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Long-chain alkanes preserved in a Martian mudstone

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Organic molecules preserved in ancient Martian rocks provide a critical record of the past habitability of Mars and could be chemical biosignatures. Experiments conducted by the Sample Analysis at Mars instrument onboard the Curiosity rover have previously reported several classes of indigenous chlorinated and sulfur-containing organic compounds in Gale crater sedimentary rocks, with chemical structures of up to six carbons. Here, we report the detection of decane ($C_{10}H_{22}$), undecane ($C_{11}H_{24}$), and dodecane ($C_{12}H_{26}$) at the tens of pmol level, released from the Cumberland drilled mudstone sample, using a modified SAM analytical procedure optimized for the detection of larger organic molecules. Laboratory experiments support the hypothesis that the alkanes detected were originally preserved in the mudstone as long-chain carboxylic acids. The origin of these molecules remains uncertain, as they could be derived from either abiotic or biological sources.

organic molecules | Mars | Curiosity rover

Significance

Decane, undecane, and dodecane were detected in a Martian sample at the tens of pmol level and may originate from long-chain carboxylic acids. The detection of long-chain alkanes in the Sheepbed mudstone is important for extending studies of habitability on Mars as the Curiosity rover continues to map out windows of high preservation potential for chemically reduced organic compounds. The provenance and distribution of these molecules are of high interest in the search for potential biosignatures on Mars.

Curiosity's primary scientific goal is to quantitatively assess the habitability potential of Mars, past or present. The Sample Analysis at Mars (SAM) instrument suite onboard the rover is devoted to this task by taking an inventory of the organic and inorganic compounds present at Mars' near-surface as potential chemical biosignatures and exploring the nature of their preservation. The two wet chemistry experiments on SAM were designed for the extraction and identification of refractory and polar organic molecules in solid samples using gas chromatography–mass spectrometry (GCMS) (1). Despite the contribution of one of the chemical derivatization agents used, *N*-methyl-*N*-tert-butyldimethylsilyl-trifluoroacetamide (MTBSTFA), to the background signal in SAM (2), several dichlorinated alkanes, chlorobenzenes, benzoic acid, thiophenes, and other sulfur-containing organic compounds of Martian origin have been identified by GCMS above background levels in several analyses of a drilled mudstone sample called Cumberland (CB) collected at Yellowknife Bay and in some subsequent samples (3–7). While initially trying to reduce the contribution of MTBSTFA onto the samples, the high versatility of the SAM instrument suite allowed the team to subsequently optimize a GCMS experiment for the detection of MTBSTFA derivatized organic compounds and other molecules present in a CB mudstone sample, in a so-called "opportunistic derivatization" (OD) experiment (*Materials and Methods*). The sample was first heated to ~475 °C to release molecular oxygen from oxychlorine decomposition in order to limit the combustion of possible organic molecules and their MTBSTFA derivatives (ODa), placed back in the sample manipulating system for the readsorption of residual MTBSTFA vapor, and subsequently reheated to the maximum temperature (~850 °C) for subsequent derivatization with MTBSTFA vapor and GCMS analysis (ODb—*Materials and Methods*, *SI Appendix*, Fig. S1). Although the prime motivation for the OD experiment was derivatization of polar and refractory Martian organics in the CB sample (*SI Appendix*, section 1), the method was also developed to minimize the oxidation of organics by the O_2 derived from perchlorate salts and other oxychlorine phase(s) present in CB (8).

Pyrolysis of the CB Sample in the Absence of O_2: Detection of Long-Chain Alkanes

The CB sample was of particular interest as it yielded elevated abundances of chlorobenzene, the first definitive evidence of an organic molecule of nonterrestrial origin at the Martian surface (3, 5), and additional evidence of sulfur-containing molecules, including thiophenes released at high temperature (4) (*SI Appendix*, section 2). The CB sample in the Sheepbed mudstone contained ~20 wt % smectite clay (9) and Fe- and Mg-sulfates (8, 9). Clays involve high surface areas and negatively charged interlayer that adsorb and

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protect molecules over geological period of time. They also possess cations associated with water in the interlayers, that retards water flow and help accumulating organics over time (10). Terrestrial phyllosilicates like smectite serve to adsorb, transport, and protect organic compounds when rapidly deposited under reducing chemical conditions (11). Exceptional structural preservation can be enhanced due to long-term hyperaridity (12). Laboratory analysis of terrestrial sulfates indicates that sulfate minerals can preserve organic molecules (13). Considering that decarboxylation of organic matter is also retarded in terrestrial sulfates, reduced organic matter could also be preserved in sulfates for billions of years on Mars (14) and be released during mineral thermal breakdown (15). The thermal breakdown of sulfate minerals occurring at a higher temperature than the thermal breakdown of oxychlorine phases, the organic compounds released from the sulfate are thus preserved from oxidation and chlorination during the sample pyrolysis (8, 15, 16). This all suggests that the Sheepbed mudstone contains minerals that are ideally suited for preserving organics that can be extracted and identified by the pyrolysis GCMS technique used by SAM. The multiple SAM experiments performed on CB before OD, using a broad range of conditions (3, 17), afforded detailed knowledge of the volatile release characteristics of the sample. This enabled determination of the ideal temperature to reach in the first heating step (ODa) in order to release most of the oxygen while still preserving high-temperature organics and not degrading sulfates (*SI Appendix*, section 3). Although the temperature reached by the sample in the ODa heating step (475 ± 25 °C) was slightly higher than expected and did initiate the sulfate decomposition (*Materials and Methods* and *SI Appendix*, section 3), the key purpose of the step was fulfilled and all of the CB sample O_2 (to detection limits) was evolved in the first step ODa (*Materials and Methods*). The O_2 in CB is thought to originate mostly from the decomposition of magnesium perchlorates, present at 1.1 wt. % in the CB sample (18) (Fig. 1).

