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The synthesis of an oxytetracycline derivative

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ABSTRACT

Antibiotics have been used for the past few centuries, and their misuse and overuse has contributed to the rise of antibiotic-resistant pathogens. Therefore, it is of great importance to synthesize novel antibiotics that may be effective against these new superbugs, which is the aim of this research. For this research project, my objective was to take a known antibiotic and change one or more of its functional groups to synthesize a novel antibiotic. Oxytetracycline was used as the starting material for the reaction with hydrochloric acid and n-chlorosuccinimide as reagents. The product of the reaction was characterized using analytical techniques such as NMR and IR spectroscopy.

Keywords: *antibiotics, organic synthesis, oxytetracyline, spectroscopy.*

INTRODUCTION

From the beginning of our existence on earth to today, we have been living with an infinite number of microorganisms. Those microorganisms are found all over the place and are considered omnipotent. Some of them are good for us to consume and work to assist us, while others are harmful and can cause an individual to get sick and even die. Bacteria are a type of the many microorganisms which can fall in both groups. Some bacteria are harmful and can cause infections which are the development of an organisms where they do not usually grow (Madigan et al. 2015). In order to deal with those infections various researchers observed diverse antimicrobial agents. Antibiotics are substances that are used to inhibit the growth of or destroy bacteria (Madigan et al. 2015). Without antibiotics, our life expectancies would have been greatly reduced. It is important to use the proper antibiotics to deal with the specific bacterial infections since out of all the antibiotics produced in nature, only about one percent can be used (Madigan et al. 2015), the rest of the antibiotic are potent to human consumption (Kuperman and Koren 2016).

There are multiple families of antibacterial drugs with various purposes. Some of the antibiotics are able to disrupt the bacterial DNA or affect its protein synthesis which leads to the bacteria not being able to properly reproduce (Getino et al. 2015). The main antibiotic family that I am planning on working with is Tetracycline, specifically the derivative Oxytetracyline. The structure of Oxytetracycline is composed of four rings containing different substituents such as numerous hydroxyl groups, amides. While looking to modify that structure, I observed most of the alterations happening at the location of the carbon C_6, C_7, C_8 and C_9.

Tetracycline and its derivatives are a part of a family known as broad-spectrum antibiotics because they are usable against both gram positive and gram negative bacteria, as well as Chlamydia and Rickettsia (Madigan et al. 2015). Gram-negative bacteria possess a peptidoglycan wall unlike gram positive. It

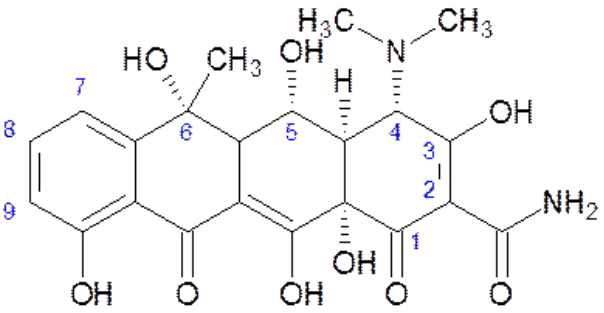

Figure 1. Oxytetracylcine

is currently known that bacteria have two different ways to resist Tetracycline, the efflux pump and the ribosomal protection (Chopra and Roberts 2001). The efflux pump is used by some bacteria to pump the antibiotics out of the cell and the ribosomal protection consists of the bacteria making a protein which it uses to stop the Tetracycline from biding to the 30s ribosomal site causing the cells to have both the antibacterial to become ineffective. The family of tetracycline's were named that way because they are made out of four six membered rings with different substituents attached to them (Salyers and Dixie 2005). Tetracycline derivatives include Doxycycline, Methylcycline, Democlocycline, and Oxytetracycline (Salyers and Dixie 2005).

It is important that researchers are able to come up with new derivatives of antibiotics. When they were first discovered in the 40's, as well as today in developing countries, they were not properly monitored (Mensah and Ansah 2016). Today, one big issue that the world is dealing with is the fact that the bacteria became resistant to many antibiotics which are overused to get rid of infections. This is the reason why today we have resistant bacteria such as MRSA which stands for Methicillin-Resistant *Staphylococcus aureus*.

MATERIALS AND METHODS

For this research, Oxytetracycline, which is a derivative of Tetracycline, was used, while adapting the method from United States Patent (Blackwood and Stephens 1978)

Synthesis

Oxytretracycline hydrochloride, 40g was dissolved in a mixture of 4 ml of concentrated hydrochloric acid and 2000 ml of water while stirring in a 3000 ml round bottom flask. 14 g of N-chlorosuccinimide was added to the flask and stirred for 4 hours at room temperature. After stirring for 4 hours, the mixture changed color from a black to a brick red color. This was placed in a centrifuge for 10 mins. The solid was pelleted at the bottom, and the supernatant was poured off and discarded. The solid was then washed with DI water and placed in a Buchner funnel attached to a filter flask and was attached to an air pump and left to air dry overnight.

Purification

To purify the product, 5.0 g. of crude was dissolved in water in a 250 ml beaker while stirring at room temperature. After being dissolved, it was placed in a separatory funnel and 320 ml of ether was added. The water layer was discarded, and the ether layer was washed with 60 ml x 5 of DI water which was decanted. The ether solution was placed in a 125 ml Erlenmeyer flask and Sodium sulfate was added to dry the solution and the ether layer was decanted in a round bottom flak where a simple distillation was set up and pure ether was collected leaving the product in the round bottom flask. A 100 ml of water was added to the product to remove it from the round bottom flask, which was placed in a 125 vml Erlenmeyer flask and left to stir for 2 hours and filtered using a Buchner funnel and a filter paper. The product was left to air dried overnight.

