SPECTRAL CHARACTERIZATION OF PTERIN MOLECULES: IMPLICATIONS FOR DETECTING LIFE ON MARS. C. L. Cook[1], A. J. Williams[1,2], K. E. Kautzman[3], M. M. Floyd[2], D. Emerson[4], [1]Department of Physics, Astronomy, and Geosciences, Towson University, 8000 York Rd, Towson, MD 21252 (ccook19@students.towson.edu), [2]NASA Goddard Space Flight Center, [3]Department of Chemistry, Towson University, [4]Bigelow Laboratory for Ocean Sciences

Introduction: The search for microbial life on Mars involves the identification of physical and molecular biosignatures. Molecular biosignatures are signs of past or present life based on the presence of molecules that formed from biologic processes. A class of organic, aromatic, and heterocyclic molecules, likely pterins that are essential to the biologic activities of some enzymes [1], was detected in a variety of terrestrial samples of or related to iron-oxidizing bacteria (FeOB) using trimethylsulfonium hydroxide (TMSH) thermochemolysis gas chromatograph – mass spectrometry (GCMS). Terrestrial, iron-rich environments that contain FeOB can serve as analogs to iron-rich environments on Mars, such as the Vera Rubin Ridge at Gale Crater [2], that may have presented a habitable environment to FeOB, if present [3]. The terrestrial samples tested for the presence of the putative pterin-bearing molecule included marine and freshwater FeOB isolates and microbial mats, freshwater environmental iron seep samples, modern biogenic iron precipitates, and iron rock 100s to 1000s of years old.

If the same pterin-bearing molecule is found in both FeOB and modern and older oxidized iron rocks, those rocks may have formed due to FeOB metabolic processes. The reliable detection of this pterin-bearing molecule in iron-dominated rocks may provide a new avenue to identify FeOB activity in even older iron-dominated environments on Earth and Mars.

Various spectroscopic techniques are useful in determining the structures of organic molecules, and will be used to identify the specific pterin-bearing molecule(s) present in the FeOB samples. This study created a reference for identifying the specific pterin-bearing molecule(s) present in the FeOB samples by analyzing the Fourier transform infrared (FT-IR) and ultraviolet-visible (UV-Vis) spectra of pterin standards and a pterin precursor standard.

The pterin standards under study include pterine ($C_6H_5N_5O$), 6-biopterin ($C_9H_{11}N_5O_3$), and pterine-6-carboxylic acid ($C_7H_5N_5O_3$) (Fig. 1A-C). The pterin precursor guanine ($C_5H_5N_5O$) (Fig. 1D), an organic-free iron oxide (*Sigma Aldrich*), and the reagent TMSH (*TCI America*) were also characterized. FT-IR spectra were collected from the pterin standards, guanine, and iron oxide before and after reaction with TMSH. UV-Vis spectra were collected from the pterin standards and guanine. The spectra of the unknown pterin molecule(s) in the FeOB samples will be compared to the spectra of pterin standards to identify the specific pterin molecule(s) present.

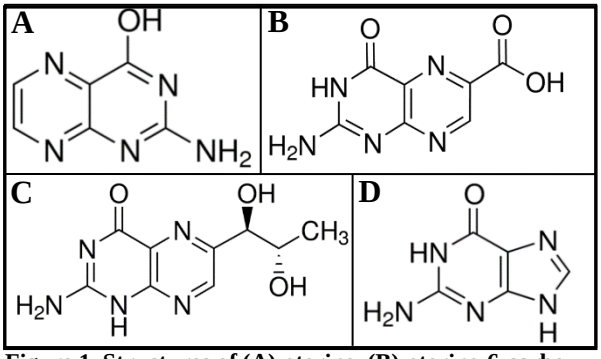

Figure 1. Structures of (A) pterine, (B) pterine-6-carboxylic acid, (C) 6-biopterin, and (D) guanine.

Methods: The standards, in the form of powders, were reacted with TMSH at a 1mg:6µL ratio and dried down in a hood. The standards and reacted standards were analyzed directly on a Nicolet 320 FT-IR spectrometer. The standards were dissolved in a pH6 10mM phosphate buffer for analysis on a Varian Cary 60 UV-Vis spectrophotometer. The UV-Vis sample spectra were background substracted from the buffer-only spectra.

Results and Discussion: The FT-IR spectra of the pterin standards and guanine did not undergo any significant shifts or shape changes after the standards' exposure to TMSH at room temperature (Fig. 2A-B). The standards are known to react with TMSH based on previous GCMS results, and the lack of change between the spectra indicated that the standards need to be heated to achieve full thermochemolysis. The spectra of the iron oxide changed after exposure to TMSH, but this spectra likely corresponds to unreacted TMSH (Fig. 2C).

The FT-IR spectra of the pterine, pterine-6-carboxylic acid, 6-biopterin, and guanine standards displayed similar features. These features included N-H stretching in the range of 3350 cm^{-1} to 3200 cm^{-1}, C-H stretching in the range of >3000 cm^{-1}, and characteristic peaks positioned at ~1500 cm^{-1} and ~1600 cm^{-1} (Fig. 2A-B). The N-H stretching likely corresponds to the -NH_2 group present in each structure [1]. C-H stretching in the range of >3000 cm^{-1} indicates the presence of an aromatic system. The peaks positioned at ~1500 cm^{-1} and ~1600 cm^{-1} further support the presence of aromatic systems in

each structure [4]. Some discrepancies between the spectra of the pterin standards and guanine were also identified. The peaks presumed to correspond to N-H stretching and C-H stretching in the spectra of the 6-biopterin were weaker than those peaks present in the other pterin standards (Fig. 2B).

