EXPERIMENT 1
SYNTHESIS OF XANTHENE DYES USING ELECTROPHILIC AROMATIC SUBSTITUTION

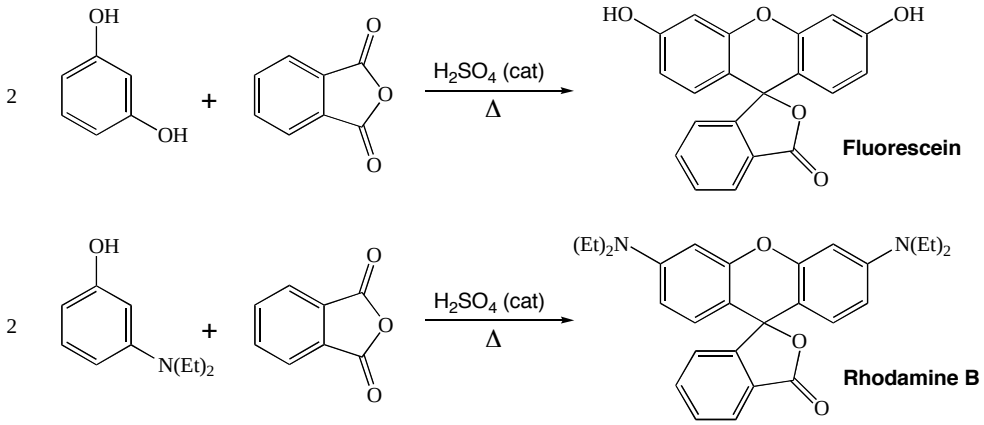
This procedure is adapted from: J.V. McCullagh, K.A. Daggett, *J. Chem. Ed.* **2007**, *84(11)*, 1799.

Reading Assignment: Smith Sections 18.5 – 18.9

Pre-lab Questions:

1. Draw a complete mechanism for formation of fluorescein.

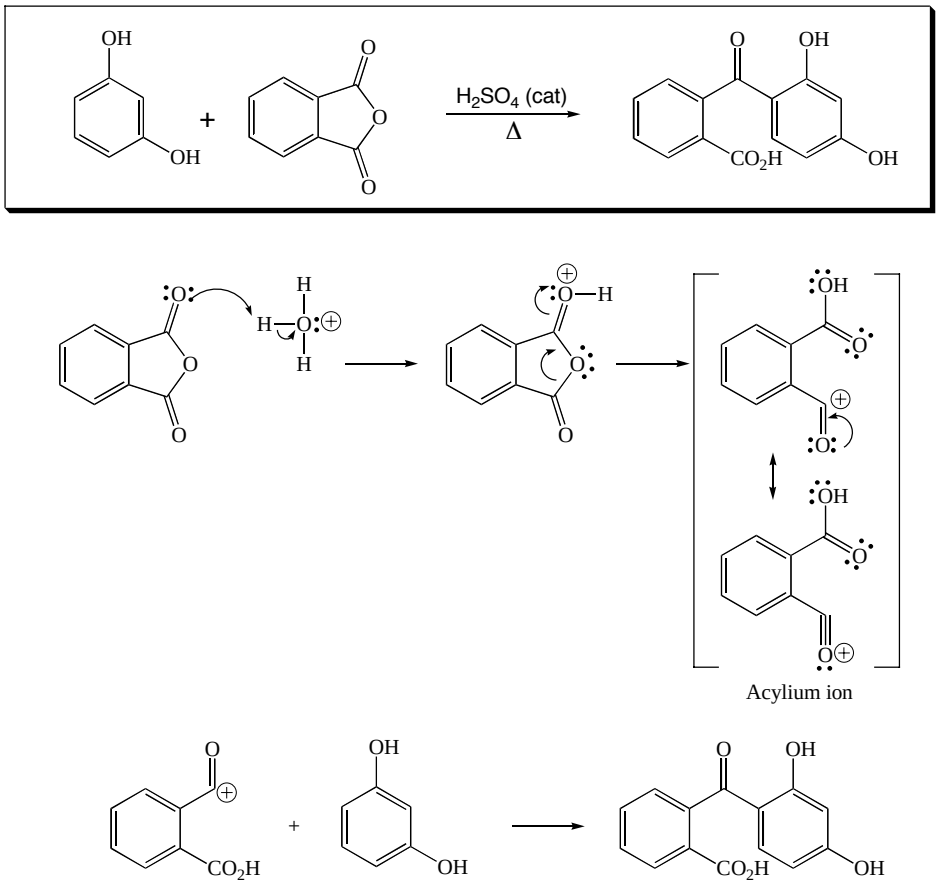
In this experiment, xanthene dyes are synthesized using Friedel-Crafts acylation. Xanthene dyes are triarylmethane dyes in which the two of the aromatic rings are also connected by an ether linkage to form a fused ring system. We will be making two xanthene dyes: **Fluorescein** and **Rhodamine B**. Fluorescein, due to its strong fluorescence and low toxicity, is used to examine wells, trace stream flow, to label biological compounds, to detect corneal abrasions, and as to dye drugs, cosmetics, and markers. Rhodamine B is used as a dye for lasers, inks, papers, cosmetics, crayons, wool, cotton and it is also used as a biological stain.



