoisomer.

In this series of lab experiments, piperine will be obtained from two different sources, one synthetic and one natural. The synthetic route will be a three-step synthesis utilizing the Horner-Wadsworth-Emmons reaction. The crude synthetic product should be sufficiently pure to complete its purification by recrystallization. The natural isolation route will use an extraction procedure to isolate piperine from ground black pepper. The crude piperine from pepper will be purified using flash column chromatography. You will then compare the piperine from the two different sources using spectroscopic characterization techniques (*i.e.*,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR).

## SYNTHETIC SCHEME

The following scheme summarizes the reactions that will be used to synthesize piperine:



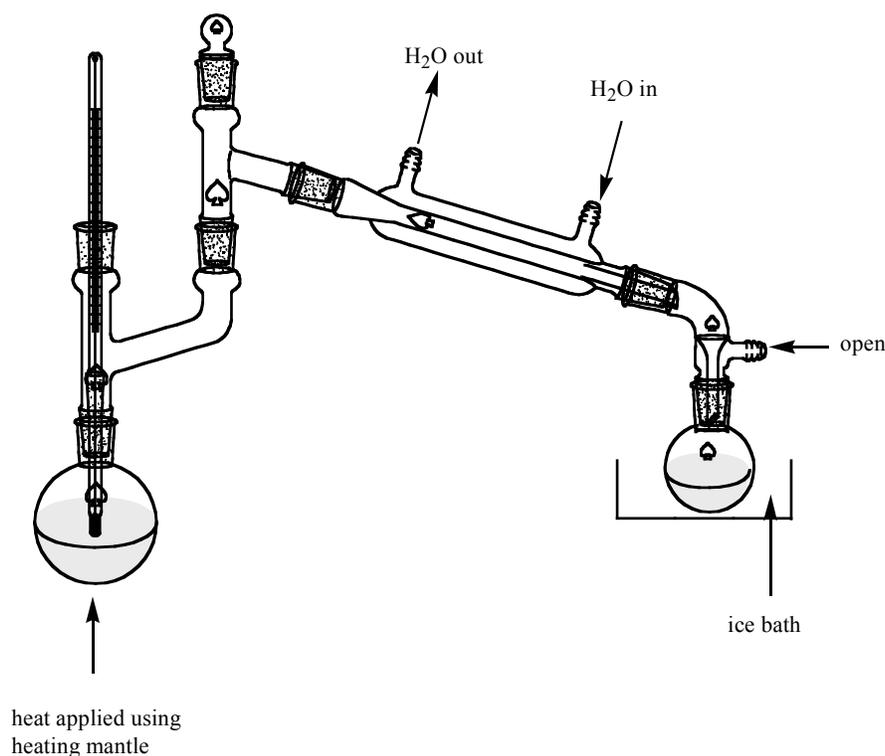
In the first reaction, the phosphonate ester is prepared from methyl 4-bromo-2-butenoate and triethylphosphite. The product, methyl 4-(diethoxyphosphinyl)-2-butenoate, is reacted in the second step with sodium methoxide to generate the phosphonate carbanion in the presence of piperonal. The phosphonate carbanion undergoes a Wittig type reaction with the piperonal to form the trans alkene, methyl piperate ((E,E)-5-(3,4-methylenedioxyphenyl)-2,4-pentadienoate). Finally, reaction of methyl piperate with piperidine in the presence of sodium methoxide in refluxing methanol solution gives piperine.

## EXPERIMENTAL

### Synthesis of Methyl 4-(diethoxyphosphinyl)-2-butenate

**Caution:** *Because of the toxicity of ethyl bromide and the stench of triethylphosphite, this experiment should be carried out in a fume hood.*

Methyl 4-bromo-2-butenate (3.5 mL, 5.3 g, 30 mmol) (see Note 1) is placed in a 25 mL round bottom flask equipped with a magnetic stirrer. To the neck of the flask is attached a Claisen head adapter fitted with a thermometer adaptor allowing the thermometer to extend down into the liquid in the flask (see Figure 1 below). The side arm of the Claisen adapter is connected to a simple distillation apparatus. Triethylphosphite (5.0 mL, 4.8 g, 30 mmol) (see Note 2) is added to the stirred methyl 4-bromo-2-butenate. The stirred mixture is gently heated. After a brief induction period, an exothermic reaction takes place and ethyl bromide (bp 37-40 °C) distills from the reaction mixture. The mixture is heated to 120-130 °C and maintained in this temperature range for 20 min or longer if necessary until ethyl bromide ceases to distill over. The crude product is characterized by IR and <sup>1</sup>H NMR and saved as starting material for step two.



