Cross-Disciplinary Collaborative Laboratory Activities that Reinforce Chemistry Content Knowledge

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Abstract

Biology majors have difficulty applying their chemistry knowledge to research topics in biology. Here we describe a problem-based laboratory activity that requires biology majors taking microbiology to apply their knowledge of organic chemistry and to collaborate with organic chemistry lab students to study *p*-aminobenzoic acid (PABA) derivatives. The organic chemistry students synthesize and characterize an array of novel PABA derivatives using the Fischer esterification reaction. They present their derivatives to the microbiology class and both classes predict which derivative would make the best UV light blocker. The microbiology students complete an experiment to test which derivative acts as the best UV light blocker.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

Learning Objectives.

After completing this activity, the student will be able to:

- formulate a testable hypothesis about the relative ability of various *p*-aminobenzoic acid (PABA) derivatives (or other organic compounds) to absorb UV light.
- design an experiment that effectively tests a hypothesis about the ability of organic compounds to absorb UV light.
- sufficiently analyze the PABA derivatives dataset and formulate appropriate conclusions from that dataset.
- explain the Fischer esterification reaction, the basic structure of esters and carboxylic acids, and the properties of organic compounds that aid in their ability to absorb UV light.
- explain the effects of UV light on bacterial cells.
- recite the wavelength range for germicidal light.
- clearly present results and conclusions from the experiment.

Background.

The students in the microbiology class should have had organic chemistry and should have conjugated organic compounds and learned the Fischer esterification reaction (1), the UV spectrum, and properties of organic compounds that affect UV absorption. If they have not had organic chemistry, the instructor can provide a brief introduction to PABA, benzocaine, the Fischer esterification reaction, and conjugated organic compounds and their ability to absorb UV light. We suggest *Organic Chemistry* as a good reference (2). The microbiology students should have also learned spread plating technique and the effects of UV light on cells. It is helpful if the students know how to use PowerPoint and Excel and understand how to create and present graphs.

PROCEDURE

Materials.

Microbiology lab materials

- *Serratia marcescens* broth cultures (1 culture per group). These cultures should be as turbid as a McFarland standard #7 (i.e., an overnight culture)
- Tryptic soy agar plates (at least 10 per group)
- 1 roll of plastic wrap
- Empty petri dish (1 per group)
- Mineral oil or petroleum jelly (approximately 3 ml per group)
- Sterile swabs (at least 10 per group)
- PABA derivatives created by the organic chemistry class or purchased (1 g of each derivative per group)
- Pipettes for dispensing 0.1 ml and 3 ml (at least 10 per group)
- Spread plating materials for each group (hockey stick plate spreaders, 95% ethanol, and glass petri dish with lid)
- Bunsen burners (1 per group)
- Short range (germicidal) UV lights within the range 200 to 290 nm (one per group is ideal, but at least one per two groups is workable)
- A balance (alternatively the instructor can weigh out 1 gram of each derivative for each group)

Organic lab materials

- See instructor version protocol

Instructor Version.
Instructor Version

Student Version.

- Organic student version
- Microbiology student version
- Microbiology student version without organic chemistry

Safety Issues.

The microbiology students will use UV lights which can be dangerous to soft tissue, especially the eyes. Care should be taken to not look directly into UV lights when in use. Students will be working with novel organic chemicals and should wear latex or plastic gloves. The compounds should not come into contact with the skin as many of these compounds can be classified as irritants or allergens. The microbiology students will be working with *S. marcescens*, which is a biosafety level 2 microorganism. Students should wear safety glasses, gloves, and laboratory coats. The organic chemistry students should exercise caution when using the strong acid catalyst during the esterification reaction. Safety glasses, aprons, and gloves should be worn at all times.

Assessment.

The following was used to determine student learning in the microbiology and organic labs. Results of these assessment methods are presented and discussed in the Field Testing section.

- Pre- and postquizzes or questions on lab practicals or exams to assess content knowledge
- Oral presentations (the microbiology students presented their results to the organic chemistry students)
- Reports
  - Organic chemistry class created derivative information sheets explaining the properties of their novel derivatives
  - Microbiology class wrote a laboratory report about their experiment

Field Testing.

This exercise was field tested with biology majors in two upper-division microbiology lab sections and one organic chemistry lab section.

Oral presentations
The microbiology students presented their results to the organic chemistry students. The presentations required the students to prepare their results visually. Students critiqued each other at the end of the presentations so they could improve their lab reports. The presentations also helped to generate a lot of discussion about experimental design and data analysis. The interesting part was that the discussion occurred between the organic chemistry class and the microbiology students. The microbiology students were challenged by presenting their experiment to a class who has never had microbiology. We have included a sample oral presentation grading rubric, which we created using Rubistar (http://rubistar.4teachers.org/index.php), in the appendix.

Lab reports
The laboratory reports were very creative and the scores were higher than what is typical for this course. Students were able to develop creative and sound hypotheses. Students actually listened to the class discussion about the data analysis and incorporated some of those thoughts into their own papers. Students often have trouble with the results and conclusions sections. The class discussion helped the students to analyze the results and make interesting conclusions. We have

included a sample laboratory report grading rubric, which we borrowed from LabWrite (http://labwrite.ncsu.edu/instructors/excelsheets.htm), in the appendix.

Quizzes

The microbiology classes were given quizzes over the material before and after the activity (n = 41).

- The average on the prequiz was 49% and the average on the postquiz was 72%.
- 87% of the students received a higher score on the postquiz than on the prequiz.
- 74% scored at least 10% higher on the postquiz
- 61% scored at least 20% higher on the postquiz.

The organic chemistry class was given quizzes over the material before and after the activity (n = 25).

- The average on the prequiz was 23% and the average on the postquiz was 53%.
- 80% of the students received a higher score on the postquiz than on the prequiz.
- 80% scored at least 10% higher on the postquiz
- 64% scored at least 20% higher on the postquiz.

Student surveys

The microbiology classes were surveyed after completing the activity (n = 41).

- 98% of the students surveyed felt that the activity helped them learn about PABA derivatives and their ability to block UV light.
- 68% of the students surveyed felt the activity helped them to understand the importance of teamwork.
- 100% felt they understood the experimental design created by the class.
- 68% enjoyed the activity and only 7% did not like it at all. The remaining 25% were undecided.
- 45% felt that collaborating with the organic chemistry class made the project more interesting.
- 65% felt that collaborating with the organic chemistry class enhanced their learning.

The organic chemistry class was surveyed after completing the activity (n = 25).

- 100% of the students surveyed felt that the activity helped them learn about PABA derivatives and their ability to block UV light.
- 76% of the students surveyed felt the activity helped them to understand the importance of teamwork.
- 76% felt that writing the PABA derivative information sheet helped them to better understand and remember the material.
- 80% enjoyed the activity and 20% were undecided.
- 72% felt that the collaborative aspect of this project made the project more interesting and enjoyable.

Instructor feedback

We have provided statements from three instructors below:

- Microbiology lab instructor 1: “The students appeared to be much more engaged in this activity as compared to others. Several asked me if they could do microbiology research with me after completing the lab.”
- Microbiology lab instructor 2: “Before this learning activity, students did not understand PABA derivatives despite previous exposure to the topic in organic chemistry. Once the activity was completed, students’ overall understanding of PABA derivatives and effect of UV light on microorganisms was much better since they were able to formulate a hypothesis and design and execute an experiment allowing them to collect and analyze data.”
- Organic chemistry lab instructor: “This lab was a great learning experience for the organic chemistry students. The synthesis of novel compounds made learning the esterification reaction more interesting, and the students really enjoyed the collaboration and discussion with the microbiology class. Since it is a prerequisite, none of the organic chemistry students had taken the microbiology class yet, and this activity was a great introduction to the microbiology lab.”

Student Data.

We have provided the following in the Appendix section:

- Example quiz taken by a microbiology student
- Example derivative information sheet created by an organic chemistry student
- Two example laboratory reports made by groups in the microbiology lab
- Example PowerPoint of a student presentation (combined several groups into one PowerPoint)
- Example student data collected from our labs

Possible Modifications.

- It is important to have some back-up derivatives in case the organic students are not successful at obtaining a product. We had 14 groups in the organic chemistry lab and 15 groups in microbiology. Two groups in organic chemistry did not successfully get a product. We gave three groups in microbiology PABA (pure). In the end this compound appeared to have worked the best at blocking UV light, which was interesting to the students. We discussed possible reasons for this, including the purity of the compound and the fact that it has been successfully used as a UV light blocker.
- If it is not possible to have the microbiology class present to the organic chemistry class, then a presentation within the microbiology class is also very useful. This presentation can be videotaped and you can add a streaming video clip to your course in WebCT or Blackboard for the organic chemistry and microbiology students to view. The students can use an online chat room function for the discussion about the data that is presented.
- You may want to try suspending the derivatives in something other than mineral oil. Students had trouble spreading the compound evenly.
- It was difficult to count colonies, so students used numbers to indicate levels of growth (0 = no growth and 3 = most growth). If the culture was diluted and spread plated, it may be easier to quantify the number of colonies on the plate.
- The students did a control where they put the plastic wrap covered with mineral oil over an inoculated plate and

exposed the plate to UV light so that they could see if the plastic wrap and oil affected the transmission of UV light. We opted to subtract any growth on these control plates from growth on the experimental plates. The students called this value the "compound efficiency number" (the higher the number the better the compound blocked the UV). The students felt that it was important to account for any affect the plastic wrap and oil may have had on the transmission of UV light.

- You can have the organic students test the purity of the compounds using spectroscopy, such as mass spectrometry, infrared spectroscopy, or nuclear magnetic resonance. This information would be interesting to consider when the data is analyzed and conclusions are made. It may also help the microbiology students with their experimental design as they are attempting to standardize the methods.
- If PABA derivatives cannot be obtained, a possible modification might include obtaining several sunscreens on the market, having the students research the active ingredient, and test hypothesis on which compound should block the most UV light.
- This laboratory focused on testing the ability of these compounds to block UVC light in the 200 to 290 nm range. We have also tried to use UVB light in the 290 to 320 nm range and it worked well. UVB kills *S. marcescens* within 3 to 5 minutes. Some of the compounds we tried out actually absorb in the UVB range. It may be interesting to have your class look at both wavelengths and compare, if you have the time to do both. We did not try to test the ability to block UVA light in the 320 to 400 nm range, because UVA does not harm *S. marcescens* cells.

