Synthesis and Characterization of Mauveine and its Substituted Aniline Derivatives

Charles A. Wolfe ’20 and Paul H. Mueller

Department of Chemistry, Hampden-Sydney College, Hampden-Sydney, VA 23943

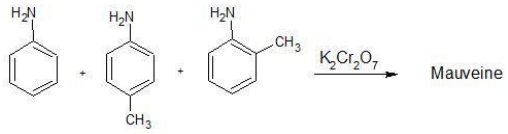
Abstract

The year was 1857 in colonial Britain, a vast empire aggressively expanding well into the tropics. In their conquest however, the British colonies began to fall ill to malaria thus halting their expansion. To treat their sickly colonies, the British began to import quinine from the Brazilian colonies owned by Portugal: an imperial adversary. To avoid this dependency British science went to work looking for a new way to synthesize quinine in a lab.

In his quest to find quinine a young William Henry Perkin, a 19-year-old student of von Hofmann at the Royal College of Chemistry, discovered an odd black tar in his flask after another failed quinine synthesis attempt. Upon rinsing the flask, Perkin noticed the tar turned into a deep purple color. The excited Perkin then promptly patented the mauve colored dye as “aniline purple” or as we call it “mauveine.” Mauveine is more than just an dye however, for it greatly contributed to the history of modern industrial chemistry by paving the way for other famous aniline dyes such as fuchsia and the microbial stain methylene blue. Early microbiologists recognized these aniline dyes for their ability to target cells, and early methods of chemotherapy were also developed using aniline dyes.

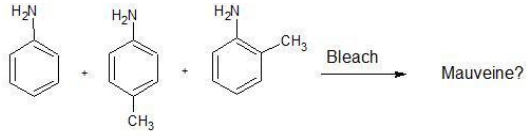
But why is there a newfound interest in mauveine in particular? Knowing the usefulness of other aniline derivatives whether they be microbial stains or chemotherapy drugs, means that the exploration of new possible aniline derivatives is very important whether it be tampering with older synthesis methods or even performing new synthesis methods. For example, Dr. Kevin Dunn invented a new synthesis method for creating purple (mauve) like dyes by replacing a dichromate oxidant with clorox bleach. In doing this, the “mauve” was much less tarry than the perkin method’s synthesis--suggesting the process will yield more mauve. However, the “mauve” created by the Dunn method has not yet been identified as actual Mauveine, as the compound has not yet been fully characterized.The Dunn method uses bleach and the Perkin method uses $Kr_2Cr_2O_7$.

Perkin’s synthesis:



"Aniline mixture"

Dunn’s synthesis:



"Aniline mixture"

Introduction

The project involved performing both the Dunn and Plater synthesis methods each with three different aniline mixtures and then characterize the products. The three aniline mixtures each contained two anilines.

Dunn Method Synthesis:

Synthesis 1	N-phenyl-p-phenylenediamine + aniline HCl
Synthesis 2	N-phenyl-p-phenylenediamine + bromoaniline
Synthesis 3	N-phenyl-p-phenylenediamine + aminoindan

Plater Method Synthesis:

Synthesis 4	N-phenyl-p-phenylenediamine + aniline HCl
Synthesis 5	N-phenyl-p-phenylenediamine + bromoaniline
Synthesis 6	N-phenyl-p-phenylenediamine + aminoindan

After the total of 6 synthesis methods were performed at least once, the goal was to identify and characterize the “mauve” created by each method. The characterization methods included liquid chromatography and mass spectrometry (LC/MS), thin layer chromatography (TLC), nuclear magnetic resonance spectrometry (NMR), and UV visible spectrometry (UV-vis).

Experimental: Synthesis Methods

The three variant Dunn method syntheses were conducted; for synthesis 1, 0.65g of N-phenyl-p-phenylenediamine to 0.88g of aniline hydrochloride were measured and dissolved in 36ml of rice wine vinegar to dissolve reactants. Then a soln of 170ml EtOH with 17ml of clorox bleach was prepared separately. Once the bleach solution was added, the reaction was stirred for 1 hour. The reaction was then rotovapped and dissolved in MeOH to remove possible pseudo mauve products.