**Figure 1:** Apparatus for Synthesis of Methyl 4-(diethoxyphosphinyl)-2-butenate

## Notes:

1. Methyl 4-bromo-2-butenate is usually contaminated to some extent with 2(5H)-furanone, a side product of its synthesis and purification. The boiling points of the two compounds are nearly the same, so it is not possible to separate them by distillation, but they are easily distinguishable by  $^1\text{H}$  NMR. The starting material used in this experiment, purchased from Sigma-Aldrich, may contain as much as 15% of the 2(5H)-furanone which does not interfere with the reaction but might be present in the product as a significant impurity.
2. Triethyl phosphate has an extremely disagreeable odor. It should be handled at all time in a fume hood.

## Synthesis of Methyl Piperate

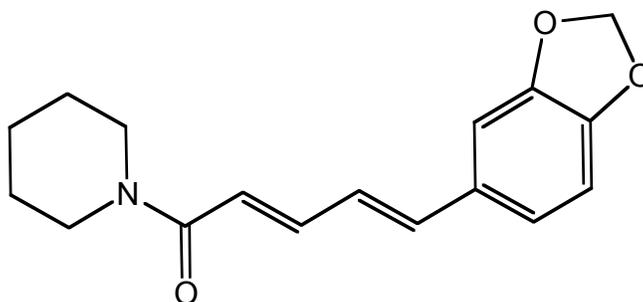
Sodium metal (0.5 g, 22 mmol) cut into small pieces is placed in a dry 50 mL round bottom flask equipped with a water-cooled reflux condenser and a magnetic stirrer placed in a fume hood. Absolute methanol (25 mL) is rapidly added to the flask through the condenser. Vigorous bubbling commences as hydrogen gas is evolved. (**Caution: make sure there are no open flames or ignition sources in the area.**) After all of the sodium metal has been consumed, the solution of sodium methoxide is allowed to cool to room temperature.

Methyl 4-(diethoxyphosphinyl)-2-butenate (5.0 g, 21 mmol), piperonal (3.2 g, 22 mmol) and 50 mL of dimethoxyethane are placed in a 250 mL three-neck round bottom flask equipped with a magnetic stirrer, a thermometer adapter, a drying tube, and a dropping funnel. The sodium methoxide solution is added to the dropping funnel. The reaction flask is cooled in an ice bath and the sodium methoxide solution is added **slowly drop wise** to the mixture with continuous rapid stirring. After addition of the sodium methoxide solution is complete, the ice bath is removed and the mixture is allowed to warm to room temperature. The mixture is stirred at room temperature for 2 h, and then stored in a refrigerator until the next lab period.

The reaction mixture is poured into 200 mL of cold water and the resulting mixture is stirred for 45 min. The solid that forms is isolated by vacuum filtration and washed with 50 mL of cold water. The crude solid is recrystallized from ethyl acetate to afford methyl piperate (methyl (*E,E*)-5-(3,4-methylenedioxyphenyl)2,4-pentadienoate) as light yellow crystals, mp 146-148 °C. The recrystallized product is characterized by IR and  $^1\text{H}$  NMR.

## Synthesis of Piperine

Sodium metal (0.2 g, 9 mmol) cut into small pieces, is placed in a clean, dry, 250 mL round bottom flask fitted with a water-cooled reflux condenser. Absolute methanol (75 mL) is rapidly added to the flask through the condenser. Vigorous bubbling commences as hydrogen gas is evolved. (**Caution: make sure there are no open flames or ignition sources in the area.**) After all the sodium has been consumed, methyl piperate (3.5 g, 15 mmol) and freshly distilled piperidine (17 mL, 170 mmol) are added to the sodium methoxide solution. The solution is refluxed for 40 h and then allowed to stand at room temperature until the next lab period. The resulting red-brown solution is poured into 300 mL of cold water and stirred for 1 h. The precipitate that forms is collected by suction filtration to afford piperine as a beige powder. The solid is recrystallized from ethyl acetate/hexane to give fine, yellow-beige needles (mp 123-124°C). The recrystallized piperine is characterized by IR, <sup>1</sup>H NMR, and <sup>13</sup>C NMR.

