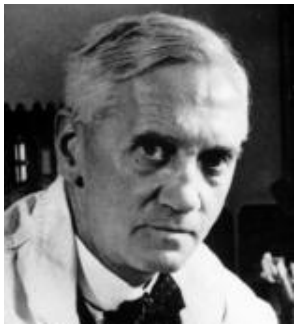
Experiment 41

SYNTHESIS AND TESTING OF ANTIBIOTICS

Fig. 41-1



Sir Alexander Fleming shared the Nobel Prize for medicine with Howard W. Florey and Ernest Chain in 1945.
http://nobelprize.org/nobel_prizes/medicine/laureates/1945/

Text Topics and Comments

Preparation of amides and/or methyl salicylate and testing of antibacterial activity. The artificial boundaries between organic chemistry and biochemistry are disappearing. These experiments were derived from a recent articles by R. D. Whitaker, et. al. (see references) on penicillin synthesis and E. M. Sega, et.al (see references) on methyl salicylate synthesis. A. M Sousa et. al., has very recently published a much simpler antimicrobial test. This experiment has not received any testing in our laboratory. Instructions for the synthesis portions are included below but the original articles and the online supporting information will need to be consulted for instructions on the assay of the antibiotic activity of the products.

Discussion

Antibiotics play an extremely important role in human history. In the mid 1930's, sulfa drugs such as Prontosil became powerful tools to use against bacterial infections. Technically, sulfa drugs are bacteriostatics as contrasted with bacteriocides. Sulfa drugs prevent the growth and reproduction of bacteria but do not kill them. The need for drugs that could fight infection from wounds in World War II accelerated the search for antibiotics. Alexander Fleming had noticed the antibacterial action of Penicillin G in 1928 but it was not until the middle of World War II that Florey and Chain were able to produce a deliverable form of the drug. Penicillin G is sensitive to stomach acid and had to be delivered by injection. Since that time, pharmaceutical chemists, based on acid-base concepts, have been able to design and subsequently synthesize penicillins that are resistant to stomach acid and can be taken orally.

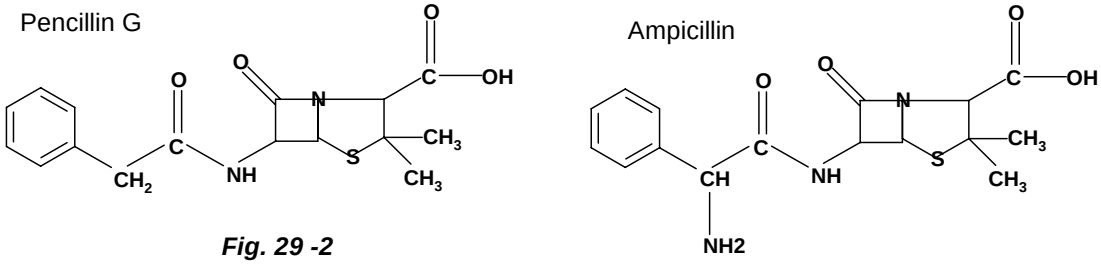
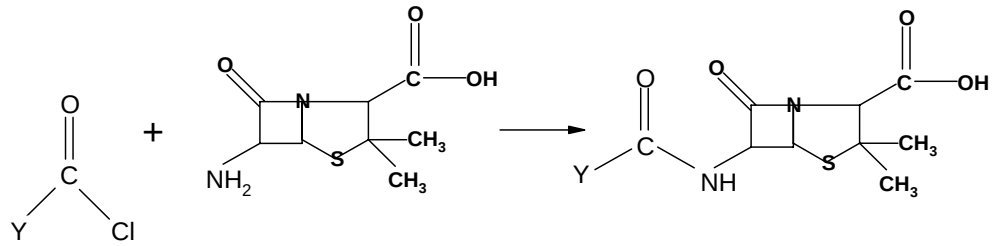


Fig. 29 -2

Ampicillin has an amino acid group that is protonized in acid media. The protonized form of ampicillin is stable in acid enabling the administration of ampicillin by oral means. Another amino containing penicillin, amoxicillin, seems to be even more commonly prescribed by doctors.

You learned in your studies of the reactions of carboxylic acid derivatives that one of the easiest laboratory methods for the synthesis of amides involves the reaction of an acid chloride with an amine. This type of reaction will be used to synthesize several different penicillins as illustrated in the next scheme.

Fig. 29-3

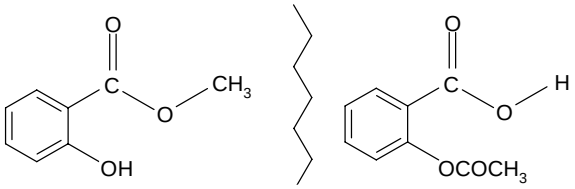


acyl chloride 6-aminopenicillanic acid penicillin derivative

Your instructor will assign an available acyl chloride to you or your group and your goal will be to synthesize a penicillin derivative, identify the derivative and test it for antibacterial activity. Some of the acyl chlorides that could be available are listed below. The original article included 15 acyl chlorides but that list has been pared down here for various reasons including price. One addition to the list is phenylglycine chloride hydrochloride as it could potentially yield ampicillin. This acyl chloride was not tested in the original article and there is a significant chance that it will not work in this synthesis.

benzoyl chloride
cinnamoyl chloride
phenylacetyl chloride
phenoxyacetyl chloride
phenylglycine chloride hydrochloride

Fig. 29-4



methyl salicylate aspirin

Another much simpler option is to convert aspirin to methyl salicylate and test the aspirin and methyl salicylate for antibiotic properties.

