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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 excited at 410 nm. However, on the basis of its behavior in acid and its spectral properties, we do not believe that this compound is a porphyrin. We therefore believe that porphyrins were not present in the lunar sample above the detectability limits of our method—that is, above $10^{-12}$ mole/g of sample (linear energy spectrofluorometer) or $10^{-13}$ mole/g of sample (conventional spectrofluorometer).

We have, however, found other fluorescent materials in the lunar sample but not in the solvent control blanks. Thus the benzonitrile and methane sulfonic acid–naphthalene extracts exhibit absorption maxima at 310 and 350 nm with a fluorescence maximum at 410 nm. This material is present in the lunar sample at concentrations of the order of 10 parts per billion (quinine sulfate equivalent). Because of the low concentration of the fluorescent material in the sample we have been unable to identify it by high resolution mass spectrometry. Extraction of an ancient terrestrial sample (Gunflint chert) by the methods outlined above yields a fluorescent material similar in its properties to those of the material present in the lunar sample.

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## Search for Organic Material in Lunar Fines by Mass Spectrometry

*Abstract.* *Three kinds of experiments were performed in an effort to detect and identify organic compounds present in the lunar material (fines 10,086): (i) vaporization of the volatilizable components directly into the ion source of a high-resolution mass spectrometer, and (ii and iii) extraction of the material with organic solvents before and after dissolving most of the inorganic substrate in hydrochloric and hydrofluoric acid. The extracts were investigated by a combination of gas chromatography and mass spectrometry. Although a number of organic compounds or compound types have been detected, none appears to be indigenous to the lunar surface.*

In an effort to characterize the organic compounds present in the surface covering the Apollo 11 landing site, a portion of fines (sample 10,086) has been subjected to mass spectrometric analysis. Three different types of experiments were undertaken: (i) vaporization of organic compounds present in lunar material; (ii) solvent extraction of lunar material; and (iii) dissolution of lunar material in acid followed by extraction of the aqueous phase.

The first experiment involved placing the lunar material (0.4 g) in a small glass bulb mounted directly on the ion source chamber of a high-resolution, double-focusing mass spectrometer (CEC 21-110B). The bulb was positioned in such a manner that any material evolving from the sample would vaporize directly into the ionizing electron beam (*1*). This approach involves the least risk of contamination and provides maximum sensitivity. The mass spectra were recorded in a number of consecutive exposures on a photographic plate (*2*); thus an integrated permanent record was obtained of all ions produced in the 20-minute period during which the sample was heated from ambient temperature to 400°C. Comparison of the resulting exposures with the spectra obtained from known amounts of *n*-decane introduced through the batch-inlet system of the spectrometer indicates that the 0.4 g of lunar material produced about 0.1 to 1.0 $\mu$g of organic material—that is, about 0.25 to 2.5 parts per million.

The composition of the ions produced is given in Table 1. Most of the species are hydrocarbon ions of various degrees of unsaturation. In addition to these, a few series of ions were found that contained one, two, or three oxygen atoms (mono-oxygenated ions predominating), and some ions containing one nitrogen atom. Furthermore, a few ions contained nitrogen and oxygen, and some rather abundant ions contained sulfur either alone or in combination with oxygen. The sulfur-containing species were either free of carbon (elemental sulfur, hydrogen sulfide, and sulfur dioxide) or had only one carbon atom (carbon disulfide and carbon oxysulfide).

A few of the ions may represent compositions with three nitrogen atoms, but these differed by only 1.3 millimass units from those having $C_2H_2O$ instead of $N_3$, and the two species were therefore difficult to resolve. The absence of species with two nitrogens would argue against the presence of molecules containing more than one nitrogen atom.

Although we must emphasize that the species listed in Table 1 represent ions formed in electron impact and do not necessarily indicate molecules present as such, one can draw a number of conclusions from these data. First, there seems to be present a series of hydrocarbons having up to nine carbon atoms and ranging from almost completely saturated species ($C_8H_{16}$ corresponds to a hydrocarbon with one double bond or ring) to more unsaturated ones. The aromatic ions ($C_9H_7$, $C_8H_9$, $C_7H_8$, and $C_6H_6$, which would correspond to an indenyl ion, styrene, toluene, and benzene) appeared to be present in much lower abundance, despite the high relative intensity of these ions in the mass spectra of the corresponding pure compounds.

Aside from the large quantities of $CO_2$, the most abundant oxygen-containing ions consisted of one or two carbons and one or two oxygens, as they may be produced upon ionization of small alcohols or glycols. Such ions might have come from either ethylene oxide or its hydrolysis product, ethylene glycol. (Ethylene oxide was used as a sterilizing agent at the Lunar Receiving Laboratory.) Similarly, the $C_2H_4O_2$ (the elemental composition of acetic acid) may be a reduction product of peracetic acid, also used as a sterilizing agent at LRL, whereas the ions containing three carbon atoms and one oxygen atom may have been derived from acetone which in turn may have been produced by pyrolysis of salts of acetic acid. The $C_8H_5O_3$ ions were