The Miller Volcanic Spark Discharge Experiment

Adam P. Johnson,[1] H. James Cleaves,[2] Jason P. Dworkin,[3] Daniel P. Glavin,[3] Antonio Lazcano,[4] Jeffrey L. Bada[5]*

In 1953, Miller (*1*) published a short paper describing the spark discharge synthesis of amino acids from a reducing gas mixture thought to represent the atmosphere of the early Earth. This experiment showed that the basic molecules of life could be synthesized from simple molecules, suggesting that Darwin's "warm little pond" was a feasible scenario.

After Miller's death on 20 May 2007, we found several boxes containing vials of dried residues. Notebooks (*2*) indicated that the vials came from his 1953–54 University of Chicago experiments that used three different configurations (*3*, *4*). One was the original apparatus used in (*1*). Another incorporated an aspirating nozzle attached to the water-containing flask, injecting a jet of steam and gas into the spark. The third incorporated the aspirator device but used a silent discharge instead of electrodes. Although Miller repeated his experiment in 1972 with use of the original architecture (*5*), the others were never tested again.

We were interested in the second apparatus because it possibly simulates the spark discharge synthesis by lightning in a steam-rich volcanic eruption (*6*) (Fig. 1A). Miller identified five different amino acids, plus several unknowns, in the extracts from this apparatus (*3*). Product yields appeared somewhat higher than those in the classical configuration, although Miller never confirmed this. We reanalyzed 11 vials in order to characterize the diversity of products synthesized in this apparatus.

The residues in the vials were resuspended in 1-ml aliquots of doubly distilled deionized water and characterized by high-performance liquid chromatography and liquid chromatography–time of flight mass spectrometry that allows for identification at the sub-picomolar ($<10^{-12}$ M) level (*2*). We identified 22 amino acids and five amines in the volcanic experiment (Fig. 1C), several of which had not been previously identified in Miller's experiments. Vials from the other two experiments were also reanalyzed and found to have a lower diversity of amino acids (table S1). The yield of amino acids synthesized in the volcanic experiment is comparable to, and in some cases exceeds, those found in the experiments Miller conducted (*1*, *3*, *5*). Hydroxylated compounds were preferentially synthesized in the volcanic experiment. Steam injected into the spark may have generated OH radicals that reacted with either the amino acid precursors or the amino acids themselves (*7*).

Geoscientists today doubt that the primitive atmosphere had the highly reducing composition Miller used. However, the volcanic apparatus experiment suggests that, even if the overall atmosphere was not reducing, localized prebiotic synthesis could have been effective. Reduced gases and lightning associated with volcanic eruptions in hot spots or island arc–type systems could have been prevalent on the early Earth before extensive continents formed (*8*). In these volcanic plumes, HCN, aldehydes, and ketones may have been produced, which, after washing out of the atmosphere, could have become involved in the synthesis of organic molecules (*3*, *4*, *8*). Amino acids formed in volcanic island systems could have accumulated in tidal areas, where they could be polymerized by carbonyl sulfide, a simple volcanic gas that has been shown to form peptides under mild conditions (*9*).