The ODb experiment, corresponding to the pyrolysis of the CB sample depleted in O_2 and other volatiles, revealed in the chromatogram the presence of the long-chain alkanes decane ($C_{10}H_{22}$), undecane ($C_{11}H_{24}$), and dodecane ($C_{12}H_{26}$) (Fig. 2). The mass spectrum of decane corresponds to that expected based on laboratory data from the SAM testbed (TB), a high-fidelity laboratory mockup of SAM (19), and differs from the National Institute of Standards and Technology (NIST) reference spectrum as a result of SAM's unique MS scanning properties (Smartscan) and SAM's lowered scan rate for *m/z* 123 and above in this particular experiment (*SI Appendix*, sections 4 and 7). As a result, the diagnostic molecular ion at *m/z* 142 for decane, *m/z* 156 for undecane, and *m/z* 170 for dodecane were not measured in SAM MS spectra. SAM GCMS measurements are being performed using a unique instrument on Mars, with an MS that highly differs from a laboratory MS. The results, as well as the data treatments, are significantly different than the usual methods used on laboratory data and have been worked and optimized over the thirteen years of the exploration phase of the mission (2–6, 20, 21). They are prone to further adjustments and have a part of unique personal expertise that is thoroughly explained in *SI Appendix*, *Supplementary Materials* and previous publications. As an example, the presence of coeluting isobars could be either removed from a fit or subtracted as a background leading to different resulting mass spectra.

The retention times of the long-chain alkanes detected in SAM (20.9 min for decane, 23.7 min for undecane, and 25.8 min for dodecane) correspond to the retention times of standards of decane, undecane, and dodecane analyzed with the TB under the same operating conditions (20.1 min, 23.3 min, and 25.1 min, respectively). The slightly longer retention time on the SAM flight instrument compared to the SAM TB is expected, due to a lower inlet pressure and a lower initial GC temperature in the flight instrument (22). The combination of both retention time and mass spectrum strictly confirms decane, undecane, and dodecane as being present among the gases released from the sample, likely under their straight-chain form (*n*-alkanes) as other structural isomers would have lower retention times (*SI Appendix*, section 5). The detection of only C_{10}, C_{11}, and C_{12} alkanes in this experiment may be due, in part, to a bias in the instrument sensitivity under the conditions of this experiment toward detection of alkanes in

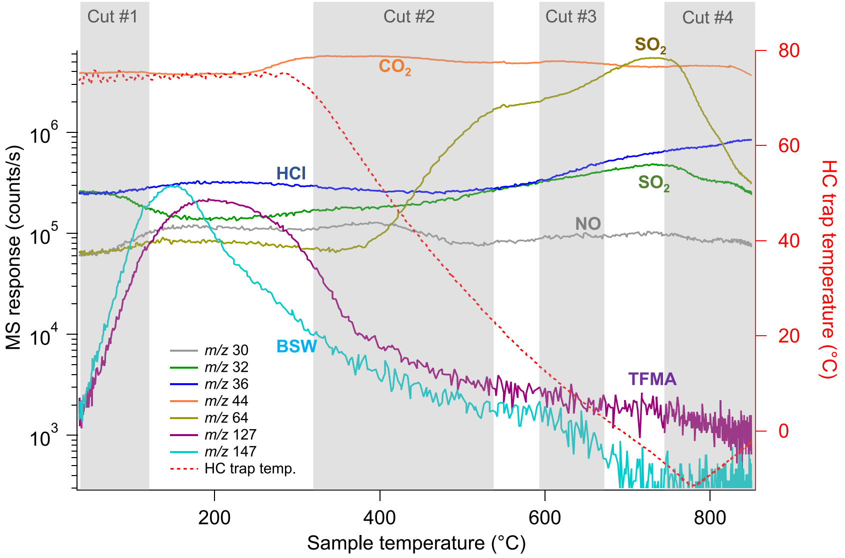

Fig. 1. Evolved major gases *versus* temperature as detected by SAM-evolved gas analysis (EGA) of the drilled CB mudstone sample in the second heating step (ODb) of the opportunistic derivatization analysis. The gray boxes show the four temperature cuts selected to send the gases to the hydrocarbon trap for subsequent GCMS analysis. The dashed red line corresponds to the SAM hydrocarbon trap temperature during the run. Identification of the chemical species was performed from specific ions produced by electron impact in the ion source of the mass spectrometer. The characteristic mass over charge (*m/z*) value for these specific ions is given for each chemical species. BSW: 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyldisiloxane, known as "bisilylated water." TFMA: trifluoro-*N*-methylacetamide. *m/z* 32 is mainly a contribution from SO_2—no O_2 was detected. The rationale for the selection of the four temperature cuts is to detect the following: Cut#1 Derivatization products from MTBSTFA and its byproducts sent to the hydrocarbon trap Cut#2 Organics released from $FeSO_4$ thermal decomposition Cut#3 Organics released from $MgSO_4$ thermal decomposition + suspected release of thiophene Cut#4 Possible refractory organic matter fragments.

