

Chemistry R.E.U. Program

Tracey-Ann Samuels, Syracuse University, Syracuse, NY

INTRODUCTION

As a participant in the Chemistry R.E.U. program of Syracuse University I conducted research with Johannes Steinreiber of the Applied Biocatalysis Research Center at the Technical University of Graz, Institute for Organic Chemistry. His research was being carried out in order to successfully complete his doctoral degree. I became involved with his project due to my interest in conducting research within the biochemical field, in addition to Mr. Steinreiber's request for a student to assist him with his research project. Although his research was based in the field of Organic Chemistry, his project was specifically focused on catalyzing reactions with the aid of enzymes which had a translational connection to biochemistry. Specifically, I worked on his project that was centered on producing the synthetic amino acid, phenyl serine. This was achieved by reacting benzaldehyde with the naturally occurring amino acid, glycine. This reaction belongs to the class of aldol reactions that involves the condensation of two carbonyl compounds with one compound acting as the electrophile and the other as the nucleophile. Because of the mechanism of the reaction, two different products can be produced that differ because of their stereochemistry. In the case of the reaction with glycine and benzaldehyde, the hydroxyl group from the benzaldehyde and the amino group from the glycine can be oriented in different positions relative to each other that result in the production of two different products. If both groups are oriented in the same plane then the syn product is formed. If they are oriented in opposite planes then the anti product is formed. In this reaction, a mixture of both products is usually formed. The main goal of this research project was to therefore find reaction conditions that would cause selectivity for either the syn product or the anti product. The reaction was

catalyzed either by the enzyme threonine aldolase or by the strong base, sodium hydroxide. The reaction was carried out using two different catalysts in order to determine which would better produce the desired outcome. The reactions were tracked using thin layer chromatography and the products were analyzed using nuclear magnetic resonance (NMR) spectroscopy.

Additionally, different halogen substituted benzaldehydes were reacted with glycine to produce halogen substituted phenylserine. The purpose was to determine reaction conditions that would have stereochemical selection for only one product.

HYPOTHESIS

If glycine is reacted with benzaldehyde with the aid of enzymatic or chemical catalysts, then the synthetic amino acid, phenyl serine can be produced.

MATERIALS AND METHODS

Chemicals

glycine, benzaldehyde, pyridoxal -5'-phosphate (PLP), L-threonine aldolase (L-TA), D-threonine aldolase (D-TA), dimethylformamide(DMF), O-bromobenzaldehyde, sodium hydroxide (NaOH), O-fluorobenzaldehyde, 3-Phenoxy benzaldehyde, DMSO(dimethyl sulfoxide), DMF(dimethyl formamide).

Aldehydes: benzaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, hexanal, octylaldehyde, decanal, dodecanal.

Experimental Methods

The phenyl serine reaction involved combining glycine with benzaldehyde. The glycine/PLP mixture was prepared by dissolving 0.375 g glycine in 0.25 ml PLP and 2.25 ml buffer in order to produce the required concentration. For each reaction 2.5 ml of this mixture was added to each of the reaction flasks. 1.4 ml of buffer was added to half of the reaction flasks and 0.4 ml of buffer was added to the remaining half. 1ml of DMF (additive) was added to the flasks containing 0.4ml of buffer. 1 ml of L-TA enzyme was added to each flask and each of the aldehydes (listed above) was added to the flasks so that every aldehyde was reacted with and

without DMF. The flasks were left on a mixer overnight at a speed of 600 rpm. Thin-layer chromatography (TLC) was used to analyze the products of each reaction using CH_2Cl_2 [75]MeOH[20]NH₃[5] as a solvent. The TLC plates were developed using both the TLC solvent and ninhydrin. For the reaction using D-TA, a 1 mM solution of manganese chloride (MnCl_2) was made by dissolving 0.0471 g of the MnCl_2 in 374.3 ml water. The glycine/PLP mixture was made using 6 g glycine, 4 ml PLP and 32 ml buffer. 2.5 ml of the glycine/PLP mixture was added to each flask in addition to 4 ml of the MnCl_2 solution. Buffer and/or additive (DMSO) was added to the respective flasks and 1 ml of D-TA enzyme was added to each. The aldehydes were added and the flasks were left in a mixer overnight at 600 rpm. The products of the reaction were analyzed using TLC. The TLC solvent used was the same as that used in the L-TA reaction.