Friedel-Crafts reactions are a class of EAS reactions that involve reaction of an aromatic ring with an electrophile that bears an electron-deficient carbon center. There are two variations on this reaction. One variant involves generating a carbocation from an alkyl chloride and a Lewis acid catalyst (*alkylation*). The other involves an acyl halide or anhydride and a Lewis acid catalyst to form an aromatic ketone (*acylation*). Protic acids can also be used to generate carbocations. In this experiment, a Bronsted acid catalyst (*sulfuric acid*) will be used to generate xanthene dyes from substituted phenols and phthalic anhydride. Two successive Friedel-Crafts

acylations will take place to produce each xanthene dye. Shown below is the reaction to form fluorescein:

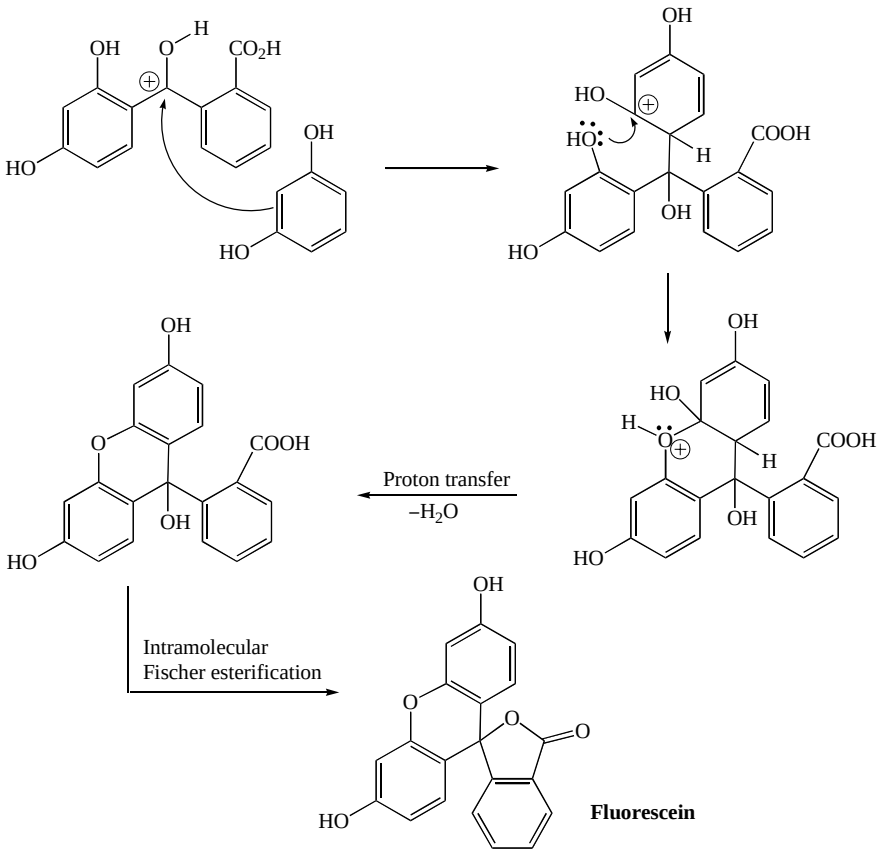
First Friedel-Crafts:



The first step in the formation of fluorescein is a Friedel-Crafts acylation reaction. In this reaction, the acyl cation is generated by protonation of the phthalic anhydride as shown above. The acyl cation will then react with the substituted phenol (*resorcinol*). This produces a substituted benzophenone.