References.

1. **Pavia, D. L., G. M. Lampman, G. S. Kriz, and R. G. Engel.** 1998. Introduction to organic lab techniques: a small scale approach. Saunders College Publishing, Philadelphia, PA.
2. **Wade, L. G.** 2006. Organic chemistry, 6th ed. Pearson Prentice Hall, Upper Saddle River, NJ.

Appendices.

Appendix 1. Example quiz taken by a microbiology student

Appendix 2. Example derivative information sheet created by an organic chemistry student

Appendix 3. Two example laboratory reports made by groups in the microbiology lab:
Example Report A
Example Report B

Appendix 4. Example PowerPoint of a student presentation (combined several groups into one PowerPoint)

Appendix 5. Example Excel file with results from our class.

Appendix 6. An example rubric borrowed from LabWrite for grading laboratory reports

Appendix 7. An example rubric created in Rubistar for grading oral presentations

Appendix 8. The instructor PowerPoint that was used to introduce the topic to the students

Recipes.

Difco tryptic soy agar can be purchased from most biological supply companies such as Fisher Scientific.

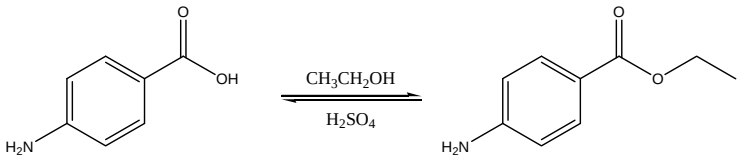
Overview of our approach

We approached this activity as a collaboration between an organic chemistry laboratory class and a microbiology laboratory class. The organic chemistry class synthesized several (about five in our case) *p*-aminobenzoic acid (PABA) derivatives and created information sheets about each novel derivative. The derivatives and the information sheets were given to the microbiology laboratory students. Both groups of students made predictions about which derivative would block the most ultraviolet (UV) light based on the structures of the compounds. The microbiology students were instructed to design an experiment to test their hypothesis. They performed the experiment, collected data, and analyzed the results. The microbiology class then worked together to prepare a presentation about their experiment. Each group prepared a different part of the presentation. The microbiology class presented to the organic chemistry class and after the presentation both classes discussed the results.

Below we explain how to use this activity in your courses. First, we explain how to complete the activity using our approach as we explained it above. Next, we explain how to adapt the activity if you cannot collaborate with an organic chemistry class.

Organic chemistry lab

During Organic Chemistry II students learn about the Fischer esterification reaction, which is an acid-catalyzed reaction of a carboxylic acid with excess alcohol to yield an ester. One well-known reaction is the reaction of PABA with ethanol to yield benzocaine.



para-Aminobenzoic acid Benzocaine

The instructor should place students into groups of two, give each group PABA (a carboxylic acid), let them choose from a selection of alcohols, and then run the esterification reaction to yield an ester. Each ester is considered a different PABA derivative; the structures differ based on the alcohol used for the reaction. Students obtain the melting temperature and spectroscopic data (optional), infrared spectroscopy as well as gas chromatography and mass spectrometry, to characterize their derivative and assess the purity of their product. Each group then creates an information sheet about their novel PABA derivative for the microbiology class including its structure, synthesis, melting temperature, and other noted physical properties. Students then predict which compound (from the selection of compounds created by all groups) will absorb the most UV light in the UVC range (200 to 290 nm). The instructor should explain that the ozone layer blocks UVC light, but UVA (320 to 400 nm) and UVB (290 to 320 nm) light actually reach the surface of the earth; therefore, most sunscreens contain compounds that block UVA and UVB light only. Due to the depletion of the ozone layer, it may be necessary to develop sunscreen that blocks UVC light. The students have previously learned about UV absorption and properties of a compound that increase its ability to absorb UV light. Students learn early in organic chemistry that certain properties of compounds improve the ability of those compounds to absorb UV light. For instance, compounds with many conjugated bonds, alternating single and double bonds (e.g., benzene), absorb UV light well, and longer chain hydrocarbons absorb more light than shorter chain hydrocarbons. The instructor should explain that the objective of this lab is to investigate the ability of novel PABA derivatives to block UVC light rays. There is an instructor version containing specific information for the organic chemistry instructor included later in this document.

Microbiology lab

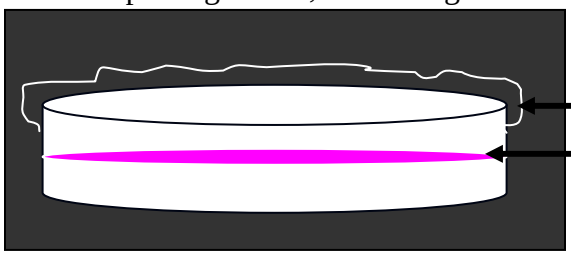
The microbiology students should have previously taken organic chemistry and already learned about germicidal UV light effects on cells. If the students have not taken organic chemistry or have not retained the information well, then the instructor can explain some of the organic chemistry to the students during a prelab lecture. Any organic chemistry textbook would be a good resource for the instructor (see reference section). We have included a PowerPoint in the appendix that contains the basic information that the students would need to understand the lab. The students would have to know the following:

- Fischer esterification reaction
- Background on PABA and benzocaine
- Properties of a compound that increase its ability to absorb UV light
- The UV spectrum and germicidal range

The instructor should also explain that the ozone layer blocks UVC light, but UVA (320 to 400 nm) and UVB (290 to 320 nm) light actually reach the surface of the earth; therefore, most sunscreens contain compounds that block UVA and UVB light only. Due to the depletion of the ozone layer, it may be necessary to develop sunscreen that blocks UVC light. Since UVC light is germicidal, bacteria such as *Serratia marcescens* can be used to investigate the ability of PABA derivatives to block UVC light. The objective of this lab is to investigate the ability of novel PABA derivatives to block UVC light rays.

Lab session 1 (lab session, recitation session, and/or homework)

1. Divide students into groups of three to four and give each group a copy of all of the PABA derivatives information sheets.
2. Explain the overall project, students' role, and the role of the organic chemistry class.
3. Ask each group to predict which compound will absorb the most UV light.
4. Show a basic set up for doing a biological assay that would work to test the ability of each compound to absorb UV light. This set up involves suspending the compound in mineral oil, spreading *Serratia marcescens* on an agar plate, covering the plate with plastic wrap (no lid), spreading the suspended compound on the plastic wrap, placing the plate under UV light for 3 minutes, removing the wrap, replacing the lid, incubating for 24 hours, and recording growth.



After suspending the compound in oil or petroleum jelly, it can be evenly applied to the plastic wrap using a swab.

5. Ask students to design an experiment that would test their hypothesis. Each group should work out the details of how they would conduct the experiment with the proper controls and replicates. Remind students that they will actually have to complete the experiment during the next two lab periods.
6. After individual group discussions, initiate a class discussion about experimental design. Allow each group to share ideas. The class should eventually agree upon an experimental design to be performed collectively by the class, including details about how much of each compound to use, the amount of oil to use to suspend the compound, how much *S. marcescens* to inoculate, how to set up controls, etc.
7. Assign one compound to each group to assay.

Note: we took 10 minutes at the end of a lab period to present this lab to the students. We instructed them to go home and think about it individually. During the next lab period we used 1 hour for group discussion, class discussion, and experimental design development. If students reside on campus, it seems reasonable to expect

them to meet with their groups to discuss the experiment and come up with a design as homework, and then only 20 to 30 minutes would be needed in class to have the class discussion.

Lab sessions 2 and 3

During lab session two, each group sets up the experiment with their assigned derivative according to the experimental design determined by the class. The plates are incubated for 24 hours. A detailed protocol can be found in the student version.

Notes:

- You will need to provide students with germicidal UV lights in the 200 to 290 nm range. It is preferable to have one light per group, but you can have two or three groups share a UV light too. Remind students to protect their eyes from the UV light. Hand-held UV lights can be purchased from Fisher Scientific for approximately $40.
- We had the students suspend their compounds in mineral oil. In some cases this worked well, but in other cases it did not. We tried petroleum jelly and it appeared to work well, but we did not have our students try it. Lotion may work too as long as it does not contain sunscreen.
- Students will be using the spread plate technique. An excellent spread plate protocol is found in the ASM MicrobeLibrary. Remind students to keep the ethanol away from the flame while performing this technique.
- We chose to use *S. marcescens* so that we could more easily see contamination. We expected that our *S. marcescens* would form pink colonies and contaminants would not. Any culture could be used as a substitute.
- One of the PABA derivatives was a sticky solid that had a paste-like consistency. The students had trouble weighing this one and suspending it in oil.
- This laboratory tested the ability of PABA derivatives to block UVC light in the 200 to 290 nm range. We have also used UVB light in the 290 to 320 nm range and it worked well. UVB kills *S. marcescens* within 3 to 5 minutes. Some of the compounds we tried actually absorb in the UVB range. It may be interesting to have your class look at both wavelengths and compare, if you have the time to do both. We did not test the ability of the PABA derivatives to block UVA light in the 320 to 400 nm range because UVA does not harm *S. marcescens* cells.

During lab session three, each group records their results by observing growth on their plates and assigning a value that reflects the amount of growth. (We have provided an example of our data collection in the appendix.) Each group reports their data to the instructor and the class data is added to a spreadsheet. The collective data is sent to the class via email. Each group analyzes the data and draws conclusions. Groups discuss what type of statistics they could use to show significance. They also discuss and plan how they will present the data. The class prepares a presentation for the organic chemistry class. Each group is assigned a topic for the presentation. Topics may include:

1. Background on PABA, benzocaine, and PABA derivatives and on UV light and absorption of UV by organic compounds
2. Explanation of the experimental design
3. Description of the biological assay set up
4. Results
5. Conclusions

Notes:

- Our students used numbers to represent the amount of growth on the plates (0 for no growth and 3 for the most growth). This is not the best quantitative way to do this; however it was too difficult to

accurately count colonies on the plates when they started with overnight cultures (i.e., colonies were too numerous to count). It may be interesting to have students dilute the *S. marcescens* culture to a concentration of 1,000 cells/ml prior to spread plating and plating 0.1 ml. This may result in plates that can be counted.

- Most students chose to use error bars in their graphs. Some chose to use a student *t* test to test for significance. We were disappointed with the students' level of statistical knowledge.