The reaction to produce phenyl serine was also done using 45% NaOH as a catalyst. The NaOH solution was prepared by dissolving 33.5g of NaOH pellets in 50ml distilled water. 0.566g of glycine was dissolved in 5ml water. 1.9ml of cold benzaldehyde was added to the reaction flask using a syringe. 2ml of NaOH was added dropwise while the flask was on an ice-bath over a magnetic mixer. The reaction was left on the mixer for an hour until the mixture solidified. The product of the reaction was isolated using a solvent extraction technique.

Approximately 4 drops of concentrated HCl were added to a vial containing the product.

Distilled water and dichloromethane were added and the flask was shaken. The top layer that formed contained the product. This layer was analyzed using TLC and reference spots of glycine and phenyl serine were included to be used for comparison to the formed product. The TLC solvent used was CH_2Cl_2 [16]MeOH[10]NH₃[1].

A new experiment was done using 2.2ml O-bromobenzaldehyde added to 0.57g of glycine that was dissolved in 5ml water. 2.2ml of the aldehyde was added using a syringe. 2ml of 45% NaOH was added dropwise and the mixture was placed in an ice bath. The ice bath was removed after 5 minutes while the flask remained over the magnetic mixer. The product was isolated using the same solvent extraction procedure as above. TLC was used to analyze the product and the solvent used was $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3(30\%)$ [16:10:1].

The reaction products from the benzaldehyde and bromobenzaldehyde reactions were worked up using 2ml HCl. The HCl was added to the reaction flasks and an additional 1ml of concentrated HCl was added to ensure that the entire solid had dissolved. The mixture was then put into a separatory funnel. For the bromobenzaldehyde reaction, the product formed the top layer and the benzaldehyde formed the lower, yellow colored layer. After extraction of each product, several drops of NaOH were added to adjust the pH of the solution to 7. The mixtures were put in the fridge overnight. The mixture from the benzaldehyde reaction was filtered and the solid residue was washed with cold water. The same procedure was done for the bromobenzaldehyde mixture and both of the products were weighed. The filtrates from each of the reactions were evaporated using the rotary evaporator (rotovap) apparatus. These products were weighed and a TLC of each was done. The bromo-phenylserine product that was obtained from the reaction was tested for solubility in water and DMSO. 40mg of the product was placed in a small glass vial.

Approximately 1ml of DMSO was added to the vial and the mixture was shaken thoroughly to dissolve the entire product. The solution was transferred to an NMR (nuclear magnetic resonance) tube using a pipet. The sample then underwent analysis by NMR spectroscopy.

0.566g of glycine was dissolved in 5ml water and the reaction flask was placed in an ice-bath over a mixer. 2.33 g of O-fluorobenzaldehyde was added using a syringe and 2ml of 45% NaOH

was also added. The ice bath was removed after 5 minutes and the reaction was allowed to continue above the mixer until the product solidified. The product was extracted using HCl and CH_2Cl_2 . TLC was done using $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3$ [16:10:1] as the solvent. The mixture was worked up using 2ml HCl and an additional 1ml was added to make the solution acidic. The mixture was placed in a separating funnel and the lower layer that formed was separated and discarded. The top layer was collected and several drops of NaOH were added in order to make the solution neutral. The product was left in the fridge for two nights in order to solidify and was then filtered.

0.28 g of glycine was dissolved in 2.5 ml of water and the flask was put above a mixer. 1.863g of 3-phenoxybenzaldehyde was added using a syringe and 1ml NaOH was also added. The reaction was allowed to proceed and when a yellow, waxy solid formed the reaction was stopped. The mixture was worked up using 2ml HCl along with an additional 1ml to make the mixture acidic.

