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Formation of Titan aerosol from CO and CH₄

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FORMATION OF TITAN AEROSOL FROM CO AND CH₄

A Thesis Submitted
in Partial Fulfillment of the
Requirements for the
Designation University Honors

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This Study by: Tate Christensen

Entitled: Formation of Titan Aerosols from CO and CH₄

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University Honors

Date

Josh Sebree, Honors Thesis Advisor, Chemistry and Biochemistry

Date Dr. Jessica Moon, Director, University Honors Program

Introduction:

In 1952 Stanley Miller and Harold Urey conducted an experiment that would simulate the conditions of early earth (Miller, 1953). The purpose was to observe the chemical origins of life, and the conclusion supported an earlier theory that such conditions favor the synthesis of more complex organic compounds from simpler inorganic molecules. The research conducted in Dr. Sebree's lab is an extension of these early findings. It is believed that early Earth was composed mostly of nitrogen, carbon monoxide, carbon dioxide and methane as opposed to our atmosphere of nitrogen and oxygen today.

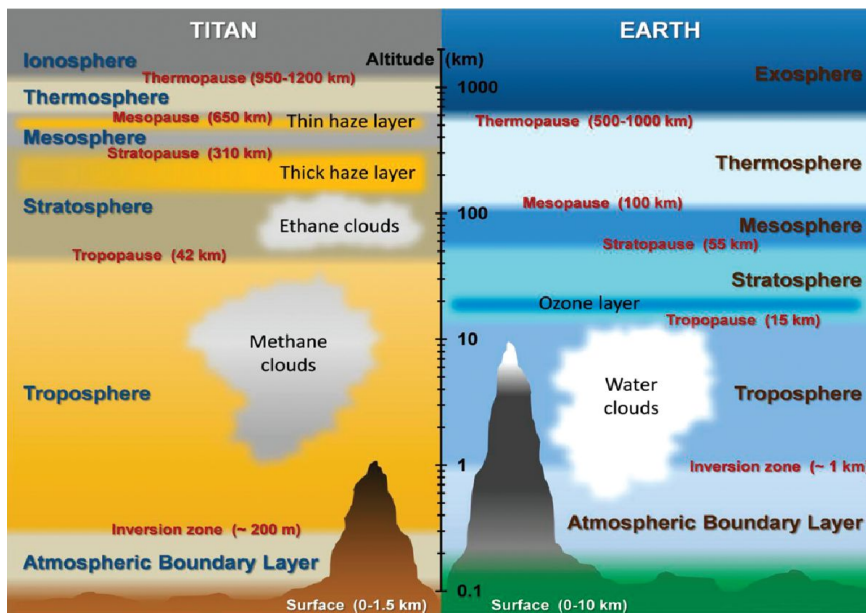


Figure 1. Atmospheric Comparison of Titan and Modern Earth

Saturn's largest moon, Titan, has qualities relatively comparable to those of Earth. It is the only body in our solar system, besides Earth, that displays clear evidence of surface liquid. Titan also is unique due to its dense atmosphere. Titan's atmosphere is largely composed of nitrogen, like Earth, with methane and other minor components leading to the formation of

organic hazes. Where Earth has water cycles, Titan has similar methane cycles. In fact, it is believed that Titan's atmospheric behavior is similar to that of early Earth (Cable, 2011).

Table 1. Components of the Atmosphere of Early Earth and Titan	
Titan	Early Earth
98.4% Nitrogen	Nitrogen
1.4% Methane	Carbon Dioxide
0.1% Hydrogen	Hydrogen
<0.1% Carbon Monoxide	Water Vapor

Titan's atmosphere can only provide oxygen through carbon monoxide in relatively small quantities, contrary to the abundant amounts of molecular oxygen on Earth. Through new studies it has been shown that biological molecules can possibly form from Titan's trace oxygen present (Horst, 2012). If biological molecules, such as amino acids, can be photochemically synthesized in laboratory settings from carbon monoxide and other components of Titan's atmosphere, this may open up a new understanding of the earliest earth.

However there is not a lot of recent research being done in this area, except for what we are doing currently. Hence, there is a smaller selection of related pieces of literature out there that would aid in our research. In a way, the research that is being conducted is filling a void for this field of study. In fact many of the the projects taking place in the lab are going on to be published, because of the new information that they are providing. One major driving force for

this research is to provide the scientific community with the data that this study is able to collect in the lab, because there is not a lot of similar work being done elsewhere.

This research project will modify concepts developed during my previous research with Dr. Sebree to study the sources of oxygen on Titan that could possibly produce aerosols in our lab. For the majority of this work, the sources of oxygen will be carbon monoxide, carbon dioxide, and molecular oxygen. These components will be individually combined with methane in order to form products. The goal of this research project is to observe glyoxylic acid and glycolic acid derivatives, which will be done by manipulating the methane/oxygen ratios. A lot of this research will be conducted using the GM/MS instrument in the biochemistry department. The results of this research project will include diagrams and tables showing the various compounds discovered in the aerosol product, as well as comparison of the oxygen reactants tested throughout the proposed method.

Literature Review:

As noted above, the atmospheric composition of Titan is comparable to Earth's proposed prebiotic atmosphere. The study of Titan aerosols gives researchers a glimpse at what conditions were like early in Earth's history. At the heart of this study are questions about the initial compounds that were at play. These initial compounds would have reacted and formed more complex compounds. As proposed by (Eschenmoser 2007) in the "glyoxylate scenario," the starting blocks of all complex biological pathways may be glyoxylate along with its formal dimer dihydroxyfumarate.