Lab session 4

Students turn in a lab report and present an assigned topic using PowerPoint. During this lab session the microbiology students present to the organic chemistry students and a class discussion about the results follows.

Instructors should use a grading rubric to assess the lab reports. An example grading rubric is provided in the appendix. You can create a rubric quite easily using the LabWrite website at North Carolina State University, http://labwrite.ncsu.edu/instructors/excelsheets.htm.

Notes:

- Many of our students were very creative and chose to pretend that they were a biology (microbiology class) or chemistry (organic chemistry class) division of a drug company. Some groups even created a name for their hypothetical drug company. Their creativity can actually be seen in their laboratory reports and derivative information sheets.

Modifications

It may not be possible for collaboration between a microbiology and organic chemistry class. Here are some suggestions on how the microbiology activity can be completed without the organic chemistry class.

- The chemicals can be purchased from a chemical supply company. PABA (4-aminobenzoic acid), benzocaine (ethyl 4-aminobenzoate), and a limited variety of PABA ester derivatives (methyl 4-aminobenzoate, butyl 4-aminobenzoate, isobutyl 4-aminobenzoate, and *tert*-butyl 4-aminobenzoate) are available for purchase from Sigma-Aldrich. It is important to note that with the limited selection of PABA derivatives available for purchase, investigations into the relationship between chemical structure and UV absorption will also be limited.
- The microbiology instructor will have to create the information sheets about the chemicals. A sheet showing the structure of the chemical, its name, melting temperature, solubility, and purity would be useful or a sheet simply showing its structure and name would suffice.
- If the microbiology students have not had an organic chemistry class, then the instructor would have to explain some of the chemistry. Information about the Fischer esterification reaction, esters, carboxylic acids, and the properties of compounds that influence their ability to absorb UV light can be found in any organic chemistry textbook. We have indicated a good reference in our references section. The students should understand that the presence of conjugated bonds greatly increases a chemical's ability to absorb UV light and that longer chain hydrocarbons absorb UV light better than shorter chain hydrocarbons.
- There are numerous sunscreens on the market that contain a variety of different UV blocking organic compounds (not necessarily related to PABA). Students can make predictions based on structures of the compounds found in those commercial products and test those products. Most commercial products contain more than one active ingredient at different concentrations, so it may be more difficult to control the experiments and make direct comparisons. We have not tried this modification with our students.

Organic Chemistry Instructor Version

If the microbiology instructor does choose to collaborate with an organic chemistry instructor for this project, the following notes and guidelines can be used for the organic chemistry portion of the project (the synthesis of PABA derivatives and preparation of the information sheets).

Before the experiment. Prior to the lab period in which the organic chemistry students will synthesize the PABA derivatives, the organic chemistry instructor will need to do the following:

- Collect the necessary materials for the experiment. The Fischer esterification reaction is a common organic reaction found in most organic chemistry laboratory textbooks. An example procedure is included in the student version of the procedure and can easily be adapted if necessary.
- Choose the alcohols that will be used in the experiment. It is recommended to select alcohols with a range of structures, including some that are conjugated, in order to investigate the correlation between structure and UV absorption.
- Assign each student (or group of students) an alcohol and assign codes for each compound that will be synthesized (Derivative A, etc). We used groups of two students in our activity.
- Distribute the procedure for the experiment to the organic chemistry class and discuss the esterification reaction.
- Explain the objectives of the project and the responsibilities of both the microbiology students and the organic chemistry students to the organic chemistry class.
- Guide the organic chemistry class in making predictions about which of the derivatives will be most effective at blocking UV light based on structure.

The experiment. The following points may be helpful to consider for this reaction:

- The Fischer esterification reaction procedure is not complicated and can be completed by students in either semester of the traditional two-semester organic chemistry lab sequence. Typically the synthesis of carboxylic acid derivatives is discussed in the second semester course, and the experiment provides a good opportunity to review this reaction mechanism. If this project is completed in the first semester course, it can be used to review percent yield calculations as well as introduce the reflux technique, functional groups, UV absorption, and spectroscopy techniques (infrared, gas chromatography and mass spectrometry, nuclear magnetic resonance, etc.).
- Standard laboratory safety rules should be followed. Special attention should be paid to the safe use of concentrated sulfuric acid.
- Isolation of the ester product can sometimes be challenging, depending on which alcohols are used. Methanol, ethanol, 1-butanol, and 1-octanol all resulted in solid ester compounds when reacted with PABA. Benzyl alcohol produced a sticky solid that was difficult to isolate by filtration.
- The procedure for the synthesis and isolation of the ester can easily be completed in one 3-hour laboratory period. Although it is also possible to determine the melting point and spectroscopy during the same laboratory period, waiting until the next period, when the products have had a chance to air dry, can result in better data.
- The spectroscopy is useful, but optional. The activity will still work if there is not enough time to gather spectroscopic data for each compound.

- Occasionally a group of students is unable to isolate their ester product. If this occurs, the students can take data (melting point, spectroscopy) and prepare an information sheet for PABA, which can also be provided to the microbiology class.

After the experiment. After the synthesis of the PABA derivatives is complete, the organic chemistry instructor will need to do the following:

- Collect the information sheets prepared by the organic chemistry students for each derivative. Determine which information sheets will be provided to the microbiology class.
- Prepare samples of each compound to distribute to the microbiology class. Each group in the microbiology class will need about 1 gram of sample. Label each vial with the derivative code. You may also want to include samples of PABA.
- Schedule time for the microbiology class to present their results to the organic chemistry class.
- (Optional) Conduct a follow-up discussion with the organic chemistry class based on the microbiology student presentations and discussion with the microbiology class. Sample questions to discuss may include the following:

    1. Which compound was predicted to be the best UV light blocker and why? According to the data from the microbiology lab classes, was the hypothesis supported? Explain.

    2. What controls were used in the microbiology portion of this experiment and why?

    3. What modifications could be made to this experiment to improve data collection or overall results?

The *para*-Aminobenzoic Acid Derivatives Lab
Organic Chemistry Student Version

This project is a collaborative project between the organic chemistry laboratory and the microbiology laboratory classes. This project introduces the synthesis of *para*-aminobenzoic acid (PABA) derivatives and the use of these compounds as ultraviolet (UV) light blockers.

Responsibilities of the organic chemistry students:

- Synthesize some derivatives of PABA
- Explain the known properties of your synthesized compound to the microbiology class by creating an information sheet about your PABA derivative
- Predict which of the synthesized compounds will be the best UV light blocker
- Discuss the results of the experiment with the microbiology students

Responsibilities of the microbiology students:

- Predict which of the synthesized compounds will be the best UV light blocker
- Design and implement an experiment to test which compound is the best UV light blocker
- Report results to the organic chemistry class
- Discuss the results of the experiment with the organic chemistry students

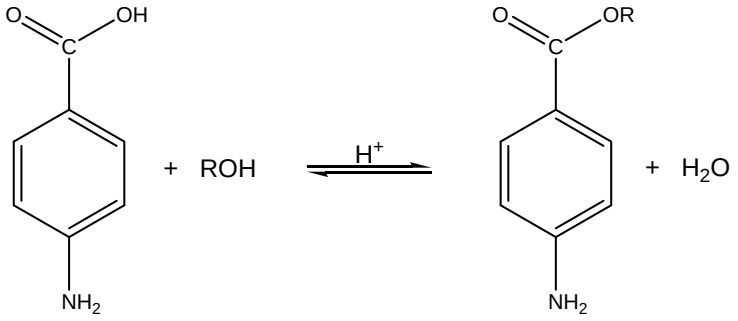
Synthesis of PABA derivatives.

Benzocaine is an ester synthesized from PABA and ethanol through a process known as Fischer esterification. It is used medicinally in a variety of forms, most notably as a local anesthetic and a topical pain reliever, and research has indicated special properties of benzocaine for use in ultraviolet light blockage. PABA is a carboxylic acid that can also block UV light. It has been used in sunscreens in the past. It is not commonly found in sunscreens today due to its tendency to cause allergic reactions.

In this laboratory experiment, we will be synthesizing benzocaine and a series of additional PABA derivatives. Then, we will take these compounds to the microbiology lab, where the microbiology class will examine the UV-blocking properties of your esters by measuring the effects of UV light on bacterial growth with and without the esters present.

Reaction.

In this experiment, a procedure is given for the preparation of an ester by direct esterification of *p*-aminobenzoic acid with an alcohol (ROH), according to the reaction below. When ROH is ethanol, the product of this reaction is benzocaine.



Procedure.

1. Each group will be assigned an alcohol for this experiment. Please see your instructor for your assigned alcohol.
2. Place 1.2 g of PABA and 12 ml of your assigned alcohol in a 100-ml round bottom flask. Swirl the mixture until the solid dissolves completely.
3. While gently swirling, add 1.0 ml of concentrated sulfuric acid dropwise. A large amount of solid may form as you add the sulfuric acid; this will dissolve as you reflux in the next step.
4. Add a few boiling stones to the flask, attach a reflux condenser, and heat the mixture at a gentle reflux for 60 to 75 minutes. During this time you should occasionally swirl the reaction mixture.
5. At the end of the reflux period, remove the reaction mixture and allow it to cool for several minutes.
6. Transfer the contents of the round-bottom flask to a beaker containing 30 ml of water.
7. When the liquid has cooled to room temperature, add about 10 ml of 10% sodium carbonate solution dropwise to neutralize the reaction mixture. Stir frequently as you add the sodium carbonate solution. Extensive gas evolution (frothing) will occur until the solution is nearly neutralized. You should also observe precipitation of the ester product as a white or off-white solid.
8. When gas no longer evolves as you add sodium carbonate, check the pH of the mixture. Continue adding sodium carbonate until the pH is about 8.
9. Collect the ester by vacuum filtration. Wash the solid (in the funnel) with a small portion of cold water. Allow air to pull through the funnel to dry your product.
10. Weigh your product.
11. Determine the melting point of your product.
12. Obtain spectroscopic data for your product.
13. Submit your product to the instructor.

PABA derivative information sheet.