Synthesis 2 substituted 0.86g aniline hydrochloride for 1.114g of bromoaniline. first, 0.65g of N-phenyl-p-phenylenediamine to 1.114g of bromoaniline were measured and dissolved in 36ml of rice wine vinegar to dissolve reactants. The bromoaniline was not soluble in vinegar, so 30ml of EtOH was added before the bleach EtOH solution in order for the bromoaniline to dissolve. Then a soln of 175ml EtOH with 20ml of clorox bleach was prepared separately. The bleach solution was then added to the flask on top of the reactants and left for 1 hour with gentle stirring and heating, after 1 hour the solution turned very dark. The rotovapped contents were then filtered with acetone and MeOH, but the acetone was appeared crimson and the MeOH wash remained a burgundy color.

Synthesis 3 substituted 0.86 aniline hydrochloride for 1ml of aminoindan. First, 0.65g of N-phenyl-p-phenylenediamine to 1ml of aminoindan were measured and dissolved in 37ml of rice wine vinegar to dissolve reactants. The aminoindan was also not soluble in vinegar, so 40ml of EtOH was added before the bleach EtOH solution in order for the bromoaniline to dissolve. Then a soln of 160ml EtOH with 18ml of clorox bleach was prepared separately. The bleach solution was then added to the flask on top of the reactants and left for 1 hour with gentle stirring and heating, after 1 hour the solution turned very dark.The rotovapped contents were then filtered with acetone and MeOH, but unlike the bromoaniline iteration. All of the solvent washes ended up violet in color.

Then the three plater synthesis methods were conducted. For synthesis 4: 250mg, 1.36mmol of N-phenyl-p-phenylenediamine, 352mg, 2.72mmol of aniline hydrochloride, and 535mg, 1.82mmol of Potassium dichromate were weighed out on a top loading balance. Both the N-phenyl-p-phenylenediamine and aniline hydrochloride were both macerated in a mortar and pestle. Separately, a solution of 25ml H_2O and 1ml of H_2SO_4 was prepared. The H_2SO_4 solution was then added to a small amount of H_2O in a 250ml flask and then heated to 75C° with gentle stirring. When the H_2SO_4 H_2O solution was at 75C°, the ground N-phenyl-p-phenylenediamine and aniline hydrochloride were added and dissolved. Upon dissolving the reactants, 225ml of DI H_2O was added and heated to 75C°. Once the solution was finally at 75C°, 535mg potassium dichromate was added to begin the reaction. The reaction was then left for 2.4 hours at 75C° with gentle stirring. The solution turned a burgundy color.

Synthesis 5 swapped the 352mg, 2.72mmol of aniline hydrochloride for 0.7ml of bromoaniline. 372mg of N-phenyl-p-phenylenediamine and 535mg, 1.82mmol of potassium dichromate were weighed out on a top loading balance. The N-phenyl-p-phenyl enediamine was macerated in a mortar and pestle. Separately, a solution of 25ml H_2O and 1ml of H_2SO_4 was prepared. The H_2SO_4 solution was then added to a small amount of H_2O in a 250ml flask and then heated to 75C° with gentle stirring. When the H_2SO_4 H_2O solution was at 75C°, the ground N-phenyl-p-phenylenediamine and bromoaniline were added and dissolved. Upon dissolving the reactants, 225ml of DI H_2O was added and heated to 75C°. Once the solution was finally at 75C°, 535mg potassium dichromate was added to begin the reaction. The reaction was then left for 2 hours at 75C° with gentle stirring. The solution turned a deep brown. The solvent was removed from the wash products via vacuum and the solid products were retained for analysis as listed above.