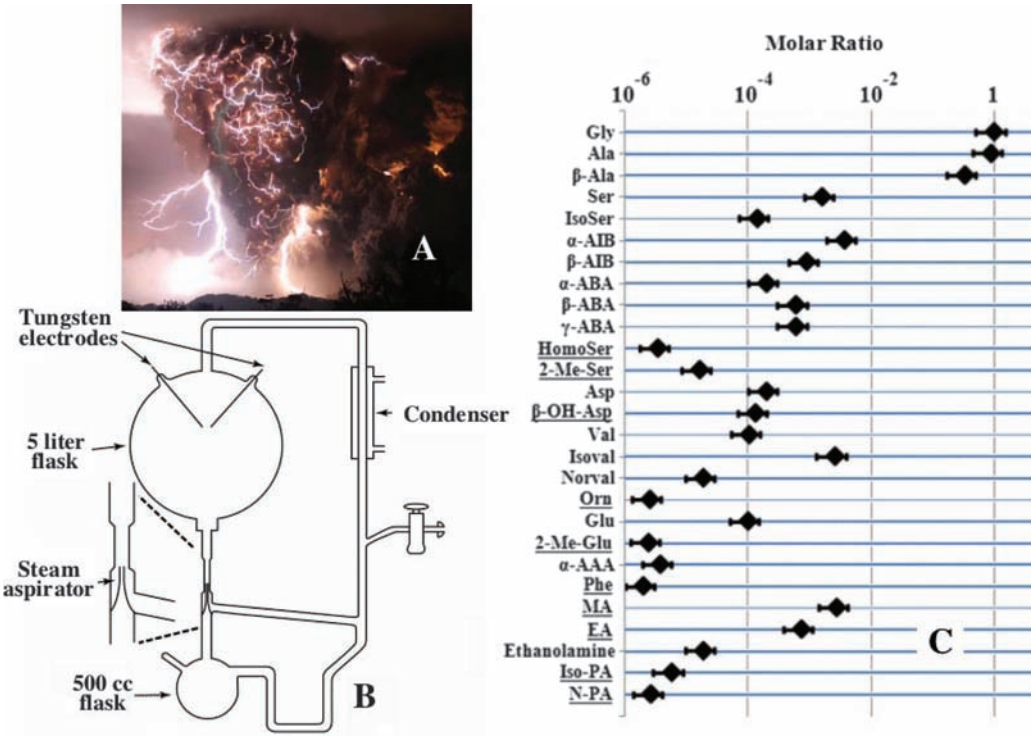

Fig. 1. (**A**) Lightning associated with the 3 May 2008 eruption of the Chaiten volcano, Chile. [Photo credit: Carlos Gutierrez/UPI/Landov] (**B**) The volcanic spark discharge apparatus used by Miller (*3*). Gas quantities added were 200 torr of CH_4, 200 torr of NH_3, and 100 torr of H_2 [these would have dissolved in the water according to their solubilities (*2*)]. Water was added to the 500-cm^3 (cc) flask and boiled, and the apparatus sparked with a Tesla coil for 1 week; (**C**) Moles (relative to glycine = 1) of the various amino acids detected in the volcanic apparatus vials [see (*2*) and table S1 for abbreviations]. Amino acids underlined have not been previously reported in spark discharge experiments. Values for amines are minimum values because of loss due to their volatility during workup.

References and Notes

1. S. L. Miller, *Science* **117**, 528 (1953).
2. Analytical details and additional data are available as supporting material on *Science* Online.
3. S. L Miller, *J. Am. Chem. Soc.* **77**, 2351 (1955).
4. A. Lazcano, J. L. Bada, *Origins Life Evol. Biosph.* **33**, 235 (2003).
5. D. Ring, W. Yecheskel, N. Friedmann, S. L. Miller, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 765 (1972).
6. S. R. McNutt, C. M. Davis, *J. Volcanol. Geotherm. Res.* **102**, 45 (2000).
7. D. Ring, S. L. Miller, *Origins Life Evol. Biosph.* **15**, 7 (1984).
8. R. D. Hill, *Origins Life Evol. Biosph.* **22**, 277 (1991).
9. L. Leman, L. Orgel, M. Reza Ghadiri, *Science* **306**, 283 (2004).

Supporting Online Material

www.sciencemag.org/cgi/content/full/322/5900/404/DC1
Materials and Methods
Figs. S1 and S2
Table S1
References and Notes

9 June 2008; accepted 8 August 2008
10.1126/science.1161527

[1]Interdisciplinary Biochemistry Program, Indiana University, Bloomington, IN 47401, USA. [2]Carnegie Institution of Washington, Washington, DC 20015, USA. [3]NASA Goddard Space Flight Center, Solar System Exploration Division, Greenbelt, MD 20771, USA. [4]Facultad de Ciencias, Universidad Nacionale Autónoma de México, Mexico Distrito Federal 04510, Mexico. [5]Scripps Institution of Oceanography, La Jolla, CA 92093–0212, USA.