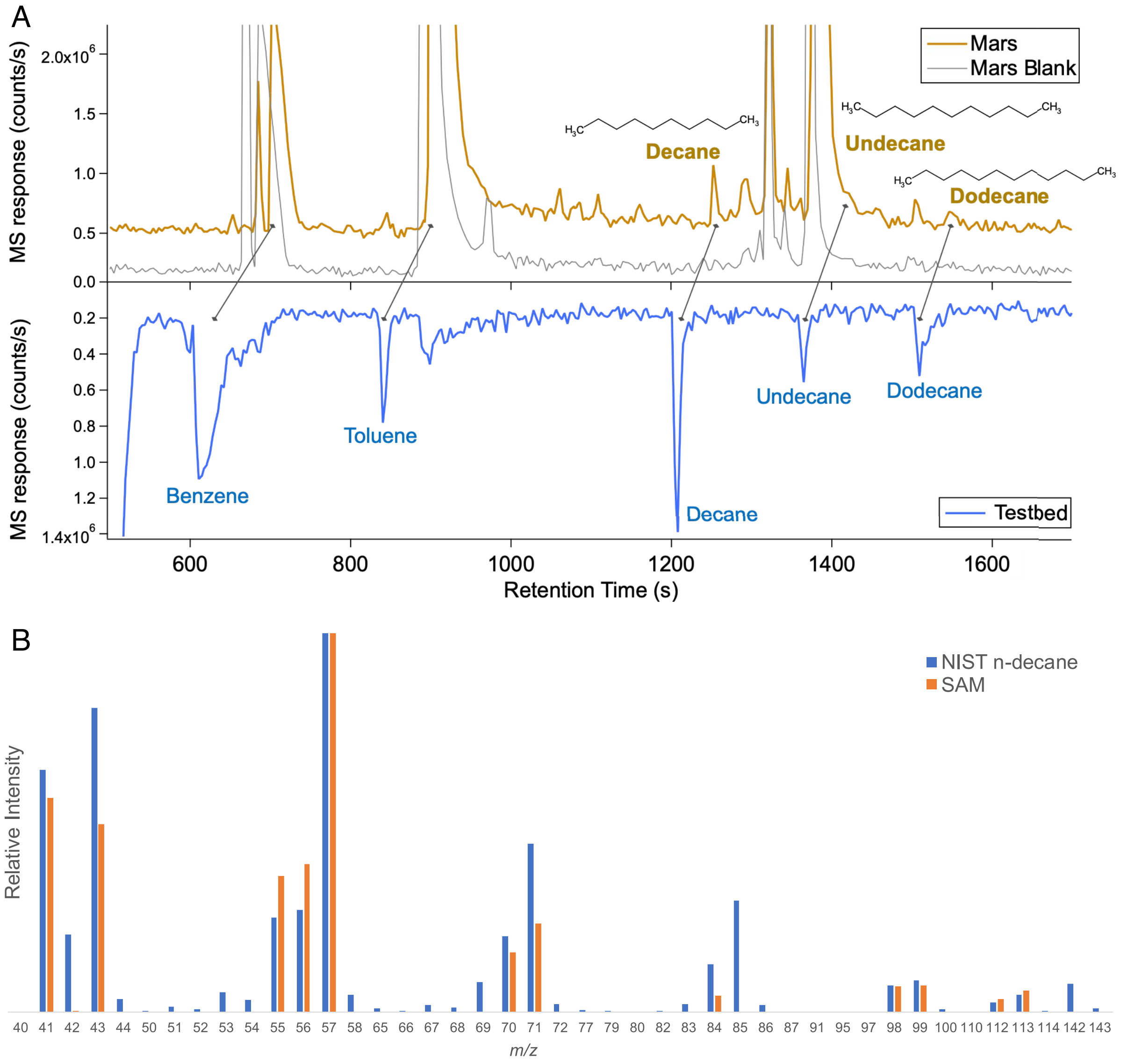

Fig. 2. (*A*) Identification of decane, undecane, and dodecane in the ODb chromatogram. Reconstructed chromatograms from *m/z* 77 × 2 + *m/z* 91 × 5 + *m/z* 57 × 50 + *m/z* 71 × 50. The decane, undecane, and dodecane detected in ODb Mars run (orange) were not present in the subsequent OD blank experiment (gray) carried out under identical experiment conditions. The retention times are compared with the C_{10}–C_{30} *n*-alkanes analyzed in the SAM TB (blue), with GCMS conditions similar to those used by the SAM flight model on Mars (*SI Appendix*, section 4). (*B*) The mass spectrum of decane from the SAM GCMS data (orange) is compared to the one of *n*-decane in the NIST database. This spectrum also is in better agreement than those of branched C_{10} alkanes. The lack of decane, undecane, and dodecane in the procedural blank and the correlation of the retention times and mass spectra of the SAM GCMS peaks with SAM TB and NIST confirm that the CB sample is the source of decane, undecane, and dodecane in the ODb GCMS run.

