Search for Alkanes of 15 to 30 Carbon Atom Length

Abstract. *A 50-gram sample of lunar fines was subjected to stepwise extraction in a mixture of benzene and methanol while intact, after being pulverized, and after being digested in hydrofluoric acid. None of these three extracts contained detectable quantities of C_{15} to C_{30} alkanes. No C_{15} to C_{30} alkane was present in this lunar sample at a concentration exceeding 1 part per billion by weight.*

Alkanes are saturated organic compounds that are composed solely of carbon and hydrogen atoms. These hydrocarbons are ubiquitous but minor constituents of the waxes, fats, and oils of plants or animals (*1*). Because alkanes are less readily assimilated than other food substances and are chemically less reactive than most biological compounds, alkanes are preferentially preserved relative to other organic materials in sedimentary environments. Natural gas and petroleum deposits commonly appear to be concentrates of alkanes partially derived from preexistent organisms (*2*). Many alkanes from petroleum have molecular structures that are either identical or similar to alkanes and related compounds in plants, animals, soils, marine sediments, and sedimentary rocks (*3*). The marked structural and distributional resemblances between certain alkanes in rocks and compounds in organisms have led to the widespread use of alkanes as molecular or chemical fossils (*4*) and to the recommendation that C_{15} to C_{30} alkanes be employed in exobiological research (*5*). This report deals with a search for C_{15} to C_{30} alkanes in a lunar sample.

All of the lunar fines allocated for organic analyses came from the 15-kg bulk sample. This sample was collected near the side of the landing module that faced to the lunar northeast (*6*). A 50-g portion of lunar fines with grain sizes in the 20 μm to 1 mm range was used. Repeated tests established that all solvents, reagents, and apparatuses were free of detectable amounts of organic contaminants with vapor pressures equivalent to C_{15} to C_{30} alkanes. The gas-liquid chromatograph at the sensitivity setting used to monitor contamination gave 5 to 12 percent of full scale deflections with 10^{-7} g of pristane (2,6, 10,14-tetramethylpentadecane), a C_{19} alkane. Sensitivity checks were run on standard solutions of pristane before and after each gas-liquid chromatographic analysis of a lunar extract sample.

The lunar fines were initially extracted with an azeotropic mixture of benzene and methanol in a specially designed combination Soxhlet extractor and ball mill (*7*). Throughout these extractions, a positive pressure of nitrogen was maintained in the system. Nitrogen flowed through a drying tower filled with silica gel into the extractor, and the nitrogen stream was exhausted through a bubbler tube with a 1 cm head of mercury. After approximately 500 ml of solvent per hour had been refluxed through the lunar fines for a period of 170 hours, a 200 ml sample of solvent was collected as it flowed from the extractor. The volume of this sample was reduced to several microliters by removal of solvent in a stream of filtered nitrogen at 40°C, and all of the residual sample that could be taken into a 10-μl syringe was injected into a Apiezon L coated capillary gas-liquid chromatographic column. No organic material except solvent was detected in this sample. The extractor was then disconnected from the reflux condenser and distillation flask, sealed with two glass stoppers, and rolled for 48 hours on a ball mill. All the extraction solvent was transferred from the distillation flask, and the volume of the extract was reduced by removal of solvent in a stream of filtered nitrogen at 40°C to approximately 100 μl. After a 2-μl portion of the remaining extract failed to yield detectable C_{15} to C_{30} alkane peaks in the gas chromatograph, the volume of the extract was further reduced to a few microliters, which were injected into the gas chromatograph. Only a solvent peak was observed in this chromatogram.

The extractor, containing the ball-milled lunar sample, was reconnected to the reflux condenser and distillation flask, and azeotropic benzene and menthanol were refluxed through the pulverized sample at a rate of approximately 500 ml per hour for 72 hours. At the end of this period, the solvent bumped, causing the mercury within the bubbler to be drawn into the extractor. The extraction was discontinued at the time of this mishap, and gas chromatographic analyses of the extract of the pulverized lunar sample indicated an absence of detectable quantities of organic materials with vapor pressures equivalent to C_{15} to C_{30} alkanes.

All contents of the extractor, except for the stainless steel grinding balls, were transferred to a 2-liter Teflon beaker. Mercury was removed from the pulverized lunar minerals with an acid cleaned gold wire, and 500 g of 48 percent hydrofluoric acid was poured slowly with stirring into the beaker. The hydrofluoric acid layer in the beaker was covered with a 5-cm layer of benzene, and the contents of the beaker were stirred periodically until reactions ceased. The beaker was then warmed for a period of approximately 2 hours to 80°C. After most of the benzene layer evaporated, the reaction mixture was transferred to a 2-liter separatory funnel containing 400 ml of distilled water. This mixture was shaken thoroughly with three successive 200-ml portions of benzene. These benzene extracts were composed and concentrated. These extracts did not contain detectable quantities of volatile organic materials.

The lunar fines analyzed contained no C_{15} to C_{30} alkane at a concentration exceeding 1 ppb by weight, whereas the concentrations of C_{15} to C_{30} alkanes in rocks from the surface of the earth commonly exceed 100 ppm by weight.

