Organic amine biomarker detection in the Yungay region of the Atacama Desert with the Urey instrument

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[1] The Urey in situ organic compound analysis instrument, consisting of a subcritical water extractor (SCWE) and a portable microchip capillary electrophoresis instrument called the Mars Organic Analyzer (MOA), was field tested in the Atacama Desert, Chile, in June 2005. Soil samples from the most arid Yungay region were collected, biomarkers were extracted by the SCWE, and organic amine composition and amino acid chirality analysis was performed by the MOA. Samples collected from the top 1 cm of duracrust soil but shielded from the ambient environment by rocks were compared to the exposed duracrust. The shielded duracrust yielded amines and amino acids ranging from 50 to 100 ppb, while amino acid signals from the exposed duracrust were below blank levels. Samples from buried gypsum deposits located directly above a water flow channel contained amino acids ranging from 13 to 90 ppb. Chiral analysis revealed D/L ratios of 0.39 ± 0.08 and 0.34 ± 0.07 for alanine/serine and 0.78 ± 0.06 for aspartic acid, indicating significant racemization of biologically produced amino acids. On the basis of the D/L ratios, we estimate sample ages ranging from 10^3 to 10^5 years. These results demonstrate the successful field testing of the Urey instrument, as well as the detection of biomarkers from past terrestrial life in one of the most arid and Mars-like regions on Earth.

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1. Introduction

[2] There is intense interest in the possibility that life once existed or still exists on Mars [*Formisano et al.*, 2004; *Malin and Edgett*, 2000, 2003; *McKay et al.*, 1996; *Squyres et al.*, 2004]. Orbiters and landers have discovered geological and mineralogical evidence that liquid water existed on the surface perhaps as recent as 300 000 years ago [*Christensen et al.*, 2004; *Fassett and Head*, 2006; *Klingelhofer et al.*, 2004; *Malin and Edgett*, 2000, 2003; *Reiss et al.*, 2004; *Squyres et al.*, 2004]. In addition, methane, which can be biologically produced, has been detected in the Martian atmosphere but the source remains controversial [*Formisano et al.*, 2004]. These results indicate that Mars once had liquid water, a requirement for life on Earth, and hint that Mars could have or may still support life. Viking landed on Mars in 1976 and performed the first experiments designed to investigate whether Mars contains either viable organisms or organic molecules. No organics were detected by the Gas Chromatograph Mass Spectrometer (GCMS) and the biology experiments are generally interpreted as inconclusive, possibly due to interference by oxidants [*Benner et al.*, 2000; *Biemann et al.*, 1977; *Levin and Straat*, 1977; *Oyama and Berdahl*, 1977; *Zent and McKay*, 1994]. Recent simulations and experiments with Mars analog soils have supported the idea that the Martian surface is highly oxidizing [*Quinn et al.*, 2005, 2006]. The presence of oxidants and the Viking pyrolysis procedure may have degraded any organic molecules into compounds such as carboxylic acids and amines that are not easily detectable by the GCMS instruments on Viking [*Benner et al.*, 2000; *Glavin et al.*, 2001]. In addition, it has been argued that coelution of target analytes and solvents in the GC column would have yielded a true GCMS sensitivity above 0.01 μg/g (10 ppb) for methylamine or ethylamine [*Glavin et al.*, 2001]. Careful selection of target analytes and development of high sensitivity instrumentation capable of detecting low-level products is necessary for future in situ organic and life detection experiments on Mars.

[3] We previously reported on an instrument called the Mars Organic Analyzer (MOA) which was developed for the in situ detection of biomarker compounds, especially organic amines that are indicative of a biological process

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[*Skelley et al.*, 2005]. Initially amino acids were targeted for analysis because these small organic amines are resistant to degradation and can thus be used to look for extant or ancient sources. Furthermore, amino acid chirality is strongly indicative of biological origin. Biologically produced amino acids are homochiral or exhibit a chiral excess while racemic amino acids indicate an ancient source or abiotic synthesis [*Miller*, 1953; *Oró and Kamat*, 1961]. With this goal in mind, a labeling method and sensitive capillary electrophoresis (CE) separation technique were developed to analyze amino acids and their enantiomers [*Skelley and Mathies*, 2003], and a portable capillary electrophoresis instrument and multilayer microfabricated device was constructed [*Skelley et al.*, 2005]. The instrument was first field tested in the Panoche Valley, CA along with the Mars Organic Detector (MOD), an instrument for extraction of biomarkers using sublimation [*Glavin and Bada*, 1998; *Kminek et al.*, 2000]. Amines and amino acids were sublimed from jarosite samples using MOD, and then detected by the MOA revealing that jarosite contains amino acids at part-per-billion (ppb) levels [*Skelley et al.*, 2005]. Further studies broadened our range of target analytes to include the detection of monoamines, diamines, amino sugars, nucleobases and their degradation products [*Skelley et al.*, 2006; *Skelley and Mathies*, 2006]. Subsequently, a subcritical water extractor (SCWE) has been developed to further increase the extraction efficiency of bioamines from soil samples [*Amashukeli et al.*, 2006, 2007]. The SCWE and MOD together with the MOA microchip CE instrument constitute the biomarker analysis instrument now proposed as Urey [*Bada et al.*, 2005].

[4] Further advancement of the technology readiness of Urey requires testing the robustness and sensitivity of these instruments in a Mars-like environment on representative samples. The site chosen for this field test was the Atacama Desert, Chile. The Atacama was formed approximately 14 million years ago and experienced a period of increased aridity approximately 6 million years ago [*Hartley and Chong*, 2002; *Hartley et al.*, 2005]. The Atacama is classified as an Ea23 desert, indicating that it is extremely arid (E), has no season for precipitation (a), has average temperatures between 10 and 20°C during its winter season (2), and average temperatures between 20 and 30°C during its summer season (3). During a 4 year observation of the Atacama Desert, the average temperature was ~16°C, with minimum and maximum temperatures of −5.7°C to 37.9°C [*McKay et al.*, 2003]. Over this period there was only one significant rain event of 2.3 mm. As a result, liquid water was present under surface stones for only 65–85 hours over the entire observation period [*McKay et al.*, 2003]. Because this region of the Atacama has the lowest recorded rainfalls on Earth it is an excellent Mars analog test site.