For this assignment you need to prepare a handout to be given to the microbiology class along with a sample of your derivative. In this handout, explain to the microbiology class what you need them to accomplish. Be creative. In the handout, you should include the following:

- Derivative code (A, B, C, D, or E)
- Name and structure of compound
- Reaction scheme showing the synthesis of this compound (include important details, such as yield)
- Properties of the compound that you observed (e.g., melting point, solubility, appearance)
- Available spectroscopic data (infrared, nuclear magnetic resonance, mass spectrometry) that you obtained
- Other information you think is appropriate and relevant

The *para*-Aminobenzoic Acid Derivatives Lab
Microbiology Student Version

This project is a collaborative project between the organic chemistry laboratory and the microbiology laboratory classes. This project will introduce you to the synthesis of *para*-aminobenzoic acid (PABA) derivatives and the use of these compounds as ultra violet (UV) blockers.

Responsibilities of the organic chemistry students:

- Synthesize some derivatives of PABA
- Explain the known properties of your synthesized compound to the microbiology class by creating an information sheet about your PABA derivative
- Predict which of the synthesized compounds will be the best UV blocker
- Discuss the results of the experiment with the microbiology students

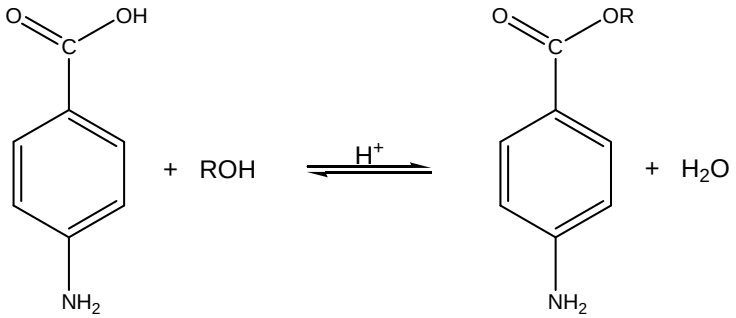
Responsibilities of the microbiology students:

- Predict which of the synthesized compounds will be the best UV blocker
- Design and implement an experiment to test which compound is the best UV blocker
- Report results to the organic chemistry class
- Compare actual results to predicted results
- Discuss the results of the experiment with the organic students

Synthesis of PABA Derivatives:

Benzocaine is an ester synthesized from PABA and ethanol through a process known as Fischer esterification. It is used medicinally in a variety of forms, most notably as a local anesthetic and a topical pain reliever, and research has indicated special properties of benzocaine for use in ultraviolet light blockage. PABA is a carboxylic acid that can also block UV light. It has been used in sunscreens in the past. It is not commonly found in sunscreens today due to its tendency to cause allergic reactions.

The organic chemistry class will synthesize several PABA derivatives, including benzocaine, using a procedure for the preparation of an ester by direct esterification of *p*-aminobenzoic acid with an alcohol (ROH), according to the reaction below. When ROH is ethanol, the product of this reaction is benzocaine. By changing the ROH in the reaction different derivatives will be produced.



Once the organic chemistry students synthesize the PABA derivatives they will test some of the physical properties and provide us with the compounds and information sheets about the compounds (including their structure). You will be expected to make predictions and develop a hypothesis about which derivative can block UV light the best based on the structure of each derivative. Then you will work with your lab group and the entire class to design an experiment to

test your hypothesis. After you have completed the experiment you will present and discuss your results.
Names of group members:_____

During the first lab period you will receive information sheets about each PABA derivative. You will meet with your group to discuss and predict which compound would theoretically block the most UV light. You will then come up with an experimental design to test your hypothesis.

Consider the following questions while designing your experiment:

1. What properties of an organic compound determine its ability to block UV light and why would one compound block UV better than another?

2. Predict which compound would best block UV light and develop a hypothesis. Explain your predictions.

3. Describe your controls and how will you set them up.

4. Explain why each control is important to the experiment.

5. How many replicates will you consider and why?

6. Look at the basic protocol on the next page. Based on the protocol and on your experimental plan, how many TSA plates are you going to need for your group?

Once your group comes up with a hypothesis we will have a class discussion and choose one hypothesis to test as a class. Each group will be assigned a different compound to assay and we will collaborate as a class to select which controls would be appropriate. While each individual group will be responsible for a derivative we will pool the class data so that you can analyze the data for all of the derivatives.

Day one protocol:

1. Obtain enough TSA plates to complete your experiment (remember your controls).
2. Obtain the following:
    a. 1 *Serratia marcescens* broth culture
    b. pipettes for dispensing 0.1 ml (1 per each plate you obtained above)
    c. pipettes for dispensing 3 ml (1 per group)
    d. plastic wrap (1 for each plate)
    e. Mineral oil or petroleum jelly (approximately 10 ml)
    f. Sterile swabs (1 for each plate)
    g. PABA derivative (weigh out 1 g)
    h. Spread plating materials (hockey stick plate spreaders, 95% ethanol, glass Petri dish with lid)
    i. UV light.
    j. Empty Petri dish
3. Pour your PABA derivative sample into an empty Petri dish. Add 3 ml of oil and stir your compound to make a creamy paste that has a uniform consistency.
4. Pipette 0.1 ml of *S. marcescens* culture onto each plate and use the spread-plate technique to aseptically prepare your plate cultures. Make sure that you completely cover the surface of the plate.
5. Put a piece of plastic wrap around each plate. If you choose to leave the lid on your culture (as a control) then simply add the plastic wrap around the plate with the lid. If you are planning to expose the plate to the UV then remove the lid before wrapping in plastic wrap. *Remember: UV cannot penetrate the lid of the Petri dish.*
6. Add your compound mixture to the plastic wrap using a swab. Apply it liberally. Try to be consistent with how much you apply to each plate as appropriate (some of your controls may not need the compound).
7. Place your plates underneath the UV light (about 5 inches above) for 3 minutes.
8. Remove plastic wrap and return lids.
9. Incubate at 25 degrees C or room temperature for 48 hours

Day two protocol:

1. Remove plates from incubator.
2. Record growth in the data collection chart provided to you. Score the growth on your plates using the numbers 0-3, where 0 is no growth and 3 is abundant growth. Provide comments about any strange things you notice on your plates (i.e. contamination, strange growth patterns, etc.). *Remember: S. marcescens is pink. If you see any colonies that are not pink you may have contaminants.*
3. Discuss your results with your group. Did the UV light penetrate your compound? How did your experimental plates compare to your controls? What could you do differently to improve this experiment?
4. When finished provide your data chart to you instructor so that he/she can record your data in collective data chart for the class.

Name of your Compound:_____

Group members:_____

Table 1. Group Data

Experimental plates	Growth (0-3)	Comments
1		
2		
3		
Average		
Control Plates (No UV)		
1		
2		
3		
Average		
Control Plates (UV, no compound)		
1		
2		
3		
Average		

5. Once your instructor has provided you with the collective data from the class, work with your group members to analyze the data. Create graphs and/or tables that represent a summary of the data. You will be asked to present your results and conclusions from your experiment and from the collective data from the class. Consider using a statistical test or error bars when analyzing your data.
6. Consider the following questions in your data analysis and group discussions:
    a. Was your original hypothesis supported? Explain.
    b. Was your data consistent between replicates? Was your experiment reproducible? If it was not, then provide an explanation.
    c. Did your results differ from other groups who had the same compound? Explain.
    d. What conclusions can you draw from your controls? Were there any additional controls that you should have done? Explain.
    e. What modifications could be made to this procedure?
    f. Based on the collective results from the class was there one compound that clearly blocked the most UV light? If so, provide an explanation for this phenomenon.

The *para*-Aminobenzoic Acid Derivatives Lab

This project introduces *para*-aminobenzoic acid (PABA) derivatives and the use of these compounds as ultraviolet (UV) light blockers.

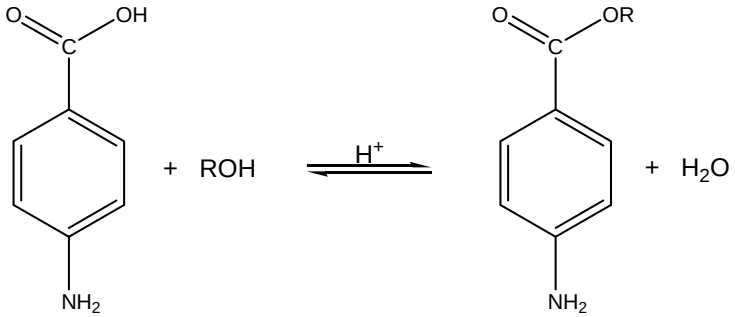
Responsibilities.

- From a selection of various PABA derivatives, predict which one will be the best UV light blocker based on its structure.
- Design and implement an experiment to test which compound is the best UV light blocker.
- Report results to the class.
- Discuss the results of the experiment with the class

Synthesis of PABA derivatives.

Benzocaine is an ester synthesized from PABA and ethanol through a process known as Fischer esterification. It is used medicinally in a variety of forms, most notably as a local anesthetic and a topical pain reliever, and research has indicated special properties of benzocaine for use in ultraviolet light blockage. PABA is a carboxylic acid that can also block UV light. It has been used in sunscreens in the past. It is not commonly found in sunscreens today due to its tendency to cause allergic reactions.

Your instructor will introduce you to several PABA derivatives, including benzocaine. These derivatives were synthesized using a procedure for the preparation of an ester by direct esterification of PABA with an alcohol (ROH), according to the reaction below. When ROH is ethanol, the product of this reaction is benzocaine. By changing the ROH in the reaction, different derivatives will be produced.



You will make predictions and develop a hypothesis about which derivative can block UV light the best based on the structure. Then you will work with your lab group and the entire class to design an experiment to test your hypothesis. After you have completed the experiment, you will present and discuss your results.

Names of group members:_____

During the first lab period you will receive information sheets about each PABA derivative. Meet with your group to discuss and predict which compound would theoretically block the most UV light. Then come up with an experimental design to test your hypothesis.

Consider the following questions while designing your experiment:

1. What properties of an organic compound determine its ability to block UV light and why would one compound block UV light better than another?

2. Predict which compound would best block UV light and develop a hypothesis. Explain your predictions.

3. Describe your controls and how will you set them up.

4. Explain why each control is important to the experiment.

5. How many replicates will you consider and why?

6. Look at the basic protocol on the next page. Based on the protocol and on your experimental plan, how many tryptic soy agar plates are you going to need for your group?

Once your group comes up with a hypothesis, we will have a class discussion and choose one hypothesis to test as a class. Each group will be assigned a different compound to assay, and we will collaborate as a class to select which controls would be appropriate. While each individual group will be responsible for a derivative, we will pool the class data so that you can analyze the data for all of the derivatives.