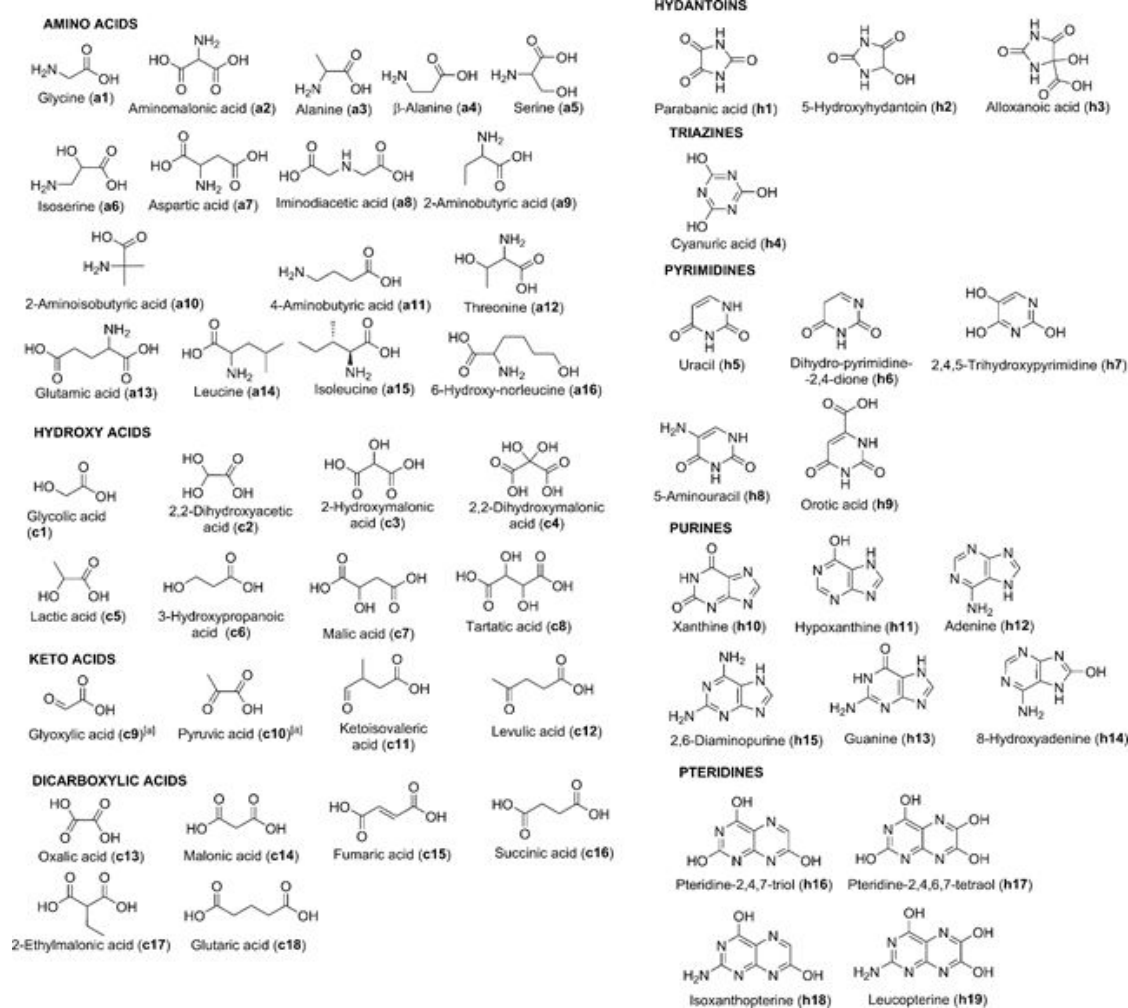


Figure 3. Structures of plausible oxyacids in aerosol product (*Role of Aqueous Aerosols/ “Glyoxylic Acid Scenario”*).

For the first time, glyoxylic acid was identified from the oligomerization of HCN. According to Marin-Yaseli (2016), “Additionally, pyruvic acid, malic acid, fumaric acid and succinic acid were identified in HCN polymers, experimentally proving a well-established relationship between HCN and the constituents of the rTCAC. Several amino acids were identified, which may come from the reductive amination of the corresponding keto acids. These keto acids were identified in some cases and in other ones they remained unidentified...” but their

“...results seem to confirm this hypothesis of the production of amino acids, also raised in the ‘glyoxylate scenario’”. These initial compounds would serve as the chemical basis for a variety of metabolic processes leading to formation of essential biological monomers such as amino acids, sugars, constituents of the carboxylic acid cycle, and pyrimidines (Yaseli, 2016).

Glyoxylic acid is the base compound for the formation of many sugars. Whereas glycolic acid leads to the formation of DNA compounds.

As seen in Figure 3, there are many glyoxylic acid derivatives. All of these derivatives play an essential role in biological processes necessary for life on Earth. In previous studies, I was able to identify these structures after they were formed in our aerosol mixtures. In fact, the “*Glyoxylic Acid Scenario*” article is a very accurate representation of what a lot of my earlier research pertained to. Using an optimal gas ratio, that was very similar to Titan’s atmosphere, I spent a semester last year searching for and identifying organic compounds that could be detected in the mixture. The figure taken from the “*Glyoxylic Acid Scenario*” article roughly summarizes the list of biological derivatives that we were able to identify.

In their study Coll et al. (1999) stated the following: “It is easier to compare the tholin spectrum with infrared spectra of known compounds, which may play a key role in aerosol formation. We have made comparisons with spectra of HC₃N polymers, HC₃N/C₂H₆ and HC₃N/C₂H₂ polymers and with spectra of different poly-HCN compounds”. As demonstrated by Coll and associates, structural analysis of Titan aerosol products has been going on since 1999. In their research article they mention a few methods of accomplishing structural analysis. Coll et al. (1999) go on by adding that: “Another way to study the chemical structure of tholins is by their pyrolysis. In the frame of the Cassini-Huygens mission, pyrolysis-GC-MS of tholins

have been carried out, using a special instrument: a prototype of the Huygens ACP experiment” (P. Coll et al. / *Planetary and Space Science*).

It is important to emphasize that this article was published in 1999, even at that time researchers were able to use current technology to analyze their samples. In their article, Coll et al. (1999) explained that structural analysis can be accomplished with a few instruments, a few of which are cited from their article. The researchers associated with Coll primarily used infrared (IR) when determining structural analysis, they also made mention of utilizing some of the instruments that would eventually be used for space exploration missions. While the research team did not have access to instruments such as the Huygens ACP prototype, we did occasionally use IR like they did. In fact, other branches of the Titan research team use instruments such as IR and nuclear magnetic resonance spectroscopy (NMR) on a regular basis to determine specifics of compound structure.

For this research, however, very little of these other instruments were put into use. Although they are important to mention because they play a crucial role in structural analysis. Instead of IR and NMR techniques like those used in studies by Coll and associates, GC/MS instrumentation were used. These techniques were used by the Horst research team as well as in the Yaseli *Glyoxylic Acid* research, both of which are represented in excerpts from their literature.

