RESEARCH FOR AMINO ACIDS IN LUNAR SAMPLES*

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Abstract. Water extracts of lunar fines were analyzed for amino acids by a gas-liquid chromatographic technique whereby amino acids were converted to the N-trifluoroacetyl *n*-butyl esters prior to analysis. The lunar material studied included both Apollo 14 (14240 SESC and 14298) and Apollo 12 (12023) samples. The water extract of the 'special' Apollo 14 sample (14240 SESC) was analyzed both for free and bound amino acids (hydrolysis with 6 N hydrochloric acid). In both the hydrolyzed and unhydrolyzed extracts, the amino acids were not observed above background levels.

The analysis of Apollo 12 and 14 samples (12023, 14298) yielded similar results. Detection limits were established at 300 pg to 1 ng for different amino acids. A large chromatographic peak with a retention temperature of 126°C was observed on analysis of sample (12023); it was identified as oxalic acid by GC-MS. The concentration of amino acids in the Apollo 14 SESC samples processed and analyzed in the joint experiments at Ames by GLC and IEC were found to be extremely low (glycine at 3 to 4 ng g^{-1}). As the quantities were so minute, these identifications could not be confirmed by GLC-MS and therefore should still be considered as tentative. Other studies included the analysis of performance standards at the 2 to 6 ng level of each of 17 amino acids, and the analysis of 5 ml of H_2O containing 2 ppb of each amino acid. Recovery of amino acids added to lunar fines were conducted at the 10, 50, and 70 ng level of each amino acid with 50 to 70 mg of lunar material. The recoveries varied from as high as 80 % for some of the aliphatics to complete loss of the amino acids ornithine and lysine.

1. Introduction

The examination of the lunar material acquired by the Apollo missions for indigenous, biologically significant substances has been of primary concern to several research groups. These studies are of obvious importance as they provide a unique possibility for gaining insight into the nature of chemical evolution. The important roles played by amino acids in terrestrial life and the possibility to determine them at a trace level by recently developed methodologies, have made these compounds prime targets of scrutiny in extraterrestrial material.

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This study was primarily directed toward the examination of Apollo 14 lunar fines for indigenous amino acids or materials which could be converted to amino acids on hydrolysis with 6 N hydrochloric acid.

Previous gas chromatographic studies conducted by Gehrke *et al.*, (1971) on unhydrolyzed and hydrolyzed water extracts of Apollo 11 and 12 samples have resulted in none of the protein amino acids being detected. Hare *et al.* (1970) and Nagy *et al.* (1970) reported the presence of glycine and alanine in unhydrolyzed water extracts of Apollo 11 fines, though analyses of a subsurface Apollo 12 samples (12033) resulted in no free amino acids being detected by either Harada *et al.* (1971) or Nagy *et al.* (1971) both of whom used ion-exchange chromatographic techniques. However, glycine, alanine, glutamic acid, and leucine were reported by Harada *et al.* (1971) to be present after hydrolysis of the aqueous extract of sample 12033 at a total concentration of 19 ppb.

In this investigation, a special sample of fines was returned by Apollo 14 astronauts from Fra Mauro to the Space Sciences Laboratory, Berkeley, opened, and made available to the three groups for analysis for amino acids. This sample (14240 SESC) was opened and distributed from a clean facility at the Space Sciences Laboratory in Berkeley, California. Earlier experiments by the three investigating teams had shown this facility to be free of any detectible amino acid contamination. This was proven by 'sand blanks', which were taken through the opening and distribution procedures, and subsequently analyzed by both gas-liquid and ion-exchange chromatography.

2. Experimental

A. APPARATUS AND REAGENTS

A Varian 2100 Series gas chromatograph with a Varian Model 20 dual pen strip chart recorder was used. The electrometer output was attached to a voltage divider which allowed the signal to be displayed on both pens of the recorder with a four fold difference of amplification. The ethylene glycol adipate (EGA) column packing and solvent vent system described earlier by Zumwalt *et al.* (1971) were used.

The derivatization reagents, *n*-butanol·3 N HCl, dichloromethane, and trifluoroacetic anhydride (TFAA) were of the quality described by Gehrke *et al.* (1971). The derivatization of the amino acids to the N-trifluoroacetyl *n*-butyl (N-TFA) esters was conducted in pyrex micro reaction vials with all teflon screw caps. The derivatization reagents were added to the samples in the vials via 25, 50, and 100 μl micropipets attached to glass syringes with teflon tubing.

The following precautions were observed to reduce difficulties during the derivatization and chromatography of the samples.