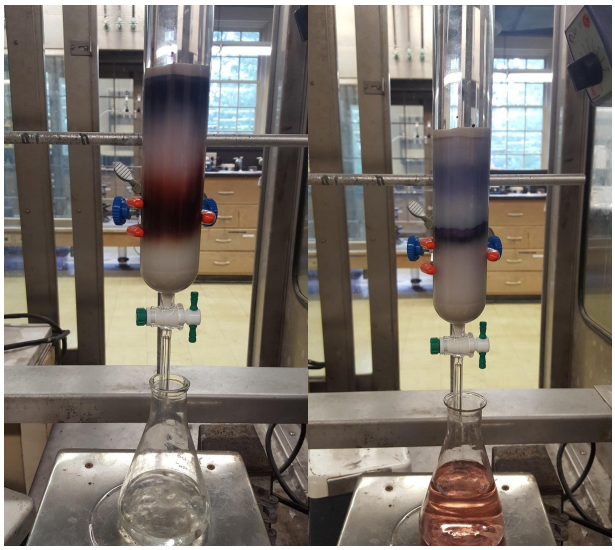
Synthesis 6 switched the 352mg, 2.72mmol of aniline hydrochloride for 1.2ml of aminoindan. 281mg of N-phenyl-p-phenylenediamine and 567mg of Potassium dichromate were weighed out on a top loading balance. The N-phenyl-p-phenylenediamine was macerated in a mortar and pestle. Separately, a solution of 25ml H_20 and 1ml of H_2SO_4 was prepared. The H_2SO_4 solution was then added to a small amount of H_20 in a 250ml flask and then heated to 75C° with gentle stirring. When the H_2SO_4 H_20 solution was at 75C°, the ground N-phenyl-p-phenylenediamine and aminoindan were added and dissolved. Upon dissolving the reactants, 225ml of DI H_20 was added and heated to 75C°. Once the solution was finally at 75C°, 535mg potassium dichromate was added to begin the reaction. The reaction was then left for 2 hours at 75C° with gentle stirring. The solution turned a deep brown. The solvent was removed from the wash products via vacuum and the solid products were retained for analysis as listed above.

Once all 6 products were rotovapped, synthesis 5 was chosen to begin the characterization process. The synthesis 5 product was suspended in MeOH and TLC was run. The product was then prepared for HPLC. The first HPLC separated the brown product into thick red and blue bands. The blue band once isolated was subjected to another HPLC for further separation. The contents of the blue

band were then separated into individual vials and concentrated for UV-vis and NMR spectroscopy.

Results and Discussion

The synthesis 5 was characterized by TLC, HPLC and C13 NMR. Synthesis 5 was chosen because the TLC tests showed that synthesis 5 had the darkest and most complex coloration. The HPLC was run with a concentrated sample of synthesis 5 product infused with silica gel. Once the synthesis 5 purple bands were isolated, a UV-Vis spectrum was taken which showed that the synthesis 5 blue bands were not Mauveine. Further characterization was provided by a subsequent C13 NMR spectrum which indicated that the synthesis 5 product was decomposed during the synthesis reaction.



The image on the left was the filtered product from synthesis 5. Synthesis 5 yielded a brown tarry liquid which once separated revealed a thick red and a thick purple band. The image on the right shows the separation of exclusively the blue band from the prior synthesis 5 HPLC.

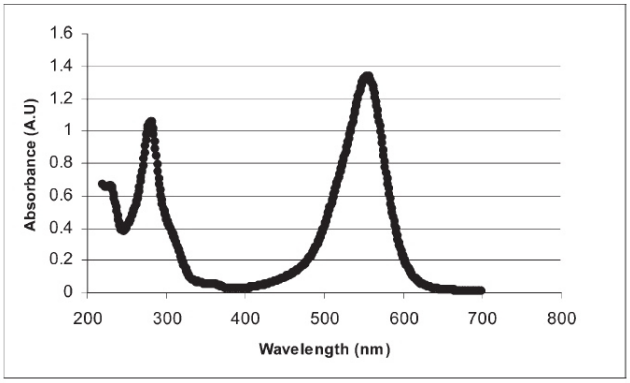


Fig. A

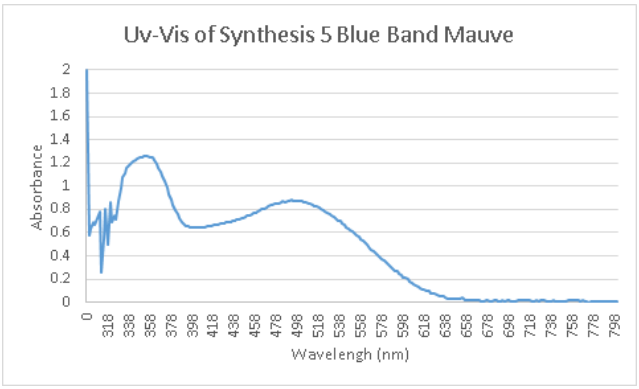


Fig. B

We expected to see sharp peak absorbances at around 560nm and 390nm from the Uv-vis, yet there is a wide peak at 358nm and an even wider peak at 498nm which does not correspond with the mauveine Uv-Vis spectrum. The NMR helps illustrate the cause of these differing products. With the majority of the NMR peaks existing in the alkane region it can be concluded that the Synthesis 5 NMR sample was fragmented during the oxidation process, perhaps the Potassium dichromate was too powerful an oxidant or the reaction was left too long over heat.