TABLE 1. SUMMARY OF OBSERVED PREBIOTIC MOLECULES

				P2CO	P2COi	P5CO	P5COi
				%N ₂	96.2	96.2	93.2
				%CH ₄	2	2	5
				%CO	1.8	1.8 C ¹⁸ O	1.8
Name	Mass	Formula	Fig.				
Nucleotide base							
cytosine	111	C ₄ H ₅ N ₃ O	2	OT	OT	OT/GC-MS	OT
uracil	112	C ₄ H ₄ N ₂ O ₂		OT	OT	OT/GC-MS	OT
thymine	126	C ₅ H ₆ N ₂ O ₂		OT	OT	OT/GC-MS	OT
adenine ^a	135	C ₅ H ₅ N ₅	2	OT		OT/GC-MS	
guanine	151	C ₅ H ₅ N ₅ O		OT		OT/GC-MS	
Biological amino acid							
glycine	75	C ₂ H ₅ NO ₂		OT		OT/GC-MS	
alanine	89	C ₃ H ₇ NO ₂		OT		OT/GC-MS	
serine	105	C ₃ H ₇ NO ₃		OT		OT	
proline	115	C ₅ H ₉ NO ₂		OT		OT	
valine	117	C ₆ H ₁₁ NO ₂		OT		OT	
threonine	119	C ₄ H ₉ NO ₃		OT			
isoleucine/ leucine ^b	131	C ₆ H ₁₃ NO ₂		OT		OT	
asparagine	132	C ₄ H ₈ N ₂ O ₃		OT		OT	
glutamine	146	C ₅ H ₁₀ N ₂ O ₃		OT		OT	
lysine	146	C ₆ H ₁₄ N ₂ O ₂		OT		OT	
histidine	155	C ₆ H ₉ N ₃ O ₂	2	OT		OT	OT
phenylalanine	165	C ₉ H ₁₁ NO ₂		OT		OT	
arginine	174	C ₆ H ₁₄ N ₄ O ₂		OT		OT	

OT indicates that the molecular formula was present in the Orbitrap spectrum. GC-MS indicates confirmation of structure from GC-MS measurements. In all cases, presence in the isotopic samples (P2COi, P5COi) refers only to the isotopic molecule. GC-MS measurements were not performed on P2CO and P2COi.

^aDue to the lack of oxygen, adenine is a special case, and additional measurements were made using P2 and P2i. Orbitrap measurements show clear incorporation of ¹³C into ¹³C₅N₅H₅ (as shown in Fig. 2), and GC-MS measurements provide structural confirmation that the ¹³C-containing molecule is adenine (as shown in Fig. 3).

^bNote that isoleucine and leucine are isomers (mass 131) and cannot be differentiated based on Orbitrap measurements.

Figure 4: Table from Horst, S. Formation of Amino Acids 2016

Horst and his team were interested in identifying prebiotic molecules on Titan, similar to what we aimed to do in our research project. As the figure above shows, they were successful in identifying a variety of significant organic molecules using their method. These molecules included nucleobases that make up DNA, as well as the amino acids that are so essential for life. Horst (2016) states: “For the purposes of this work, the molecules of interest are the five nucleotide bases (adenine, cytosine, guanine, thymine, uracil) and the nineteen amino acids utilized by life on Earth (biological amino acids) composed only of C, N, O, and H. Detailed analysis of these spectra resulted in the identification of over 8,000 different molecular formulae, which corresponds to a much greater number of molecules if structural isomers are taken into

account. Here, we focus on the definitive detection of a handful of species that are interesting for prebiotic synthesis. Eighteen peaks with masses that correspond to the molecular formulae of biological amino acids (14—iso-leucine/leucine are isomers) or nucleotide bases were detected in the spectra. Formulae that correspond to nonbiological amino acids and purine bases were also identified” (Horst, 2016).

As Horst (2016) described in the above passage, they were focused solely on using chemical components that were necessary for life on Earth. According to his research, these elements, which are also found on Titan, are all that are needed to create biological compounds. The research they conducted experienced a few challenges in the form of contamination. While running a lab like this a constant threat is oxygen contamination. This means that atmospheric oxygen from the lab gets into the reaction and distorts the source of oxygen which can ruin the product. To avoid this issue it is important to keep the process in a sealed environment at all times. The research team foresaw this in the research, partly due to the contamination issues in the Horst method. One way to observe the source of oxygen, and to identify whether the oxygen in the product is from the reaction or contamination, is to use an isotopic oxygen. This is also mentioned in the Horst project, in which oxygen 18 would be used instead to follow the oxygen through the reaction.

In the glyoxylic acid article by Yaseli (2016), they discuss the effects of reaction time on the final products. It is well known to those in the scientific community that there is a relationship between the reaction time, the temperature and the process of the cyanide polymerization. High temperatures improve the degree of polymerization and/or cross-linking ratio, and it is proposed that experiments that are conducted at higher temperatures are a

reasonable model of the reactions that take place at low temperatures on a geological time scale (Yaseli, 2016). Their team found it necessary to run the reaction over the course of a few days at high temperatures. As they explained in this excerpt, the point to this was to simulate a lengthy geological time at low temperatures by using higher temperatures for a relatively brief time period. This has been taken into consideration into consideration as we have prepared for our own research project. However, their method is different than ours, and ultimately this is because our labs and end goals are different.

In the lab where our research project took place, we were able to produce aerosol products at a more rapid pace. It is accurate to say that we were able to run reactions anywhere from 36-48 hours and collect an ample amount of product. In the “*Glyoxylic Acid*” article, they let their reaction come to its full before starting the process of analysis. Comparing that to our project, however, we found that if we do not prepare our samples for analysis within an hour of completing the derivatization process it is likely that we will get weak detections. Basically this is another area where our research parameters are different than our literary comparatives; they let their aerosol reactions come to their full before analysis, whereas we found it most efficient to put the samples into analysis as soon as possible.

Method:

A simulation chamber has been developed under the supervision of Dr. Joshua Sebree and has the capabilities to recreate either Titan's atmosphere or the early Earth. Experiments are running using mixtures of nitrogen, methane, and oxygen species in a stainless steel mixing chamber. The mixture is flowed into a reaction chamber and gas flow is constantly regulated by an Alicat mass flow controller. The content of the reaction chamber is exposed to a 115-400 nm wavelength range ultra-violet lamp to simulate solar radiation. Products are gathered underneath the reaction chamber, and aerosols sizing 30 μm and larger are collected for further analysis. Collection takes place on a glass fiber filter in the collection chamber.