Procedure

Synthesis of a penicillin. This reaction must be carried out in a hood. Because of limited hood space and the cost of the starting materials, it might be advisable to work with a partner on this experiment. Using a magnetic stirrer in an Erlenmeyer flask, dissolve 1.05 g of $NaHCO_3$ in a mixture of 3 mL of acetone and 9 mL of water until the solution is homogeneous. Continue stirring and add 0.54 g of 6-aminopenicillanic acid to the solution.

In a separate small container, prepare a solution of 0.0050 moles of your assigned acyl chloride in 1 mL of acetone. After mixing, add acetone dropwise only if necessary until a homogeneous solution is achieved.

Add the acyl chloride/acetone solution dropwise to the 6-APA solution over a period of five minutes. Stir for an additional 40 minutes with the magnetic stirrer. While stirring, place 3 test tubes in an ice bath containing 10 mL of deionized water in the first, 2 mL of 5 M H_2SO_4 in the second and 6 mL of n-butyl acetate in the third. After 40 minutes, transfer the reaction mixture to a small separatory funnel (about 50 mL) and extract twice with two 6 mL portions of room temperature n-butyl acetate. Discard the organic layers as instructed. Transfer the aqueous layer to a small beaker and cover with the 6 mL of cold n-butyl acetate. Using the cold 5 M H_2SO_4, add the acid dropwise and cautiously to the aqueous layer while monitoring the pH with pH paper until the pH drops to 2. Return the mixture to the separatory funnel, shake and draw off the aqueous layer. Wash the n-butyl acetate layer with cold water and dry it over anhydrous sodium sulfate for several minutes. Decant or filter to remove the drying agent and transfer to a small beaker. Add 1 mL of a 50% solution of potassium 2-ethylhexanoate in butanol. Cool in an ice bath and scratch to induce

crystallization. If crystallization occurs, collect the crystals using vacuum filtration and wash with a small amount of dry acetone. Store the crystals in a labeled vial. If crystallization does not occur, store the solution in a refrigerator in a stoppered flask for several days. Hopefully, crystallization will occur. Collect the crystals as above. Perform tests to determine the identity of your product.

Synthesis of methyl salicylate. Add three aspirin tablets to a mortar and grind them into a powder with the pestle. Add 5 mL of methanol to the pestle and mix. Gravity filter the slurry to remove the tablet binding material. Very carefully, add 5 drops of concentrated sulfuric acid to the filtrate and heat in a hot water bath for 30 minutes. After cooling, add the mixture to a small separatory funnel and add 5 mL of saturated aqueous $NaHCO_3$. Extract the mixture twice with dichloromethane and combine the organic layers. Dry the organic layer with anhydrous sodium sulfate. Evaporate the solvent with a rotary evaporator. Determine the residual liquid mass and verify its identity using at least two of the three ir, nmr and refractive index. Based on the amount of acetylsalicylic acid in aspirin tablets, calculate the percent yield of the process.

Biological Assay of the synthesis products. For instructions on this topic, see the references below and the online supporting material. Only *Journal of Chemical Education* subscribers have access to these sites but the journals (see References) should be available in your library.
http://pubs.acs.org/doi/pdf/10.1021/ed100194v
http://pubs.acs.org/doi/suppl/10.1021/ed100194v/suppl_file/ed100194v_si_001.pdf
http://pubs.acs.org/doi/abs/10.1021/ed086p475
http://pubs.acs.org/doi/suppl/10.1021/ed086p475
http://pubs.acs.org/doi/pdf/10.1021/ed200701w
http://pubs.acs.org/doi/suppl/10.1021/ed200701w/suppl_file/ed200701w_si_001.pdf

Extensions. Test other materials such as over the counter topical antibiotics for activity.

References

Whitaker, R. D.; Truhlar, L. M.; Yuksel, D.; Walt, D. R.; Williams, M. D. *J. Chem. Educ.*, **2010**, *87*, 634-636.
Sega, E. G.; Clarke, J. *J. Chem. Educ.*, **2013**, *90*, 1658-1661.
Hartel, A. M.; Hanna, J. M. *J. Chem. Educ.*, **2009**, *86*, 475-476.
Sousa, A. M.; Waldman, W. R. *J. Chem. Educ.*, **2014**, *91*, 103- 106.

Prelaboratory Preparation - *Experiment 41*

First, be sure to list all the goals of the experiment. Use the Internet to determine if there are any physical properties that can be used to determine if your penicillin synthesis has been successful. Also use the Internet to see if you can find a reason why amoxicillin is more commonly used than ampicillin. Draw the structure of amoxicillin. Locate the synthesis used for this experiment on the *Reaction-Map of Organic Chemistry* in ***Appendix C*** and include the reaction number in your report. Methyl salicylate is commonly called oil of wintergreen. What are some of its uses and why is it called oil of wintergreen?

Observations

Report all relevant observations including masses, melting ranges and ^{1}Hnmr.

Conclusions

This section should include the following:

1. Were the goals of the experiment achieved? Explain your answer.
2. Was your synthesis successful? Provide all available evidence in support of your conclusion.
3. If a biological assay was performed, rank the activities of the penicillins or other products synthesized by your class. Were any of the other penicillins more effective than Penicillin G?
4. Is it possible from the very limited class results to make any comments about a structure activity relationship?