Day one protocol:

1. Obtain enough tryptic soy agar plates to complete your experiment (remember your controls).
2. Obtain the following:
    a. 1 *Serratia marcescens* culture (broth)
    b. Pipettes for dispensing 0.1 ml (1 per each plate you obtained above)
    c. Pipettes for dispensing 3 ml (1 per group)
    d. Plastic wrap (1 for each plate)
    e. Mineral oil or petroleum jelly (approximately 10 ml)
    f. Sterile swabs (1 for each plate)
    g. PABA derivative (weigh out 1 g)
    h. Spread-plating materials (hockey stick plate spreaders, 95% ethanol, glass petri dish with lid)
    i. UV light
    j. Empty petri dish
3. Pour your PABA derivative sample into an empty petri dish. Add 3 ml of oil and stir to make a creamy paste that has a uniform consistency.
4. Pipette 0.1 ml of *S. marcescens* culture onto each plate and use the spread-plate technique to aseptically prepare plate cultures. Make sure to completely cover the surface of the plate.
5. Put a piece of plastic wrap around each plate. If you choose to leave the lid on your culture (as a control) then simply add the plastic wrap around the plate with the lid. If you are planning to expose the plate to the UV light, then remove the lid before wrapping in plastic wrap. Remember: UV light cannot penetrate the lid of the petri dish.
6. Add your compound mixture to the plastic wrap using a swab. Apply it liberally. Try to be consistent with how much you apply to each plate, as appropriate (some of your controls may not need the compound).
7. Place your plates underneath the UV light for 3 minutes (have the light set about 5 inches above the plates).
8. Remove plastic wrap and return lids.
9. Incubate at 25ºC or room temperature for 48 hours.

Day two protocol:

1. Remove plates from incubator.
2. Record growth in the data collection chart provided to you. Score the growth on your plates using the numbers 0 to 3, where 0 is no growth and 3 is abundant growth. Provide comments about any strange things you notice on your plates (i.e., contamination, strange growth patterns, etc.). Remember: *S. marcescens* is pink. If you see any colonies that are not pink, you may have contaminants.
3. Discuss results with your group. Did the UV light penetrate your compound? How did experimental plates compare to controls? What could you do differently to improve this experiment?
4. When finished, provide your data chart to the instructor so your data can be recorded in a collective data chart for the class.

Name of your compound:_____

Group members:_____

TABLE 1. Group data

Experimental plates	Growth (0-3)	Comments
1		
2		
3		
Average		
Control plates (no UV)		
1		
2		
3		
Average		
Control plates (UV, no compound)		
1		
2		
3		
Average		

5. Once your instructor has provided the collective class data, work with your group members to analyze the data. Create graphs and/or tables that represent a summary of the data. You will be asked to present results and conclusions from your experiment and from the collective class data. Consider using a statistical test or error bars when analyzing your data.
6. Consider the following questions in your data analysis and group discussions:
    a. Was your original hypothesis supported? Explain.
    b. Was your data consistent between replicates?
    c. Was your experiment reproducible? If it was not, then provide an explanation.
    d. Did your results differ from other groups who had the same compound? Explain.
    e. What conclusions can you draw from your controls? Were there any additional controls that you should have used? Explain.
    f. What modifications could be made to this procedure?
    g. Based on the collective results from the class, was there one compound that clearly blocked the most UV light? If so, provide an explanation for this phenomenon.

CHEM 2412L/BIOL 3250L
Clower/Furlong/McFarlane
Benzocaine project
PostQuiz

Participant CodeName __

11/12

This Post quiz is not for a grade. This is simply to assess your knowledge after you have completed the benzocaine project.

1. Wavelengths of the UV region in the electromagnetic spectrum are:
   a. 200 – 400 nm
   b. ~~400 – 800 nm~~
   c. 2.5 – 25 μm
   d. 30 – 900 MHz

2. Fill in both blanks about the reaction involved in producing benzocaine derivatives:

   a. A carboxylic acid + a(n) alcohol yields an ester in the presence of a(n) acid catalyst.

3. Which compound would theoretically absorb more UV and why?

   D becaose it has 2 aromatic conjugated rings

4. Give two reasons why *Serratia marcesscens* was a good model organism to test the affects of UV light. because they don't produce spores

5. UV obviously kills bacteria when they are exposed. What specific effect does UV light have on cells that leads to their demise?
   a. It causes free radicals and/or superoxide anions to form in the cells, which damages cellular macromolecules and causes cell death
   b. It causes thymine dimers to form in the DNA, which causes mutations and possible cell death
   c. It causes the cell walls to disintegrate, which results in cell death
   d. It causes peptide bonds to break, which results in protein denaturation and cell death.

6. If you had the opportunity to redo the experiment briefly describe one thing you would do differently.

   devide compound up evenly b/w group

CHEM 2412L/BIOL 3250L
Clower/Furlong/McFarlane
Benzocaine project
PostQuiz

Participant CodeName _____

7. You wish to run an experiment to test the effectiveness of a sunscreen on cells. You set up your experiment by applying the sunscreen to the cells and exposing the cells to UV light. The effects of the UV light on the cells are then observed. Describe all of the appropriate controls that you would have to run with this experiment to make certain that the results are valid.

run cells w/ no sunscreen & epose to U.V
run cells w/ no sunscreen & no U.V

8. Benzocaine is a(n) ~~dentistic~~ ester and PABA is a(n)

carboxcylic acid.

24/30

SA Company

142 South Main Street
Nashville TN 499529 USA

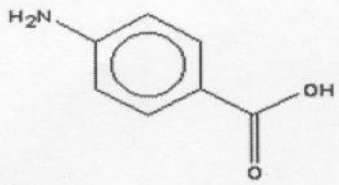
phone 1 (615) 555-1432
fax 1 (615) 555-1433

March 24, 2008

To whom it might concern,

Please analyze the PABA and compare its effectiveness in blocking UV light to all other products available in the market.

Below are the information regarding PABA.

CHEMICAL NAME	p-Aminobenzoic acid, Aniline-4-carboxylic acid, *p*-Carboxyaniline.
FORMULA	$C_7H_7NO_2$
STRUCTURE	
MOLECULAR WEIGHT	137.1378
MELTING POINT	189°C
DENSITY	1.374
SOLUBILITY IN WATER	Slight soluble
SOLVENT SOLUBILITY	Insoluble: Chloroform, Petroleum ether, Benzene. Soluble: Ethyl acetate, acetic acid, Ether.
pH	3.5
NFPA RATINGS	Health: 1; Flammability: 1; Reactivity: 0
STABILITY	Stable under ordinary conditions
APPEARANCE	White crystalline powder
PURITY	99.5% min
WATER	0.2% max
ASH	0.1% max
HEAVY METALS	20ppm max
DESCRIPTION	Light sensitive. Discolors on exposure to air or light.
USED	Common ingredient in sunscreen

Sincerely,

TDA
Project Manager

GLOBAL TECH INC.

YOUR #1 SOUCRCE FOR ALL YOUR NEEDS !

Benzocaine Derivative Experiment

April 17, 2008

Performed By:

2000 Clayton State Blvd. Morrow, GA 30260 770-555-3000

Introduction

The purpose of this experiment was to determine which one of the five derivatives was an effective UV blocker. We performed our experiment on novel compounds, which were provided to us by the chemistry department of our company. We were asked to analyze the 5 different compounds, namely PABA, Benzocaine, n-Octyl p-aminobenzoate, Benzyl alcohol and Butyl- PABA to determine their effectiveness at blocking UV light for a new sunscreen. 4-Aminobenzoic acid (also known as *para*-aminobenzoic acid or PABA) is an organic compound with the molecular formula $C_7H_7NO_2$. PABA, a white crystalline substance that is slightly soluble in water, consisting of a benzene ring substituted with an amino group and a carboxylic acid has been in our markets for several years now. We designed an experiment with controls to help us determine which compound is more effective.

Hypothesis

We hypothesized that due to the structures of the compounds, compound D would be best at blocking UV light due to the presence of 2 benzene rings.

Materials and Methods

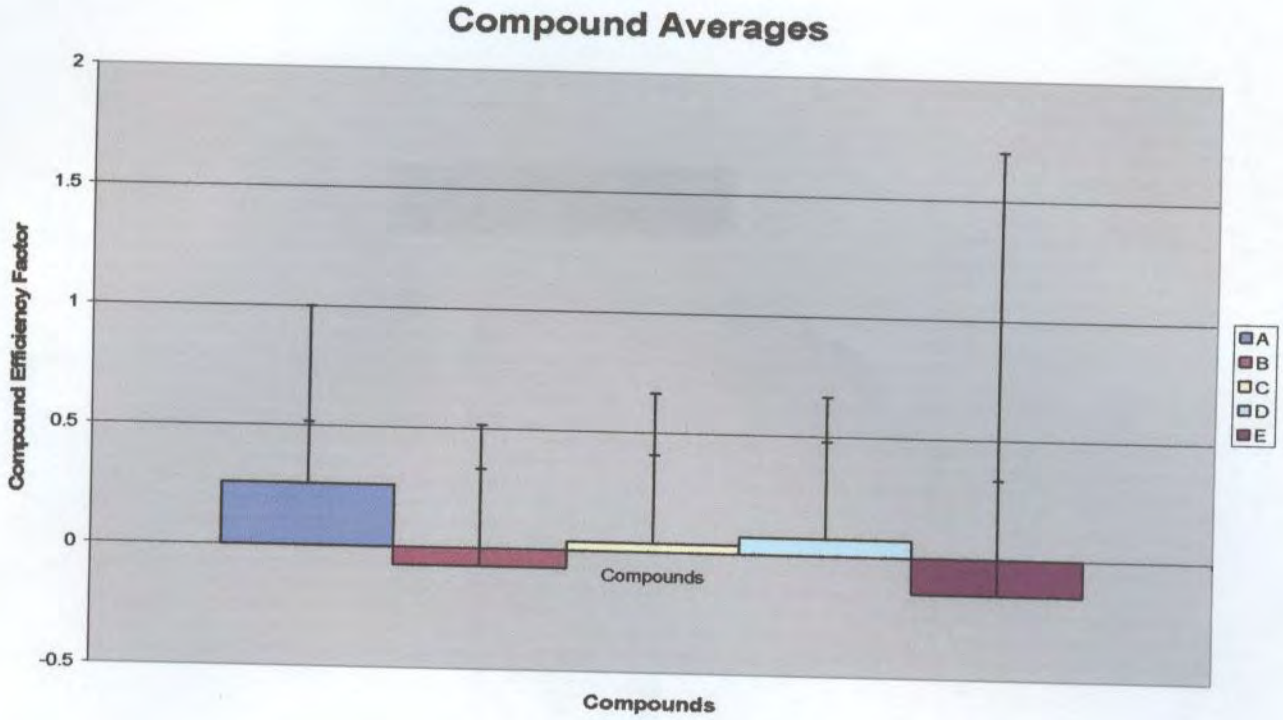
Nutrient Agar Plates	A vial of mineral oil
Petri dish's	Sterile cotton swabs
Serratia Marcescens (SM) Culture	Samples of Benzocaine Derivates (A-E)
Pipette (1 mL)	UV light source
Automatic pipettor	Incubator at 25° C

We used 4 Nutrient Agar Plates and applied SM bacteria to the entire surface of the agar. We labeled each plate with the respective controls that were applied to it. The

controls we used were one with No UV, the second with UV only, the third with saran wrap and mineral oil and UV, and lastly the experimental plate, which contains bacteria, saran wrap and a mixture of mineral oil (approximately 2 mL) and the different compounds. These were then placed in the incubator for 48 hours.