The reaction does not stop at this point. Under the reaction conditions used, the substituted benzophenone formed in the first reaction is protonated to form a cationic intermediate that can participate in a reaction similar to the Friedel-Crafts alkylation reaction. The resulting cation then undergoes alkylation to complete the assembly of the triarylmethane carbon skeleton. Subsequent acid catalyzed esterification (lactonization) then yields flourescein (*next page*).

Second Friedel-Crafts:



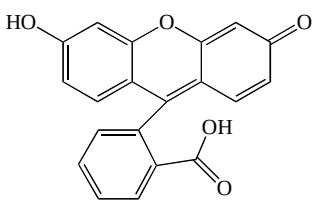
Fluorescein

Optical Properties: Dye color

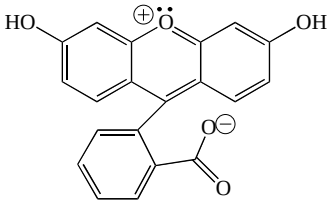
Light in the ultraviolet and visible regions can cause an excitation of electrons within a molecule. The electrons move from bonding or nonbonding orbitals into higher energy antibonding orbitals. For many organic compounds, these relatively high energy transitions correspond to light in the UV region leading to uncolored compounds. In general, increasing the amount of conjugation in a molecule will cause the energy gap between the highest occupied π bonding orbital and the lowest unoccupied π^* antibonding orbital to decrease. This results in a shift in absorbance to lower energies (longer wavelength) with increasing amounts of conjugation. If a sufficient amount of conjugation is present, absorbance of light will fall in the visible range of the spectrum leading to colored organic compounds.

The xanthene dyes synthesized in this experiment all are sufficiently conjugated to absorb in the visible range of the spectrum provided they are in a form where the central carbon between all three aromatic rings is sp^2 hybridized. This allows conjugation between the different aromatic rings. We can see this if we consider the dye fluorescein. Fluorescein has been shown to have at least 3 reasonably stable neutral tautomers, each of which have different physical characteristics. This has been demonstrated by the fact that 3 distinct crystalline forms of fluorescein with different colors (red, yellow and colorless) can be formed depending on how the crystals were

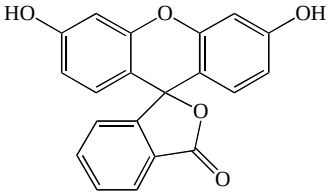
formed. Likewise, the solutions of many of the dyes change or even lose their color in different solvents. For example, fluorescein is orange in acetone and nearly colorless in ether. This is presumably due to differing tautomer stability in each of these solvents. In the colorless form of fluoroescein, the central carbon is sp^3 hybridized so the π- systems of the aromatic rings are all isolated from each other. In this form, the molecule does not have a large enough extended conjugated system to absorb in the visible region of the spectrum, and so is colorless. In the yellow and brick red forms have a central carbon that is sp^2 hybridized. The increase in conjugation causes an absorbance shift into the visible region, resulting in the observed colors.



