Compound-Specific Isotope Analysis of Amino Acids for Stardust-Returned Samples. J.E. Elsila, J.C. Stern, D.P. Glavin, and J.P. Dworkin, Goddard Center for Astrobiology, NASA Goddard Space Flight Center, Greenbelt, MD 20771, Jamie.Elsila@nasa.gov

Introduction: Significant portions of the early Earth's prebiotic organic inventory, including amino acids, could have been delivered to the Earth's surface by comets and their fragments [1]. Analysis of comets via spectroscopic observations has identified many organic molecules, including methane, ethane, ammonia, cyanic acid, formaldehyde, formamide, acetaldehyde, acetonitrile, and methanol [2,3]. Reactions between these identified molecules could allow the formation of more complex organics such as amino acids.

Analysis of samples of silica aerogel and aluminum foil exposed to comet 81P/Wild2 and returned to Earth by the Stardust spacecraft indicated the presence of several amines and amino acids at levels exceeding those found in controls, as shown in Figure 1 [4,5]. The detected compounds included methylamine (MA), ethylamine (EA), and the amino acid glycine. The most abundant amine present in both the controls and the comet-exposed samples was ε-amino-*n*-caproic acid (EACA), most likely originating from exposure to Nylon-6 [5].

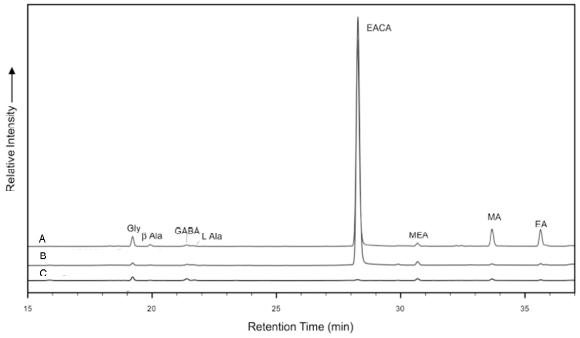

Figure 1. Fluorescence chromatograms from liquid chromatography-fluorescence detection-time of flight mass spectrometry analyses showing amines detected in: A, Stardust comet-exposed aerogel; B, Stardust aerogel witness tile (shielded from comet exposure); and C, unflown aerogel [6].

Although the MA, EA, and glycine were present in comet-exposed samples at levels significantly higher than observed in controls (non-flight aerogel and the Stardust-flown but not comet-exposed witness coupon aerogel), there is as yet no proof that these compounds are cometary in nature and not the result of terrestrial contamination. Extraterrestrial organic molecules frequently have carbon, nitrogen, and hydrogen stable isotopic ratios that are well outside the range of terrestrial compounds. Isotopic analysis could reveal whether an extraterrestrial signature is present in the Stardust-exposed amines and amino acids. Although bulk isotopic analysis would be dominated by the terrestrial signature of contaminants such as EACA, compound-specific isotope analysis (CSIA) could determine the signature of each of the other individual amines.

Here, we report on progress made towards CSIA of the amino acids glycine and EACA in Stardust-returned samples.

Analytical Techniques: CSIA of carbon in standards and samples was carried out using Thermo hybrid gas chromatography-combustion-isotope ratio mass spectrometry instrumentation with additional gas chromatography-quadrupole mass spectrometry (GC-QMS/GC-C-IRMS) capabilities. Compound separation occurred in a Trace GC with a Restek Rxi-5ms column (30 m, 0.25 mm ID, 0.5 μm film thickness). The output of the GC was split, with approximately 10% directed to a DSQ quadrupole mass spectrometer. The DSQ provided mass and fragmentation information for compound identification. The remaining 90% of the GC output passed through a Thermo GC-C III combustion interface and then into a MAT 253 isotope ratio mass spectrometer, where the $^{13}C/^{12}C$ ratio was measured.

Standards consisted of a solution of glycine (Sigma Aldrich) and EACA (Acros) dissolved in Millipore 18.2 MΩ water. Two methods were used for creating volatile amino acid derivatives: (1) reaction with N-methyl-N-[tert-butyldimethyl-silyl]trifluoroacetimide (MTBSTFA) [7] and (2) isopropyl esterification followed by reaction with trifluoroacetic anhydride (TFAA-IPA) [8].

Stable carbon isotope ratios of the underivatized glycine and EACA standards were meansured on a Costech ECS4010 elemental analyzer coupled through a Thermo Conflo III interface to the MAT 253. The isotope ratios of the underivatized standards are used with the values of the derivatized standards and samples to calculate the isotope ratios in the underivatized samples [9].

Stardust-returned samples consisted of aluminum foils that backed comet-exposed aerogel cells. Each sample was sealed in a borosilicate glass test tube with 1 mL of Millipore water for 24 hr in a heating block set at 100ºC. Half of the water supernatant was transferred to a separate test tube, dried under vacuum, and hydrolyzed under 6 M HCl vapor at 150ºC for 3 hr. A

small aliquot of the hydrolyzed sample was used in previous liquid chromatographic analyses [4,5]; the remainder was derivatized by one of the two methods described above and analyzed with GC-QMS/GC-C-IRMS.