this mass range (*SI Appendix*, section 6). Laboratory supporting investigations, combined to the results of a subsequent OD experiment on the Rock Hall Martian sample, excluded SAM internal background as the origin of the long-chain alkanes detected in CB OD experiment (*SI Appendix*, section 8). Quantification of the chromatographic peak areas and calculation of the alkanes abundances based on calibration standards yielded 37 ± 7 pmol of decane, 41 ± 8 of undecane and 19 ± 4 pmol of dodecane in the ODb GCMS run (*SI Appendix*, section 7). With a sample mass of 135 ± 31 mg of the three portions of CB (3), this corresponds to 43 ± 17 ppb(w) of decane, 53 ± 22 ppb(w) of undecane and 27 ± 11 ppb(w) of dodecane. The detected abundance of alkanes is in the same order of magnitude than the dichloroalkanes [up to 70 ppb(w) (3)] or dichlorobenzenes [0.5 to 17 ppb(w) (5)], and lower than chlorobenzene [150 to 300 ppb(w) (3)]. The alkane values have high uncertainties and correspond to lower limits of the abundances initially present in the sample, due to potential thermal degradation which can occur during pyrolysis, losses during gas transfer and GC columns splitting, and use of few ion in the MS signal. Estimation of the initial abundance in the sample leading to such level of detection is in the hundreds of ppm(w) range, for chlorobenzene (23), and the same range of concentration if all the CO2 detected with SAM in Mars samples could be explained by oxidation and decarboxylation of organic matter (8). This high abundance estimation would reconcile the experiments with the predicted levels of up to 500 ppm(w) of organic matter (benzenecarboxylates) from meteoritic infall (24).

Unlike laboratory GCMS data, the limits of detection and quantification are adjusted to this unique set of data from Mars. It is internally accepted by the SAM team that a signal can be assessed from a S/N of 2 and the quantification is tentatively made from any detected *m/z*. Specifically, while decane *m/z* 57 has a S/N of 7 and is aligned with typical laboratory limits for detection and quantification, the dodecane *m/z* 55 has a S/N of 2.8. Because the ion at *m/z* 55 is sufficiently separated from the background on both sides and is combined with a set of other masses presenting the same shape and retention time, we use it for both detection and quantification measures (*SI Appendix*, section 7).

Possible Origin of Long-Chain Alkanes

It cannot be excluded that the alkanes detected in CB were present as such in the sample and were released intact from the mineral matrix during pyrolysis. Indeed, alkanes can be produced in many abiotic chemical reactions such as Fischer–Tropsch processes. However, the generic oxidation pathway proposed at or near the surface of Mars makes the case for the formation of more metastable products and demonstrates a conversion of organic matter of abiotic or biotic origin to aromatic and aliphatic carboxylic acid derivatives (24). In SAM ovens, the sample is heated up to 850 °C and the organic molecules detected may have a different chemical precursors; hence, benzoic acid is the preferred precursor for the identified aromatic chlorohydrocarbons (23), macromolecular hydrocarbons or nonsulfurized volatiles could be the precursors of the thiophenes and other sulfur-bearing compounds (4, 7). Carboxylic acids and their salts are known to readily decarboxylate in pyrolysis experiments at high temperatures when chemically bound to or embedded within a mineral matrix. It was shown, for example, that benzoic acid decarboxylates to benzene at 500 °C in the presence of a CB analog soil (23, 25). A similar decarboxylation of phthalic acid was observed in the presence of hematite (15). In order to test whether such clay mineral-catalyzed decarboxylation occurs with aliphatic carboxylic acids, a laboratory experiment was performed with the long-chain carboxylic acid *n*-undecanoic acid ($C_{11}H_{22}O_2$). A solution corresponding to 5 wt. % *n*-undecanoic acid was added to organic-free montmorillonite [used as a proxy for CB smectite clay (9)] and heated with a SAM-like ramp set at 35 °C·min^{-1} (*SI Appendix*, section 9). During the sample heating ramp, the MS signal was recorded, similar to a SAM EGA run. The results (Fig. 3*A*) confirm the decarboxylation of *n*-undecanoic acid to *n*-decane at elevated temperature (490 °C), coincident with a CO_2 release. A GCMS analysis of the products confirms decane as the main alkane product of the pyrolysis (Fig. 3*C*). Although pure undecanoic acid was also observed in this laboratory setup, a follow-up experiment using less organic material showed a complete decarboxylation of the substrate. Regardless of its presence or absence, the extended retention time of undecanoic acid under SAM-like GC conditions resulting from the size of the molecule and the polarity of the acidic functional group would preclude its detection in the SAM OD experiment. The decarboxylation reaction observed when undecanoic acid was in the presence of the clay may be due either to the higher temperature seen by the adsorbed or embedded molecule, a temperature much higher than that required to volatilize pure organics *via* pyrolysis (Fig. 3*A*), to the formation of a strong ionic bond with active sites in the mineral matrix that prevented sublimation and promoted thermal decarboxylation (26), or to a catalytic effect promoted by the mineral itself. The decarboxylation was observed at temperatures up to ~600 °C.