Results

After carrying out our experiment and taking the plates from incubator, we analyzed the growth on each plate using our scale as 0 for no growth, +1 for some growth, +2 for more growth, and +3 for the highest amount of growth we had. We then compiled our data (amount of growth) from the several replicates that were ran and compiled into a spreadsheet of "Raw Data" and then removed the control that we deemed unnecessary, the control with the oil and wrap only, because we had assumed that the UV light would not be able to penetrate it anyways. We then compiled our new averages into a spreadsheet called "Normalized Data" and made a graph to compare the average growth for each compound and its ability to absorb UV radiation. A standard deviation for each compound was included in the graph to help compare compounds. According to the graph, the plate with compound A had the highest growth, then compound D, followed by C, then B and lastly E, which had the lowest growth when the derivative was applied.



Discussion:

UV radiation causes DNA to form thymine dimers which in humans, results in skin cancer. Due to the high risk of using humans as test subjects, we used a form of bacteria called *Serratia Marcescens*. This particular bacterium was used because it is easier and faster to experiment on. Also, it has shorter generation time which helps us get quicker results as opposed to humans that would take a long time for the effects to show up. In addition to this, its distinct color, pink, helps us easily identify whether UV had an influence on its growth or not.

On performing the experiment and analyzing our results, we found that our results refuted our hypothesis, which stated that compound D would work the best. However compound D did work well compared to the other compounds. This discrepancy may have been due to the fact that compound A was in fact PABA in its purest form. PABA

is an active ingredient in sunscreens and is known to absorb UV radiation very well, as we can see in the above bar graph. By looking at the graph, we can tell that compound D was the next best option after compound A to act as a sunscreen.

Other discrepancies that may have caused the fluctuations in the data are unevenly applied compound paste to the saran wrap, also, the distance between the UV light and the plate itself, and the bacteria may not have been spread out evenly in all cases. Also, the compounds did not properly dissolve into the mineral oil, and therefore, when applied to the saran wrap the compound may not have been on the entire surface and may have absorbed UV light at some spots, and not at others, killing the bacteria. The derivative and oil combination could also have collected at the center of the saran wrap due to the weight, which may have caused more growth at the center, where the compound was, hence killing off the bacteria around the edges. The compounds that we received from our chemistry department contained impurities, which then, compared to the pure PABA, created a larger deviation.

For future experiments, we should consider purifying the compounds as much as possible, and running more replicates of the experiment. Other things we could do in order to get better results could be, using pure samples of benzocaine derivatives, and avoiding unnecessary controls, which allow us to do more replicates for the controls that actually do count towards the experiment. The actual experiment and the control which consisted of the oil, saran wrap and UV could have had better results if we used consistent amounts of UV on the samples and distances of UV light from the plates. Since this experiment was done by different students, their techniques of streaking would be different too. This creates a lot of room for error.

Comparison of the Sun-Screening Capabilities of *Para*-amino benzoic acid (PABA) with some of its novel derivatives

A COMPLETE REPORT COMPILED BY

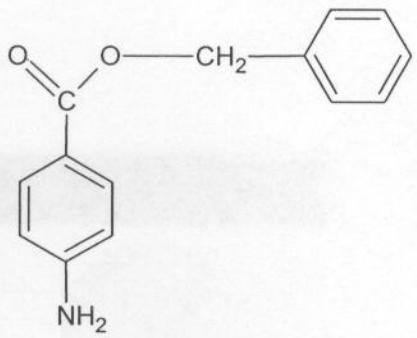
Comparison of the Sun-Screening Capabilities of *Para*-amino benzoic acid (PABA) with some of its novel derivatives

A COMPLETE REPORT COMPILED BY

INTRODUCTION

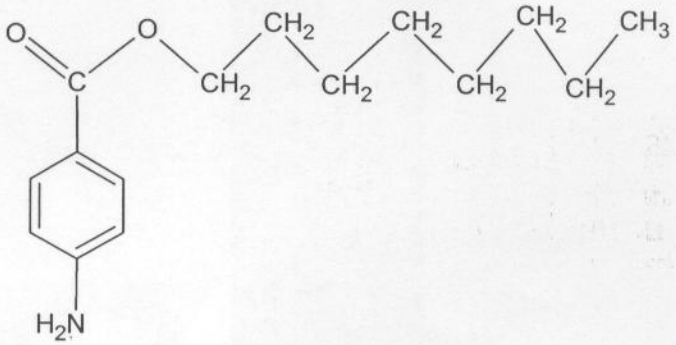
Our Company, NusoPro®, was commissioned by the United States Department of Defense to examine and compare the sun-screening capabilities of five compounds: *para*-aminobenzoic acid (PABA) and four novel derivatives of PABA (ethyl *para*-aminobenzoate (or Benzocaine), n-octyl *para*-aminobenzoate, benzyl *para*-aminobenzoate and n-butyl *para*-aminobenzoate (labelled as Comound A, B, C, D and E respectively). The Defense Department would like to develop a compound which is better than PABA, since the decreasing ozone layer is causing more ultraviolet radiation to enter earth's atomosphere. This in turn is causing the incidence of skin cancer to rise, especially among soldiers. We examined the sun-screening capabilities of PABA and the four PABA derivatives using microbiology techniques. Our experimental procedure was based on the fact that ultraviolet radiation interferes with DNA replication. Extended exposure can lead to cell death, by causing the formation of pyrimidine dimmers. We used this characteristic of UV radiation to test the effectiveness of the various compounds. If there were bacterial growth, then the compound was successful at blocking UV radiation. If there were no bacterial growth, then the compound was not successful at blocking the UV radiation. We list below the *IUPAC* names and structures of the given compounds, based on the order in which we hypothesize them to be effective in absorbing UV light:

Compound D



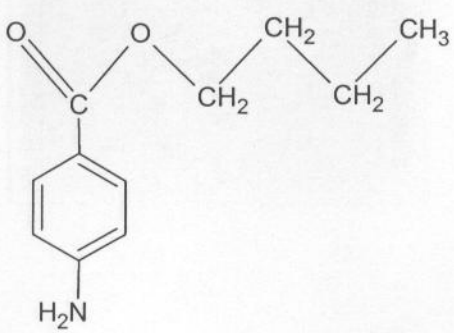
benzyl *para*-aminobenzoate

Compound C



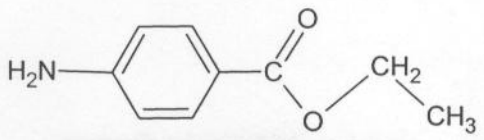
n-octyl *para*-aminobenzoate

Compound E



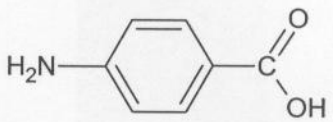
n-butyl *para*-aminobenzoate

Compound B



ethyl *para*-aminobenzoate (Benzocaine)

Compound A



para-aminobenzoic acid (PABA)

OUR HYPOTHESIS

We proceeded by looking at the various structures as shown above and hypothesized that compound D will likely have the most beneficial effect because it has a more conjugated structure than the others, because it has two benzene rings. Our reasoning was predicated on the fact that the more conjugated a compound is, the better its ability to block UV radiation. Other factors we looked at were chain lengths and various levels of saturation. Before beginning our experiment, we ranked the compounds in the following order: D, C, E, B, and A, with D being the most effective and A the least.

MATERIALS AND METHODS

To carry out the experimental process, one group (three students) used the following materials: 1 *Serratia marcescens* culture, 1 P-100 pipettor, a box of yellow tips, 1 1-ml pipettor and a pi-pump, 1 empty Petri dish, Compound C (1 of the 5 benzocaine derivatives assigned),

Petri plates containing nutrient agar (12), cotton swabs, and a tube of mineral oil. First, the compound that was given was poured into an empty Petri dish and then 3-ml of mineral oil was added into the dish using a 1-ml pipettor. Next, the oil and the compound were mixed using the stick end of the cotton swab so that it had a uniform consistency on the plate. The sunscreen is now prepared to be tested on the bacteria. Three of the plates will be control groups: No UV exposed, only UV exposed, and oil and saran wrap exposed to UV. Pipette 100-ul of *Serratia marcescens* culture on to each plate. Then the culture was smeared so that the surface of the nutrient agar plate was completely covered using a sterile cotton swab. Next, for the control group with no UV exposed, the plate was incubated at 25°C. For the other control groups, a piece of saran wrap was placed on top of each plate. For the control group with UV exposed, the plate was placed under UV light for 2 minutes. For the control group with oil and saran wrap exposed to UV, 1-ml of mineral oil was added on top of the saran wrap and smeared using a cotton swab. The plate was then exposed to UV light for 2 minutes. Next the sunscreen was placed on the saran wrap and exposed to UV light for 2 minutes. After all control groups and experimental plates were exposed to UV light, the plastic wraps were removed and the lids were placed back onto the plate. Then the plates were incubated at 25°C for 48 hours.