*To whom correspondence should be addressed. E-mail: jbada@ucsd.edu

www.sciencemag.org/cgi/content/full/322/5900/404/DC1

Supporting Online Material for

The Miller Volcanic Spark Discharge Experiment

Adam P. Johnson, H. James Cleaves, Jason P. Dworkin, Daniel P. Glavin, Antonio Lazcano, Jeffrey L. Bada*

*To whom correspondence should be addressed. E-mail: jbada@ucsd.edu

Published 17 October 2008, *Science* **322**, 404 (2008)
DOI: 10.1126/science.1161527

This PDF file includes:

Supplementary Online Material
The Miller Volcanic Spark Discharge Apparatus

Adam P. Johnson[1], H. James Cleaves[2], Jason P. Dworkin[3], Daniel P. Glavin[3], Antonio Lazcano[4] and Jeffrey L. Bada[5]*

[1] Interdisciplinary Biochemistry Program, Indiana University, Bloomington, IN 47401

[2] Carnegie Institution for Science, Washington, DC 20015–1305, USA

[3] NASA Goddard Space Flight Center, Solar System Exploration Division, Greenbelt, MD 20771

[4] Facultad de Ciencias, UNAM, Mexico D.F. 04510, Mexico

[5] Scripps Institution of Oceanography, La Jolla, CA 92093-0212, USA

* To whom correspondence should be addressed: E-mail: jbada@ucsd.edu

MATERIALS AND METHODS

Identification of Vials and Experimental Description

The original samples were found stored in labeled four-dram vials; they were catalogued and identified by consulting Miller's original laboratory notebooks [S1]. The samples chosen for analysis came from the three experiments reported in Miller's PhD thesis at the University of Chicago [S2] and published in 1955 [S3]. The three runs represent three different apparatus used in the experiment [S3, S4]. One was the classic Miller spark discharge apparatus; another incorporated an aspirating nozzle to increase the gas flow rate through the apparatus and the third incorporated the aspirator device, but in place of the spark discharge electrodes it used a silent discharge device. The experimental design used in the original experiments includes the gases CH_4, NH_3, and H_2 at 200, 200 and 100 torr, respectively, with 500mL water. The gases would have dissolved in the water according to their individual solubility [S5] in the order $NH_3 >> CH_4 > H_2$. The solution was boiled and the apparatus sparked with a Tesla coil for one week.

Chemicals and Reagents

All glassware and sample handling tools were rinsed with Millipore water, wrapped in aluminum foil, and then heated in air at 500ºC overnight. All of the chemicals used in this study were purchased from Sigma-Aldrich and Fisher Scientific. A stock amino acid solution (~10^{-6} M) was prepared by mixing individual standards (97-99% purity) in Millipore (18.2 Mohm) water. The o-phthaldialdehyde/N-acetyl-L-cysteine (OPA/NAC) reagent used as a chemical tag for enantiomeric separation and fluorescence detection of primary amine compounds was prepared by dissolving 4 mg OPA in 300 μl methanol (Fisher Optima), and then adding 685 μl 0.1M sodium borate buffer (pH 9) and 15 μl 1 M NAC. The sodium tetraborate decahydrate ($Na_2B_4O_7*10H_2O$) powder (SigmaUltra, 99.5-100% purity) used to prepare the sodium borate buffer was heated in air at 500ºC for 3 h to reduce amine contamination in the reagent. A 0.1 M hydrazine (NH_2NH_2) solution used to remove excess OPA after derivatization was prepared by double vacuum distillation of anhydrous hydrazine (98% purity) and subsequent dilution in water. The 6M HCl was double-distilled, and the ammonium formate buffer used in the LC-ToF-MS analyses was prepared by NH_4OH titration of a 50mM formic acid solution to pH 8. A 1 μM phenolphthalein solution in acetonitrile with 0.1% formic acid was used for mass calibration of the Time-of-Flight Mass Spectrometer (ToF-MS) via an independent electrospray emitter.

