The Technique of Column Chromatography and Salicylic Acid Chemistry

Introduction

Salicylic Acid and its Derivatives as Pain Relievers

Aspirin is among the most fascinating and versatile drugs known to medicine, and it is among the oldest. The first known use of an aspirin-like preparation can be traced to ancient Greece and Rome. Saligen, an extract of willow and poplar bark, has been used as a pain reliever (analgesic) for centuries. In the middle of the last century it was found that saligen is a glycoside formed from a molecule of salicylic acid and a sugar molecule. Salicylic acid is easily synthesized on a large scale by heating sodium phenoxide with carbon dioxide at 150°C under slight pressure (the Kolbe synthesis):

Salicylic acid is a white crystalline compound that is commonly used in ointments and plasters for the removal of warts. Unfortunately, however, salicylic acid attacks the mucous membranes of the mouth and esophagus and causes gastric pain that may be worse than the discomfort it was meant to cure. Felix Hoffmann, a chemist for Friedrich Bayer, a German dye company, reasoned that the corrosive nature of salicylic acid could be altered by addition of an acetyl group. In 1893, the Bayer Company obtained a patent on acetylsalicylic acid, despite the fact that it had been synthesized some 40 years previously by Charles Gerhardt. Bayer coined the name Aspirin for their new product to reflect its acetyl nature and its natural occurrence in the Spiraea plant. Over the years the company has allowed the term aspirin to fall into the public domain so that it is no longer capitalized. The manufacturers of Coke and Sanka work hard to prevent a similar fate befalling their products.

In 1904, the head of Bayer, Carl Duisberg, decided to emulate John D. Rockefeller's Standard Oil Company and formed an *interessen gemeinschaft* (IG, a cartel) of the dye industry (Farbenindustrie). This cartel completely dominated the world dye industry before World War I, and it continued to prosper between the wars, even though some of their assets were seized and sold after World War I. After World War I, an American company, Sterling Drug, bought the rights to aspirin. The company's Glenbrook Laboratories division still is the major manufacturer of aspirin in the United States (Bayer Aspirin).

Because of their involvement at Auschwitz, the top management of IG Farbenindustrie was tried and convicted at the Nuremberg trials after World War II, and the cartel broken into three large branches, Bayer, Hoechst, and BASF (Badische Anilin and SodaFabrik), each of which now does more business than DuPont, the largest American chemical company.

By law, all drugs sold in the United States must meet purity standards set by the Food and Drug Administration, so all aspirin is essentially the same. Each 5 grain tablet contains 0.325 g of acetylsalicylic acid held together with a binder. The remarkable difference in price for aspirin is primarily a reflection of the advertising budget of the company that sells it.

Aspirin is an analgesic (painkiller), an antipyretic (fever reducer), and an anti-inflammatory agent. It is the premier drug for reducing fever, a role for which it is uniquely suited. As an anti-inflammatory, it has become the most widely effective treatment for arthritis. Patients suffering from arthritis must take so much aspirin (several grams per day) that gastric problems may result. For this reason, aspirin is often combined with a buffering agent. Bufferin is an example of such a preparation.

The ability of aspirin to diminish inflammation is apparently due to its inhibition of the synthesis of prostaglandins, a group of C-20 molecules that enhance inflammation. Aspirin alters the oxygenase activity of prostaglandin synthetase by moving the acetyl group to a terminal amine group of the enzyme.

If aspirin were a new invention, the U.S. Food and Drug Administration (FDA) would place many hurdles in the path of its approval. It has been implicated, for example, in Reyes syndrome, a brain disorder that strikes children and young people under 18. It has an effect on platelets, which play a vital role in blood clotting. In newborn babies and their mothers, aspirin...
can lead to uncontrolled bleeding and problems of circulation for the baby- even brain hemorrhage in extreme cases. This same effect can be turned into an advantage, however. Heart specialists urge potential stroke victims to take aspirin regularly to inhibit clotting in their arteries, and it has been shown that one-half tablet per day will help prevent heart attacks in healthy men.

Aspirin is found in more than 100 common medications, including AlkaSeltzer, Anacin ("contains the pain reliever doctors recommend most"), APC, Coricidin, Excedrin, Midol, and Vanquish. Despite its side effects, aspirin remains the safest, cheapest, and most effective nonprescription drug. It is made commercially employing the same synthesis used here.

