CHM 114: Bioorganic Molecules
Week 6 Laboratory

Azosulfonamides: Part 1. Synthesis of a Ligand for Protein Binding Studies[1]

Dyes and Serendipity:

In the Persian fairy tale *The Three Princes of Serendip*, the title characters were forever discovering things they were not looking for at the time—thus, *serendipity* is the aptitude for for making happy discoveries by accident. The preparation of the first commercially important synthetic dye by William Henry Perkin (not of Perkin's restaurant fame) in 1854 is a good example of a serendipitous discovery in science. During the nineteenth century quinine was the only drug known to be effective against malaria, and it could be obtained only from the bark of the cinchona tree, which grew in South America. French chemists had isolated pure quinine from cinchona bark in 1820, but the inaccessibility of the tree made natural quinine very expensive. Perkin, then an 18-year-old graduate student working for the eminent German chemist August Wilhelm von Hofmann, realized that anyone who could make synthetic quinine might well become rich and famous. Perkin knew nothing about the molecular structure of quinine—structural organic chemistry was in its infancy in the mid-1800's—but he knew that quinine's molecular formula was $C_{20}H_{24}N_2O_2$. Perkin prepared some allytoluidine ($C_{10}H_{13}N$), apparently thinking that two molecules of allyltoluidine plus three oxygen atoms minus a molecule of water would magically yield $C_{20}H_{24}N_2O_2$ –quinine! (Figure 1).

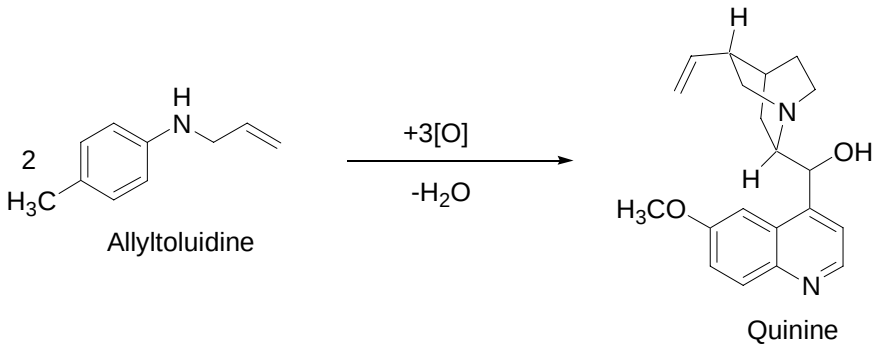

Figure 1. Perkin's Idea for the Synthesis of Quinine

Of course Perkin had attempted the impossible. The molecular structure of allyltoluidine bears no resemblance to that of quinine, which was not synthesized until 1940. But he hopefully oxidized allyltoluidine with potassium dichromate and came up with a reddish-brown precipitate that he quickly realized was not quinine. Most chemists would have thrown out the stuff and started over, but the product had properties that interested Perkin, so he decided to try the reaction with a simpler nitrogen base, aniline. This time he obtained a black precipitate that, when extracted with ethanol, formed a beautiful purple solution that greatly impressed some of the local dyers. Perkin knew a good thing when he saw it, so he promptly gave up his study of chemistry and went into the business of manufacturing "aniline purple," or mauve as the dye soon came to be known (Figure 2). Ironically, Perkin became rich and famous by failing to synthesize quinine. His dyestuffs plant was so successful that he was able to retire at the age of 36, finish his chemistry studies, and devote the rest of his life to pure research.

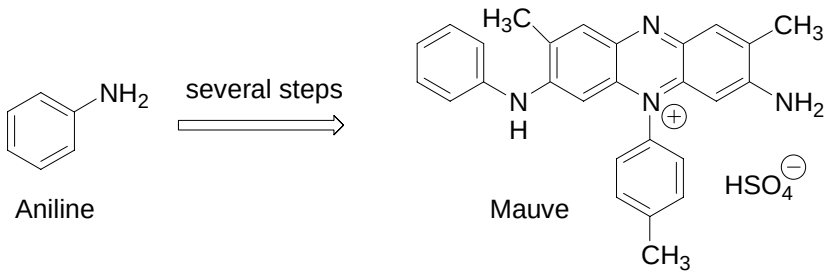

Figure 2. Perkin's Synthesis of Mauve

[1] Manalang, M. G.; Bundy, H. F. *J. Chem. Educ*. **1999**, *66*, 609-611.