High Performance Liquid Chromatography with UV Fluorescent Detection

The residues in the various vials were re-suspended by vortex stirring and sonication into 1mL aliquots of doubly-distilled water and diluted from initial stock concentrations according to optimal fluorescent signal response.

Amino acid primary amines were separated and detected using a 5 μm x 250 μm C-18 reverse phase HPLC column (*Phenomenex*) with a Shimadzu RF-535 fluorescence detector (λ_{ex}=340 nm, λ_{em}=450 nm) as their o-phthaldialdehyde/N-acetyl-L-cysteine (OPA/NAC) derivatives [S6]. OPA/NAC was prepared as follows; 4 mg OPA was dissolved in 300 μL methanol prior to addition of 250 μL 0.4M sodium borate (pH 9.4), 435 μL water and 15 μL 1M NAC. Buffer flow rate was 1 ml/min with gradients optimized for separation of glycine, β-alanine and several isomers of amino-butyric and amino-iso-butyric acids. Buffers were Optima grade Methanol (A) and 0.05M sodium acetate with 8% methanol (pH 9) (B).

Fractions of each sample were dried down twice with 0.4 M sodium borate buffer (pH 9.4) and re-suspended in 20 μL distilled water prior to 1 and 15 minute derivatization with 5 μL OPA/NAC; increase in reaction time from 1 to 15 minutes ensured complete recovery of α-substituted amino acids [S6]. Reactions were quenched with 0.05 M sodium acetate buffer (pH 5.5) to a final volume of 500 μL and immediately analyzed. Recovery of DL norleucine internal standard was >99%. Analyses of secondary amines such as sarcosine, imino-diacetic acid and imino-acetic propanoic acid, which are not reactive with OPA/NAC, are not reported. Concentrations of unknowns were determined from bracketed calibrations of known amino acid standards based on peak area.

Liquid Chromatography with UV Fluorescence Detection and Time of Flight-Mass Spectrometry (LC-FD/ToF-MS)

A fraction of each residue was prepared and derivatized as described above. The derivatization reaction was quenched after 1 min at room temperature with 75 μl of 0.1M hydrazine hydrate, and the solution was loaded into the LC-FD auto sampler carousel at 4ºC within a few minutes of analysis.

Amino acid derivatives and their enantiomeric ratios in the Miller extracts and procedural blanks were analyzed after chemical derivatization by LC-FD/ToF-MS. The LC-FD was a Waters ACQUITY system with a 1.7 μm particle 2.1x150 mm Waters BEH Phenyl-Hexyl column at 10°C and a flow of 0.15 mL/min. For additional details on the Waters LCT

Premier ToF-MS instrument and operation parameters used in these analyses see [S6]. The gradient was A (pH 8 50 mM ammonium formate with 8% methanol buffer) into B (methanol) with the 0-35 min, 0-55% B; 35-45 min, 55-100% B. The instrument was optimized for maximum sensitivity of OPA/NAC primary amine derivatives in the 300-450 m/z range with detection limits in the sub-femtomole (~10^{-15} to 10^{-16} moles) range. In addition to identifying the major fluorescent peaks present in the LC-FD/ToF-MS chromatograms by retention time and mass, we also searched for the masses corresponding to C2-C6 amino acids and C1-C6 amines by plotting the exact mass of each compound, with a ~20 ppm window, over the elution time.

RESULTS AND DISCUSSION

Example chromatograms of the analyses of the extracts from the various apparatus are shown in **Fig. S1**. The greater diversity of amino acids synthesized in the volcanic spark discharge apparatus is clearly apparent. The extracts from apparatus one may not be complete. **Fig. S2** shows the identification of D, L-2-methylglutamic acid in the Apparatus 2 extract using LC-FD/ToFMS analysis. A summary of the amounts (relative to glycine = 1) of the amino acids and amines detected in the extracts from each apparatus is given in **Table S1**. Besides the amino acids listed, several 6-carbon amino acids were also detected but these were not quantified. Amino acids with chiral centers were racemic within the precision of the measurements.