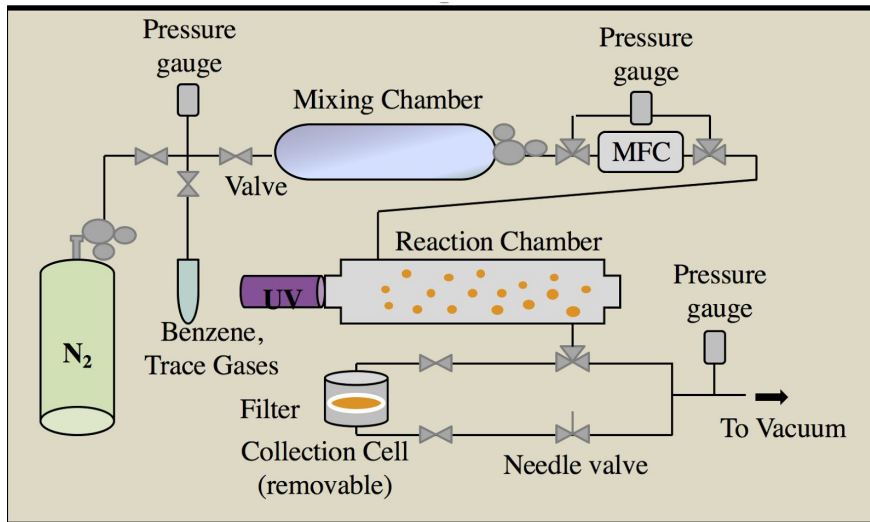


Figure 2. Representation of the Titan and Early Earth Atmosphere Apparatus

The aerosols were studied using gas chromatography and mass spectrometry (GC/MS). Any prebiotic molecules were derivatized using a literature method (Horst, 2012). As discussed previously, Titan's atmosphere does not contain elemental oxygen. Oxygen on Titan is only existent in carbon monoxide. This makes laboratory control of oxygen important.

The research projects method included individually testing varying ratios of oxygen and carbon containing gases such as carbon monoxide, carbon dioxide, and oxygen gas. The following mixtures listed below were the standard gas mixtures run through the chamber. The ratios of carbon to oxygen are listed as well.

1. Aerosol A: CO/CH₄
2. Aerosol B: CO₂/CH₄
3. Aerosol C: O₂/CH₄

The initial plan was to arrange each aerosol product so that the ratio of methane to oxygen species was 1:1. We thought this may change, however, if analysis shows that that mixture is unfavorable, and so the ratio was modified in the first part of the research project. It became necessary for us to adjust the ratio and concentrations so that the methane to oxygen ratio was 0.1% to 0.1%. The designated quantities of compound will be piped into the reaction chamber, and allowed to come to completion over the course of a few hours to days. The product aerosols will be collected and placed in a sealed water free glovebox to prevent contamination. This is a crucial aspect of the research, since the purpose is to establish differences in present compounds based on oxygen sources.

When the aerosol was ready, it was prepared for a derivatization process. This process includes the addition of MTBSTFA and DMF, along with continuous N₂ flow in the oven. MTBSTFA (N-Methyl-N-tert-butyltrimethylsilyltrifluoroacetamide) and DMF (dimethylformamide) are chemical that make the process of derivatization more efficient. Titan aerosol analogs were produced *in situ* via UV photolysis of various gas mixtures. Then we

analyzed the Titan aerosol analogs for the presence of amino acids, the aerosols must undergo a derivatization procedure. The goal of any derivatization procedure is to yield a sample that is more volatile and less reactive, so that it can be detected using analytical methods. In the case of amino acids, the derivatization replaces active/acidic hydrogens on hydroxyl, amino, and thiol polar functional groups with a nonpolar group. The derivatization agent used was N-tert-butyldimethylsilyl- N-methyltrifluoroacetamide (MTBSTFA). MTBSTFA forms tert-butyl dimethylsilyl (TBDMS) derivatives when reacted with polar functional groups containing active hydrogen of the aerosol.

Analysis:

Analysis of the aerosol took place once the derivation process is complete. It has been found, from earlier research projects, that analysis is most effective within thirty minutes of the derivation process. Analysis will be conducted using a GC/MS method used in prior analysis of aerosols products in Dr. Sebree's lab. Gas chromatography (GC) is an analytical separation technique used to analyze volatile compounds in the gas phase. For analysis, the TBDMS derivatives are dissolved in a methylene chloride solvent for optimum selectivity of the compounds of interest and vaporized in order to separate the components through unique interactions with the column.

When the sample exits the GC column, it is passed into a mass spectrometer (MS), where the sample is ionized and fragmented into charged ions by electron-impact. The fragments are then separated according to their mass to charge ratio (M/Z). The components of the sample are then identified using the characteristic fragmentation pattern. Carboxyl groups are essential in

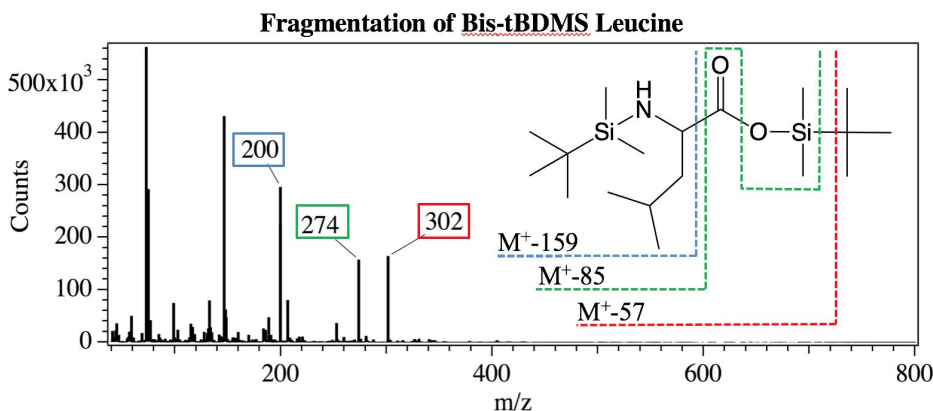


Figure 4: Example of a fragmentation pattern as it occurs in MS/MS.

the production of biologically significant molecules, such as amino acids. Components of the aerosols are identified using an internal spectral library, based on the fragmentation pattern of best fit. However, difficulty arises in determining if the compound is a fragment of a larger compound and/or not in the library. Formulas of the components must then be identified manually and further analysis is required to identify the structure of the compound.