While Perkin was getting the synthetic dye industry under way, other chemists were experimenting with aniline and the vast array of other compounds that could be extracted from coal tar. One of these was a brewery chemist named Peter Griess, who took time off from the brewing of Alsopps' Pale Ale to discover the azo dyes. Undiscouraged by the fact that many of the diazo compounds he prepared had a tendency to explode, Griess did some fundamental research into the diazotization of aromatic amines (aniline is an aromatic amine) and went on to discover a coupling reaction by which virtually all azo dyes are now synthesized. The azosulfonamides that we will synthesize will use this same coupling reaction discovered by Griess (Figure 3).

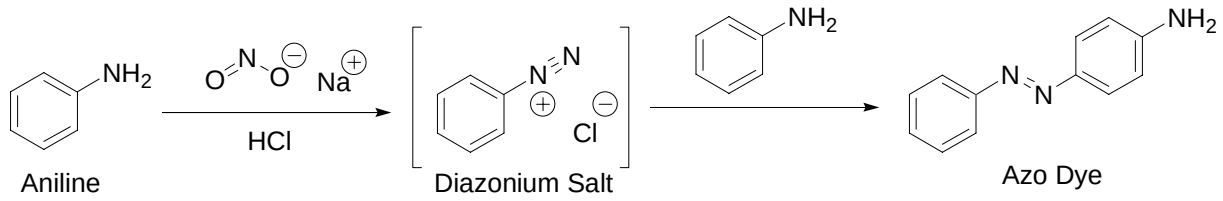

Figure 3. Griess' Coupling of Diazonium Salts to form Azo Dyes

Sulfonamides[2]*:*

The discovery of antibacterial sulfonamides in the early 1930's ushered in the modern era of effective chemotherapy of infectious diseases. Sulfonamides were the first real success in treating bacterial infections with relatively safe, nontoxic chemical compounds.

The decades preceding the mid-1930's saw the development of some chemotherapeutic agents that included organic arsenic compounds such as p-amino-phenylarsonic acid, wishfully named "Atoxyl" (because it was slightly less toxic than inorganic arsenic compounds), which showed some useful activity in managing certain diseases. Paul Ehrlichs development of arsphenamine in 1910 as an antisyphilitic drug can now, with over ninety years of hindsight, be viewed as the beginning of a revolution that changed the treatment of all infectious diseases. Other early drugs were the alkaloids emetine from Ipecac root, and the acridines euflavine and proflavine (Figure 4).

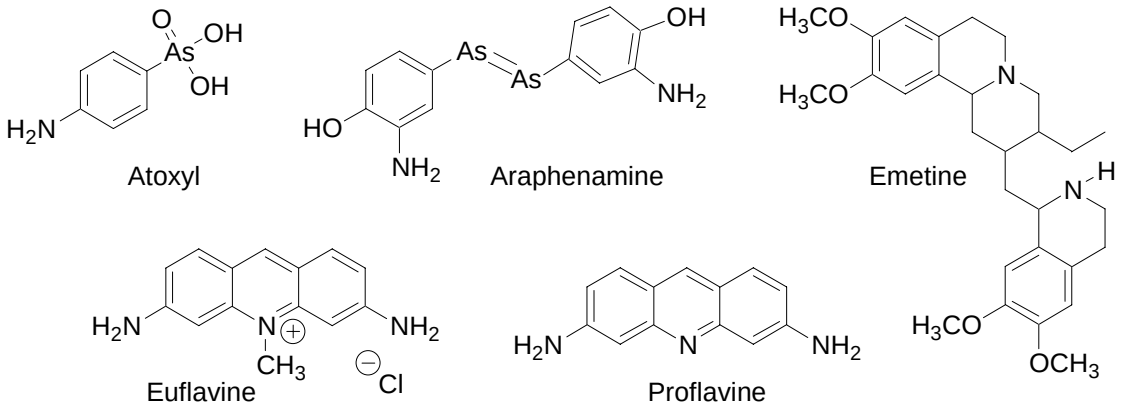

Figure 4. Structures of Some Early Drugs

For awhile, it looked as though effective treatment of infections using small synthetic organic molecules might not be achievable. The major breakthrough came when it was decided to test over 1000 azo dyes synthesized by the Bayer laboratories. The sulfonamide dye prontosil (Figure 5) was shown to have outstanding antistreptococcal and staphylococcal activity in mice. The rational for screening sulfonamide (-SO_2NH_2)-containing dyes for possible antibacterial activity was based on the known binding propensity of these dyes to wool fibers that are protein (fast dyes). Analogous strong attachment (covalent bonding?) was assumed to be likely with bacterial proteins. The drug was introduced into clinical medicine on the basis of successful animal experiments. The curious observation that prontosil had no effect on bacterial growth in culture and the basis of its molecular activity were soon solved.