DATA AND RESULTS

Normalized Data. Calculated by subtracting the value for the oil/wrap control from experimental value and determining the average from the 3 experimentals /group.

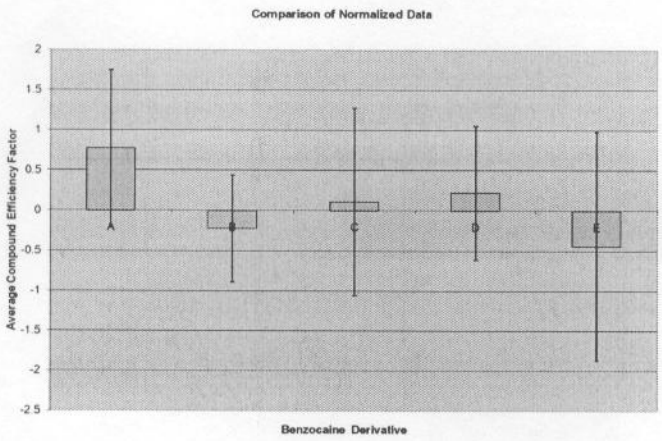
Abbreviations:		
	Exp =	Experiment
	Obs =	Observed
	Val =	Value
	Avg =	Average, Averages
	Comp =	Compound

Expect. = Expected

SD = Standard Deviation

Benz. Deriv's = Benzocaine Derivatives

Benz. Deriv's	Exp. Runs	Exp. 1 (Obs. Value)	Exp. 2 (Obs. Value)	Exp. 3 (Obs. Value)	Avg	Comp. Avg's (EXPECT. VALUE)	Comp. SD's	Benz. Deriv's	T-Test Trials	T-Test Calculations p < 0.05
A	G1A	0	2	1	1.0000			A	A-B =	0.0216
	G2A	1	1	2	1.3333	**0.7778**	0.9718		A-C =	0.2063
	G3A	-1	0	1	0.0000				A-D =	0.2114
									A-E =	0.0493
B	G4B	1	0	0	0.3333			B	B-C =	0.4675
	G5B	0	0	-1	-0.3333	**-0.2222**	0.6667		B-D =	0.0856
	G6B	0	-1	-1	-0.6667				B-E =	0.6772
C	G7C	0	0	0	0.0000			C	C-D =	0.8191
	G8C	0	1	1	0.6667	0.1111	1.1667		C-E =	0.3787
	G9C	-1	2	-2	-0.3333					
D	G10D	-1	1	1	0.3333			D	D-E =	0.2430
	G11D	1	1	0	0.6667	0.2222	0.8333			
	G12D	0	0	-1	-0.3333					
E	G13E	-3	-1	-2	-2.0000			E	E-D =	0.2430
	G14E	1	-1	1	0.3333	-0.4444	1.4240			
	G15E	0	1	0	0.3333					



DISCUSSION

According to the graph, compound A performed better at absorbing UV radiation in this experiment than D, C, B, and E in that order. We were told after finishing the experiment, that compound A was in its pure form. All the other compounds had not been purified. We, therefore, cannot conclude that compound A is the best sun-screening agent in this experiment. As in all experiments, there are always experimental errors. One type of error was that the concentration of compound B, C, D, and E are not the same as compound A when we mixed it in oil to create the sunscreen because they were impure derivatives of compound A, which were made by the Organic Chemistry class. Also, as the compounds were mixed into oil and spread onto the saran wrap by different groups, we must assume that the amount of sunscreen varied by each trial. Our hypothesized product D, might have been a better sunscreen if we compared it with compound B, C, and E. Another experimental error in this experiment is that some of the compounds were not completely soluble in the oil during the experimental process carried out by

different groups. In summary, compound D may have been more effective than shown, but we would need to redesign the experiment to decrease the random and systematic errors in order to show this.

PABA Derivatives

Student Presentations

INTRODUCTION

- UV light is harmful to most bacteria.
- There are three different types of UV: UVA, UVB, and UVC:
  - Wavelength varies in the range of 100-400nm.
  - UVA - wavelength 320-400nm
  - UVB - wavelengths 290-320nm
  - UVC - wavelength 100-290nm.
- The shorter the wavelength, the more penetrating it has on bacteria and therefore the more harmful it is.

Why UV is harmful?

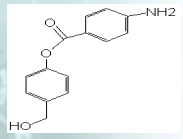
- It caused mutation in bacteria by creating pyrimidine dimers by forming extra bond between adjacent pyrimidine bases in DNA.
- It specifically targets thymine in DNA which can lead to cell death.
- The UV light from the sun can be harmful to human skin.
- The purpose of this experiment is to determine the ability to block UV light of the compounds supplied by different firms.
- In this experiment, different compounds are used to test for its ability in blocking UV light from penetrating bacteria.

Hypothesis

- After carefully reviewing all of the derivatives from the various companies, the derivative formulated by BIOTECH Inc. seems to be most efficient compound for blocking UV light.

Hypothesis

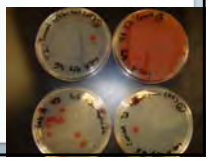
- The PABA derivative “D” will provide the best protection against UV light, because its two benzene rings will absorb the most UV light.



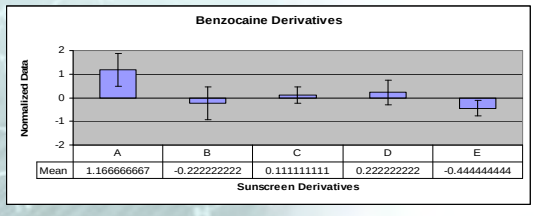
Chemicals drawn using ChemDraw Software

Photo Credit: Michelle Furlong

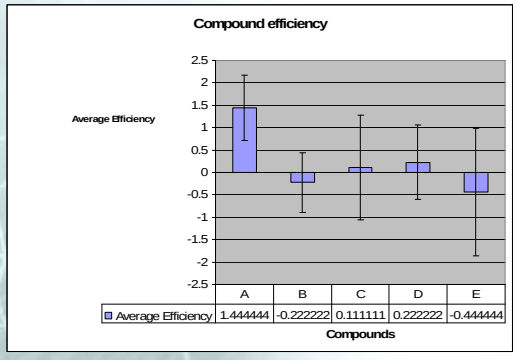
12 TSA Plates
1 P-100 pipettor w/Box of yellow tips
1 ml pipettor w/pi pump
Several Cotton Swabs
1 empty Petri dish
Compound A-D
Tube of mineral oil
Saran wrap
UV light
25 degree incubator



Graph Data



Data Analysis



Discussion

- Hypothesis
  - D>C>E>B>A
- Actual Results
  - A>D>C>B>E
- Hypothesis Rejected

- Effectiveness of Controls
- UV, no oil/wrap
  - Cell death occurred
- UV, Oil/wrap
  - Cell death occurred
- No UV, no oil/wrap
  - No death, high growth

Possible Error

- Purity of compounds is unknown
- Concentration of Compound (g compound/ml oil) between groups was disregarded
- Application thickness of compound was disregarded
- Results could have been affected by these errors.

Methodology: The Alternative Approach

Achieving experimental efficiency with greater reliability

- Standard Scale
- Profitable controls
- Uniform techniques
- Purification of derivatives
- Amount and application of UV light

Notes

1. 1) 1-20 2) 21-60 3) >60
2. Control 3. apply saran wrap. Then remove it. Served no purpose. Should just grow colony normal.
3. Same means of application of mixture, streaking technique, and consistency.
4. Same compound from different exp? Diff. exp. Conditions and possibly did procedures wrong. Sets of data per compound aren't comparable due to this.
5. Exact amount of wattage UV light, distance from specimen, type of light source (lamp, handheld)

TABLE 4. Normalized data. Calculated by subtracting the value for the oil-wrap control from experimental value and determining the average from the three experimental

Derivative	Exp 1	Exp 2	Exp 3	Average	Compound averages	Compound Standard Deviations	
G1A	0	2	1	1			
G2A	1	1	2	1.333333333			A
G3A	0	0	0	0			
G4B	1	0	0	0.333333333			
G5B	0	0	-1	-0.333333333			B
G6B	0	-1	-1	-0.666666667			
G7C	0	0	0	0			
G8C	0	1	1	0.666666667			C
G9C	-1	2	-2	-0.333333333			
G10D	-1	1	1	0.333333333			
G11D	1	1	0	0.666666667			D
G12D	0	0	-1	-0.333333333			
G13E	-3	-1	-2	-2			
G14E	1	-1	1	0.333333333			D
G15E	0	1	0	0.333333333			

TABLE 1. Experimental raw data. Each number represents the level of growth on experimental plates. In most cases each group ran three replicates. Group 3A is missing; Groups 4B, 6B, 11D, and 13E only had two replicates that were reliable.

	Growth			
Derivative	Exp 1	Exp 2	Exp 3	Average
G1A	1	3	2	2
G2A	2	2	3	2.333333333
G3A				#DIV/0!
G4B	2	1		1.5
G5B	1	1	1	1
G6B	2	1		1.5
G7C	1	1	1	1
G8C	1	2	3	2
G9C	2	3	1	2
G10D		2	2	2
G11D	2	2		2
G12D	2	1	1	1.333333333
G13E		2	1	1.5
G14E	1	0	1	0.666666667
G15E	1	3	2	2

Missing data from afternoon class

Discussion questions pertaining to data above.

By looking at the raw data can you point to one compound that appears to block the most UV light? Explain.

Would it be appropriate to average different groups averages for their experiments? Why or why not? How can the data be normalized?

TABLE 2. Average control data for all three controls

Derivative	Control		
	UV only	UV wrap and oil	No UV
G1A	1.33	1	2.33
G2A	3	1	3
G3A			
G4B	1	1	3
G5B	2	1.333333333	3
G6B	0.33	1.666666667	3
G7C	1	1	3
G8C	1.5	1.333333333	3
G9C	1.6	2.333333333	3
G10D	1.5	1	3
G11D	1.3	1	3
G12D	2.3	1.666666667	3
G13E	2.33	3	3
G14E	1	0.333333333	3
G15E	2.33	1.666666667	3
Average	1.608571429	1.380952381	2.952142857

Discussion questions for the above data

Did the controls work as expected? Explain.

Did the combination of wrap and oil affect the cells' UV light exposure? Explain.

How did the controls help you in determining a number value for your experimentals?