Prior to the project we had estimated that each mixture will take two weeks to make. The gases have to be prepared, sent through the reaction chamber and then collected once the process is completed. Once the aerosol is formed, it would go through the derivatization process, therefore each aerosol would require two weeks to produce. Again, we estimated that analysis of the aerosols would require a week and a half of attention each. The aerosols can be analyzed between aerosol production runs every two weeks. Starting in January, this research project

began running aerosols through the method mentioned above. Below is an example of our initial estimated schedule of events, and the process throughout the research was roughly similar to it.

Table 2. Preliminary Schedule for Spring Semester taken from Fall Semester

Dates	Task to be completed
January 20th	GC/MS run for aerosol #1 (CO/CH ₄)
February 3rd	GC/MS run for aerosol #2 (CO ₂ /CH ₄)
February 10th	GC/MS run for aerosol #3 (O ₂ /CH ₄)
February 17th	Analysis of aerosol #1 (CO/CH ₄)
February 24th	Analysis of aerosol #2 (CO ₂ /CH ₄)
March 3rd	Analysis of aerosol #3 (O ₂ /CH ₄)
March 10th	Modification of method or ratios if needed

Results:

In Figure 6 below, the graph shows the chromatograms of three separate experimental aerosol runs. The bottom chromatogram is a blank run that contains zero produced aerosol, showing only background peaks from the GC column. A blank run is always used so that we can discern which compounds detected by the GC are from the actual aerosol product compared to being column run off of the GC itself. The middle chromatogram is the 8mg/ μ L sample, and shows the peak for acetic acid at a retention time of 8.8 min. The top chromatogram is 1 mg/ μ L sample, also showing the peak for acetic acid at a retention time of 8.8 min.

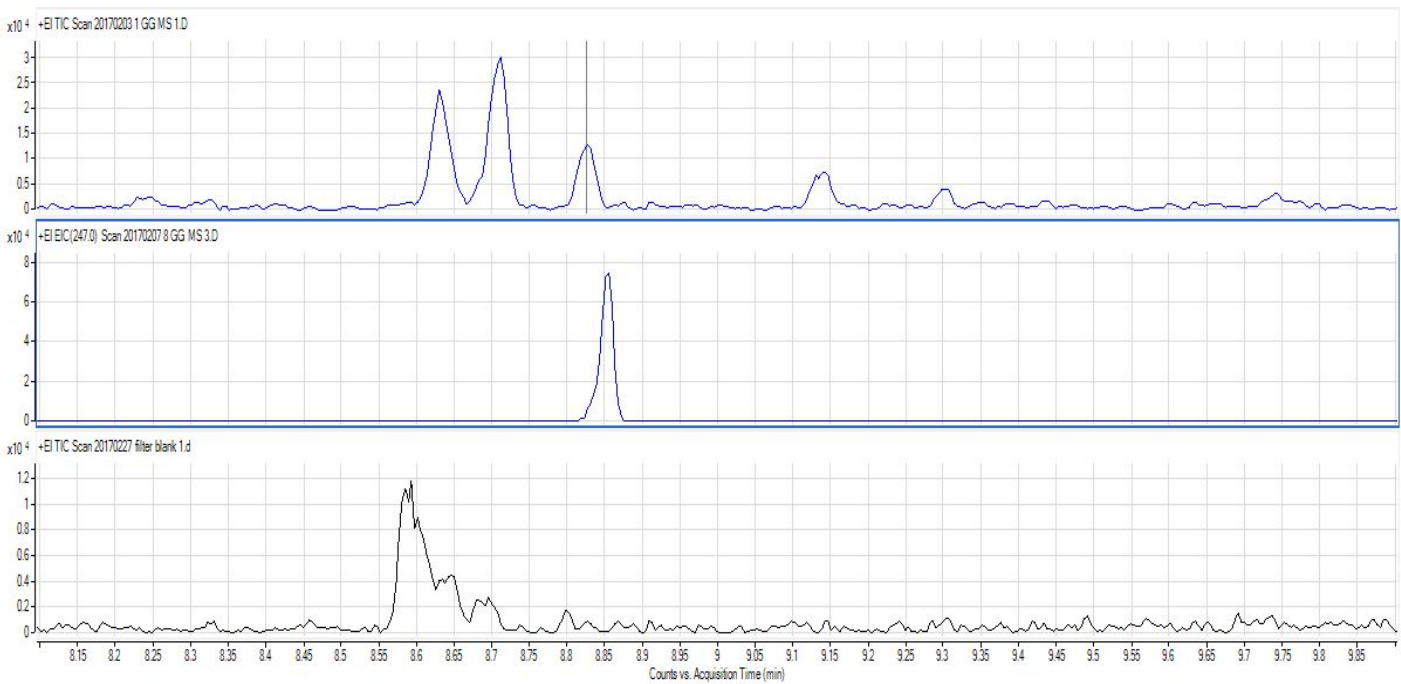


Figure 6: Chromatogram showing relative retention times of three separate mixtures.

As Figure 6 illustrates, the peak identified with acetic acid appears around the retention time of 8.8 minutes. The peak is observed in both the 8mg/ μ L and 1mg/ μ L samples, but is not observed in the blank run. Results such as this show us that we are making biologically organic molecules in our mixtures despite their varying composition and concentration. This is a major finding for this research project, because we have proved that whether we are using a high carbon monoxide to methane concentration or a low carbon monoxide to methane concentration we get the same products.

Furthermore, these desired biological compounds are not found in the control blank mixtures. As the blank run shows, there are still other peaks. These peaks are just background detections coming from the GC column, and while they are still identifiable chemical compounds, they do not hold any significance to our research project. These background peaks are observed in both the blanks and also in the aerosol mixture runs. The peaks that are significant to us though are the ones like acetic acid in Figure 6, in which they are detected in the aerosol mixture runs but not in the blank control run.