[2] Gringauz, A. *Introduction to Medicinal Chemistry: How Drugs Act and Why*, Wiley-VCH, New York, 1997.

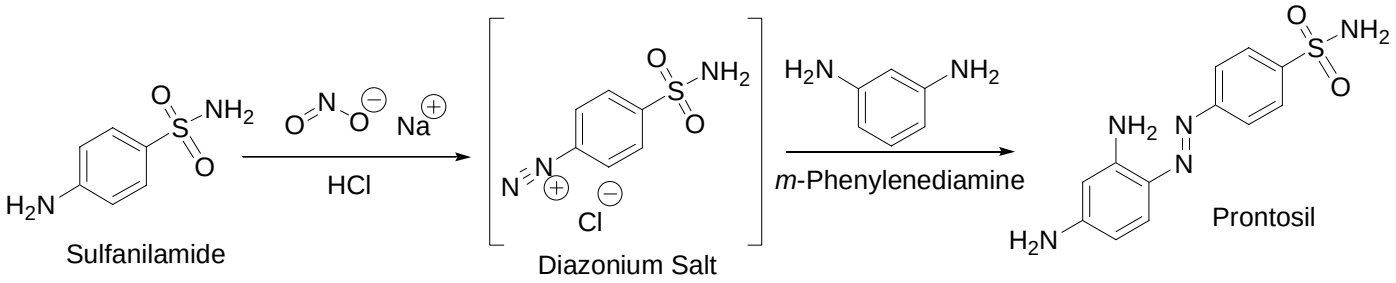

Figure 5. Synthesis of Prontosil Using Diazo Coupling

It bothered chemists that prontosil showed antibacterial activity in patients (in vivo) but not in a test tube. They started tinkering with the structure of prontosil to see if they could increase its antibacterial activity. It was found that structural modifications to the diaminobenzene half of the molecule (the half originating from phenylenediamine) had no significant effect on antibacterial activity. Furthermore, urine analysis of patients treated with prontosil showed that most, if not all, of the ingested drug was excreted with the azo linkage reductively cleaved (Figure 6), yielding the compounds sulfanilamide and triaminobenzene. Sulfanilamide was able to be established as the active antibacterial agent. The mystery of prontosil's in vitro inactivity could now be explained. Prontosil itself had no intrinsic antibacterial action. However, in the body, the azo dye was reductively cleaved by azo-reductase enzymes to produce the anatibacterial compound sulfanilamide. When looking for antibacterial activity in a test tube, no reductase enzymes were present so no antibacterial activity was observed.

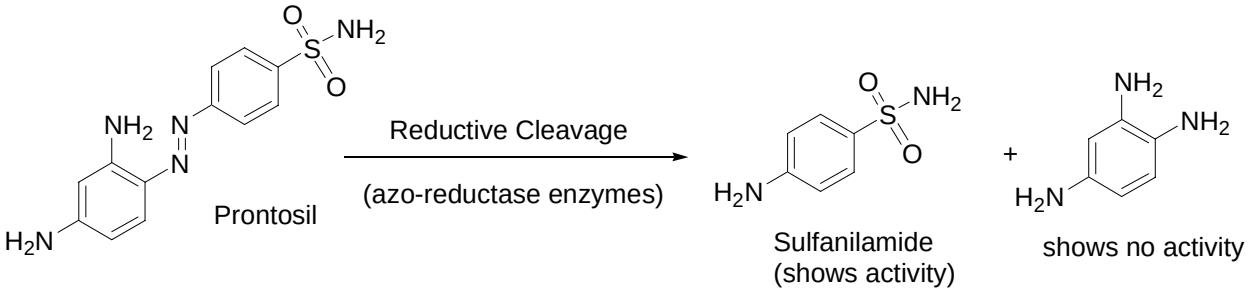

Figure 6. Reductive Cleavage of Prontosil

Soon after this discovery, there was an explosion of sulfonamide drug tests. The impetus for this was twofold. One reason was to capitalize on the lead compound—sulfanilamide—in order to obtain even more effective compounds with fewer side effects. Another factor was that the chemical sulfanilamide was *not* a patentable drug, since it had been synthesized years earlier. The new commercial competition spawned numerous new and clinically better "sulfas," as they came to be known.