We were not able to calculate actual yields for each experiment because there was no record of how much of the water from various experiments was saved. However, Miller (S2, S3) gave the yields of glycine (based on carbon added as methane) from the three apparatus as 2.1 %, 1.8 %, and 0.4 %, respectively. The actual yields of the various amino acids can thus be calculated based on these values.

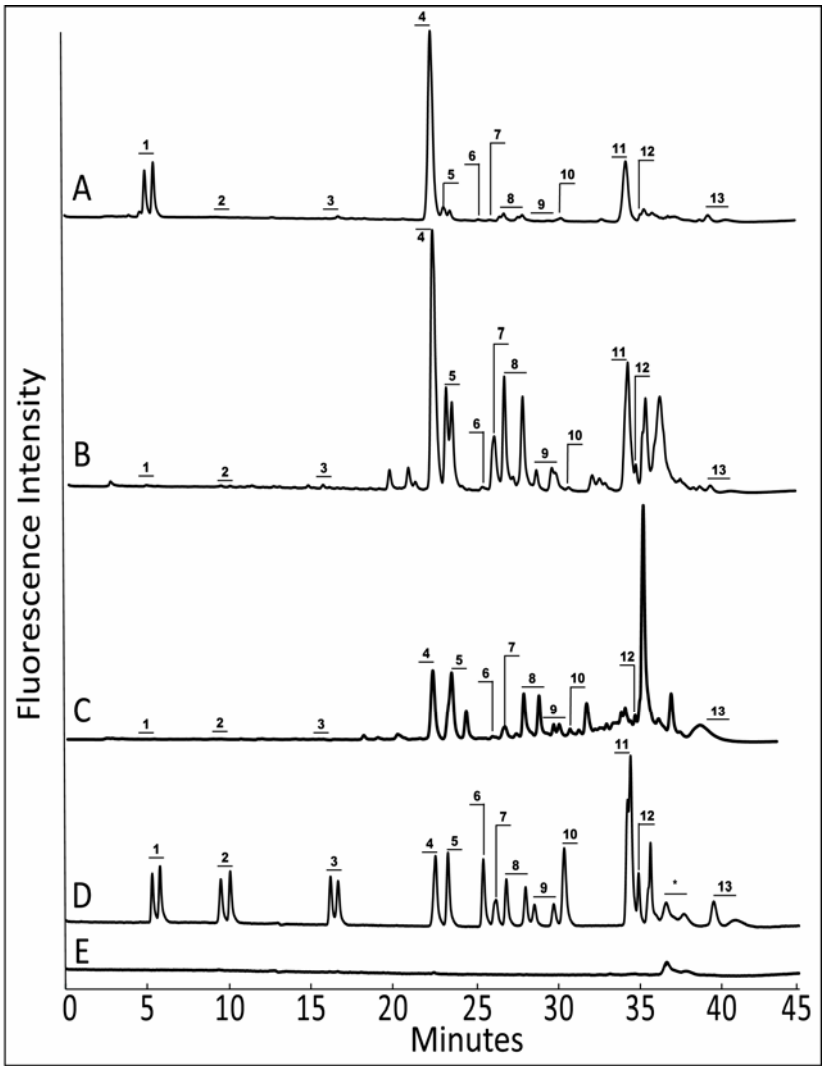

Fig. S1: Representative cumulative HPLC chromatograms for: (A) classic apparatus design: (B) volcanic apparatus design; (C) silent discharge apparatus design: and (D) an amino acid standard: peak identifications 1-D,L-Asp; 2-L,D-Glu; 3-D,L-Ser; 4-Gly; 5-β-Ala; 6-γ-ABA; 7-D,L-β-AIB; 8-D,L-Ala; 9-D,L-β-ABA; 10-α-AIB; 11-D,L-α-ABA; 12-D,L-Isovaline; 13-D,L-Norleucine. (* are unidentified peaks); and (E) a procedural blank. Note: (A), (B) and (C) are summed signals from multiple fractions and various dilutions chosen in order to optimize signal intensity; peak heights and/or areas are not representative of total recovery and are meant only as a qualitative analysis of diversity between the three.