[5] Previous studies in the Atacama by *Navarro-Gonzalez et al.* [2003] have used both GCMS and culture techniques to profile a north-south transect both in terms of detectable organics and culturable bacteria. In the northern-most Rock Garden site (Yungay region), extremely low levels of culturable bacteria were found, and the GCMS was only able to detect benzene and formic acid, indicating highly oxidized soil samples. Further oxidant studies in the Yungay region have suggested that the dry-deposition of acids may be a major contribution to the oxidants detected [*Quinn et al.*, 2005, 2006]. The absence of obvious indigenous life, the low levels of organics and the high levels of oxidants confirm that the Yungay region is an ideal Mars analog.

[6] Here we report the results of a two-week field test performed from June 1st to 15th, 2005 in the Yungay region of the Atacama Desert. The specific goals of this field test were to demonstrate the operation of the Urey instruments in the field, to develop an optimized protocol for extraction of bioamines using the SCWE followed by MOA analysis, and to demonstrate the detection and characterization of biomarkers in one of the most Mars-like regions on Earth.

2. Materials and Methods

2.1. Assembly of the Field Laboratory

[7] All instrumentation was flown within packing containers, to Antofagasta, Chile and then trucked ~100 km to the field lab located in the Yungay region. The University of Antofagasta field lab is a collection of shelters with no power or air handling systems so instruments were run from generators in the ambient desert environment.

2.2. Selection of Sampling Sites

[8] Detailed sampling of the hill called Yungay1122 (S 24° 3.6423; W 69° 5.2082; elevation 1122 m, Figure 1) was performed. Yungay1122 was chosen because of its proximity to the Rock Garden site previously studied [*Navarro-Gonzalez et al.*, 2003; *Skelley et al.*, 2005] and because it exhibited few signs of recent human activity on the south and west faces. Differential GPS mapping was used to survey Yungay1122, and all of the 20 sampling sites are shown on the map in Figure 1b. The sampling sites were selected on the south and west faces, avoiding a low saddle that exhibited more anthropomorphic activity. The sites were chosen with different altitudes and slopes, and had different rock coverage and exposure to sunlight. All sites were marked prior to sampling, and a path around the hill connecting the sites was established to avoid contamination. The soil to be sampled was not disturbed until after all the sites had been chosen and GPS mapping was completed.

[9] The Soil Pit site was also studied because it is located in the plains immediately southwest of the Yungay site but is still within the hyper-arid region (S 24° 6.131; W 70° 1.087; elevation 1023 m). The site had been previously studied for organic carbon and mobile ions with depth [*Ewing et al.*, 2007]. Three different samples were collected at the surface, at 88 cm and 136 cm deep. Material was first cleaned off the walls of the soil pit (~5 cm removed horizontally) before the soil samples were collected.

2.3. Procedure for Sampling Soils

[10] Sampling was performed from a position below each site on Yungay1122. Trowels were sterilized with ethanol-soaked technicloths and different depth soil samples were collected from each site. Typically a duracrust sample (the cohesive soil crust) was taken from the top 1 cm of an ~25 cm × 25 cm area. Immediately below the duracrust a