In this laboratory period, you will synthesize prontosil and 4-hydroxyazobenzene-4'-sulfonamide and purify these compounds by recrystallization. These compounds will be saved for use next week, when we will investigate the binding between these compounds and the enzyme carbonic anhydrase.

Experimental Procedure:

Synthesis of prontosil:

Dissolve 0.6 g (0.006 mol) *m*-phenylenediamine in 30 mL of 1.0 *M* sodium acetate, and cool the solution in an ice bath. Prepare a solution of 1.0 g (0.006 mol) sulfanilamide and 2 mL (0.012 mol) 6 *M* HCl in 10 mL of deionized water. Cool the sulfanilamide solution in an ice bath with magnetic stirring. Once the solution has cooled, form the diazonium salt by slowly adding 10 mL of ice cold 0.7 *M* $NaNO_2$ solution to the stirred sulfanilamide solution. After the addition is complete, let the solution stir for an additional 3-4 minutes. Remove the diazonium salt solution from the stir plate and keep it ice cold. Begin stirring the phenylenediamine solution while it is still in its ice bath. Add the cold diazonium salt solution dropwise, with constant stirring, to the cold diamine solution. Midway through the addition, and again when the addition is complete, adjust the pH to 8 (pH paper) with 5%

KOH. After the final pH adjustment, continue stirring for 15 minutes. Filter the product and wash the solids with cold water until the filtrate registers neutral pH (~200 mL). Maintain suction until the filter cake appears dry. Remove the filter cake (use gloves if needed to prevent your hands from becoming dyed) weigh the solid. crystallize the product from 50% ethanol-water solution. You will need about 25 mL of solution for each gram of solid. Add the solid to the appropriate amount of ethanol-water solution. Heat on a hot plate to dissolve the solids. If the solution is nearing a boil with solids still present, quickly filter while hot. Allow the hot solution to cool to room temperature for 15 minutes and then in an ice bath for 30 minutes. Vacuum filter the crystals and let dry until next week. Obtain a melting point, GC and UV-Vis spectrum for your crystals.

4-hydroxyazobenzene-4'-sulfonamide

Prepare a solution of 0.56 g (0.006 mol) of phenol and 0.5 mL 5% KOH in 10 mL of water. Prepare a solution of 1.0 g (0.006 mol) sulfanilamide and 2 mL (0.012 mol) 6 *M* HCl in 10 mL of deionized water. Cool the sulfanilamide solution in an ice bath with magnetic stirring. Once the solution has cooled, form the diazonium salt by slowly adding 10 mL of ice cold 0.7 *M* $NaNO_2$ solution to the stirred sulfanilamide solution. After the addition is complete, let the solution stir for an additional 3-4 minutes. Remove the diazonium salt solution from the stir plate and keep it ice cold. Begin stirring the phenol solution while it is still in its ice bath. Add the cold diazonium salt solution dropwise, with constant stirring, to the cold phenol solution. Midway through the addition, and again when the addition is complete, adjust the pH to 8 (pH paper) with 5% KOH. After the final pH adjustment, continue stirring for 15 minutes. Filter the product and wash the solids with cold water until the filtrate registers neutral pH (~200 mL). Maintain suction until the filter cake appears dry. Remove the filter cake (use gloves if needed to prevent your hands from becoming dyed) weigh the solid. crystallize the product from 60% methanol-water solution. You will need about 40 mL of solution for each gram of solid. Add the solid to the appropriate amount of methanol-water solution. Heat on a hot plate to dissolve the solids. If the solution is nearing a boil with solids still present, quickly filter while hot. Allow the hot solution to cool to room temperature for 15 minutes and then in an ice bath for 30 minutes. Vacuum filter the crystals and let dry until next week. Obtain a melting point, GC and UV-Vis spectrum for your crystals.