Quinoid tautomer
Brick red colored crystals



Zwitterionic tautomer
Yellow colored crystals

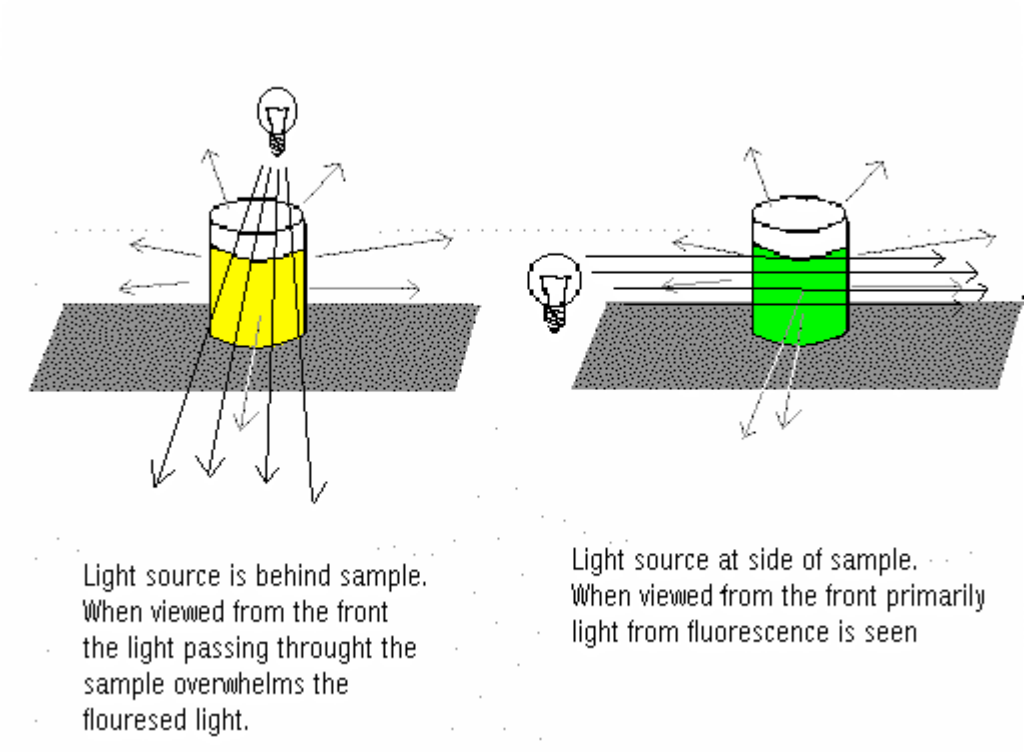


Lactone tautomer
Colorless crystals

Fluorescence

Both fluorescein & rhodamine B exhibit the phenomenon known as fluorescence. Fluorescence occurs a molecule re-emits a portion of absorbed energy as light. The emitted light is always of lower energy than the light that was absorbed. For many fluorescent compounds, the absorbed light is in the ultraviolet region and the emitted light is in the visible region. Since we can only see the emitted light, these compounds glow when exposed to a UV light source. If the solution also absorbs light in the visible region of the light spectrum, then some interesting phenomena can be observed when examining these compounds. Solutions of these compounds can look as if part of the solution is one color while the rest of the solution is a different color. This is particularly noticeable if we examine dilute solutions of fluorescein. These solutions can look entirely orange, a mixture of orange and glowing green, or all glowing green depending upon the angle between a viewers line of sight and the beam of the light source illuminating the sample. Fluorescein absorbs light in both the UV and blue region of the visible spectrum. So, a fluorescein solution will look most orange if the light source illuminating the sample is on the opposite side from the viewer due to the absorption of light in the visible part of the spectrum. On the other hand, the green light from fluorescence is released equally in all directions. This means that to optimally see green with the least interference orange fluorescein should observed at right angles to the light source (see figure below).

At a 90º angle to the light source against a non-reflective surface, like a black lab bench, the solution will appear to be green. The fluorescence properties of fluorescein can also be seen if we illuminate a dilute solution of the compound with a black light (long wave UV lamp). If fluorescein or rhodamine B is synthesized, their fluorescent properties will be observed as part of the experiment.



CAUTION

Wear gloves and safety glasses and conduct all aspects of this synthesis in a hood. The organic solvents used in this laboratory experiment (acetone, diethyl ether are volatile flammable solvents of moderate toxicity, but should prove little hazard if handled in a fume hood and disposed of properly after use. Methylene chloride is a volatile solvent that is toxic and potentially carcinogenic. Students must work in a fume hood and wear disposable butyl nitrile gloves when using this solvent. The organic reagents used in this experiment are also classified as irritants with moderate toxicity. Some of the strong acids and bases used in this experiment (H_2SO_4 and NaOH, respectively) are toxic and corrosive. Any students handling these materials should again be wearing safety glasses and butyl nitrile gloves.

1. Synthesis of Fluorescein:

(a) Reaction Setup

The sand bath temperature for the first step of this reaction should be between 180° and 200°C. To a large test tube (15 X 150 mm) add 153 mg of resorcinol and 100 mg of ground powdered phthalic anhydride. To this mixture of powders add 3 drops of 4N H_2SO_4 (DO NOT ADD MORE THAN 3 DROPS). Stir the mixture briefly with a spatula. Place the test tube in

the preheated sand bath deep enough such that its contents are just slightly (0.5 cm) below the surface of the sand. The reaction should be run at a temperature between 180° and 200°C. **Periodically adjust the heater setting to keep the temperature within this range**. *(Note: It is extremely important to monitor the temperature and keep it within this range. Overheating will cause the product to decompose.)* Start timing the reaction once it reaches 180°C. The reaction should run for 30 minutes within this temperature range. By the time the reaction reaches this temperature, the solids should have melted to form a solution which should develop an orange-brown color as it progresses. Once the reaction time is up, remove the test tube from the sand bath and allow it to cool for about 5 min. Turn the sand bath heater to a lower setting. The sand bath will need to be near 100°C for the rest of the experiment.