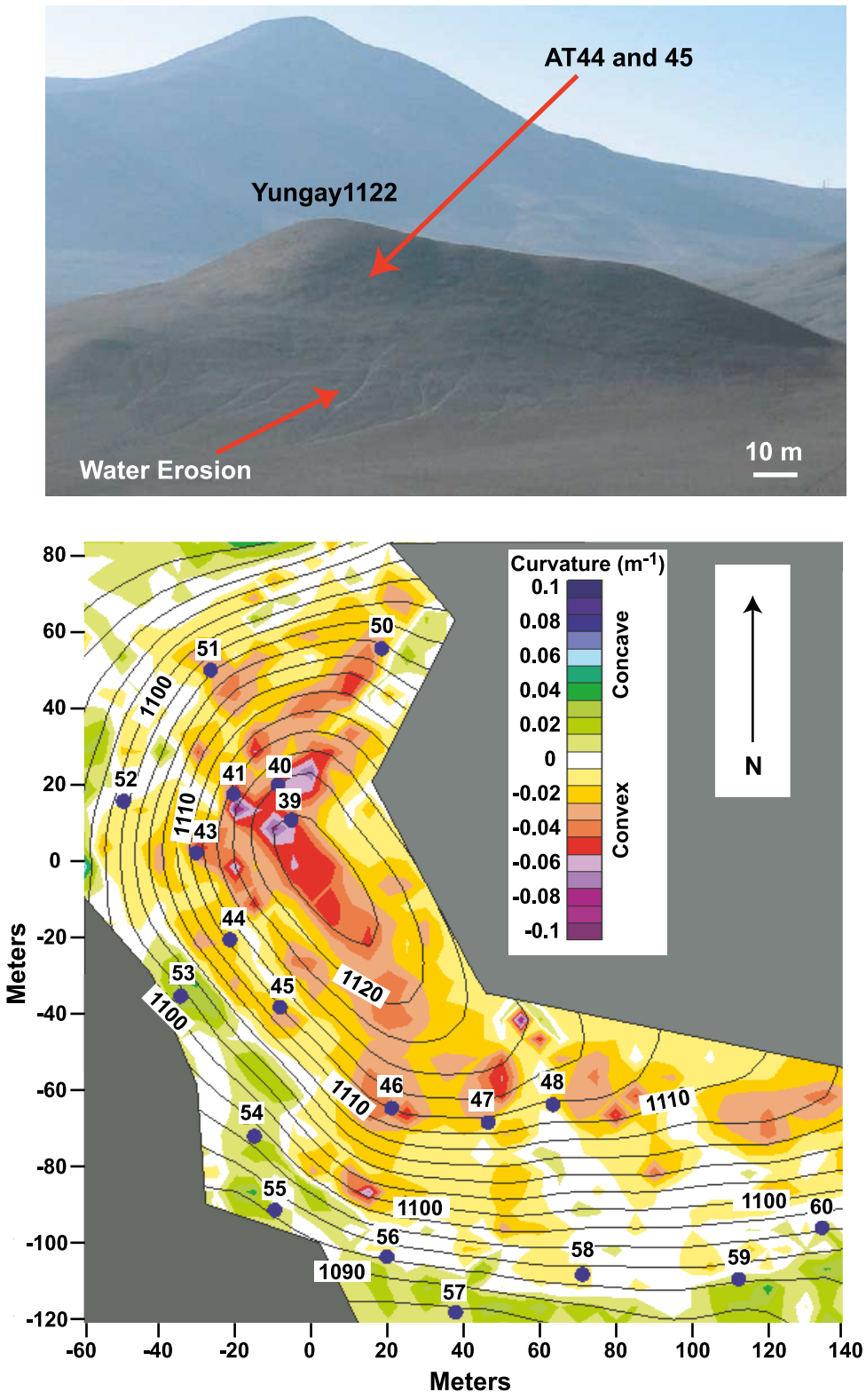


Figure 1. (a) Photograph of hill Yungay1122 in the Atacama Desert taken from the Rock Garden site. View towards the north/east. Water erosion gullies extend down from Sites 44 and 45. (b) Differential GPS map of Yungay1122 showing sampling sites (black dots) that were chosen on the southern and western slopes.

second sample was taken with depths ranging from 2–5 cm. At different sites, samples were also collected from gypsum mounds present below and at the surface. Approximately 100 to 500 g of soil was removed for each sample and placed directly into sterile plastic bags. The labels (for example AT45A4_08 (i)) described the area (AT = Atacama Yungay1122, SP = Soil Pit), the site (45), the location at the site (A or B indicating areas of different rock coverage or

mineralogy), the depth fraction (4 = the fourth depth fraction, with exact depth recorded), the date in June 2005 that the sample was collected, and the fraction collected from the SCWE (here the first fraction (i)). All of the samples collected, with the exception of the soil used for field analyses, were shipped back to JPL for further study.

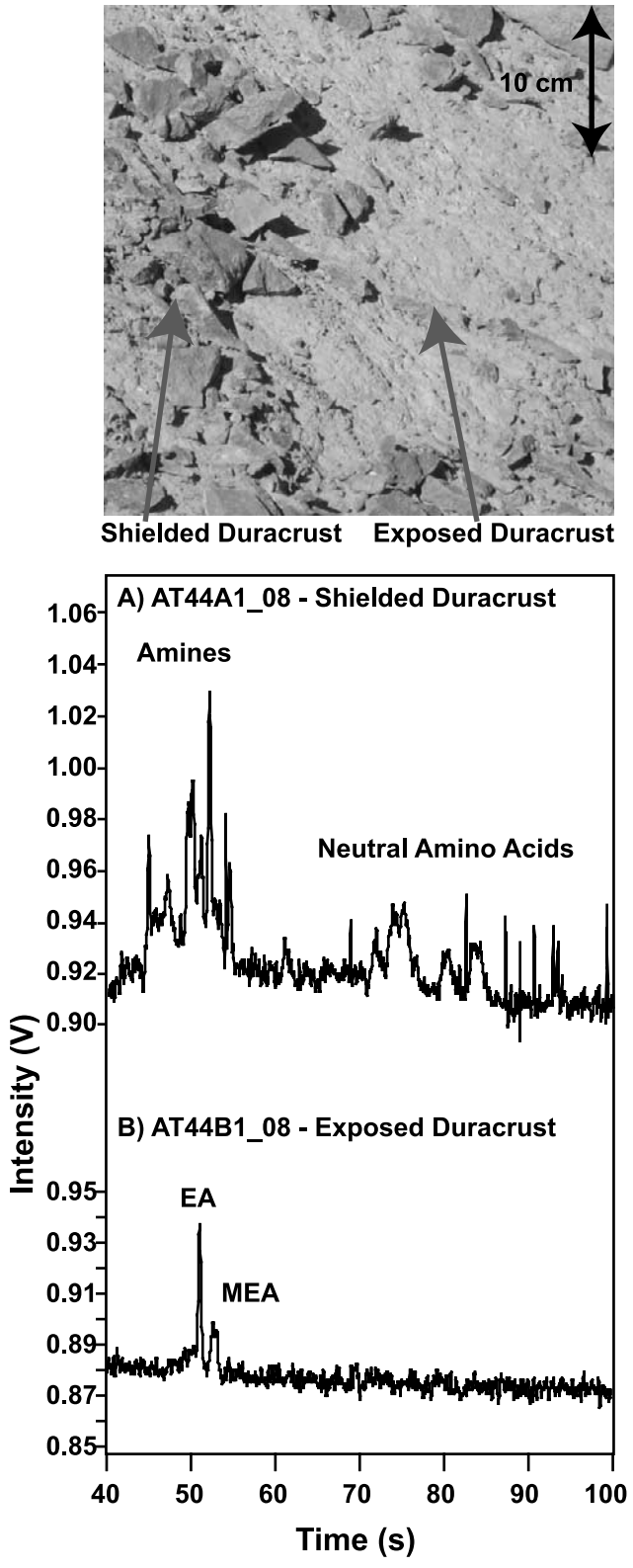


Figure 2. (top) Photograph of Site 44, showing the shielded and exposed duracrust areas. Samples were taken from the shielded duracrust and from the exposed duracrust at different depths at this site. (bottom) CE analyses of the surface extracts (<1 cm) from this site analyzed in the field. The (a) shielded duracrust contained higher levels of neutral amino acids and amines, while the (b) exposed duracrust exhibited low levels of simple amines at the level of the solvent blank.