Methysalicylate is a component of oil of wintergreen and is used in flavoring candies.

**Synthesis**

Synthetic organic reactions are rarely rapid or quantitative, that is, they do not give a 100% yield of product. Organic reactions can take place quite slowly, even at elevated temperatures and since time is money, reactions are stopped after a reasonable amount of time even if some starting material remains. More importantly, organic reactions often yield unwanted by-products. These tend to increase with time again limiting the length of time a reaction should be run. Therefore, typical yields or organic products are about 80% with yields of 95% being hard to achieve. Organic chemists spend a lot of time searching for that optimal combination of reaction conditions – time, temperature, concentrations of reactants or catalysts, etc. – to give the best yield possible and then devising a way to separate this yield from by-products or starting materials.

In their search for optimal reaction conditions, organic chemists use a number of different methods to measure or monitor the formation of products or by-products and the disappearance of starting materials. In the two reactions involved in this experiment, we will be using thin layer chromatography (TLC) to monitor the progress of the reactions. We will also use column chromatography to purify a mixture of product and by-products. The reactions are shown in Scheme I and Scheme II below:

**Scheme I – Hydrolysis of methyl salicylate (oil of wintergreen) to salicylic acid.**

\[
\begin{align*}
\text{methyl salicylate} & \xrightarrow{\text{NaOH}} \text{2} \\
\text{salicylic acid} & \xrightarrow{\text{H}_2\text{SO}_4} \text{4}
\end{align*}
\]

**Scheme II – Acetylation of salicylic acid to yield acetylsalicylic acid (aspirin) and methyl salicylate to methyl acetylsalicylate.**

\[
\begin{align*}
\text{salicylic acid} & \xrightarrow{\text{acetic anhydride}} \text{5} \\
\text{methyl salicylate} & \xrightarrow{\text{acetic anhydride}} \text{6}
\end{align*}
\]
Methyl salicylate has two functional groups: an ester and a phenol. Esters can be hydrolyzed with base to the corresponding carboxylic acid. When a strong base such as NaOH is added to methyl salicylate three reactions occur: 1) the phenolate ion, 2, is formed, 2) ester hydrolysis to produce the sodium carboxylate 3 (Scheme I). Addition of a strong acid protonates both the phenolate and the carboxylate anions to give salicylic acid, 4.

The hydrolysis reaction is very slow at room temperature but the reaction time can be shortened when the mixture is heated. A general rule of thumb is that the reaction rate approximately doubles for every 10°C rise in temperature. The difference between room temperature (~20°C) and boiling water (~100°C) is 80°. Therefore, the reaction rate increases by a factor of 2^3 or 256.

Different students will use different amounts of NaOH and/or different reaction times for the hydrolysis reaction (Scheme I) and will determine the relative amount of salicylic acid product, 4, and methyl salicylate starting material, 1, for each set of conditions. The crude product from this reaction, containing both product 4 and reactant 1, will then be acetylated it using acetic anhydride (Scheme II). The crude reaction product from the reactions in Scheme II will be analyzed by TLC to determine the relative amounts of the four possible products, 5 and 6, and any residual starting materials, 1 and 4.

Although the reactions in Schemes I and II are standard introductory organic chemistry experiments, the variations of reaction conditions and examination of crude mixed products have been done only a few times before, and so we will have to carefully follow each step, making adjustments as necessary. There are no guarantees that this experiment will go completely as outlined. This is research, and it is good to remember that old adage, “If research were easy, they would call it search, instead of research.”

**Activities:**

- Read the Introduction sections of the Column Chromatography chapter, Chapter 9 of the Lab Guide.
- Read the background material on salicylic acid derivatives (included above).
- This study will involve three lab periods. On **Day One**, you will carry out the hydrolysis reaction (Scheme I) and isolate the crude product.
- On **Day Two**, run the acetylation reaction.
- On **Day Three**, column chromatography will be carried out on the reaction mixture to see whether you can isolate enough (~20 mg) of any of the possible compounds for spectral analysis by IR and ^1^H NMR and possibly a GC-MS.
- A special handout for the flash column chromatography procedure can be downloaded on the course website.

**PreLab:**

Your chemical data table should include the chemicals shown in Schemes I and II above unless already on the Common Shelf Chemical Data Table.