The product was characterized using IR Nicolet Avatar 320 FT-IR and NMR Varian Inova 400 MHz spectroscopy.

RESULTS

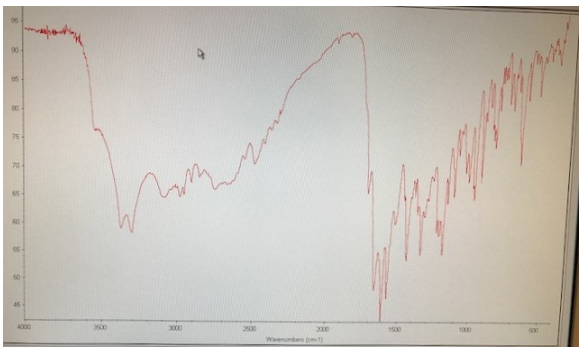

Figure 2. IR of Starting Material

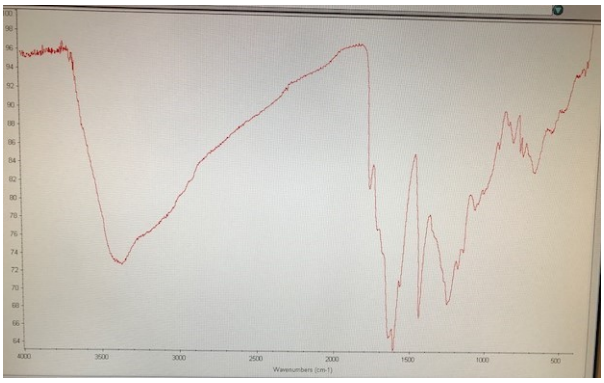

Figure 3. IR of Product

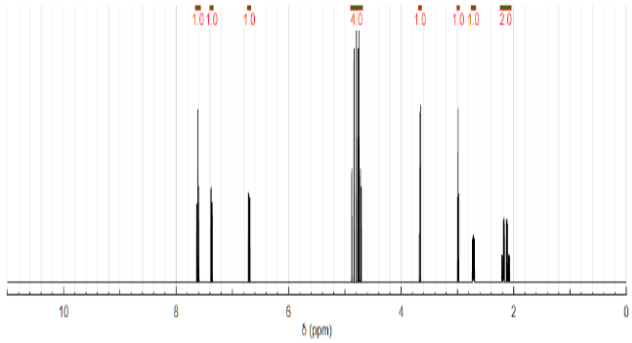

Figure 4. Predicted ^{1}H NMR of Starting Material using the website nmr.org

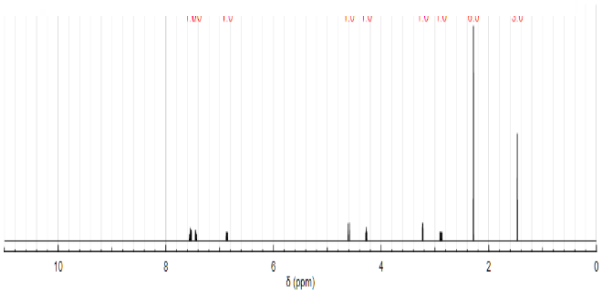

Figure 5. ^{1}H NMR of Starting Material

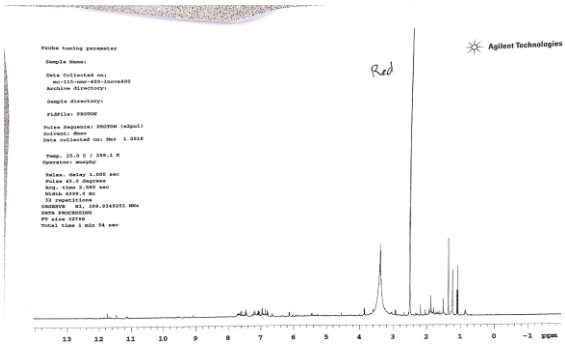

Figure 6. ^{1}H NMR of Product

DISCUSSION

A similar procedure was performed by Blackwood and Stephens (1978) with Tetracycline as a starting material instead of Oxytetracycline.

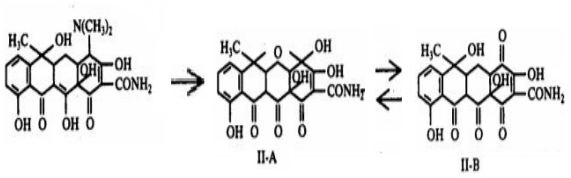

Figure 7. Tetracycline reaction leading to the formation of products identified as II-A and II-B.

Their results show: Found: C,51.96;H, 3.83;N, 3.22; K, 8.18; H_2O, 1.71, Calcd. For $C_{20}H_{16}O_9NK.O.5H_2O$: C, 52.0% H, 3.8; N, 3.2; K, 8.2; H_2O, 1.7

The reaction resulted in the structure 4-hydro-4-dedimethylaminotetracyline (Blackwood and Stephens 1978). Also, the reaction done in the patent showed a mixture of product but the structure of the tetracycline was mostly conserved, and the product collected in the patent contained the four rings which. On the other hand, my product did not show any of the similar peaks. After the NMR analysis, I found that the product NMR was different from the predicated and it looked like it was not a pure compound. I ran a TLC to see how many compounds I was observing, but it was not successful on silica gel column chromatography. The quest to discover or synthesize new antibiotics is at an all-time high. Related to this project, further research needs to be done in order to properly evaluate and study the effectiveness of this novel compound.

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