2.4. Amino Acid Extraction

[11] Approximately 1.5 g of soil, homogenized using a sterile mortar and pestle, was extracted using the portable subcritical water extractor (SCWE) described in detail by *Amashukeli et al.* [2007]. The SCWE consists of a sealed sample cell connected to a piston pump, which is capable of pressurizing water to 20 MPa. The temperatures of the sample cell and the subcritical water are maintained by the PID temperature controllers. The sample cell is equipped with 2 μm filtration frits that prevent loss of the soil and interruption of SCWE operation. The subcritical water flow is controlled by valves positioned on the input and output sides of the sample cell. The sample was loaded into a sterile sample cell, inserted into the SCWE, and sealed. Water at room temperature was flushed through the soil sample ($\sim$0.5 mL/min for 1 min) to insure that there were no leaks in the system. The flow was then stopped and the exit valve was closed. The temperature controllers were set to experimental temperatures, 150 to 250°C. The average heating rate of the water and sample cell was 30°C/min. Once at temperature, water was flushed through the sample cell at constant pressure at 0.5 mL/min and $\sim$2 mL of solution was collected for each of 3–4 fractions. At the end of the extraction experiment, the sample cell was cooled down, depressurized, and removed from the SCWE. The system was then flushed at 1 mL/min, T = 100°C for 5 minutes before loading the next cell. Blanks were run by inserting a clean, empty cell into the SCWE and following the same heating and run protocols. Three fractions (2 mL each) were collected from each cell. Aliquots (10 μL) were removed for direct analysis without any preconcentration, and 500 μL aliquots of each sample were dried on a heating block (T = $\sim$60°C) at atmospheric pressure to produce the concentrated sample. Some dried aliquots were analyzed in the field during the 2-week field trial, the remainder were flown back to the United States for analysis.

2.5. Labeling and Analysis of Extracted Samples

[12] The dried aliquots were brought up in 20 μL of 10 mM CO_3^{2-} pH 9.78 buffer and split into 2 separate volumes. To the 10 μL of sample (either from the dried aliquot or direct SCWE extract) was added either 10 μL of pH 9.78 buffer or 10 μL of standard (Mars 7 Standard (M7S), containing 13 μM valine, alanine, serine, glutamic acid and aspartic acid, 6.6 μM glycine, and 27 μM alpha-aminoisobutyric acid (AIB), 100 μM total amino acid concentration in pH 9.78 buffer). Twenty μL of 20 mM fluorescamine in acetone was added to each and the

solutions were reacted at room temperature for 10 minutes [*Skelley and Mathies*, 2003]. The acetone was removed for 3 minutes under vacuum, and the samples were diluted 1/3 or 1/9 with either pH 9.78 buffer for achiral separations or 10 mM CO_3^{2-} pH 8.4 buffer for chiral separations.

[13] The samples were analyzed on the portable CE instrument previously described [*Skelley et al.*, 2005]. The temperature of the separation channel was not controlled for the achiral separations and ranged from ~5°C to ~30°C in the field station. A regular injection was first performed, injecting sample across to the waste reservoir for 10 s at −2500 V. A 300 ms direct injection, consisting of first a 10 s regular injection followed by injecting sample directly to the waste for 300 ms, was then performed to increase the sensitivity of the assay. The samples were run at −15 kV, sample and waste biased at −2650 V, anode grounded, temperature and buffer as specified. All samples were injected in duplicate, and the average value and standard deviation are reported. Chiral separations were performed by including hydroxy-propyl-β-cyclodextrin in the run buffer (15 mM HBβCD, 10 mM CO_3^{2-} pH 8.7). The temperature during a chiral separation was kept below 10°C either by using a recirculating water bath or by running before 11 am in the field station (ambient temperature 4°C to 10°C).

2.6. Peak Fitting and Determining D/L Ratios

[14] Electropherograms were fit using Grams 32 to determine peak times, widths, heights, and areas. To account for differences in injection efficiency between the sample trace and the spiked sample (sample plus standard), all peaks were normalized to a common, unspiked peak (typically the peak eluting immediately before valine). The D/L ratios were determined by fitting the D and L peaks of the different enantiomers. The D/L alanine-serine ratio was determined by subtracting the contribution from glycine (see auxiliary material and Figure S1; glycine produces two peaks because the glycine-dye adduct is chiral).[1]

2.7. Calibration of Field Instruments

[15] Testing of the equipment was performed in the field lab on amino acid standards and on soil standards from San Diego to establish optimal extraction protocols. Different extraction temperatures were explored in the field by analyzing a South Bay salt sample collected in San Diego, CA. The sample processed at 250°C yielded the highest levels of amines and amino acids in the direct extract (auxiliary material, Figure S2). Of the three 2 mL fractions collected, the first fraction contained significantly higher levels of organic material. Subsequent samples were then extracted at 250°C and the data from the first fraction are presented.

3. Results

[16] A total of 71 soil samples were collected from Yungay1122 and 3 samples from the Soil Pit. A selection of 11 samples from 4 different sites was chosen for initial field studies to sample the variability of organics as a

