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UV Irradiation of thymine molecules and gas chromatography-mass spectrometry

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Abstract / Introduction

When irradiated with UV light, adjacent pyrimidine bases undergo a photochemical reaction to form cyclobutane pyrimidine dimers (CPD)[3]. Although this damage is often repaired, CPDs can cause issues with DNA replication and transcription, and are believed to be one of the major sources of carcinogenic mutations created through DNA replication[2]. Although CPDs can be formed between any two pyrimidine bases, the most common dimers formed are between two thymine molecules. Previous studies have shown success in the creation of thymine dimers by irradiating frozen thymine solution with UV light[4], however techniques of analysis vary. Gas chromatography-mass spectrometry (GCMS) is an effective method to easily separate and analyze organic compounds in a short amount of time, however thymine is not volatile enough to be used in GCMS on its own[1]. Because of this, thymine needs to go through derivatization in order to increase its volatility. In this study, we aim to determine a simple and cost-effective method for synthesizing and analyzing thymine dimers within an educational lab setting, using gas chromatography-mass spectrometry as our technique of analysis, and isobutyl chloroformate (IBCF) as our agent of derivatization.

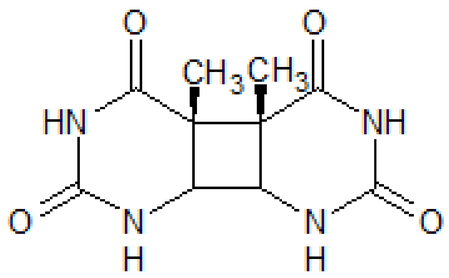

Figure 1- The thymine dimer

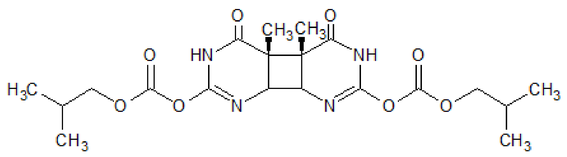

Figure 2 – Thymine dimer capped with IBCF

Materials & Methods

For our UV irradiated trials, 0.015859 M frozen thymine solution was irradiated in open petri dishes over dry ice with a germicidal UV lamp for 4 hours (after irradiation, solutions were thawed and kept covered with aluminum foil as much as possible to reduce UV exposure, which might cause a reversal). For both our thymine control and our UV irradiated trials, we then performed the capping reaction to prepare the thymine solution[1]. We did this by adding 1.0mL 16 µM thymine solution to 0.5 mL acetonitrile-water-pyridine-methanol solvent (40:40:10:10 V/V/V/V), 0.5mL carbonate buffer (pH 9), and 0.5mL IBCF. The mixture was then sonicated in an ultrasonic bath for 15 minutes at 30˚ C. 1.0 mL of solution was combined with 1.0 mL chloroform and mixed well. Organic layer was pipetted out of the sample, and diluted by a factor of four with chloroform. The solution was then injected on an HP-5 capillary column (30m x 0.32mm) film thickness 0.25µm of an HP-5890 GC (with an HP-5972 MS) using a split mode of 20:1. The injection port and transfer line were kept at 250˚ C and 280˚ C, respiectively[3]. The carrier gas (Helium) was kept at a flow of 1mL/minute. The column temperature was increased from 120˚C to 250˚C at 10˚C/minute after an initial 2 minutes at 120˚C, and then increased from 250˚C to 280˚C at 30˚/minute, finally being kept at 280˚C for 2 minutes[3].

Results

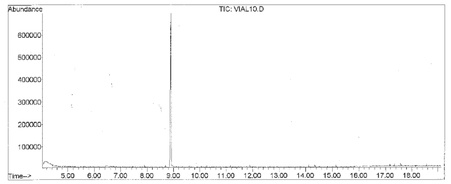

Figure 3 – GC column spectrum for our non-irradiated thymine control sample

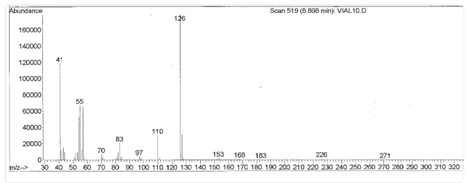

Figure 4 - Mass spectrum for our non-irradiated thymine control sample

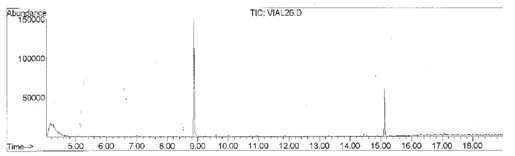

Figure 5 – GC column spectrum for our UV irradiated thymine sample

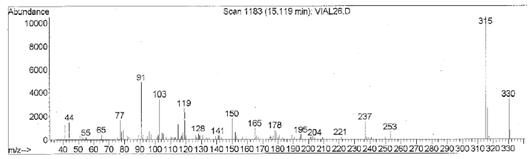

Figure 6 – Mass spectrum for our UV irradiated thymine sample

Conclusions

Our results along with previous experimental findings demonstrate that IBCF works as a successful capping agent for thymine in regards to volatility required for GC analysis[1]. Furthermore, our mass spectrum demonstrates that the fragmentation pattern of thymine is easily identified, even with the addition of the capping reagent (Figure 4). However, we do not have conclusive results on the efficacy of this process in regards to the thymine dimer. Although we do have a mass spectrum that may be representative of the thymine dimer (Figure 6), the mass fragmentation of the dimer is not clear.

The problems that we experienced in separating the thymine dimer with the GC may be due to a few different factors. IBCF may not be a useful capping reagent for the thymine dimer, because the reactivity of the molecule would be different than that of a singular thymine molecule. Furthermore, our UV lamp may not have been powerful enough to create thymine dimers in an abundance that would be detected by the GC. We could test the efficacy of our UV lamp by irradiating our sample normally, but using a derivitizing agent which is known to work on thymine dimers (such as TMS) and analyzing using GCMS. By doing this, we could accept or reject the hypothesis that our UV lamp may not be powerful enough for the experiment. Although our results are not conclusive, UV irradiation and GCMS analysis of thymine dimers shows promise as an educational tool in the study of organic and photochemistry.

References

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Acknowledgements

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