FRAGMENTATION OF TRYPTOPHAN AS A BIOMARKER ON MARS WHEN EXPOSED TO UV IRRADIATION IN THE PRESENCE OF AN OXYCHLORINE Alexandra Walter[1] and Samuel P. Kounaves[2], [1]University of Notre Dame Department of Chemistry & Biochemistry, Nieuwland Science Hall, Notre Dame, IN 46556, awalter7@nd.edu, [2]Tufts University, Dept of Chemistry, Medford, MA 02155, samuel.kounaves@tufts.edu.

Introduction: One key question in the search for life on Mars is how biogenic molecules (biomarkers) on or near the surface are altered when exposed to UV irradiation in the presence of strong oxidants such as oxychlorines (ClO_x) and their reaction products.

In 2008 the *Wet Chemistry Laboratory* (WCL) on board NASA's Phoenix Mars mission found ~ 0.6wt% perchlorate (ClO_4^-), most likely in the form of Mg- or $CaClO_4$ salts [1, 2]. The widespread presence of ClO_4^- and other oxychlorines (ClO_x), has also been confirmed by their detection in the martian meteorites EETA79001 and Tissint [3,4] and by the *Sample Analysis at Mars* (SAM) instrument on the *Curiosity* rover [5]. Under ambient Mars conditions ClO_x can be photochemically produced on Cl-bearing mineral surfaces, most likely due to silicate (SiO_2) and/or other photocatalysts [6]. During that process several ClO_x intermediates such as hypochlorite (ClO^-), chlorite (ClO_2^-), chlorate (ClO_3^-), and chlorine dioxide (ClO_2) gas, as well as radicals such as ClO^-, O_2^-, ·OCl, ·Cl, and ·OH are also likely produced [7]. These intermediate products can alter or destroy organic compounds, with UV resistant or well protected ones surviving. The intermediaries may be even more destructive than UV-driven reactions because they are not limited by screening, and through diffusion, cryoturbation, or impact reworking, could over time reach to greater depths (e.g., ClO_2 gas by diffusion) [7]. Understanding the production of oxychlorines and the processes by which they or their intermediates destroy or alter organic compounds is key to understanding the preservation and detection of biomarkers.

Tryptophan (Trp), an aromatic amino acid, has been detected in martian and chondritic meteorites [8] and is an amino acid essential for life. This makes it a good candidate molecule, serving both as a biomarker and an abiotic infall compound. It also has a high molecular weight (204) and a well-resolved UV absorbance (280 nm), as well as being a more reactive aromatic. In addition, it is relatively stable in the 190-400 nm UV wavelength range that reaches the martian surface.

Methodology: Three different Trp samples were run, one as a film on a stainless-steel disk, and the other two in silica sand (SiO_2), one without any oxychlorine, and the other with sodium chlorite ($NaClO_2$). All samples were exposed for 25 hours at 25°C and a 7 mbar Mars atmosphere. A quadrupole mass spectrometer (QMS) monitored emitted gases in real-time. Liquid chromatography-mass spectrometry (LCMS) was used for analysis of the products. The absorbance was measured at 280 (=Trp), 210, 230, and 254 nm. The three latter wavelengths were monitored to check for any new products.

Results: The LCMS chromatographs of Trp as a film (without $NaClO_2$), on the stainless-steel disk, and silica (SiO_2) sand, all gave results similar to Figure 1, eluting at t = 2.212 min with an accompanying MS ion peak at 205 *m/z* (mass/charge ratio), indicating that no new products were formed.

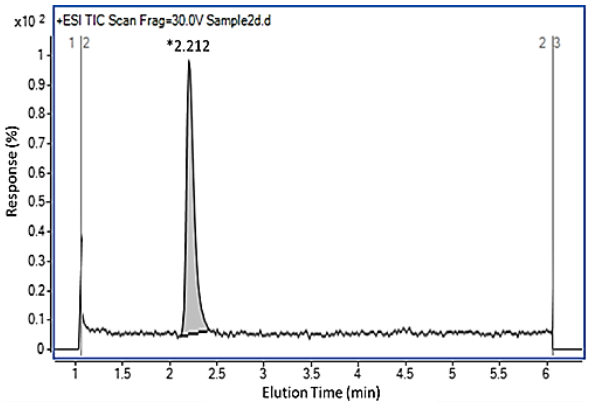

Figure 1*. LCMS TIC chromatograph of Trp.*

When Trp was irradiated with UV in a sample containing silica sand and $NaClO_2$ the results were in stark contrast to the standard and to those in the literature. Figure 2 shows the LCMS chromatogram of the UV+ $NaClO_2$ sample, and instead of having one single Trp peak at t = 2.212 min, it has 4 additional broader and shorter peaks. The QMS scans from the silica with $NaClO_2$ sample are also different when compared to the other samples, as it produced far more CO_2. The four additional peaks in the chromatogram at t = 1.362, 4.604, 4.902, and 5.421 min each appear to correspond to a single compound.