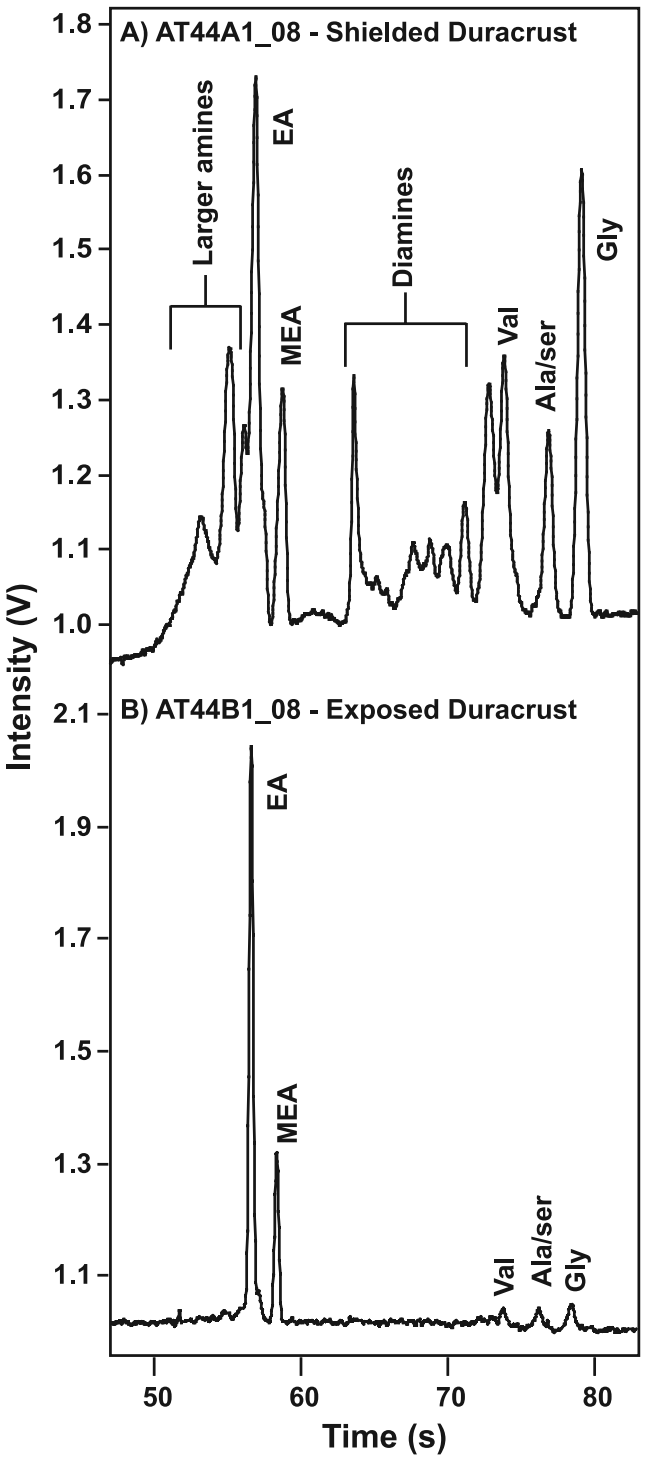


Figure 3. MOA analysis of concentrated Site 44 extracts (extracted in the field, analyzed in the United States). The (a) shielded duracrust (<1 cm) contained high levels of neutral amino acids and amines, while the (b) exposed duracrust exhibited amino acids and amines at the level of the blank. The sample was run at a 1/9 dilution from the labeled mix and is ~25-fold more concentrated than the direct SCWE extract analyzed in Figure 2.

[1]Auxiliary materials are available in the HTML. doi:10.1029/2006JG000329.

Table 1. Amino Acid Concentrations in Atacama Subcritical Water Extracts[a,b]

Sample	Val	Ala/ser	Gly	Glu	Asp
AT40AB1_08	9 ± 4	10 ± 4	14 ± 5	7 ± 10	19 ± 6
AT40C1_08	3 ± 2	BB[c]	BB		
AT40A2_08	4 ± 2	5 ± 2	68 ± 23		
AT40B2_08	8 ± 3	24 ± 6	52 ± 5		
AT40B2_08	1.1 ± 0.9	2 ± 2	BB		
AT44A1_08 (Shielded Duracrust)	61 ± 8	45 ± 8	92 ± 32		
AT44B1_08 (Exposed Duracrust)	BB	BB	BB		
AT44A2_08	BB	BB	0.7 ± 0.3	2.0 ± 0.5	2.8 ± 0.6
AT45A4_08 (Gypsum Mound)	8 ± 3	19 ± 5	70 ± 24	13 ± 5	79 ± 29
SP000_10 (Soil Pit, surface)	BB	13 ± 5	BB	3.7 ± 0.6	9.7 ± 0.3
SP088_10 (Soil Pit, 88 cm deep)	BB	BB	BB	2	4
SP136_10 (Soil Pit, 136 cm deep)	BB	6 ± 3	10 ± 3		
Blank[d]	5 ± 5	11 ± 8	17 ± 7		

[a]All concentrations in ppb.
[b]Values are all blank-corrected. Errors are calculated from standard deviation of duplicate or triplicate runs. When no error is reported, the peak was only seen in one run.
[c]BB: Peaks were identified and quantified, but concentrations were below those of the blank.
[d]Average of three separate samples, duplicate runs, analyzed both the in the field laboratory and the U.S. laboratory.

function of exposure, proximity to water flows, and depths. Site 44 at Yungay1122 (Figure 2a) was chosen because of the difference in duracrust coverage observed within a 1 m^2 area. Samples from the surface duracrust were collected both in exposed regions and in regions where the duracrust was shielded by small rocks (~3 to 10 cm large axis). The rocks were removed, and the shielded duracrust was processed to determine whether the sheltered regions nucleate and preserve moisture and biomarkers better than those directly exposed to oxidants and UV. The samples were first processed through the SCWE, and then directly analyzed on the MOA without any preconcentration. The analysis of the direct SCWE extract for AT44A1 and AT44B1 is shown in Figures 2a and 2b. The shielded duracrust exhibited low monoamine and neutral amino acid signals (valine, alanine/serine, glycine) while the exposed duracrust produced amine signals at or below the level of the blank. The majority of the other samples analyzed without concentration gave signals at the level of the blank. These signals are relatively noisy because of the high gain settings needed for non-concentrated samples.

[17] To improve the limits of sensitivity, 500 μL SCWE extracts were dried on a hotplate, and rehydrated in 20 μL of run buffer before labeling with fluorescamine. The analysis of the concentrated extracts from Site 44 performed after returning to the U.S. is shown in Figure 3. At a 1/9 dilution the currents were within the operating range of the power supplies. The shielded duracrust (Figure 3a) produced amino acid concentrations of 61 ± 8 ppb for valine, 45 ± 8 ppb for alanine/serine and 92 ± 32 ppb for glycine in addition to diamines and monoamines, while the exposed duracrust exhibited lower levels of neutral amino acids and a strong ethyl and methyl amine signature which was not quantitated. The signals for the exposed duracrust were at the levels of amino acids detected in evaporation blanks, processed by flushing water through a blank sample cell, collecting fractions and then drying the samples before analysis. The concentrations of the amines and amino acids detected are summarized in Table 1.