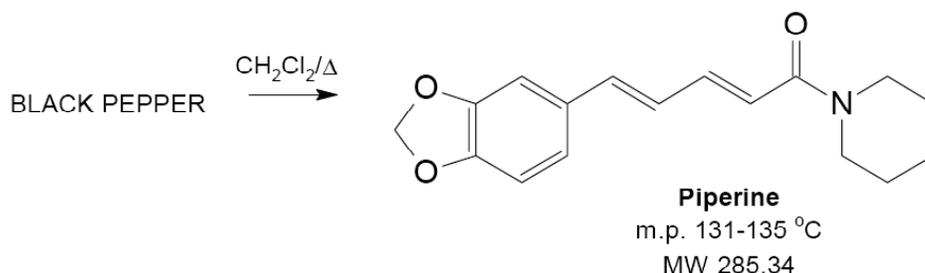


**Piperine**

## Piperine – Isolation and Purification of the Natural Product<sup>†</sup>

The chemistry of natural products is an integral part of organic chemistry. Around the turn of this century, chemists endeavored to discover the active agent in black pepper. In this project, you will isolate and purify this agent, piperine, from black pepper, study its structure with 1D NMR experiments, and compare this structure to what you synthesized in the laboratory.

### Isolation of Piperine



Place 15 g of ground black pepper in a 100 mL round-bottom flask, add 30 mL of  $\text{CH}_2\text{Cl}_2$  and at least five boiling chips. Attach a water-jacketed condenser and heat at reflux for 0.5 hr. **IN YOUR FUME HOOD**, using your large Buchner funnel, suction filter the mixture, rinse the pepper solids with  $\text{CH}_2\text{Cl}_2$  (up to 15 mL), and then concentrate the resulting filtrate by use of a rotary evaporator. Remove the bulk of solvent.

To remove excess  $\text{CH}_2\text{Cl}_2$ , cool the residue in an ice bath, add 5-10 mL of cold diethyl ether to the flask and triturate the residue until the ether is mixed in well. Precipitation may begin at this point. Remove the ether using the rotary evaporator. If the remaining residue still appears oily, repeat the cooling/trituration/evaporation process. When the residue appears to have solidified, dissolve as much of it as possible in 10-15 mL of 95% ethanol.

To 10 mL of a 10% solution of potassium hydroxide in 95% ethanol, contained in a 125 mL Erlenmeyer flask, add the ethanolic pepper extract. Be sure to wash any undissolved solid into the KOH flask with a small amount of 95% ethanol.

Warm the resulting solution (steam bath) and slowly add water with a Pasteur pipette (trickle the water down the sides of the flask). The mixture should become cloudy and a yellow precipitate should begin to form. Continue adding water in this fashion until you have added a total of 100 mL. Cover the flask with Parafilm, label it, and allow the mixture to stand until the next lab period.

<sup>†</sup> This laboratory experiment is modified with permission from the Pomona College Departmental of Chemistry 2003 Organic Chemistry Laboratory Manual.

## Purification of Piperine

Collect the solid by suction filtration using the small Buchner funnel and two pieces of filter paper (this helps avoid clogging of the filter paper). Wash the solid with two 5 mL portions of water. If you observe solid in the filtrate, collect it also. To facilitate drying, wash the solid with one 5 mL portion of cold diethyl ether. Allow the solid to dry under vacuum (use the vacuum manifold on the vacuum cart and have a TA assist you with this manipulation) before determining its mass. Determine and report the crude yield of this powder as a weight percentage of the black pepper you started with.

After you have determined the crude yield, weigh 350 mg of the piperine into a small vial. **Please note:** if you don't have 350 mg of piperine, contact your TA. Dissolve/suspend the powder in 2–5 mL of  $\text{CH}_2\text{Cl}_2$  and use TLC to determine the purity of this crude material. Be sure to use the standard “3 spot” procedure (lane 1 = crude compound, lane 3 = authentic piperine, lane 2 = co-spot of lanes 1 and 3). Use a 40% ethyl acetate/60%  $\text{CH}_2\text{Cl}_2$  mixture as the elution solvent and UV irradiation to visualize the developed plate. **Draw the plate in your notebook and record the  $R_f$  values of any significant spots.**