The mass spectrum of peak #1 at t =1.362 min had ion peaks at *m/z* 237 and *m/z* 221, which match those expected for *N*-formyl-L-kynurenine, a Trp metabolite, formed as a part of the kynurenine pathway when the five-membered ring is broken. An NMR spectrum also supported this conclusion, showing degradation of the aromatic ring in the 6.8-8.5 ppm region. There is a known mechanism for the formation of NFK in the presence of chlorine dioxide (ClO_2). Given that ClO_2 is a theorized intermediate in the oxidation of the ClO_2^- in the soil, this is a highly probable fragmentation pathway for the formation of NFK.

Peak #2 is that of Trp, and like the standard and the samples without $NaClO_2$, it had an elution time of ~2.3 min, and a major ion peak at *m/z* = 205, indicative of the addition of one H^+ during ionization.

Peak #3 at 4.604 min shows two new ion peaks at 241 and 257 *m/z* on the MS, indicative of fragmentation of the aromatic ring. The LCMS analysis software assigned the molecular formula $C_{11}H_{10}N_2O_3$ for the peak at *m/z* 241. This assigned formula indicates a loss of two hydrogens and the addition of an oxygen as Trp reacted with UV + $NaClO_2$ under Mars ambient conditions. It has been reported in a paper on water purification with ClO_2 that an isocyanate species with the same formula formed when Trp was exposed to ClO_2 and was a result of further degradation of NFK [9]. This isocyanate species matches the MS library formula and the NMR results of the degraded aromatic ring.

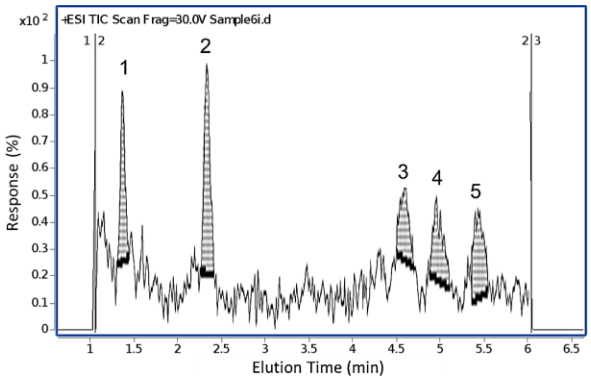

Figure 2. *LCMS TIC chromatograph of Trp in silica sand plus $NaClO_2$. The five peaks have been identified using the MS library as: (1) N-formyl-L-kynurenine; (2) Tryptophan; (3) Isocyanate; (4) Unidentified fragment with formula $C_{12}H_{14}N_2O_4$; and (5) Oxindole.*

The mass spectrum of the fourth peak in Figure 2 had a major peak at *m/z* = 251, and an MS library suggested formula of $C_{12}H_{14}N_2O_4$. In the same paper on water purification with ClO_2, it states that there was a peak found with the same *m/z* and the same formula [9]. This formula is very similar to the formula of 5-methoxy-tryptophan ($C_{12}H_{14}N_2O_3$). In addition, there are several Trp metabolites that have an oxygen added as a double bond at the point of the five-membered ring, like oxindolylalanine, indicating that the pyrrole moiety is the part of Trp most susceptible to oxidation [10], so an extra oxygen may have been added. Unfortunately, no mechanism was found to justify this product, so it is possible but unverified.

Finally, peak #5 has a mass spectrum with a major peak at *m/z* = 235. The formula given by the LCMS library matched that of an oxindole, a product of tryptophan dimerization. This product supports the two possible pathways of oxidation for tryptophan in the presence of oxychlorines. It has been theorized that radicals from ClO_2 oxidation could attack either the amino nitrogen or the indole nitrogen [11]. This would mean either the breaking of the aromatic rings, as seen with some of the previously identified products or with the changing of the amino acid functional groups, as seen with oxindole. Oxalic and fumaric acid were also identified as major products in the degradation of Trp, but were below the limit of detection for the LCMS and did not appear on any of the mass spectrums. The QMS data, from the Mars chamber gas analysis, also seems to support these results, as it indicated that CO_2 was being generated during UV exposure with the $NaClO_2$

Conclusion: The results presented here are in contrast to previous published works that reported the extent of degradation of organic compounds when irradiated with UV, but without any oxychlorines present and with pure organic samples. These results support our previous studies showing that it is the production of the ClO_4^- end product from lower oxidation-state chlorine that creates the highly reactive ClO_x and intermediary radicals that destroy or alter the organic compounds. In this preliminary work we have found that the mixture of Trp, as a candidate biomarker, with $NaClO_2$ under UV irradiation is fragmented, forming four different products. This supports previous studies showing that a salt containing any oxidation state of chlorine (from Cl^- to ClO_3^-) when exposed to Mars ambient UV has the potential to result in an outcome ranging from alteration to degradation to complete destruction of the biomarker and even to a more complex molecule of higher molecular-weight than the starting biomarker. Our preliminary results also suggest that by comparing the fragmentation products plus CO/CO_2 ratios, that the original parent biomarker could be identified.

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