



Stinkbug Contamination Knowledge Report

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Introduction

A dead “stinkbug” was collected from the Lockheed Martin (Littleton, CO) RAL highbay airlock prior to spacecraft clampband shock testing (11/6/15). Due to the daily cleaning schedule of the highbay the body should have been relatively fresh (certainly compared to amino acid degradation and racemization rates). The insect was removed from the Kapton tape it was transported in and the whole insect was taken through amino acid extraction, hydrolysis and desalting process to determine the amino acid composition. The analysis indicated a significant concentration of several D-amino acids. We assumed this was an analytical bias or error from the high concentration (for us) of protein in the sample. To further investigate our methods a “similar” insect collected in Crownsville, MD was analyzed in parallel with high concentration standards. *We confirmed that the unexpected non-zero D/L ratio for several amino acids in the hydrolyzed whole-insect extracts were also present in the MD sample and the high concentration standards did not exhibit racemization under the procedure.*

Procedure

The entire dead (presumably) Brown Marmorated Stinkbug from Crownsville, MD, procedural blank, and standard were each placed in 13 mm test tubes and extracted at 100 °C for 24 hours. 10 μ L and 990 μ L of the stinkbug sample, the 10^{-4} M standard, and the procedural blank were placed in a 10 mm test tube and dried via centrifugal evaporation. The samples were hydrolyzed over 6M HCl vapor for 3 hours at 150°C. The samples were dried again. The acid-hydrolyzed water extracts were desalted using cation-exchange resin (AG50W-X8, 100-200 mesh, hydrogen form, BIO-RAD), and the amino acids recovered by elution with 2 M NH_4OH (prepared from Millipore water and $\text{NH}_3(\text{g})$ (AirProducts, *in vacuo*). The samples were then dried with sodium borate before analysis.

Analysis

The amino acids in the NH_4OH eluates were derivatized with *o*-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC) for 15 minutes at room temperature. The abundance, distribution, and enantiomeric compositions of the two- to six-carbon aliphatic amino acids present in the non-hydrolyzed and acid-hydrolyzed water extracts of the wasp and stinkbug were then determined by ultra-performance liquid chromatography fluorescence detection and time of flight mass spectrometry (hereafter LC-FD/ToF-MS) using a Waters ACQUITY H Class UPLC with fluorescence detector and Waters Xevo G2 XS. The instrument parameters and analytical conditions used were similar to those described elsewhere (Glavin et al., 2010; Glavin et al., 2006). For the Xevo mass calibrations, an automatically applied lockmass of a fragment of Leucine Enkephalin (278.1141 Da) with a scan time of 1 second every 60 seconds is used. The capillary voltage was set to 1.2 kV. The amino acids and their enantiomeric ratios were quantified from the peak areas generated from both fluorescence detection and from the mass chromatogram of their

OPA/NAC derivatives as described previously (Glavin et al., 2006).

Results

Free amino acids in the hydrolyzed extract were dominated by those shown in Table 1. These also exhibited varying abundances of D amino acids (Table 2). It is unexpected to see such high D concentrations. These values are distinctly different from those observed in degraded organic matter in terrestrial sediments or abiotic processes (e.g. Table 3) and a few bacterial and thermophilic microbial samples. The subsurface samples exhibited far lower D-aspartic acid concentrations (the fastest amino acid to racemize), suggesting that this is a limitation for

Table 1. Amount of amino acids in Stinkbug in $\mu\text{moles/insect}$

Amino Acid	Extract Concentration ($\mu\text{moles/sample}$)
D-Aspartic acid	1280.03 \pm 155.48
L-Aspartic acid	5538.50 \pm 85.22
L-Glutamic acid	8418.38 \pm 262.18
D-Glutamic acid	580.50 \pm 12.98
D-Serine	322.35 \pm 40.70
L-Serine	3255.78 \pm 302.43
Glycine	10261.55 \pm 93.11
D-Alanine	1418.59 \pm 32.91
L-Alanine	6116.00 \pm 170.32
L-Valine	3841.83 \pm 136.60
D-Valine	16.04 \pm 22.69

Table 2. The D/L Ratios for the insect. Note that any values above zero indicate that there are D amino acids present.

Amino Acid	“Stinkbug”
Aspartic Acid	0.231 \pm 0.028
Glutamic Acid	0.069 \pm 0.004
Serine	0.099 \pm 0.037
Alanine	0.232 \pm 0.014
Valine	0.004 \pm 0.001

Table 3. Enantiomeric results from our study of various geologic samples for comparison. These samples indicative of degraded bacteria, except for Rio Tinto which also contained recent bacterial colonies and Murchison meteorite which is dominated by abiotic chemistry (Glavin et al., 2008).

Amino Acid	Atacama Desert Surface (0-1 cm)	Atacama Desert Subsurface (10 cm)	Green River Shale	Svalbard Gypsum	Panoche Valley Jarosite	JSC-1 Mars analog	Rio Tinto	Murchison Meteorite
Aspartic Acid	0.50	0.45	0.83	0.54	0.64	0.39	0.22	0.91
Glutamic Acid	0.26	0.22	0.48	0.28	0.30	0.44	0.10	0.82
Serine	0.40	0.29	0.83	0.63	0.05	0.43	0.04	0.62
Alanine	0.37	0.35	0.80	0.25	0.42	0.29	0.05	0.98
Valine	0.10	0.05	0.30	0.09	0.07	0.04	0.05	0.53

Table 4. Enantiomeric results from our subsurface microbial study (Onstott et al., 2014).

Amino Acid	<i>E. coli</i> (cultured)	DR4IPC (-0.945km)	DR938 (-2.825km)	MP104 (-3.100km)	KL739 (-3.300km)	KL441 (-3.350km)
Aspartic Acid	0.02	0.044	0.054	0.049	0.065	0.098
Glutamic Acid	0.02	0.063	0.084	0.065	0.060	0.084
Serine	0.02	0.053	0.048	0.049	0.046	0.086
Alanine	0.05	0.358	0.554	0.252	0.503	0.437
Valine	0.02	<0.03	0.205	0.025	<0.03	0.027

References

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