Answer the assigned questions in Chapter 9.

Be sure to include the “Chromatographic Behavior & Spectral Features Comparisons” section. That is, predict the Rf values of compounds 1, 4, 5, and 6 with respect to each other, that is, which is most polar, least polar. Also, do a spectral comparison (^1^H NMR and IR) of these compounds.
Day One: Procedure for the Hydrolysis of Methyl Salicylate to Salicylic Acid

First, make a 5 mL solution of 4M NaOH in water. When weighing out the NaOH pellets work quickly and do not touch them with your hands. Measure out 3.5 mL of the 4M NaOH (aq) solution and place in a large test tube, 20 x 150 mm. Drop a 1/2” stir bar into the test tube. Add 230 mg of methyl salicylate using a 100 – 1000 µL pipetor set to the correct volume. Ask for help if you don’t know how to use a pipeter. A white solid will quickly form. Clamp the tube and, while stirring, heat the solution to a gentle boil using a sand bath mounted above a magnetic stir plate. Continue heating gently for 30 minutes, then cool to room temperature.

Workup: Cool the reaction in a beaker of ice water. Add 3 M sulfuric acid to the tube in 0.5 mL increments, (about 10 drops from a Pasteur pipet) until a white precipitate forms and remains when the tube is stirred. Add an additional 10 drops of acid to ensure complete protonation of the salicylic acid. Using a clean glass stirring rod, test the pH of the reaction using pH paper; the solution should be acidic. Pour the resulting solution or slurry into a 125-mL separatory funnel, rinsing the test tube out into the separatory funnel with a small amount of CH₂Cl₂. Extract the aqueous layer with three 10 mL portions of CH₂Cl₂, combining the CH₂Cl₂ extracts in a 125 Erlenmeyer flask. Add enough anhydrous Na₂SO₄ to cover the bottom of the flask and enough such that some Na₂SO₄ does not clump up. Swirl occasionally for 5 min and decant the CH₂Cl₂ into a tared 50-mL Erlenmeyer flask. Allow the CH₂Cl₂ to evaporate in your locker until the next lab period.

Day One Two: Procedure for the Acetylation and Methyl Salicylate to their Acetyl Derivatives

Determine the weight of the crude product mixture from above. You should have at least 250 mg of crude product. Add 600 µL of acetic anhydride using a pipeter to the flask. Everyone in the class can use the same pipeter for the addition of the acetic anhydride. Add one drop of 85% phosphoric acid from a Pasteur pipet and then loosely cork the top of the flask. Swirl the flask gently to dissolve the salicylic acid and then place the tube in a beaker of hot tap water. Leave the flask clamped in the hot water for 15 minutes, replacing the hot tap water every 5 min.

Workup: Remove the flask from the water bath, add 2 mL of water and swirl for a few min to decompose the excess acetic anhydride. Pour the resulting solution or mixture into a 125-mL separatory funnel, rinsing the test tube out into the separatory funnel with a small amount of CH₂Cl₂. Extract the aqueous layer with three 10 mL portions of CH₂Cl₂, combining the CH₂Cl₂ extracts in a 125 Erlenmeyer flask. Add enough anhydrous Na₂SO₄ to cover the bottom of the flask, swirl occasionally for 5 min and decant the CH₂Cl₂ into a tared 50-mL Erlenmeyer flask. Use this solution in the TLC procedure below.

Day Three: Column Chromatography

First, find a suitable mobile phase for the column via TLC analysis. Start by trying 30% EtOAc in hexanes. Once you’ve found a suitable mobile phase (one that elutes the highest spot to an Rf of 0.4), you can start the column. Column chromatography or flash chromatography will be used to separate your crude product into its pure components. Please download the instructions of flash chromatography from the course website and read the Introduction section of Chapter 8 in the Lab Guide before beginning the column.

Final Report

Mark all compound spots on all the TLC plates with a pencil. Tape your developed TLC plates in your notebook with the wide transparent tape from the dispenser on the side shelf. Determine the weights of the pure compounds isolated. Calculate the theoretical yield and the % yield of each compound. Calculate the percent recovery of salicylic acid. Present a short discussion on the interpretation and quality of your TLC and CC results.

PostLab Questions:
Answer the assigned questions in Chapter 9.

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