Electrophilic Aromatic Substitution of L-Tyrosine

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**Abstract:**

In this experiment, an Electrophilic Aromatic Substitution was carried out on L-Tyrosine to produce an acceptable percent yield of our product, nitrotyrosine. L-tyrosine reacted with sulfuric acid and nitric acid to produce an important non-aromatic intermediate. The hydrochloric acid and the sulfuric acid were combined to create the necessary compound, which acted as the electrophile in the first step of the reaction. The intermediate that was formed created a new sigma bond due to the electrophile, giving this intermediate the name of *sigma-complex.* The intermediate then experienced a deprotonation close to the site of the new sigma bond, resulting in the regeneration of a pi bond. The theoretical product yield and experimental product yield were used to calculate and acquire an acceptable percent yield.

**Introduction:**

This reaction is widely used and is very useful. Application of this reaction can be seen in fields all the way from pharmaceuticals to chemical engineers(2,3). Specific examples of this reaction can be seen in the processes such as Xanthoproteic test and in Friedel-Crafts Alkylation (2). Electrophilic Aromatic Substitution takes the basic concepts of substitution and aromaticity and combines them. “Substitution is one of the oldest reactions in both organic and inorganic chemistry, and over time, it has become an extremely important form of transformation”(1). The main concept needed about substitution for this reaction is that there is always an electrophile waiting to kick out a potential leaving group. There are two types of substitution reactions, namely S­N1 and SN2 reactions. SN1 reactions are characterized by their two step reactions and can mainly be differentiated from the SN2 reactions by seeing what they substitute. SN1 reactions prefer to substitute primary and secondary carbons, whereas SN2 reactions prefer to substitute tertiary carbons. The electrophilic aromatic substitution is a SN1 reaction.

The aromatic compounds used for this type of reaction are generally stable, yet it does react with an electrophile. This is the first step of the reaction, which is also known as the rate determining step for an SN1 reaction (2). As the aromatic ring is quite stable, it does not immediately react with the electrophile, hence the term rate determining. As a pi bond on the aromatic ring attacks the electrophile, a new sigma bond is made. There are many possible attacks that could be done after the first step, all in relation to where an electron donating group lies in relation to the leaving group. This, however, will be discussed in detail later. The loss of aromaticity leads to higher reactivity, which then leads to the rapid second step of this reaction. As the molecule is deprotonated with the help of a base, the molecule becomes aromatic once more.

**Results:**

**Theoretical Yield**

5 g L-tyrosine x ( ) x ( ) x ( ) = 6.24 g 3-nitrotyrosine

**Actual Yield**

4.084 g

**Percent Yield**

3-nitrotyrosine x 100 = 65.45%

**Melting Point Range of Product**

161⁰C – 17⁰C

**Reaction Mechanism:**

**Diagram

Description automatically generated**

**A picture containing application

Description automatically generated**

**Materials and Methods:**

5 g of L-tyrosine was collected and dissolved in 20 mL of deionized water. This solution was kept in a 100 mL RBF, along with a stir bar. An ice bath was then prepared on top of a stirring and heating mantle. In two separate test tubes, 3.6 mL of concentrated sulfuric acid and 4.7 mL of concentrated nitric acid were collected. These acid test tubes were then kept in the ice bath to chill. While allowing this to chill, a Claisen adaptor was fitted to our clamped RBF. The neck directly above the RBF on the Claisen adaptor was fitted with a separatory funnel, and the second neck was fitted with a Westin condenser. As the acids were chilled to a desired temperature (moderately cold to touch), the sulfuric acid was poured into the nitric acid, and then left back in the ice bath to chill for a few more minutes. After a suitable time had elapsed, the well mixed acid solution was added directly into the closed separatory funnel. After all the acid solution was added to the funnel, a small-enough opening was obtained in the separatory funnel to allow 1 drop out every 2-3 seconds. The stirring mantle was also turned on during this process to allow a thorough mixture of the solution. Once all the acid was drained from the funnel, the RBF was left in the ice bath with the stir bar still stirring for about 15 minutes. Following this, the RBF was then placed in a lukewarm water bath to slowly increase the temperature of it to around 40⁰C. The temperature was then stabilized to between 40⁰C and 50⁰C for 30 minutes through the use of the heating mantle. It is important to note that the Claisen adaptor along with the separatory funnel and Westin condenser was still attached to the RBF with the stirring continued. Following these 30 minutes, the RBF was then transferred to an ice bath with the stirring turned off. After waiting for around 45 minutes, some of the solution eventually crystallized. The solids in the RBF were then extracted and kept into a Buchner funnel as part of a vacuum filtration apparatus. The solid was vacuumed filtrated as much as possible and rinsed with an unmeasured amount of ethyl acetate. The resulting gummy solid was then weighed and then sampled for melting point and proton NMR. A small portion of the product was also repeatedly rinsed in ethyl acetate to try to dry it out more for the melting point analysis.