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Fluorometric Examination of a Lunar Sample

Abstract. *We have been unable to detect porphyrins in 13 grams of the bulk fine lunar sample from the Sea of Tranquillity under conditions in which less than 10^{-13} mole per gram of lunar sample could have been detected. By appropriate extraction, however, the lunar sample yields a material which exhibits absorption maxima at 310 and 350 nanometers and a fluorescence maximum at 410 nanometers.*

Since porphyrins are essential components of all life, their presence in lunar samples would suggest that life once existed on the moon. Porphyrins have characteristic absorption spectra, which we have measured by energy-corrected fluorescence activation on nanogram quantities in organic solvents. We have examined several solvent extracts of a lunar surface sample for the presence of porphyrins by spectrophotometry and the highly sensitive linear-energy spectrophotofluorometry (LEF) (*1*). The LEF method in general permits measurement of absorption spectra of fluorescent compounds at a sensitivity of the order of 100 to 1000 times that of absorption spectrophotometry.

Our LEF (Perkin-Elmer) apparatus automatically compensates for variations of energy with wavelength of both the excitation light source and the detector sensitivity in such a way that the spectrum is free from distortion. The instrument uses a high-pressure xenon arc source, an EMI 9558 photomultiplier (trialkali photocathode tube), and a photon counter. We have used it to record the complete absorption spectrum of 10^{-12} mole of porphin in 0.2 ml of methylene chloride, and similarly spectra of the nickel mesoporphyrin IX dimethyl ester at 10^{-11} mole and vanadyl tetra-meso-beta-naphthyl porphin at 10^{-12} mole in the same volume.

The extraction scheme is based on the use of sequential Soxhlet extractions with methanol and benzonitrile (*2*) for 24 hours to remove loosely held material, followed by a 10-hour period of ball-milling with a melt of methane sulfonic acid–naphthalene (1:1, by weight) —at 50°C in order to remove any material bound in solid carbon phases (*3*). Each extract was worked up separately for fluorescent residues.

Fluorometric grade methanol (Harleco) and scintillation grade naphthalene (Packard) were used without treatment, but reagent grade benzonitrile and methane sulfonic acid were distilled freshly before use. Glassware was cleaned with hot aqua regia and rinsed with water and fluorometric grade methanol before use. Teflon sleeves were used on all ground joints. All operations on the sample were carried out in a dry box under an argon atmosphere.

Tests for possible contaminants in the extraction solvents were run as follows. Identical volumes of the solvent systems used on the sample were used in the same way to treat a 13-g sample of clean 200-mesh optical quartz. The test extracts were treated and examined exactly as those from the sample were.

Samples 10002,4 (5.2 g) and LRS-1002,4 (7.9 g) of lunar surface powder were pooled as received, and the bulk sample was layered in layers 5 mm thick separated by layers of borosilicate glass wool, about 5 mm thick, in a microporous fluorocarbon polymer Soxhlet thimble. The layering of the powdery sample was necessary in order to promote free access and percolation of the extraction solvent. The sample was placed in a double-jacketed Soxhlet apparatus so designed that the thimble and contents were bathed in the vapor of the refluxing solvent and thus extracted efficiently by solvent at its boiling point. The sample was extracted first with 200 ml of fluorometric grade methanol for 24 hours. The methanol extract was then filtered through an ultrafine-porosity glass filter to remove trace solids and concentrated to near dryness in a Buchler evaporator. The extract was dried under a stream of nitrogen, then taken up in 2 ml of methylene chloride for examination of the methanol extract. The sample was next extracted with 200 ml of benzonitrile under reflux at about 67°C and a pressure of 4 to 5 mm-Hg, filtered as before, and finally concentrated to dryness by vacuum distillation and taken up in 2 ml of methylene chloride for examination of the benzonitrile extract. The sample was freed of benzonitrile by brief Soxhlet extraction with methanol and transferred to a 500-ml glass-stoppered borosilicate reagent bottle. Alundum balls were added, together with methane sulfonic acid (28.9 g) and naphthalene (28.9 g), and the mixture was ball-milled as a melt at 54 rev/min for 10 hours at a temperature kept at 40° to 50°C by means of a heat lamp. The methane sulfonic acid–naphthalene sample slurry was transferred by water washes into a round-bottom flask, and excess naphthalene and water were removed on a Buchler evaporator at a pot temperature of 42°C and pressure of 0.5 mm-Hg. Water (200 ml) and methylene chloride (100 ml) were used, with scraping as needed to transfer the black mineral slurry to a separatory funnel equipped with a Teflon stopcock. The glass wool used in layering the sample in the thimble was removed into an ultrafine-porosity sintered glass filter apparatus and washed with water until the washes were approximately neutral; these washes were added to the main body of the acidic aqueous mineral slurry in the funnel. The aqueous phase was washed three times with 100 ml of methylene chloride, the yellow fluorescent organic phase was discarded, and the aqueous phase was centrifuged in 150-ml glass centrifuge bottles. The clear acidic aqueous supernatant was returned to the separatory funnel. The precipitate was washed twice with 50 ml of water, and these washes were added to the contents of the separatory funnel. The clear aqueous phase, with its presumed dicationic metal-free porphyrins, was brought to *p*H 8 by the addition of 1*N* KOH and extracted four times with 100 ml of methylene chloride. The methylene chloride phase was then concentrated to 2 ml under a stream of nitrogen for examination as the "methane sulfonic acid–naphthalene extract."

Spectral study of all extracts and solvent blanks before and after addition of methane sulfonic acid to demetallate any metallic porphyrins present (*4*) showed that the benzonitrile extract contained a material which exhibited a fluorescence peak at 690 nm when ex-