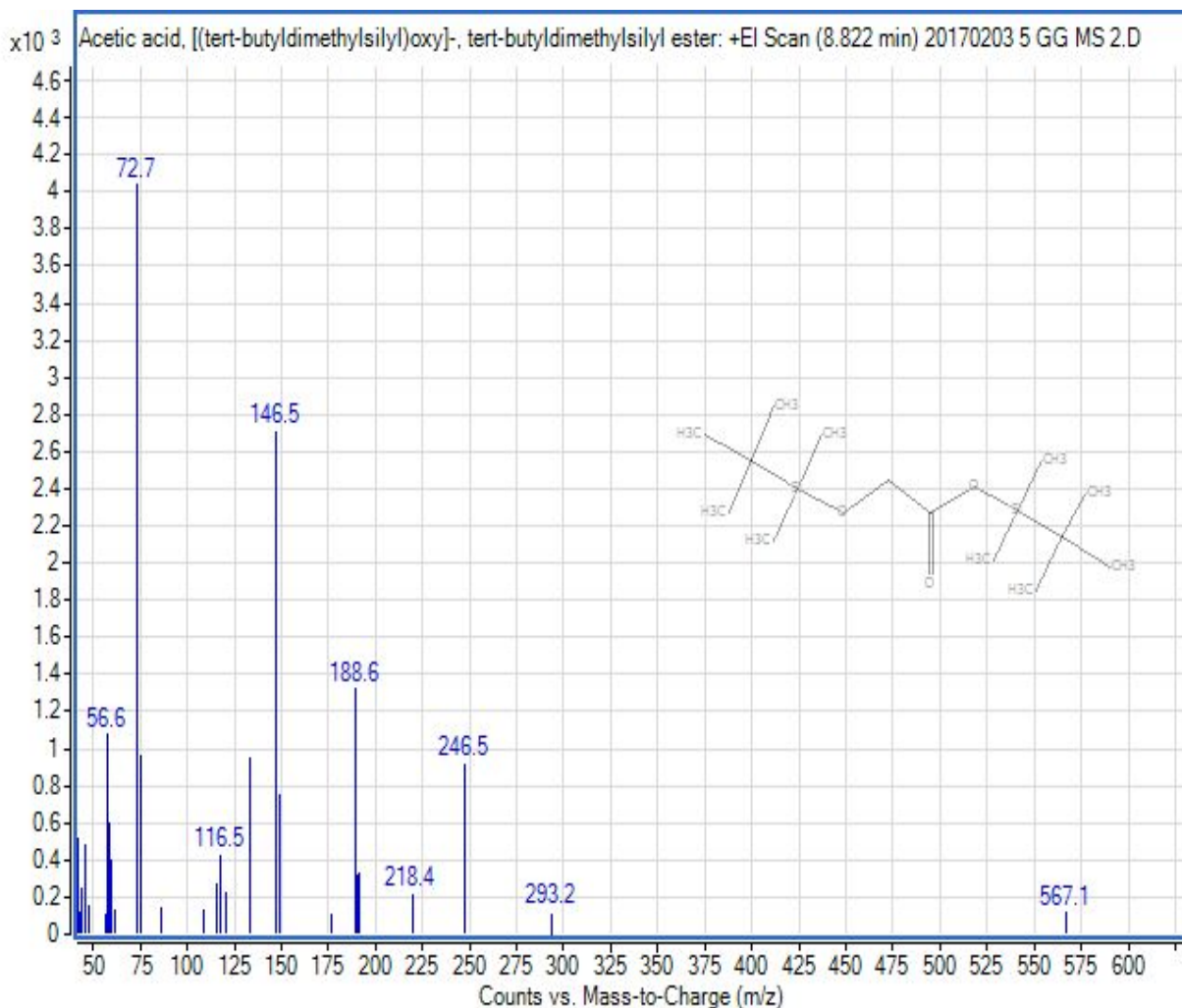


Figure 7: Mass to Charge Fragmentation of Acetic Acid

One of the major compounds that was identified was acetic acid. It is silylated in the above spectrum. The term silylated refers to the fact that it is carrying extra silicon components that reactively combine to the molecule in the derivatization process. These extra structural aspects are not important to us, because they are just a part of the derivatization process and are just as easily fragmented. The fragmentation process is described in the introduction of the thesis. To make it clear to the readers that this molecule is a fundamental organic compound the simple acetic acid structure is shown here in Figure 8.