[18] The only other site at Yungay1122 that gave significant levels of several amino acids and amines was Site 45, a mound of gypsum (Figure 4) located above a drainage channel. The mound was approximately 30 cm across, and there was a cavity below the surface. A sample of gypsum was removed approximately 4 to 8 cm uphill from the heart of the gypsum mound (AT45A4). The concentrated extract (Figure 4b) produced similar levels of amino acids as those found at Site 44 (8 to 70 ppb), but lower levels of diamines. In addition, acidic amino acids (13 ± 5 ppb glutamic acid and 79 ± 29 ppb aspartic acid) were observed at this site. Differences in chemical composition, exposure, and/or moisture levels at these sites alter the variety and concentrations of amino acids and amines that are observed (see Table 1).

[19] Figure 5 presents the chiral analysis of samples from the shielded duracrust at Site 44 (Figure 5a), the gypsum mound at Site 45 (Figure 5b), and the Mars 7 Standard (Figure 5c) as a control. The D/L ratio of alanine/serine was 0.39 ± 0.08 at Site 44 and 0.34 ± 0.07 at Site 45. The D/L ratio of aspartic acid at Site 45 was 0.78 ± 0.06. The Mars 7 Standard, a racemic mixture of amino acids, produced D/L ratios of 0.96 ± 0.09 for alanine serine and 1.0 ± 0.2 for aspartic acid.

[20] The Soil Pit samples collected at 136 cm below the surface and just above the nitrate cement layer revealed valine (below the blank) and alanine/serine and glycine at 6 and 10 ppb, respectively (Table 1, bottom). A sample taken at 88 cm below the surface exhibited lower levels of the neutral amino acids, all below the blank. The sample taken at the surface had higher levels of neutral and acidic amino acids (4 to 13 ppb) but these biomarkers were attributed to contamination of the field site, evidenced by soil disruption and anthropomorphic residues.

4. Discussion

[21] The GCMS experiments performed on Mars by the Viking Landers were later thought to have been affected by the oxidizing surface conditions [*Benner et al.*, 2000]. The soils in the Yungay region of the Atacama Desert have been shown to be similarly harsh. *Quinn et al.* [2005] performed field tests of the Mars Oxidant Instrument in the Atacama Desert and concluded that the surface is significantly influenced by dry-acid deposition and dust accumulation. When the water activity is increased, for example in a nighttime fog event, these dust particles are wetted and the acids

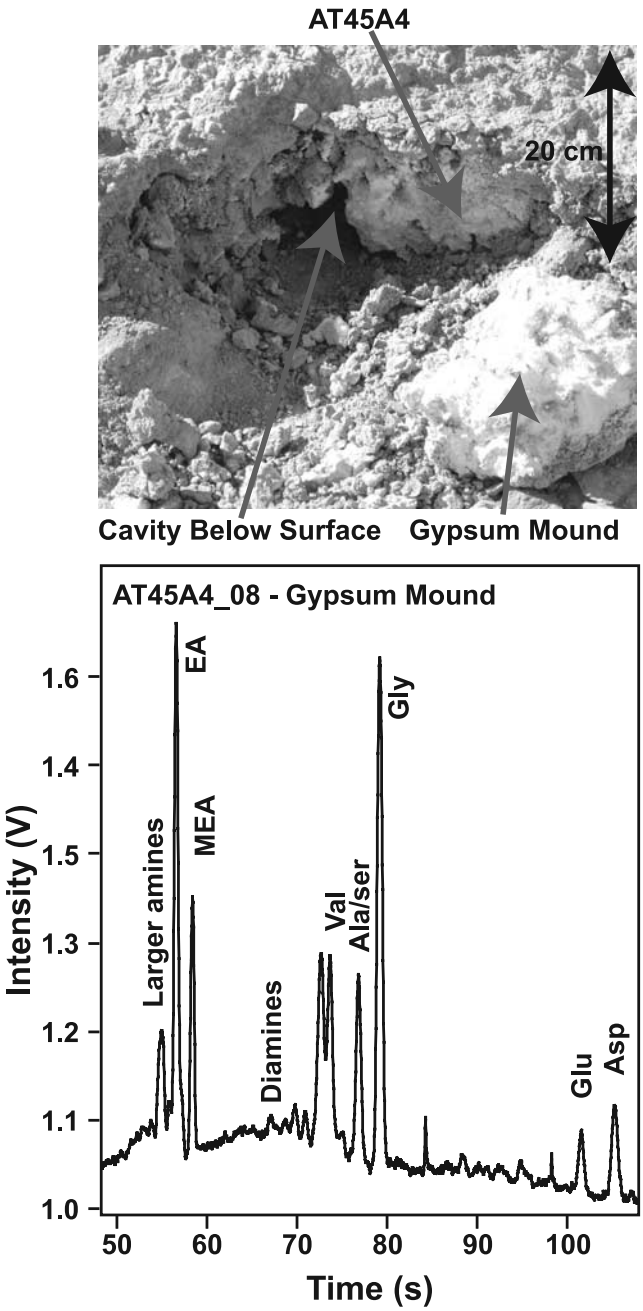


Figure 4. (top) Photograph of Site 45, showing the gypsum mound and the cavity exposed below the surface. Samples were taken at different depths, both above and below the mound, as well as towards the interior of the mound. (bottom) CE analysis of the concentrated extract from the interior of the gypsum mound (~8 cm below surface). This sample was run at a 1/3 dilution from the labeled mix and contained acidic amino acids at the 10 to 80 ppb level.

in the soil form thin acidic films capable of chemically modifying organic molecules. Transient wetting of the Atacama soils produced pH shifts similar to those observed by Viking [*Quinn et al.*, 2005]. It is likely that the accumulation of acids and oxidants in the Atacama has created a harsh environment for life and biomarker deposits, explaining the barren soils observed by *Navarro-Gonzalez et al.* [2003] in the Yungay region. The soils in this region are thus excellent targets for Mars in situ instrumentation because we can test our ability to operate in a harsh Mars-like environment as well as our ability to detect biomarkers from soil conditions that are likely to be found on Mars.