Altogether, the SAM and supporting laboratory data point toward a saturated form of straight-chain, primary carboxylic acids (C_{11}–C_{13}) present in the CB sample prior to pyrolysis heating (*SI Appendix*, section 10). This detection extends the variety of organic compound classes detected on Mars. The release at high

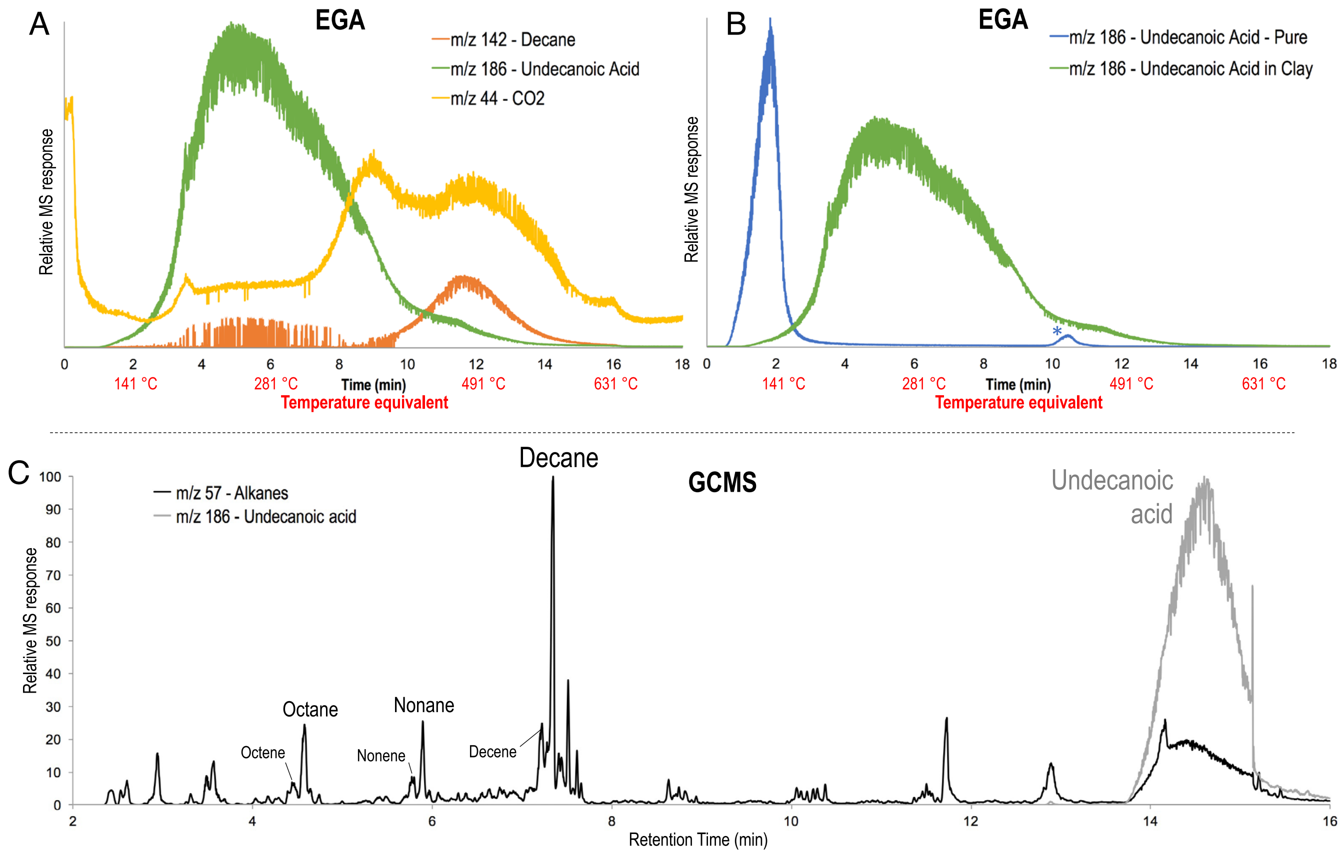

Fig. 3. Decarboxylation of long-chain carboxylic acids observed in laboratory experiments. Laboratory experiments of (*A*) 35 °C·min^{-1} EGA of undecanoic acid 5 wt % on montmorillonite. Specific *m/z* are displayed to correspond to CO_2 (*m/z* 44), decane (*m/z* 142), and undecanoic acid (*m/z* 186). (*B*) 35 °C·min^{-1} EGA of pure undecanoic acid blue) and undecanoic acid 5 wt % on montmorillonite (green). *: dimerization product (*C*) 35 °C·min^{-1} pyrolysis-GCMS analysis of undecanoic acid 5 wt % on montmorillonite. The *x* axis represents the retention time of the compounds onto the GC capillary column. Specific *m/z* are displayed to correspond to generic alkanes (*m/z* 57) and undecanoic acid (*m/z* 186). Experimental conditions are described in *SI Appendix*, section 9.