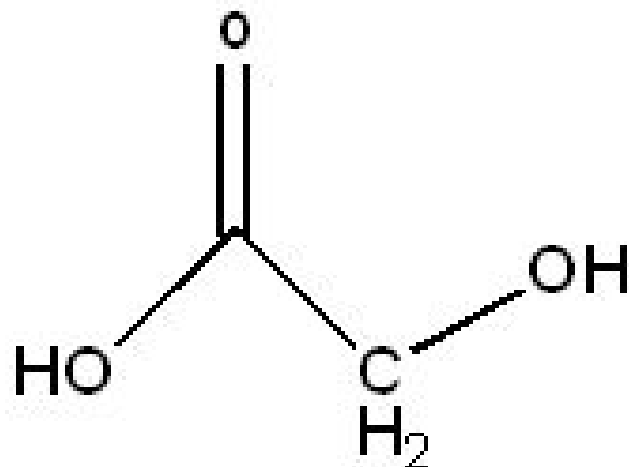


Figure 8: Structure of Acetic Acid

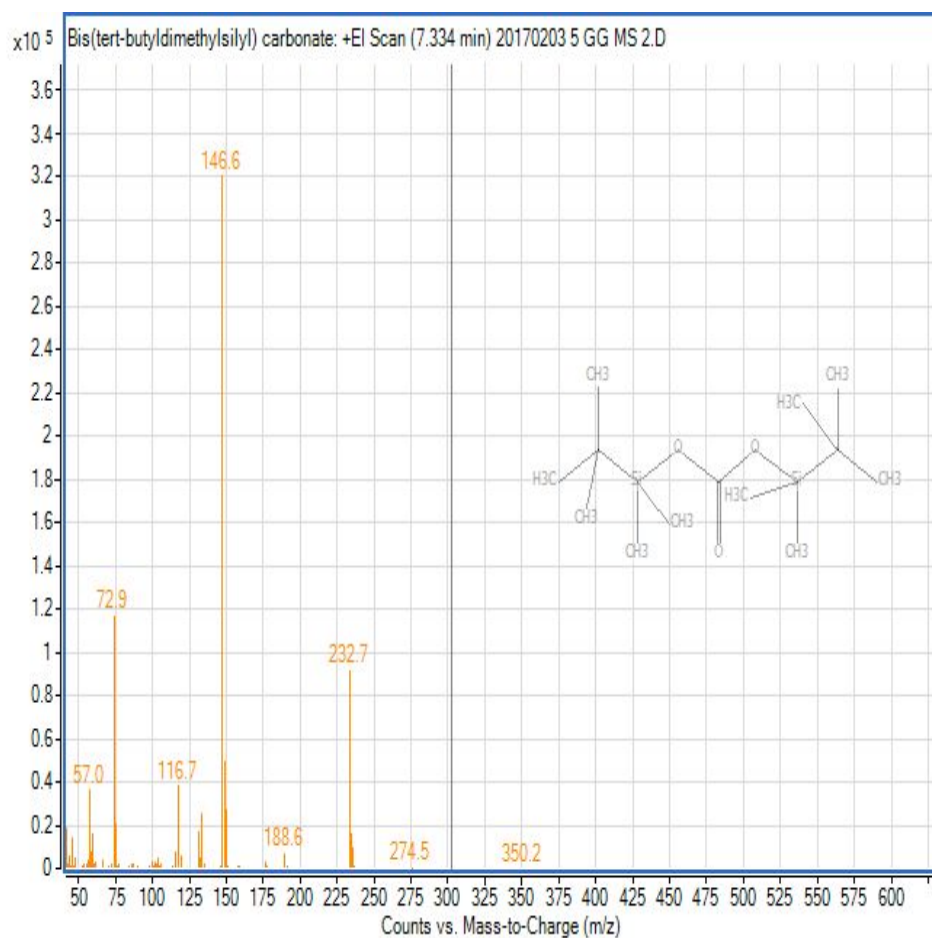


Figure 9: Mass to Charge Fragmentation of Carbonate

Another one of the major compounds that was identified was carbonate. It is silylated in the above spectrum. As stated above, these extra structural aspects are not important to us. Again, to make it clear to the readers that this molecule is a fundamental organic compound the simple carbonate structure is shown here below.

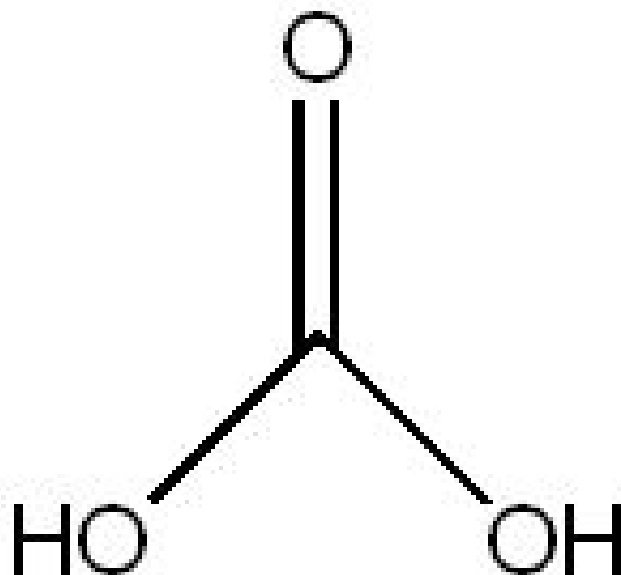


Figure 10: Simple Carbonate Structure

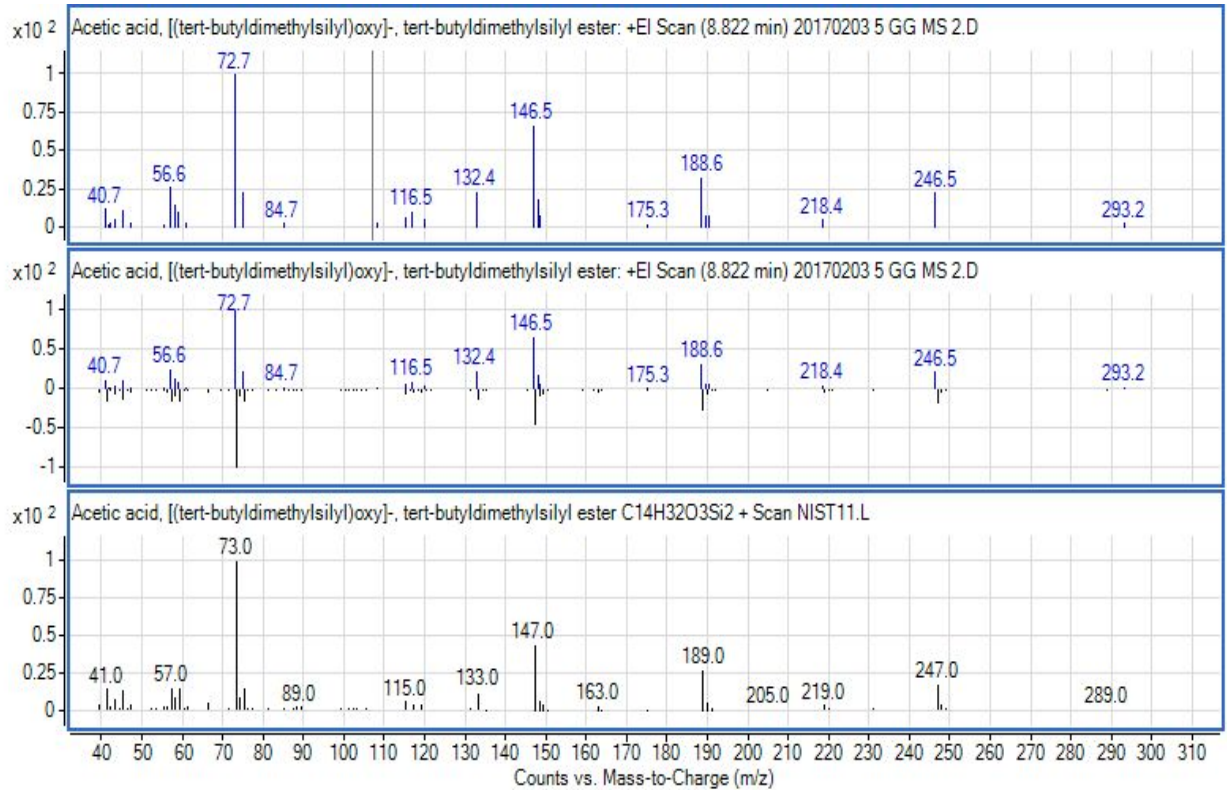


Figure 11: Mass to Charge (m/z) Count

Acetic acid was observed in samples of varying concentrations as we discussed in the earlier section. This is described by the spectra represented in Figure 6. The research team knows that a peak represents a specific molecule because of the grouping of signals on the Mass Spectrometer (MS). MS indicates that the mass charge counts as the molecule is fragmented. As the spectra above shows in Figure 11, the mass peaks that indicate the molecule is acetic acid in each run line up, which indicates that we can detect acetic acid in both large and small concentrations runs. This figure is important because it provides mass/charge quantities, which we can use to compare to older readings. This insures that we truly do have acetic acid as well as other biological compounds. Furthermore, this figure proves that, with our research method, we can produce and detect these organic molecules consistently, despite varying the concentration prior to derivatization.