Prepare your silica gel column using the following procedure. Weigh approximately 32 g of silica gel into a 250 mL beaker and suspend the silica gel in  $\text{CH}_2\text{Cl}_2$  (70 mL). Dislodge any air bubbles by stirring the slurry with a clean spatula. Clamp the column to the distillation rack in your hood. The outflow of the column should be directed to a 125 mL Erlenmeyer flask. Add a small quantity of sand (a powder funnel is handy here) so that a 0.5 cm layer rests on top of the glass frit. Add a small quantity of  $\text{CH}_2\text{Cl}_2$  so that flow is established and arrest the flow with the stopcock when there is a one-inch layer of  $\text{CH}_2\text{Cl}_2$  covering the sand layer. Swirl the silica gel slurry (some settling may have occurred) and slowly pour it down the inner surface of the column so that it disperses and settles evenly when it reaches the one-inch layer of  $\text{CH}_2\text{Cl}_2$  at the bottom of the column. After most of the silica gel slurry has been transferred to the column, open the stopcock and allow the excess  $\text{CH}_2\text{Cl}_2$  to drain through the column. As this is happening, use a Pasteur pipette and some of the excess  $\text{CH}_2\text{Cl}_2$  to rinse down any silica gel that might be adhering to the upper inside surface of the column. After the  $\text{CH}_2\text{Cl}_2$  has drained, the column should have a level upper surface. Add dry sand such that a 0.5 cm layer rests on top of the silica gel. A few mL of  $\text{CH}_2\text{Cl}_2$  can be used to wash down any sand that might be adhering to the inner surfaces of the column. A “well poured” column should be evenly packed and free of any visible air bubbles.

Use a Pasteur pipette to add the crude pepper extract (350 mg in 2-5 mL  $\text{CH}_2\text{Cl}_2$ , as described above) to the top of the column bed. Add the solution in such a manner such that it enters the column in an even manner. This can be accomplished with 1-2 smooth pipette discharges using expanding concentric circles above the top of the column bed. If any of the solution accidentally gets caught on the inner glass surface, don't worry too much — it will get washed down onto the column with subsequent rinses.

Allow the solution to enter the column and use 2 mL of fresh  $\text{CH}_2\text{Cl}_2$  to rinse the inner surface of the column and sand layer (some “misers” will use this volume of solvent to rinse the residual vial contents onto the column — this is a useful maneuver when dealing with a precious compound). Repeat this rinse twice, always allowing the excess solvent to drain from the bottom before adding new solvent to the top of the column. The goal is to load the mixture onto the column in a non-polar solvent, in as concentrated a solution as possible.

To the top of the column, carefully add the first elution solvent (100 mL of 40% v/v ethyl acetate in  $\text{CH}_2\text{Cl}_2$ ) and force it through the packed silica by applying a stream of compressed air to the top of the column. Use care when adding solvent to the column: you don't want to disturb the top of the column packing! When it appears that solvent front (i.e., the blue fraction) is about to emerge from the column (the compounds are yellow in color), begin to cut fractions with 13x100 mm test tubes (fill each ca. 3/4 full). When the 40% ethyl acetate/60%  $\text{CH}_2\text{Cl}_2$  eluent is running low, make a 100 mL batch of 60% ethyl acetate/40%  $\text{CH}_2\text{Cl}_2$  and continue the column until this eluent has been used.

Use TLC to assess the chemical identity of each fraction. This is conveniently done by spotting 4-5 fractions on one 1 inch-wide TLC plate. In order to “pack” this many spots on one plate, you must use care with your spotting technique (use tight, non-diffuse spots on a plate pre-marked with pencil spots to indicate the origin of each lane). Be sure to draw these TLC plates in your notebook.

Using a pre-weighed round-bottom flask, consolidate the piperine fractions and remove the solvents with a rotary evaporator. If the compound does not immediately form a solid, try triturating the flask contents with a few mL of ether or hexane and then remove the excess solvent again with the rotary evaporator. Collect the solid and determine its mass and melting point. Calculate and report the yield as a weight percentage of piperine contained in the crude material used for chromatography.

### **NMR Characterization of Piperine**

Using the compound purified by column chromatography, prepare a sample for NMR analysis by dissolving 10-15 mg in  $\text{CDCl}_3$ . Obtain a  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra on the sample. Compare these spectra to those you collected for synthetic piperine. Are they the same? Include these spectra and your analyses in your lab report.