(b) Reaction Workup

To the test tube add 5 mL of acetone and 1.27 cm X 0.79 cm stir bar. Using a ring stand and clamp, place the test tube over a magnetic stir plate and stir the solution for 5 to 10 minutes. The solution should turn yellow as the crude fluorescein dissolves. If the entire product did not dissolve, repeat the process with an additional 5 mL of acetone until the entire product dissolves (do not use more than 15 mL total). Combine the acetone layers in a 50 mL round bottom and remove the solvent on a rotary evaporator. Take this crude residue and dissolve it in 20 mL of diethyl ether and 1 mL of water. (*Note: Even though most of the dye will end up in the organic layer it will not dissolve unless a small amount of water is present.)* Place a stir bar in the solution and put the round botttom over a magnetic stir plate for several minutes until all the solids dissolve. Using a small separatory funnel extract the organic layer once with 10 mL water. Following this, extract the ether layer once with 10 mL of a saturated NaCl (brine) solution. Dry the organic layer over anhydrous sodium sulfate, filter, and remove the solvent on a rotary evaporator to yield the product as an orange solid. Weigh the crude product before proceeding to part 3.

2. Synthesis of Rhodamine B

(a) Reaction Setup

The sand bath temperature for the first step of this reaction should be between 180° and 210°C. To a large test tube (15 X 150 mm) add 228 mg of 3-(diethylamino)-phenol and 100 mg of ground powdered phthalic anhydride. To this mixture add 3 drops of 4N H_2SO_4 (DO NOT ADD MORE THAN 3 DROPS). Briefly stir the mixture with a spatula. Place the test tube in a preheated sand bath deep enough so that its contents are just slightly (0.5 cm) below the surface of the sand. The reaction should be run at a temperature between 180° and 210°C. **Periodically adjust the heater setting to keep the temperature within this range**. *(Note: It is extremely important to monitor the temperature and keep it within this range. Overheating will cause the product to decompose.)* Start timing the reaction once it reaches 180°C. The reaction should run for 1 hour within this temperature range. The reaction should become red-violet in color.

Once the reaction time is up, remove the test tube from the sand bath and allow it to cool for about 5 minutes. Then, place it in an ice bath and cool it to room temperature.

(b) ***Reaction Workup***

To the test tube add 3 mL of methylene chloride and a 1.27 cm X 0.79 cm magnetic stir bar. Using a ring stand and clamp, place the test tube over a magnetic stir plate and stir the solution for 5 to 10 minutes. Remove the dye solution and set it aside. Repeat this process with additional 3 mL of methylene chloride until the entire product dissolves. This should take about a total of 12 mL. Using a small separatory funnel, extract the organic layer once with 10 mL 5% sodium bicarbonate solution. Following this, extract the organic layer twice with 10 mL of water *(THINK: Which layer is on top?)* Dry the organic layer over anhydrous sodium sulfate, filter, and remove the solvent on a rotary evaporator to yield the product as a dark magenta colored solid. The product may be slightly tacky at this point due to residual solvent trapped in the product. Proceed to part 3.

3. Observation of Fluorescence

Prepare a solution of rhodamine B by dissolving **5** milligrams of the sample in **50** mL of methanol, or a solution of fluorescein by dissolving **5** milligrams of the sample in 0.1 M NaOH. Place the solution in a vial and place it on a black non-reflective surface, such as a lab bench. Place a bright light source on the opposite side of the bench from the observer and note the appearance of the solution. In this case, the color observed is primarily due to absorbance of some wavelengths of visible light passing through the sample from the light source.

Next observe the same solution at a position 90 degrees from the light source. The color observed from this perspective is primarily due to fluorescence.

Finally, in a darkened room shine a long wave UV lamp at the sample vial. The vials should visibly glow from the fluoresced light. Record your observations, then proceed to step 4.

4. IR

Take an IR of a portion of your remaining crude sample. Submit the rest to your TA in a labeled vial.

Post-lab Questions:

1. Give an explanation of why fluorescein is orange in acetone and nearly colorless in ether.
2. Based on the functional groups observed in the IR, which form of your xanthene dye predominates in your IR spectrum?