Discussion

In my proposal I stated that results and analysis will include information on the compounds present in the aerosol after derivatization. Likewise I said that proof of their presence will be provided from the mass fragmentation patterns in the analysis. The goal of this research project is to prove that there is formation of oxyacids such as glycolic acid and glyoxylic after aerosols are produced in the presence of carbon monoxide and carbon dioxide. The research was carried out using funds awarded from the Iowa Space Grant Consortium obtained this fall upon selection. The results described above were taken from a presentation poster that I created to use as a representation of my research at the Iowa Space Grant Consortium Student Research Seminar. These figures were used to demonstrate the results of my project to a number of Iowa Space Grant Consortium committee judges, as well as Iowa State University faculty who were hosting the event.

Conclusion and Future Studies:

We have adjusted our method from previous projects to analyze the effects of varying oxygen to methane ratio on aerosol composition. Our analysis shows that biological compounds can be derived, through our method, from high concentration aerosols as well as low concentration aerosols. In the coming months we would like to identify where the components of each compound are going in the product. The mechanism in Figure 12 will be used for this extended research plan.

The focus of future studies will be to track the carbons in the reaction. To do this we will use isotopic ^{13}C and follow it into the produced aerosols. This mechanism is shown in the figure to the left, in which we see the carbon labeled in the product. The significance of a procedure like this is that it will give insight into how the reaction process actually navigates. If we know where the product carbons are coming from we can determine whether they are coming from the methane source or from the carbon monoxide source. This is similar and also related to the oxygen contamination example that I introduced with the Horst research project. Just as the figure I constructed shows the origin and final placement of the isotopic carbon, so too isotopic oxygen can be used in the reaction mechanism. Using the isotopic oxygen will give the researchers an accurate idea of where the oxygen is coming from. If they observe atmospheric oxygen in the product they would know that at least a portion of their method is susceptible to contamination.

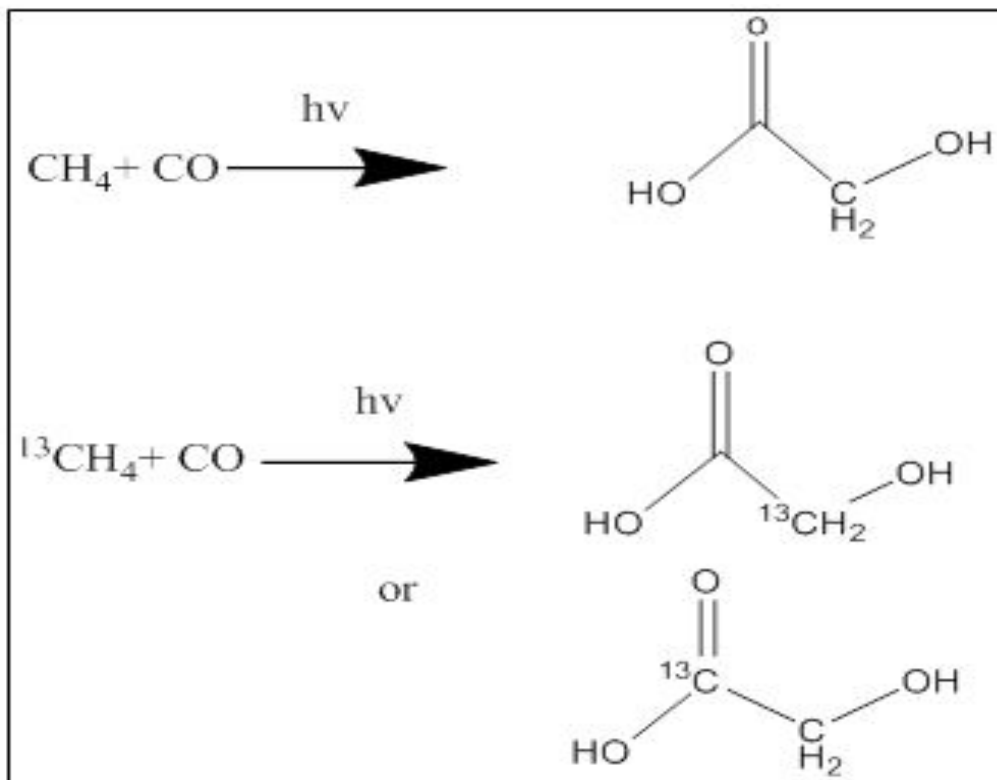


Figure 12: Reaction Process of Acetic Acid and The Use of Isotopic Carbon

I will not personally be involved in this extended research project but my work thus far will be utilized for whomever takes up the next chapter of the work. Our current finding will provide adequate background information for the isotopic studies. Also it is more than likely that the method constructed for my research will be replicated in the future or perhaps modified to fit the exact needs of that research. Finally, the model in Figure 12 will serve as a direct process representation for the future studies.

As I made mention of above, this research was made possible by funding by the Iowa Space Grant Consortium (ISGC). The Iowa Space Grant Consortium deemed this research project worthy of funding upon receiving a research proposal as well as a brief history of earlier

work accomplished in the lab. The grant from the Iowa Space Grant Consortium provided adequate funds for this research, with which we were able to purchase needed reactants such as quantities of laboratory purpose carbon monoxide and methane. This funding will be important in future studies for similar reasons. The funding will allow the research group to purchase needed quantities of expensive products, such as isotopic oxygen and isotopic carbon.

Although research in this field goes back decades to the original Urey Miller experiments, there is not a lot of research groups continuing the work. Given our ability to collect actual data from missions, such as the Cassini space mission, we are able to fill in the void. As stated in the introduction, there are a lot of findings coming out of this research lab that are eventually being published, due to the lack of new data for this specific field. This specific project is only a small piece of the puzzle when it comes to Titan research. It is not the first project that has been completed for Titan in Dr. Sebree's research lab, and it will not be the last. The majority of the background information for this research project comes from previous studies within the same lab conducted by the same students.

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