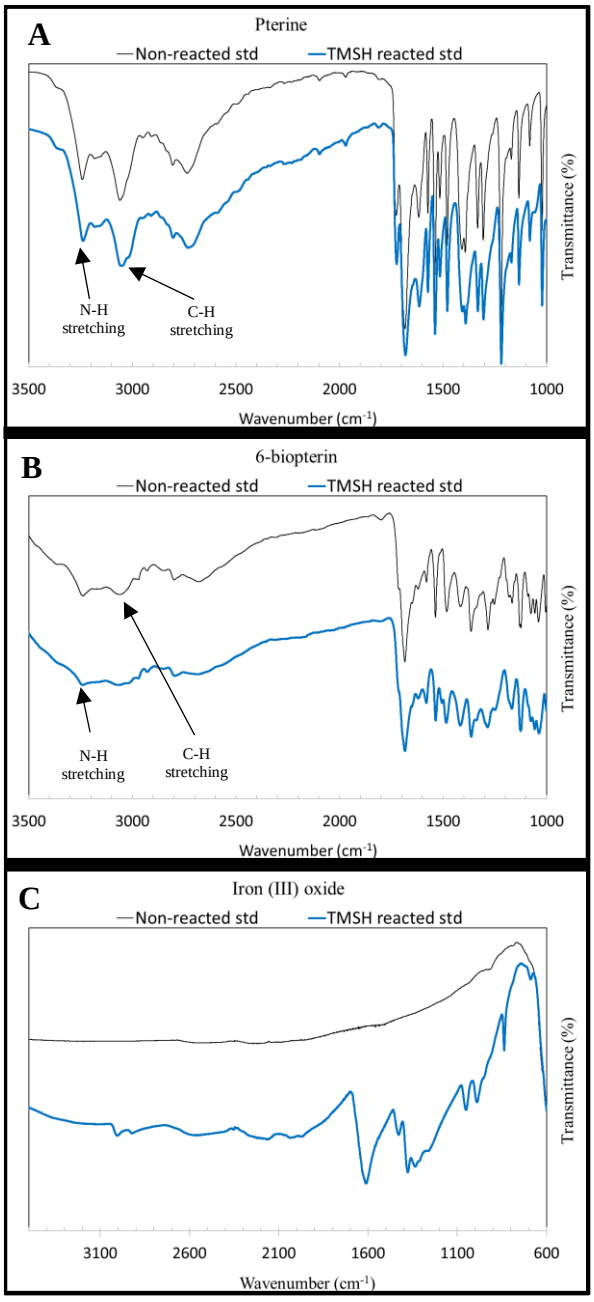

Figure 2. FT-IR spectra of (A) pterine, (B) 6-biopterin, and (C) iron oxide before and after exposure to TMSH.

UV-Vis spectra were collected from pterine, pterine-6-carboxylic acid, and guanine (Fig. 4). The spectra of the pterine and pterine-6-carboxylic acid were similar in shape, but displayed absorption maxima positioned at different wavelengths. The spectra of the pterine displayed a peak at ~274 nm, whereas the spectra of the pterine-6-carboxylic acid displayed a peak at ~288 nm. The spectra of the pterine and pterine-6-carboxylic acid also displayed peaks at ~350 nm, which is in agreement with the spectra of pterin molecules collected by other studies [1, 5]. The spectra of the guanine lacked similarly positioned peaks. These non-reacted standards were distinguishable based on their UV-Vis spectra.

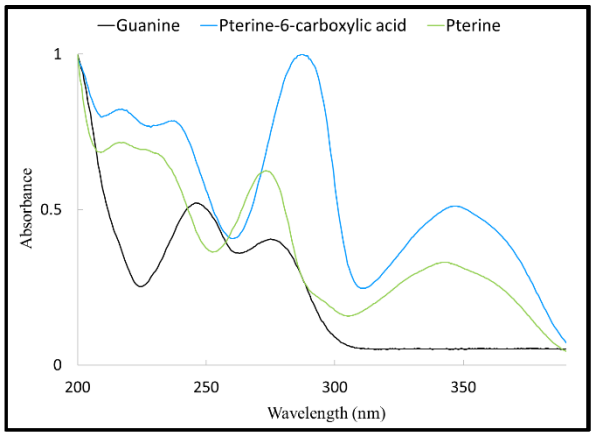

Figure 3. UV-Vis spectra of pterine, pterine-6-carboyxlic acid, and guanine.

Conclusions: The standards did not fully react with the TMSH at room temperature. How the FT-IR and UV-Vis spectra of these standards change upon full reaction with TMSH remains undetermined. Future attempts to complete a full reaction will involve heating of the reacted standards.

FT-IR and UV-Vis spectroscopy were useful in characterizing the standards, but should not be the only spectroscopic techniques used to identify different pterin molecules. A full suite of spectroscopic techniques are necessary to create a complete reference for the identification of the pterin-bearing molecule(s) present in the FeOB samples. Future work will strengthen this reference by analyzing the fluorescence and nuclear magnetic resonance spectra of the pterin standards, guaunine, and iron oxide. The completed reference will aid in the identification of pterin-bearing molecules present in FeOB-related samples on Earth and Mars.

References: [1] Nisshanthini S. D. et al. (2015) *Journal of Microbiology*, *53*, 262-271. [2] Fraeman A. A. et al. (2013) *Geology*, *41* (10), 1103-1106. [3] Williams A. J. et al. (2015) *Astrobiology*, *15*, 637-668. [4] Coates J. (2000) *Encyclopedia of Analytical Chemistry*, 10815-10837. [5] Fernandez R. et al. (2004). *Appl. Environ. Microbiology*, *70*, 121-128.