TABLE 3. Raw data for control with oil and wrap

Derivative	Growth		
	Exp 1	Exp 2	Exp 3
G1A	1	1	1
G2A	1	1	1
G3A			
G4B	1	1	
G5B	1	1	2
G6B	2	2	1
G7C	1	1	1
G8C	1	1	2
G9C	3	1	3
G10D	1	1	1
G11D	1	1	
G12D	2	1	2
G13E	3	3	3
G14E	0	1	0
G15E	1	2	2

Evaluation: Lab report

Writer: _____

Section Points		Poor F 50	D 61	C 75	B 87	Excellent A 100	Section scores
10	**Title**						0.00
	Describes lab content concisely, adequately, appropriately						
5	**Abstract**						0.00
	Conveys a sense of the full report concisely and effectively						
10	**Introduction**						0.00
	Effectively defines the research problem and states the research question						
	Successfully establishes the scientific concept of the lab						
	States hypothesis and provides logical reasoning for it						
15	**Methods**						0.00
	Gives enough details to allow for replication of procedure						
15	**Results**						0.00
	Opens with effective statement of overall findings						
	Presents visuals clearly and accurately						
	Presents verbal findings clearly and with sufficient support						
	Successfully integrates verbal and visual representations						
20	**Discussion**						0.00
	Opens with effective statement of support of hypothesis						
	Backs up statement with reference to appropriate findings						
	Provides sufficient and logical explanation for the statement						
	Gives answer to the research question and solution for unknowns						
	Effectively links answer of research question to solution of problem						
	Sufficiently addresses other issues pertinent to lab						

10	**Conclusion**						0.00
	Convincingly describes what has been learned in the lab						

5	**References**						0.00
	All appropriate sources in the report are listed						
	Citations and references adhere to proper format						

5	**Presentation**						0.00
	Format of tables and figures is correct						
	Report is written in scientific style: clear and to the point						
	Grammar and spelling are correct						

5	**Overall aims of the report:** the student…						0.00
	Has successfully learned what the lab is designed to teach						
	Demonstrates clear and thoughtful scientific inquiry						
	Accurately measures and analyzes data for lab findings						

100	**Points earned**	**0.00**
	Total possible points	100
	Percentage	**0%**

Oral Presentation Rubric: PABA Derivatives Laboratory Presentation

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Teacher name: **Dr. Furlong and Professor McFarlane**

Student name: _____

Category	Points			
	4	3	2	1
Comprehension	Student is able to accurately answer almost all questions posed by classmates about the topic.	Student is able to accurately answer most questions posed by classmates about the topic.	Student is able to accurately answer a few questions posed by classmates about the topic.	Student is unable to accurately answer questions posed by classmates about the topic.
Preparedness	Student is completely prepared and has obviously rehearsed.	Student seems pretty prepared but might have needed a couple more rehearsals.	The student is somewhat prepared, but it is clear that rehearsal was lacking.	Student does not seem at all prepared to present.
Collaboration with peers	Almost always listens to, shares with, and supports the efforts of others in the group. Tries to keep people working well together.	Usually listens to, shares with, and supports the efforts of others in the group. Does not cause "waves" in the group.	Often listens to, shares with, and supports the efforts of others in the group but sometimes is not a good team member.	Rarely listens to, shares with, and supports the efforts of others in the group. Often is not a good team member.
Stays on topic	Stays on topic all (100%) of the time.	Stays on topic most (90 to 99%) of the time.	Stays on topic some (75 to 89%) of the time.	It was hard to tell what the topic was.
Listens to other presentations	Listens intently. Does not make distracting noises or movements.	Listens intently but has one distracting noise or movement.	Sometimes does not appear to be listening but is not distracting.	Sometimes does not appear to be listening and has distracting noises or movements.
Time limit	Presentation is 5 to 6 minutes long.	Presentation is 4 minutes long.	Presentation is 3 minutes long.	Presentation is less than 3 minutes OR more than 6 minutes.
Vocabulary	Uses vocabulary appropriate for the audience. Extends audience vocabulary by defining words that might be new to most of the audience.	Uses vocabulary appropriate for the audience. Includes 1 or 2 words that might be new to most of the audience, but does not define them.	Uses vocabulary appropriate for the audience. Does not include any vocabulary that might be new to the audience.	Uses several (5 or more) words or phrases that are not understood by the audience.
Speaks clearly	Speaks clearly and distinctly all (95 to 100%) of the time and mispronounces no words.	Speaks clearly and distinctly all (95 to 100%) of the time but mispronounces one word.	Speaks clearly and distinctly most (85 to 94%) of the time. Mispronounces no more than one word.	Often mumbles or cannot be understood OR mispronounces more than one word.

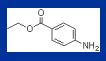
Date Created: **March 7, 2008 10:12 am**

para-Aminobenzoic Acid Derivatives

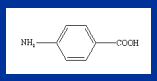
Can we design a novel UV light protector?

Esterification reactions review

- Carboxylic acid + alcohol yields ester
- Acid catalyzed
- Benzocaine is a(n) ____.



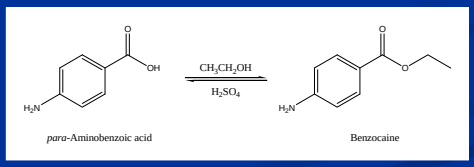
- *Para* amino-benzoic acid (PABA) is a(n) _____.



Chemicals drawn using ChemDraw Software

Benzocaine

- Synthesized from PABA and ethanol
- Fischer esterification



Chemicals drawn using ChemDraw Software

Benzocaine and PABA

- Benzocaine (ethyl *para*-aminobenzoate)
  - Common use: topical anesthetic
  - Less common use: fish tranquilizer
  - Benzocaine also used in Phenazone (anti-inflammatory)
- PABA
  - Common use: sunscreen ingredient
    - Absorbs UV light
  - Less common use: treatment for irritable bowel syndrome or fibrotic skin disorders
  - Nutritional supplement (B complex)
    - Not really a vitamin; not essential to human nutrition
    - Folic acid synthesis in bacteria and in human intestine by bacteria
  - Common allergen

PABA and PABA esters as sunscreens

- First marketed in the U.S. in early 1970s
  - First true sunscreen
- Absorbs UV radiation
- The PABA esters are padimate O, padimate A, and glyceryl aminobenzoate
  - Not water soluble; bind readily to cells and water resistant
- Problems:
  - Products containing PABA and PABA-like chemicals may need to be applied up to 2 hours before sun exposure to achieve their maximal effect
  - Contact allergies to PABA and PABA esters are common
  - Effects on DNA questionable

Ultraviolet light

- Wavelength
- Non-ionizing radiation
- The UV radiation spectrum can be divided into three bands: UVA (320 to 400 nm), UVB (290 to 320 nm), and UVC (200 to 290 nm)
  - Little UVC radiation reaches the earth because it is filtered out by the ozone layer
  - UVB radiation is the principle cause of sun damage (sunburn and skin cancer)
  - UVA radiation is responsible for causing a slow natural tan to develop and may also contribute to the cancer-causing potential of UVB radiation
- Sun damage
  - Collagen fiber damage leads to premature aging
  - DNA damage
  - Possible skin cancer

UV light

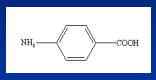
Abbreviation	Wavelength range (nm)	Energy per photon (eV)
UVA	400 to 315	3.10 - 3.94
UVB	315 to 280	3.94 - 4.43
UVC	280 to 100	4.43 - 12.4

Which is germicidal?

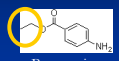
Sunscreens

- Most sunscreens on the market today absorb UV light in the UVA and UVB ranges.
- With the depletion of the ozone layer, it may be necessary to block UVC light. Our goal in this laboratory exercise is to investigate the ability of novel PABA derivatives to block UVC light.

PABA derivatives



PABA



Benzocaine

PABA derivatives that interest us will have different groups attached to the ester. Read the handouts made for you by the organic chemistry class.

Chemicals drawn using ChemDraw Software

Problem to solve

- Overview of this lab component
- Questions to consider
  - Do different derivatives of PABA absorb UV light differently?
  - What factors contribute to a compound's ability to absorb UV light?
  - Which PABA derivative absorbs UV light the best?
- Homework for each group
  - Which of the PABA derivatives will best absorb UV light and why?
  - Formulate your hypothesis and hand it in.
  - Due on _____.

Discussion questions

- What does UV light do to bacterial cells?
- Why are bacteria good for testing the effectiveness of a sunscreen?
- Have you performed an assay to determine UV effects on bacteria? What did you find out?
- What do you know about *Serratia marcescens*?

Photo credit: Michelle Furlong

Experimental design

- Bioassay
  - *Serratia marcescens* spread on plate
  - Plate covered with plastic wrap (UV light can theoretically penetrate plastic wrap, but you should probably test this)
  - Compounds suspended in mineral oil
  - Oily compounds spread on plastic wrap
  - Plate exposed to UVC light for 3 minutes
  - Plate incubated for 24 hours
  - Growth observed and scored

Clip Art

Drawing by M. Furlong

Examples

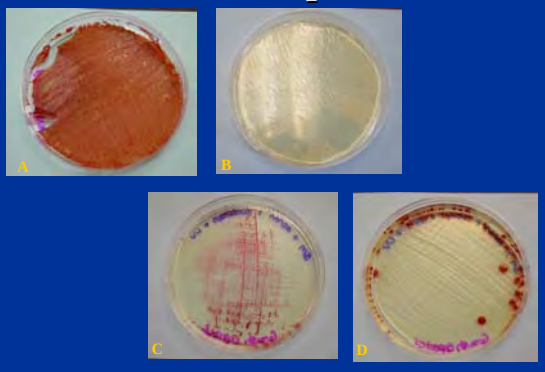


Photo credit: Michelle Furlong

Experimental design

- Get with your group and decide the following
  - Hypothesis
  - How many replicates are you going to do?
  - What controls should you run?
    - How many controls?
  - How do you set up these controls and how do you treat them?

Plan

- Lab 1
  - Discuss experimental design with your group
- Lab 2
  - Turn in experimental design and briefly discuss
  - Perform experiment (day 1 protocol and your experimental design)
- Lab 3
  - Collect results (raw data) and discuss with group
  - Share results
- Lab 4
  - Get with groups to create a presentation on data and on the experiment (assignments to come)
  - Continue with data analysis and start planning report
- Lab 5
  - Present and have discussion.
- Report due-____