temperatures and the absence of Cl- and S-moieties contrast with the previous detections of chlorohydrocarbons and thiophenes, attesting to a potentially different source. The release of aliphatic hydrocarbons also differs from the previously detected, mostly aromatic, hydrocarbons. Finally, dodecane is so far the highest molecular weight molecule definitely identified on Mars, with twelve carbons, demonstrating the potential of SAM to detect and identify compounds of higher mass than small prebiotic molecules, with *m/z* in the hundreds. The pyrolysis in the presence of the 20 wt.% smectite clay in the CB sample (9) could have triggered the high-temperature decarboxylation of carboxylic acids or their salts (25) to the alkanes detected by SAM. The presence of the carboxyl form of the observed decane, undecane, and dodecane is thus strongly suspected in the CB sample, *i.e.,* undecanoic acid, dodecanoic acid, and tridecanoic acid would be their respective precursors. Those three carboxylic acids are examples of fatty acids. Although fatty acids can be found ubiquitously in many abiotic environments (27), they are also of high interest in the search for biosignatures, as being the main constituent found in terrestrial living cell membranes. The presence of decane, undecane, and dodecane is also compatible with degradation of longer-chain saturated or unsaturated fatty acids (Fig. 3*C*), such as palmitic, stearic, or oleic acids (C_{16} and C_{18}), which can be considered biosignatures under certain conditions. The main alkane fragment C_{n-1} and other alkane fragments higher than C_{12} would not been seen on the SAM chromatogram (*SI Appendix*, section 6).

The combined results from the CB sample suggest a unique geochemical history at Yellowknife Bay compared to overlying strata; in addition to the qualitative and quantitative wider detection of organic molecules (3–5), nitrates were reported (17), a large sulfur isotopic fractionation was observed (28), and highly depleted carbon 13 signature in methane released from the sample was observed (29). Given the detection of C_{10}–C_{12} alkanes and by extension the potential presence of C_{11}–C_{13} or higher chain length carboxylic acids in the Martian sample, a discussion of possible sources (biologic and abiotic) is warranted. On Earth, long-chain carboxylic acids are usually indicative of cellular membrane lipids (fatty acids) or other biological processes, whereas abiotic (e.g., hydrothermal) processes yield primarily shorter-length carboxylic acids and decreasing amounts of carboxylic acids of longer chain lengths (30). In carbonaceous chondrites, the amount of carboxylic acids is high comparatively to other soluble species (31) but their abundance decreases with increasing chain length (32). The type II terrestrial kerogen is highly aliphatic with some aromatic character and a high proportion of sulfur-bearing units. These aliphatic features represent the biological heritage of the kerogens and challenge the merits of using these kerogen types as suitable analogues for macromolecular material in chondrites (33). Although it was not expected that Martian kerogens behave similarly than the terrestrial ones, the CB sample analyzed by SAM produced aromatic [chlorobenzene, benzoic acid (3, 6)], sulfur-bearing [thiophene (4, 7)] and aliphatic (decane, undecane, dodecane) compounds, with a stronger resemblance to type II biological kerogens than more mature, abiotically produced type III-IV kerogens found in the Murchison meteorite and carbonaceous chondrites. The distribution of organic compounds in Cumberland is also consistent with organic matter reported in Martian meteorite Tissint, with the presence of chlorobenzenes, sulfur-containing compounds, aromatic hydrocarbons, and the presence of a weak aliphatic peak associated with carboxyl groups in spectroscopic analysis (34, 35). However, pyrolysis GCMS of terrestrial biological kerogens shows a variety of alkane/alkene pairs (33) (in contrast to meteoritic macromolecular fraction), which were not observed in the SAM data where only alkanes were identified. The presence of H_2 in SAM at high temperature may nevertheless explain the saturation of the alkenes into alkanes (*SI Appendix*, section 10), leading to the detection of alkanes only. Robust confirmation of a biologic origin for long-chain fatty acids is predicated on patterns in the carbon number, specific isotopes, and relative acid abundances (36). For example, fatty acids derived from terrestrial microbial cellular metabolic processes have a strong even-over-odd carbon chain length preference due to enzymatic polymerization building blocks (C_2) ultimately derived from glucose (37). With the caveats discussed earlier and in *SI Appendix*, section 7 on quantification, if the decane (37 ± 7 pmol), undecane (41 ± 8 pmol), and dodecane (19 ± 4 pmol) arose from decarboxylation, the corresponding fatty acids would have been undecanoic acid, dodecanoic acid, and tridecanoic acid, respectively, which would correspond to a preference for even-over-odd carbon chain—the same trend as observed in terrestrial biology. Abiotic fatty acid patterns will favor shorter carbon chain lengths of Flory-Schulz distribution, with no carbon number preference (38). With SAM's detection of only three alkanes hypothesized to form from fatty acid decarboxylation and uncertain undecane relative quantification, the set of aromatic and aliphatic compounds found on Mars, to date, do not allow us to point conclusively with this analysis to a biotic or abiotic source of these compounds. Regardless of the origin of the alkanes, this detection is a major finding that demonstrates that larger organic molecules can be preserved in ancient sediments on Mars despite the intense radiation and highly oxidizing environment. Due to the intrinsic nature of in situ exploration, we cannot consider driving back to Cumberland and investigate further the CB area. Samples returned from Mars will be complementary to in situ investigation and could help to elucidate the distribution and abundances of organic compounds, with replicates and without the limitations of the embarked instruments. The current campaign of Mars sample collection with the Perseverance rover on samples containing potential aromatic organics (39) may allow future laboratory measurements on samples returned to Earth to help establish the diversity, the source, and the distribution of this family of molecules or other potential biosignatures in Martian near-surface samples.