Results and Discussion: GC-QMS/GC-C-IRMS analysis of MTBSTFA-derivatized glycine and EACA standards were possible with detection limits in the Stardust-relevant range (~1.1 nmol glycine, ~8.9 nmol EACA) [4,5]. However, problems subsequently developed with this method.

MTBSTFA derivatization was initially chosen because it is a simple one-step reaction which minimizes the potential for sample loss and contamination [7]. Although MTBSTFA derivatization produced excellent results with standards, the analysis of the MTBSTFA-derivatized acid-hydrolyzed extracts of Stardust foil sample C2092S,0 was dominated by the presence of derivatized boric acid. The boric acid appears to have originated both from the acid hydrolysis procedure, which is carried out in borosilicate ampoules, and from the Stardust sample itself. Stardust flight aerogel contains ~1.9 ppm boron [10]; it is conceivable that the aluminum foil samples picked up some boron from the adjacent aerogel. The derivatized boric acid overwhelmed the GC-QMS/GC-C-IRMS chromatograms, preventing detection of derivatized glycine or EACA. In addition, the derivatized boric acid also destroyed the GC column, resulting in high column bleed and residual boric acid peaks in later analyses. Thus, the MTBSTFA derivatization method proved unsuitable for these samples.

The TFAA/IPA derivatization method requires additional care, but does not present the same reaction aspecificity as MTBSTFA. In our tests, TFAA/IPA does not effectively derivatize boric acid. In addition, it adds fewer carbons to the amino acids, resulting in better sensitivity and precision of the isotope ratios. TFAA/IPA has been successfully used for analysis of amino acids in other extraterrestrial samples, including the Murchison meteorite [11,12] and appears promising for the analysis of Stardust amino acids. Figure 2 shows the simultaneous GC-QMS/GC-C-IRMS data from a single injection of TFAA/IPA-derivatized glycine/EACA standard solutions at Stardust-level concentrations. Although some optimization is required, these results show that the TFAA-IPA method should be suitable for CSIA of the amino acids in Stardust-returned samples for analysis of the stable isotope ratios in carbon, nitrogen, and hydrogen.

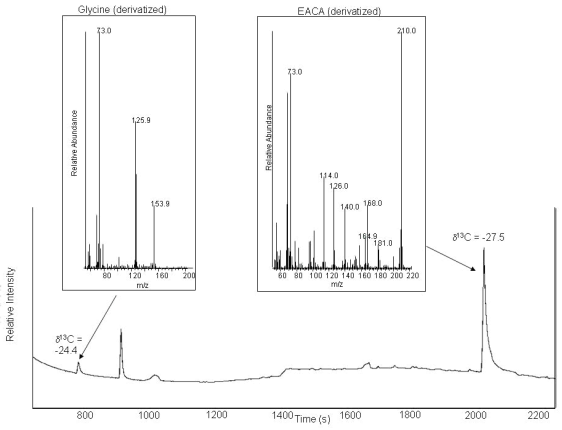

Figure 2. The GC-C-IRMS chromatogram of an injection of ~0.1 nmol TFAA/IPA-derivatized glycine and ~0.9 nmol TFAA/IPA-derivatized EACA. The $\delta^{13}C/^{12}C$ values of the derivatized standards are shown. Inserts show the simultaneous GC-QMS mass spectra of each peak, providing unambiguous compound identification.

Conclusions: GC-QMS/GC-C-IRMS of amino acids relevant to Stardust samples is possible and will help determine the origin of these compounds. TFAA/IPA derivatization is the most appropriate method. TFAA/IPA derivatization with GC-QMS/GC-C-IRMS will be applied to acid-hydrolyzed extracts of Stardust foils and the results presented.

References: [1] Chyba C. and Sagan C. (1992) *Nature, 355*, 125-132. [2] Crovisier J. and Bockele_e-Morvan D. (1999) *Space Sci. Rev., 90*, 19-32. [3] Crovisier J. et al. (2004) *Astron. Astrophys., 418*, 1141-1157. [4] Sandford S.A. et al (2006) *Science, 314*, 1720-1724. [5] Glavin D.P. et al (2008) *Met. Plan. Sci*., in press. [6] Glavin D.P. and Dworkin J.P. (2007) *LPS XXXVIII*, Abstract #1052. [7] MacKenzie S. L. et al. (1987) *J. Chromatogr., 387*, 241-253. [8] Silfer J.A. et al (1991) *Anal. Chem., 63*, 370-374. [9] Glaser B. and Amelung W. (2002) *Rapid Comm. Mass Spec., 16*, 891-898. [10] Tsou P. et al. (2003) *JGR, 108*, 3 – 16. [11] Pizzarello S. et al. (2004) *GCA, 68*, 4963-4969. [12] Pizzarello S. and Huang Y. (2005) *GCA, 69*, 599-605.

Acknowledgements: The authors would like to thank D. Brownlee, S. Sandford, G. Matrajt, C. Snead, A. Westphal, M. Zolensky, and P. Tsou for providing the samples analyzed and helpful discussions and the NASA ROSES Stardust Sample Analysis Program, the NASA Astrobiology Institute, and the Goddard Center for Astrobiology for grant support.