[22] One of our goals was to evaluate the robustness and reliability of the SCWE and MOA systems and to explore the feasibility of directly coupling SCWE extracts to CE analysis. Conditions were challenging as the temperature of the field laboratory fluctuated from below freezing at night to ~35°C during the afternoon. Extraction protocols were initially determined using a standard soil sample, and it was demonstrated that high levels of bioamines were extracted at 250°C in the first 2 mL fraction. The concentrations of salts found in this fraction were within the range tolerable by the MOA. Over the course of the 2-week field test, 340 electropherograms were run using only 3 different CE channels. Only one minor electrical continuity failure was noted and repaired. The successful operation and high sensitivity detection and analysis of biomarker amines by the SCWE/MOA suite in the field is important because it demonstrates field operation in a Mars-like environment on samples potentially relevant for Mars exploration.

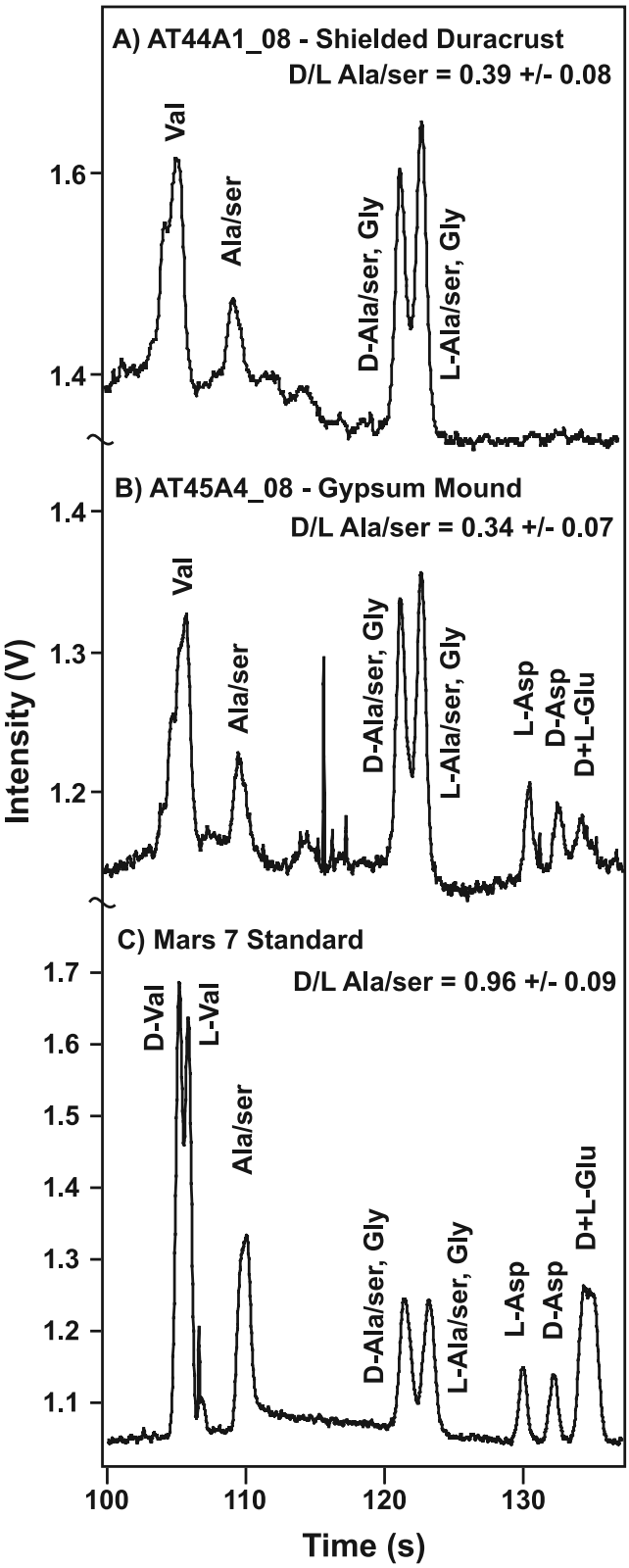
[23] Analyses performed in the field were selected to span the range of samples collected from Yungay1122 and the Soil Pit. The results from Site 44 indicated that the amount of exposure significantly affected the concentration of biomarkers. Samples from the exposed duracrust (AT44B1_08) produced amines and amino acids at the level of the solvent blank, while ~10-fold higher levels of neutral amino acids, amines and diamines were detected from the shielded duracrust (AT44A1_08). These results show that surface context and coverage significantly influence the chemical biomarker concentration found within the duracrust.

[24] The analysis of Site 45 further suggests that the amount of surface moisture is an important factor for biomarker production and preservation. Site 45 was located directly above a water flow channel. These flow channels are reminiscent of those observed on Mars by the Mars Global Surveyor [*Malin and Edgett*, 2000, 2003]. This site contained a large mound of gypsum, an evaporite of saline water [*Mancinelli et al.*, 2004], indicating water alteration of the mineralogy. The Site 45 sample produced significant levels of amines and amino acids, including acidic amino acids but lower levels of diamines.

[25] The results from the Soil Pit demonstrate that the vertical transportation of moisture is also relevant for the location of biomarkers in planar regions. Differences in solubilities of the salts create a gradient with depth, resulting in an impermeable nitrate barrier at ~140 cm [*Ewing et al.*, 2007]. Directly above this layer the highest concentrations of biomarkers were found. These results agree with previous observations of total organic carbon as a function of depth at the Soil Pit site [*Ewing et al.*, 2007].