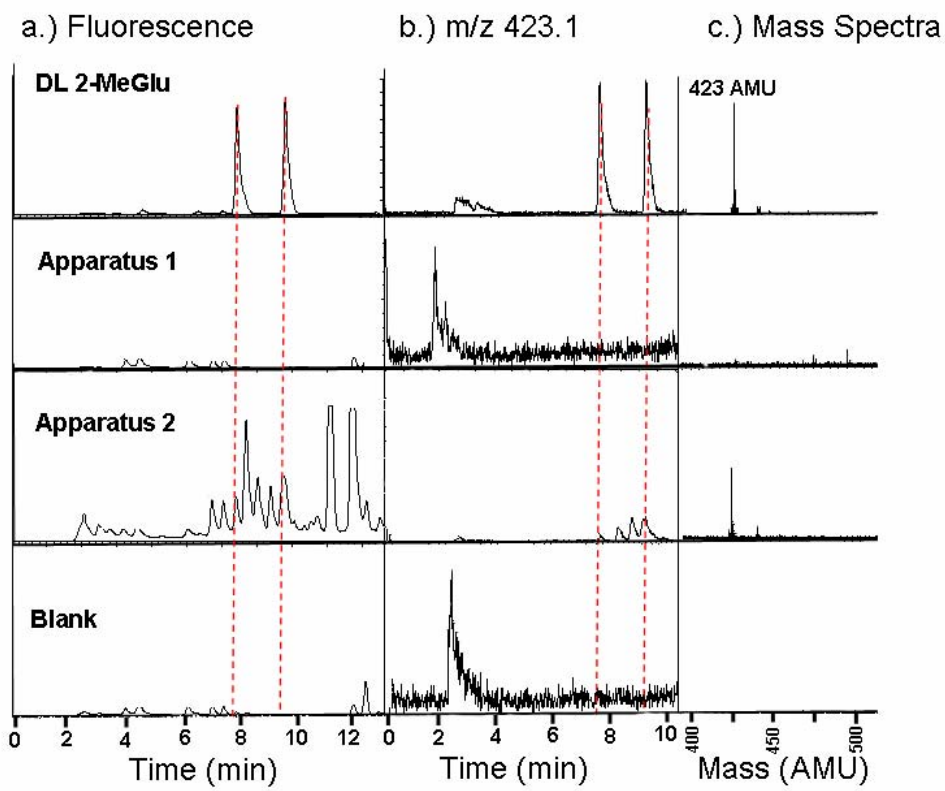

Fig. S2: An example of the use of LC-MS to identify compounds in the various extracts. a.) Fluorescence HPLC traces of the 0-12.5 min region (y-axis is fluorescence intensity) of an OPA/NAC derivatized authentic D, L-2-methylglutamic acid standard (top), extract from Apparatus 1 (second trace from top), extract from Apparatus 2 (third trace from top), and a water blank (bottom trace); b.) m/z 423.123 LC-FD/ToF-MS traces of the 0-12.5 minute region (y-axis is relative signal intensity); c.) 400-500 amu mass spectra of the 9.6 minute region of the LC-FD/ToF-MS chromatogram corresponding to 2-MeGlu (y-axis is relative signal intensity). The pure individual D and L enantiomeric standards were not available to identify the peaks corresponding to the 2-MeGlu enantiomers. Note that the extract of apparatus 3 was not analyzed by LC-ToF-MS.

Table S1: Various amino acids detected in the extracts.