0.566g of glycine was dissolved in 5ml water. 1.788g of pyrrole-2-carboxaldehyde was added to the reaction flask which was placed above a magnetic mixer. The reaction was allowed to proceed for 5 minutes and 2ml of 45% NaOH was added dropwise. The reaction was allowed to proceed and was worked up with 3ml HCl and put in the fridge overnight. CH_2Cl_2 was added to the reaction flask to extract the product. After HCl was added the mixture solidified so had to be filtered. The product was again extracted with CH_2Cl_2 and left in the fridge. All the solvent was evaporated using the Rotovap in order to isolate the product.

DISCUSSION and CONCLUSION

The results obtained from the experiments show that phenyl serine can be produced from the reaction with glycine and benzaldehyde. When this reaction is catalyzed with 45% NaOH, the product is obtained in greater yields than when the reaction is catalyzed with either conformation of the threonine aldolase enzyme. This is possibly due to the fact that the NaOH

reacts with the substrate to form an intermediate that is then further reacted to form the product. This mechanism is a possible explanation of the higher yield obtained since the reaction proceeds through intermediates formed through the interaction with NaOH. These results suggest that further experimentation should take advantage of this mechanism by using 45% NaOH as a catalytic agent in the reaction. The results of the NMR analysis verify that the phenyl serine product was in fact produced in the reaction. Further NMR analysis showed that the reaction produced both the syn and anti product of phenyl serine with no evidence that either conformation is more favorable. Therefore, further experimental manipulation is necessary in order to elucidate the reaction conditions that will favor one conformation over the other.

The procedure that was modified in order to produce the halogen substituted phenyl serine proved to be relatively unsuccessful. The yields of the products were low when ortho-substituted benzaldehyde was used. Better yields were obtained with meta- substituted benzaldehyde. This result is thought to be a result of steric hindrance caused by the positioning of the halogen group on the reactant. This placement blocks the mechanism of action of the base which results in a low yield of the final product. The meta substituted reactions produced a greater yield of product possibly due to the meta placement facilitating the mechanism of the reaction. Therefore, due to steric hindrance, the ortho substituted reactants are not well suited for this particular reaction to produce substituted phenyl serine.

In the case of 3-phenoxybenzaldehyde, a yellow waxy substance was produced from the reaction. It is postulated that this product was formed because of an unstable reaction intermediate. The yellow color of the product suggests that a conjugated product was produced which indicates that an alternate mechanism occurred to form the product. Therefore, for this

particular reaction, experimental conditions should be carefully regulated in order to produce the desired product.

The amount of manipulation required for this reaction is relatively small and therefore means that phenyl serine can be efficiently produced. Further research should be conducted in order to define the reaction conditions that would make the reaction more effective in terms of selectivity for stereochemical conformations. This is of importance so that the product, phenyl serine can be isolated in greater quantities to facilitate investigation into its chemical functions. It has been proposed that the product may have importance in pharmaceutical preparations as in the development of new drugs.

EXPERIENCE

I believe that the opportunity to participate in this research program was an invaluable one. The experience was extremely beneficial for me in terms of getting some research experience and also in terms of helping me to decide about my future career goals. During the ten weeks that I was involved in the program, I was introduced to several technical aspects of lab work that I had not been exposed to before, having never done research prior to this experience. I had the opportunity to use several pieces of lab equipment that I had never used before thus increasing my knowledge of chemistry lab techniques. Additionally, being in another country and experiencing a totally different culture, in addition to being engaged in an educational experience was completely rewarding for me. I also found the whole experience valuable as it gave me some insight about my future career plans. Prior to doing the program, I was interested in a research oriented career. However, after having a hands-on experience of what scientific research entailed, I came to the realization that I was more interested in a career such as medicine in which I have a more direct impact in facilitating change in terms of providing therapy. It is still a possibility however, that in the future, I will participate in a research scientist training program which would allow me to conduct research that has applications to clinical and medicinal fields. In this way I would be able to have two levels of involvement in patient care, that is through both medicinal research and direct patient interaction.