[26] Previous studies of bioamines released from *E. coli* cells ($\sim 4.7 \times 10^{-16}$ mol of valine per cell released by acid hydrolysis) indicate we can roughly determine the cell count equivalent for the soil samples studied [*Skelley et al.*, 2006]. Based on laboratory studies showing that the SCWE

extraction efficiencies are ~80% in comparison to HCl vapor-phase acid hydrolysis [*Amashukeli et al.*, 2007]), and assuming that all the valine observed is of cellular origin, we estimate ~1 × 10^6 cell equivalents/g at Site 44 and ~2 × 10^5 cell equivalents/g at Site 45. These levels are higher than those reported by *Navarro-Gonzalez et al.* [2003] for exposed samples at the surface (>100 CFU/G) in adjacent Yungay regions. However, our results agree with the results of Navarro-Gonzales et al. for samples taken at lower depths (1 × 10^2 to 3 × 10^5 CFU/g) [*Navarro-Gonzalez et al.*, 2004]. Since Urey extracts and detects bioamines from both the viable and non-viable degraded cells and the culture conditions used by Navarro-Gonzales et al. may not be appropriate for all cell types, it is expected that our results would produce an upper limit on cell concentration.



[27] Chirality analysis was used to examine whether the amino acids were of biological or abiotic origin. The observed D/L ratios were notably different than zero and had an average of ~0.4 suggestive of significant racemization of the sample; around 28% of the total amino acid population was present in the D-form. Amino acid racemization rates are influenced by pH, temperature and moisture. The racemization rate for aspartic acid is greater than those of alanine/serine [*Poinar et al.*, 1996], consistent with the observed higher D/L ratio for aspartic acid at Site 45. The racemization rates for aspartic acid, using the average temperature of ~16°C [*McKay et al.*, 2003], are ~7 × 10^{-6} yr^{-1} under dry conditions and ~5 × 10^{-4} yr^{-1} under wet conditions [*Bada and McDonald*, 1995] corresponding to half-lives of 5 × 10^4 to 8 × 10^2 years. Using the following expression [*Bada and McDonald*, 1995]:

$$\ln[(1 + D/L)/(1 - D/L)] = 2\ k\ t \qquad (1)$$

we calculate a sample age of 8 × 10^3 (wet) to 6 × 10^5 years (dry) based on the aspartic acid ratio (D/L = 0.78) at Site 45. Using the rate constant for alanine racemization we calculate a sample age of 2 × 10^3 (wet) to 1 × 10^5 years (dry) based on an average D/L ratio of 0.37. While the SCWE can racemize amino acids during extraction, preliminary data suggest that the extraction conditions used should not result in more than a ~15% racemization of the sample (A. Aubrey, manuscript in preparation, 2007). It is also possible that D-amino acids from certain bacterial cell walls may contribute and increase the D/L ratio but this cannot be quantified [*Glavin et al.*, 1999]. The ages calculated here are therefore over estimates and assume that the entire amino acid population is of the same age. The Atacama has been arid for 10–15 million years [*Hartley*

Figure 5. Chiral analysis of the extracts of (a) Site 44 shielded duracrust (<1 cm deep), (b) Site 45 gypsum mound (interior sample, ~8 cm deep), and (c) the Mars 7 Standard. The samples were diluted in pH 8.4 buffer, and the running buffer was pH 8.4 buffer with 15 mM hydroxyl-propyl-β-cyclodextrin (HPβCD) for chiral analysis [*Skelley and Mathies*, 2003]. Temperatures were either actively maintained below 10°C (Figures 5a and 5b) or the run was performed at an ambient temperature suitable for the chiral separation (Figure 5c, ambient temperature 4.7°C). These samples produced intermediate alanine/serine chiral ratios of 0.39 and 0.34, respectively, while the racemic Mars 7 Standard gave the expected D/L ratio of 1 (see auxiliary material for explanation of D/L ratio calculation).

and Chong, 2002; *Hartley et al.*, 2005; *McKay et al.*, 2003] and although some increased precipitation during El Niño events 10 000 to 16 000 years ago was recorded, it is not known to what extent this precipitation penetrated the Atacama [*McKay et al.*, 2003]. The ages calculated here are significantly younger than the age of the desert, but match the timeline for the formation of desert crusts (10^3 to 10^5 years) and also the period of increased aridity approximately 6 million years ago [*Hartley and Chong*, 2002; *Hartley et al.*, 2005; *McDonald et al.*, 1996; *McDonald*, 2002]. It is also possible that the sample is a combination of both viable and ancient organisms dating back to the last El Nino event.

[28] A variety of life-detection experiments have previously targeted various regions and samples from the Atacama Desert. Zoë, an autonomous rover platform [*Cabrol et al.*, 2005; *Tompkins et al.*, 2006], performed several field campaigns in the Atacama, using the area as a test of the rover platform, the instruments, and autonomous operation. Zoë studied both coastal and semi-arid areas of the Atacama, and the fluorescent imager correctly detected life in the form of DNA, proteins and chlorophyll at the sites studied where significant life is readily evident [*Weinstein et al.*, 2005]. SAM, a GCMS-based instrument [*Cabane et al.*, 2004], has performed laboratory-based analyses of Atacama samples, detecting 1 to 100 ppb levels of carboxylic acids in all samples and low levels of amino acids in some samples [*Buch et al.*, 2004]. The experience gained by such analyses and especially field tests in Atacama-like locations is crucial for developing and verifying potential instrumentation for successful Mars exploration.

5. Conclusions

[29] The primary goals of this field test were to demonstrate operation of the Urey instrument suite in a Mars analog site while analyzing Mars-like soils for relevant organic monoamine, diamine and amino acid biomarker compounds. The field test demonstrated the capability of the Urey components to operate under extreme and challenging conditions compared to typical laboratory environments. The SCWE was successfully coupled to the MOA for an end-to-end analysis, demonstrating that extracted organics are still detectable even though salts are present. More importantly, the data presented here demonstrate the successful detection of amine biomarkers through compositional analysis of soil samples and the detection of amino acids from ancient terrestrial life in the Atacama. Concerning the selection of sampling sites on Mars, our results demonstrate that examination of locations that have experienced water alteration, and locations that have been shielded from surface degradation or that are below the surface will increase the likelihood of finding detectable concentrations of biomarkers. The concept and key components of the Urey instrument have been validated as a result of this field trial in one of the most arid, Mars-like areas on Earth. Urey is being developed for the ESA ExoMars mission (see http://astrobiology.berkeley.edu).

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