**Discussion:**

This procedure went better than expected as we were not expecting to obtain any solid product. The procedure could have been executed better without a few mistakes. At the beginning of the procedure, not all of the deionized water and L-tyrosine solution was added to the RBF due to some spillage when transferring it from its prior vessel. When adding the combined acid solution to the separatory funnel, a vapor was noticeable. This indicated that we had either not let the solution chill all the way or had not mixed the solution well enough. Towards the end of the procedure, we made the mistake of not flattening the gummy solid, fearing that the filter paper would tear. This meant that we had a high chance that not all the excess acid was drained from our product. However, our fear was justified as we had seen the filter paper almost torn when extracting the remaining solid in the Buchner funnel. The product we extracted was a gummy, red solid. It is important to note that as the reaction proceeded, the mixture got darker. This likely indicates a higher degree of conjugation.

Through an analysis of our proton NMR and melting point, we could tell that our product was not particularly pure. The melting point analysis gave us a broad range of 161⁰C – 176⁰C, which was very far off from the theoretical melting point of 230⁰C. The low and broad range of the melting point was most likely due to impurities in the product. This was expected, as the dried solid still most likely contained ethyl acetate, which was used in an attempt to further solidify the product. This impurity was also seen in the H1 NMR spectra. The expected integral ratios were 3:1:2. The integrals produced, however, were a ratio of 3:3:2. This was also due to the excess amount of ethyl acetate still remaining in our solution.

Along with this, we analyzed our expected IR and C13 NMR. These all showed that the expected product of 3-nitrotyrosine was produced. This was indicated by the IR spectrum given, which indicated the presence of a NO­2 group (absorption at 1300.81 cm-1 and 1525.48 cm-1). Additionally, the H1 NMR and C13 NMR provided showed the presence of aromatic carbons, hydrogens on an aromatic ring, and an amine (on H1 NMR). Looking towards the aromatic region on the H1 NMR, one singlet and two doublets were present, indicating that the predicted product was in fact 3-nitrotyrosine through electrophilic aromatic substitution. This product was predicted because of its stability.

Following the first step of the reaction, three possible configurations of the molecule were available. In relation to our electron donating group (the hydroxyl group on the aromatic ring), there were three possible positions available for substitution. These attacks were the ortho attack (one bond away from the electron donating group), the meta attack (two bonds away from the electron donating group), and the para attack (three bonds away from the electron donating group) (1, 2). The ortho and para attacks are naturally more stable because they have more resonance forms. The para position on the aromatic ring for L-tyrosine already contained another group (2-aminopropanoic acid). This lead to our prediction of the attack happening on the ortho position, which we predicted would create the compound 3-nitrotyrosine.

**Conclusion:**

Electrophilic aromatic substitution is a very useful reaction that can be applied to many fields. The electrophilic aromatic substitution of L-tyrosine took place in two steps. The first step was an ortho attack, leading to a non-aromatic (unstable) 6 carbon ring with a nitro group attached 1 bond away from the hydroxyl group. The second step was rather quick, as the hydrogen sulfate (acting as our base) in our solution deprotonated the compound. This resulted in our predicted product of 3-nitrotyrosine. The product produced by our reaction was not pure, however it was still produced. This was proven by an analysis of the melting point, IR, H1 NMR, and C13 NMR that were taken or given at the end of the procedure. This experiment was successful in producing the desired product with a percent yield of 65.45%, however further purification would be necessary to produce a more refined product.

**References:**

(1) Liu, Shubin. “Quantifying Reactivity for Electrophilic Aromatic Substitution Reactions with Hirshfeld Charge.” *The Journal of Physical Chemistry A*, vol. 119, no. 12, 2015, pp. 3107–3111., https://doi.org/10.1021/acs.jpca.5b00443.

(2) Martin, Christopher B., Electrophilic Aromatic Substitution – Use of Organic Chemistry as a Tool for the Determination of Protein Content for Nutritionists. *Organic Chemistry II-Laboratory*, 2017.

(3) Stamenković, Nikola, et al. “An Analysis of Electrophilic Aromatic Substitution: A ‘Complex Approach.’” *Physical Chemistry Chemical Physics*, vol. 23, no. 9, 21 Dec. 2021, pp. 5051–5068., https://doi.org/10.1039/d0cp05245k.