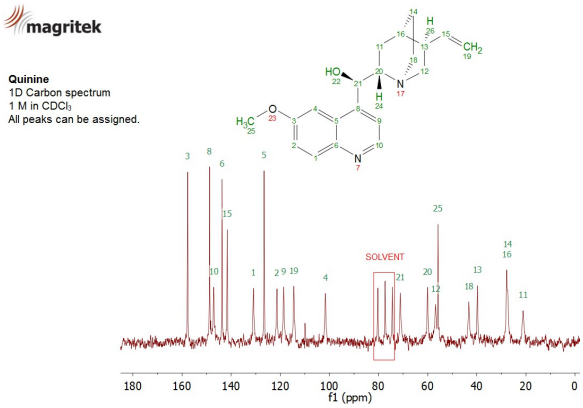


Fig. C

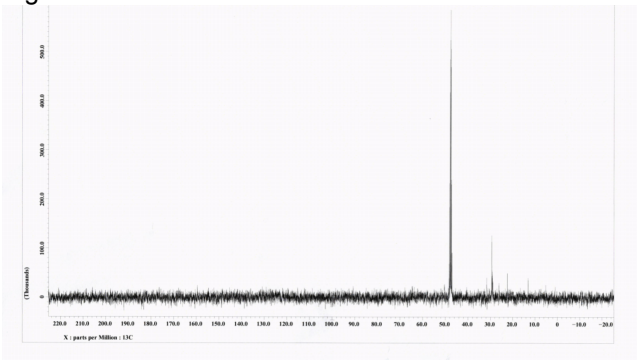


Fig. D

Mauveine has five aromatic rings, Quinine — just for comparison's sake—(Fig. C) has 3 rings meaning that the majority of the carbons should reside around the 120ppm-140ppm range. However,

the majority of the synthesis 5 peaks (Fig. D) existed between 10ppm-30ppm. This supports the theory that the synthesis 5 product decomposed into smaller particles during the reaction process.

Conclusion

Six different synthesis methods were performed. The Synthesis 5 product was partially characterized leading to the conclusion that not mauveine nor any other aniline derivatives were formed. In the future, the oxidizing agent (in the synthesis 5 case potassium dichromate) should be much weaker, and the synthesis reactions should be run for a shorter amount of time. Other variables could optimized through further exploratory synthesis.

REFERENCES

1. Omer Gunkaraa, Tahir; Bagdatlib, Emine; Ocala, Nuket. Synthesis of new pyrazolone dyes. *J. Chem. Res.* 2013, 227–231.
2. CA Glusko; CM Previtali; CA Chesta; HA Montejano. Simple procedures for analyzing and purifying methylene green. *Biotech. Histochem.* 2012, 321–324.
3. Camilla-Baratto, Maria; Juarez-Moreno, Karla; Pogni, Rebecca; Basosi, Riccardo; Vazquez-Duha, Rafael. EPR and LC-MS studies on the mechanism of industrial dye decolorization by versatile peroxidase from Bjerkandera adusta. *Environ. Pollut.* 2015, 22, 8683–8692.
4. Tsai, Chia-Fen a; Ching-Hao Kuo; Yang-Chih Shih, Daniel; Determination of 20 synthetic dyes in chili powders and syrup-preserved fruits by liquid chromatography/tandem mass spectrometry. *FDA*. 2015, 453-462.

Figure C: Crasto, Dr. Anthony Melvin. “QUININE.” http://www.allfordrugs.com, Nov 28th 2014, http://www.allfordrugs.com/tag/quinine/.

Figure A: Rzepa, Henry. “The colour of purple is … not orange but mauve?.” *http://www.ch.imperial.ac.uk,* Imperial College of London, March 12th, 2013, http://www.ch.imperial.ac.uk/rzepa/blog/?p=5248.
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