Conclusion

The opportunistic derivatization procedure was enabled by SAM's versatility, which allowed the maximization of MTBSTFA and the minimization of oxygen from the oxychlorine phase(s) of the Cumberland drill sample in a complex two-step hot-trap experiment on Mars. The first step ODa consisted of heating the sample to 475 ± 25 °C, which left a sample depleted in O_2-evolving phases for the subsequent ODb GCMS experiment. The drastic reduction of evolved O_2 decreased the oxidation of the organic material released from Cumberland in the second step and allowed the detection of decane (37 ± 7 pmol), undecane (41 ± 8 pmol), and dodecane (19 ± 4 pmol). The organic molecules detected are indigenous to the Cumberland sample, and dodecane represents to date the highest molecular mass organic molecule identified at Mars' surface. The detection of several organic compounds at different temperatures on Mars (e.g., chlorobenzene at 250 to 450 °C, long-chain alkanes at 320 to 550 °C or higher temperatures, thiophenes at temperatures > 600 °C), indicates a variety of sources of organics and preservation mechanisms in the CB sample. Clay and sulfate minerals are expected to play an important role in this long-term preservation. The detection of long-chain alkanes in the Sheepbed mudstone is important for extending studies of habitability on Mars as Curiosity continues to map out windows of possibly higher preservation potential for other, chemically reduced, organic compounds that are metastable in the current strongly oxidizing environment. The source of the long-chain alkanes remains uncertain; however, laboratory experiments support a source from the saturated form of straight-chain, primary carboxylic acids, i.e., undecanoic, dodecanoic, and tridecanoic acids for decane, undecane, and dodecane, respectively. Although abiotic processes can form these acids, they are considered universal products of biochemistry, terrestrial, and perhaps Martian. Thus, the provenance and distribution of these molecules are of high interest in the search for potential biosignatures on Mars.

Materials and Methods

The CB sample used in the OD experiment was drilled in the Sheepbed mudstone on Sol 279 of the mission, and a fresh triple portion (~135 ± 31 mg) was added on top of a previously pyrolyzed CB single portion sample (3, 40). This fresh triple portion sample was left inside SAM for about 1,260 sols and accumulated MTBSTFA vapor present in the SMS during the traverse from Yellowknife Bay to the base of Mt. Sharp, allowing the solvent to react with the organic molecules present in the sample. The OD experiment was designed to remove the majority of O_2 resulting from the thermal decomposition of oxychlorine and other perchlorate salts from the sample, in order to limit the combustion of organics and interference with MTBSTFA derivatization reactions. The OD experiment was conducted as a multisol experiment as follows (*SI Appendix*, Fig. S1):

1) **The first step (ODa**–Identification number TID 25386) consisted of a medium temperature heating of the sample from ambient (~13 °C) to 475 ± 25 °C at a rate of 35 $°C \cdot min^{-1}$ under 25 mbar helium flowing at a rate of ~0.8 $mL \cdot min^{-1}$. This step was implemented to release O_2 through the decomposition of perchlorate salts and other oxychlorine compounds present in the sample (18, 40). During the ODa step, volatiles released from the sample were analyzed in EGA mode using the quadrupole mass spectrometer (QMS). The EGA thermogram of ODa is shown in *SI Appendix*, Fig. S2. The oven temperature reached a maximum of 625 °C and is represented in the figure. This corresponds to a sample temperature of ~485 to 525 °C estimated from models. The oven temperature is plotted in the figure because of the inaccuracy of the modeled sample temperature during such an atypical run. All O_2 from the perchlorate and oxychlorine decomposition was released from the sample in this step. More specifically, 0.35 ± 0.1 μmol of O_2 was evolved during the ODa run and no O_2 was detected in the second ODb sample heating step down to SAM's limit of detection for O_2 of ~0.1 μmol (Fig. 1). The amount of O_2 evolved from this sample was significantly lower than the average observed for other standard CB runs of 9.9 ± 2.3 μmol. However, the amount of CO_2 detected in the ODa run is significantly higher than average CB (10 ± 2 μmol for ODa vs. 2.9 ± 0.5 μmol for average CB) and the sum of O_2 and CO_2 for average CB and ODa are the same within uncertainty. This is indicative of organic matter combustion during pyrolysis, which is consistent with adsorption of MTBSTFA and its byproducts on a sample over a long period of time that react with O_2 released from the thermal decomposition of oxychlorine phases. The O_2 quantification protocol is explained in ref. 41. Because of the slight overheat from the predicted maximum temperature of ODa, the $FeSO_4$ started to decompose–this was observed by an SO_2 release when the oven temperature reached 600 °C (~500 °C sample temperature in a nominal SAM temperature ramp). In addition, the corelease of *m/z* 112 and *m/z* 114 suggested chlorobenzene was released from the sample, as expected, in the temperature range it was observed in previous CB samples [430 to 600 °C oven temperature, equivalent to 180 to 350 °C sample temperature (3)], confirming the presence of indigenous organic molecules in CB samples. The cup was then removed from the pyrolysis oven and placed back into the SMS where the sample could readsorb and react with MTBSTFA vapor for 72 h, at a temperature that remained below 15 °C.