	Apparatus One	Apparatus Two	Apparatus Three
Glycine (Gly)	1	1	1
Alanine (Ala)	2.7×10^{-2}	0.9	1.4
β-Alanine (β-Ala)	0.003	0.3	0.9
Serine (Ser)	1.0×10^{-4}	1.6×10^{-3}	2.7×10^{-3}
Isoserine (IsoSer)	Not detected	1.4×10^{-4}	Not detected
α-Amino-Isobutyric Acid (α-AIB)	1.1×10^{-3}	3.7×10^{-3}	7.1×10^{-2}
β-Amino-Isobutyric Acid (β-AIB)	3.2×10^{-5}	9.0×10^{-4}	4.8×10^{-2}
α-Amino-Butyric Acid (α-ABA)	7.0×10^{-4}	2.0×10^{-4}	Not detected
β-Amino-Butyric Acid (β-ABA)	5.0×10^{-4}	6.0×10^{-4}	4.7×10^{-2}
γ-Amino-Butyric Acid (γ-ABA)	1.0×10^{-4}	6.0×10^{-4}	1.4×10^{-2}
HomoSerine (HomoSer)	Not detected	3.4×10^{-6}	Not detected
2-Methyl Serine (2-Me-Ser)	Not detected	1.6×10^{-5}	Not detected
Aspartic Acid (Asp)	6.0×10^{-4}	2.0×10^{-4}	2.5×10^{-3}
β-hydroxy-Aspartic Acid (β-OH-Asp)	Not detected	1.3×10^{-4}	Not detected
Valine (Val)	3.3×10^{-5}	1.1×10^{-4}	Not detected
Isovaline (Isoval)	4.0×10^{-4}	2.6×10^{-3}	9.9×10^{-3}
Norvaline (Norval)	5.4×10^{-5}	1.9×10^{-5}	Not detected
Ornithine (Orn)	Not detected	2.5×10^{-6}	Not detected
Glutamic Acid (Glu)	2.0×10^{-4}	1.0×10^{-4}	1.3×10^{-3}
2-Methyl Glutamic Acid (2-Me-Glu)	Not detected	2.4×10^{-6}	Not detected
α-Amino Adipic Acid (α-AAA)	Not detected	3.8×10^{-6}	Not detected
Phenylalanine (Phe)	Not detected	2.0×10^{-6}	Not detected
Methylamine (MA)	5.0×10^{-3}	2.8×10^{-3}	Not detected
Ethylamine (EA)	1.7×10^{-3}	7.4×10^{-4}	Not detected
Ethanolamine	2.5×10^{-4}	1.9×10^{-5}	7.2×10^{-2}
Isopropylamine (Iso-PA)	1.3×10^{-5}	5.7×10^{-6}	Not detected
n-Propylamine (N-PA)	3.8×10^{-6}	2.6×10^{-6}	Not detected

Reference and Notes

S1. Special thanks to the Mandeville Special Collections, Geisel Library, University of California, San Diego for their help in obtaining Miller's original notebooks.

S2. S. L. Miller, "*A production of organic compounds under possible primitive earth condition"*. PhD Thesis, University of Chicago (1954).

S3. S. L. Miller, *J. Amer Chem. Soc*. **77**, 2351 (1955).

S4. A. Lazcano, J. L. Bada, *Orig. Life Evol. Biosph*. **33**, 235 (2003).

S5. E. Wllhelm, R. Battlno, R. J. Wllcock, *Chem. Rev*. **77**, 220 (1977).

S6. M. X. Zhao, J. L. Bada *J. Chromatography A* **690**, 55 (1995).

S7. D. P. Glavin, J. P. Dworkin, A. Aubrey, O. Botta, H. Doty III, Z. Martins, J. L. Bada, *Meteorit. Planet. Sci*. **41**, 889 (2006).

S8. APJ would like to acknowledge the Marine Biology Institute's Planetary Biology Internship Program, through which funding and support for this project was provided. Support from CONACYT (Mexico, Project 50520-Q) to AL is gratefully acknowledged. Support for JPD and DPG are from the NASA Astrobiology Institute and the Goddard Center for Astrobiology.