2) **The second step (ODb**–Identification number TID 25387) consisted of heating the sample from ~33 to ~850 °C to perform pyrolysis and derivatization of molecules that evolved from the sample at elevated temperatures, with much less O_2 available for the combustion of organics. In the ODb step, the volatiles (MTBSTFA-derivatized and nonderivatized) released over four different temperature ranges, or "temperature cuts" (Main text, Fig. 1), of the sample during the heating were sent to the hydrocarbon (HC) trap [packed with silica beads, Tenax® TA, and Carbosieve G adsorbents (1)] for subsequent GC injection and separation on SAM's Chirasil-Dex and MXT-1 columns (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness) and QMS analysis. The HC trap temperature was slightly elevated to let simple MTBSTFA byproducts pass through, while still trapping heavier derivatized compounds of interest. The HC trap temperature was kept at ~75 °C during the first part of the pyrolysis, up to the sample pyrolysis temperature of ~300 °C: the temperature at which most of the MTBSTFA byproducts had been released from the sample. When the pyrolysis temperature reached 300 °C, the trap heater was turned off and allowed to passively cool down to −10 °C. However, passive cooling occurs at a slow rate, so the trap was still at 71 °C at the beginning of temperature cut #2 and was at 23 °C at its end (Main text, Fig. 1). The MXT-1 results suffered from reduced chromatographic separation of MTBSTFA reaction products and sample volatiles in this particular analysis which made the QMS data more difficult to interpret and are not discussed further.

The four temperature cuts were selected to target specific molecular species and exclude others to avoid overloading of the HC trap and GC columns with an excessive amount of volatile material. Cut#1 (~40 to 115 °C) was chosen to target MTBSTFA and its byproducts to allow additional derivatization in the HC trap; Cut#2 (~320 to 550 °C) and Cut#3 (~587 to 680 °C) were specifically chosen to target the beginning of the decomposition of iron sulfate ($FeSO_4$, first SO_2 release) and magnesium sulfate ($MgSO_4$, second SO_2 release) (18), respectively, and analyze organics released from the thermal decomposition of sulfates. The release of organic molecules at the starting temperature of sulfate decomposition is in line with laboratory analog experiments (15) and with the tentative identification of alkanes associated with sulfates in Martian meteorites (34). Cut#3 corresponds to the expected release temperature of thiophene (4). Cut#4 (~739 to 850 °C) was selected to target potential refractory molecules that require higher thermal energy to breakdown (42). After the ODa and ODb EGA and GCMS analyses, a procedural control blank experiment was carried out using the identical SAM analytical conditions on the same quartz cup containing the heated CB residue (TID 25392 and 25393).

Oven vs. Sample Temperatures

The oven used for the ODa experiment was heated by its specific heater circuit using the proportional integral differential (PID) method provided by the SAM electronics. For technical reasons, the temperatures are measured on the outside of SAM oven. Because of the low heat conductance from the oven to the sample inside the oven, the sample temperature is lower than the oven temperature, and can only be inferred through a model. The modeled sample temperature is based on common pyrolysis temperature ramps, for a specific oven, and a 35 $°C \cdot min^{-1}$ ramp rate. While the estimation of a sample temperature during the 35 $°C \cdot min^{-1}$ pyrolysis ramp is straightforward, the PID system induces a slowdown of the rate when reaching the final temperature target, in order to avoid quick and steep overshoot. Therefore, it was difficult to estimate how long was needed to wait after the theoretical end of the ramp, and the final target temperature was overshot. However, considering the high uncertainty related to these oven and sample temperatures, we had chosen purposefully to slightly overshoot the targeted temperature, rather than to miss the end of the O_2 release. Therefore, the aim of O_2 removal was fully preserved, however, the $FeSO_4$ unwillingly started to decompose in ODa run.

In the ODa experiment, the CB sample was heated to an oven temperature of 600 °C, held for 21 min. The drift in temperature brought the oven temperature to 625 °C by the end of the 21 min. The model estimated the maximum sample temperatures to be 425 °C at the beginning of the plateau, and 485 °C at the end. The sample temperature is thought to be slightly misestimated due to the specific conditions of this run. Thus, the sample temperature reached during the ODa experiment is estimated to be between 450 and 500 °C.

ODb experiment follows the EGA ramp of the nominal CB experiments, and thus the sample temperature is better constrained, although they may be underestimated because of the sample pretreatment, including water release, in ODa. The oven temperature cuts were chosen to be 200 to 360 °C + 575 to 750 °C + 790 to 875 °C + 930 to 1,000 °C (end of the run). This corresponds to estimated sample temperature cuts of 40 to 115 °C + 320 to 550 °C + 587 to 680 °C + 739 to 850 °C (end of the run).

Data, Materials, and Software Availability. Reduced data records (RDRs) from SAM flight experiments are archived in the Planetary Data System (https://pds.nasa.gov) (43) and are identifiable by the sol or test identification (TID) numbers listed in *Materials and Methods*. All other data are available in the manuscript and/or *SI Appendix*.

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