(1) Aqueous samples were placed under an infrared lamp, and allowed to dry without excessive heating. Dichloromethane was added, then evaporated, to azeotropically remove the last traces of H_2O.

(2) After formation of the amino acid *n*-butyl esters with *n*-butanol·3 N HCl, the butanol·HCl was also evaporated under the IR lamp. Care was taken to prevent

excess heating of the sample, as the more volatile aliphatic amino acid esters are subject to losses at this point. However, all the butanol·HCl must be removed, as injection of butanol·HCl may present problems in the chromatographic analysis.

(3) Careful preparation of the chromatographic column proved essential, with special attention devoted to obtaining a uniform coating on the support material, and a minimum of fractured particles after coating. Conditioning of the column to reduce liquid phase 'bleed' was conducted at 220 °C with a N_2 carrier flow of ca. 50 ml min^{-1} until the 'bleed' rate had been reduced to a satisfactory level. When not in use, the columns were kept at 200 °C in the chromatograph with a carrier flow of 20 to 50 ml min^{-1}. If the columns had to be removed, the ends were tightly capped to exclude moisture. Generally, EGA columns should not be subjected at any one time to temperatures in excess of 225 °C for longer than one to two hours.

B. PREPARATION OF EXTRACTS AND HYDROLYSATES

1. *Extraction*

The samples of lunar fines (0.5 to 2 g) were placed in pyrex glass conical centrifuge tubes (13 ml), water added (5 ml/g^{-1} of fines), and the tubes were closed with all teflon screw caps. The tubes were shaken manually, then placed in a 100 °C sand bath for 24 h with periodic shaking. At the end of this period, the tubes were allowed to cool, then centrifuged at 3300 rpm for 10 min. The supernatant was then decanted into small (10 to 20 ml) beakers for evaporation, followed by hydrolysis and derivatization.

2. *Hydrolysis*

After evaporation of the extracts to ca. 1 ml under the IR lamp, they were transferred to pyrex micro reaction vials and taken to dryness. Then, 100 μl of 6 N HCl were added, and the vials capped with teflon 'Mininert' caps with stopcock-like apertures for attachment to a laboratory vacuum line.

After partial evacuation, the vials were closed, and heated for 22 h at 110 °C in a sand bath. The samples were then allowed to cool, and the HCl evaporated. The samples were then derivatized as described by Gehrke *et al.* (1971).

3. Results and Discussion

Initial experiments were conducted to confirm the integrity of the derivatization reactions and reagents, and to optimize the GLC instrumental and chromatographic system for the separation and flame ionization detection of the amino acid derivatives. Figure 1 presents the chromatogram obtained after derivatization of a standard solution containing 6 ng of each of 16 amino acids.

To determine the recovery of low concentrations of amino acids from water, a solution containing 2 ppb of each amino acid was prepared. Five ml of this solution were taken through the entire analytical chromatographic process. Figure 2 presents

the chromatogram along with the corresponding chromatographic-reagent blank. Recoveries were generally well above 80%, thus showing that losses of amino acids due to adsorption on glass did not occur.

Interest was then centered on the analysis of the water extract of the special Apollo 14 sample (ARC 14240 SESC). This extract was divided into two equal portions; one portion was derivatized and analyzed for free amino acids, the other was hydrolyzed with 6 N HCl, then derivatized and analyzed. Figure 3 shows the resultant

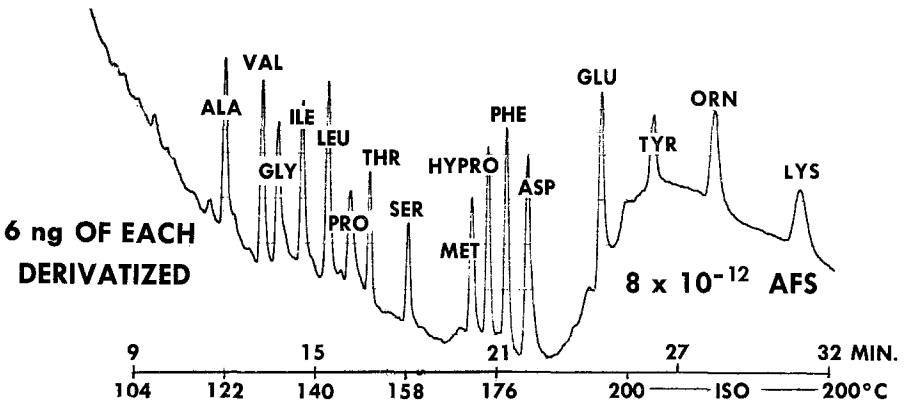

Fig. 1. Derivatization-chromatography standard 6 ng of each derivatized.

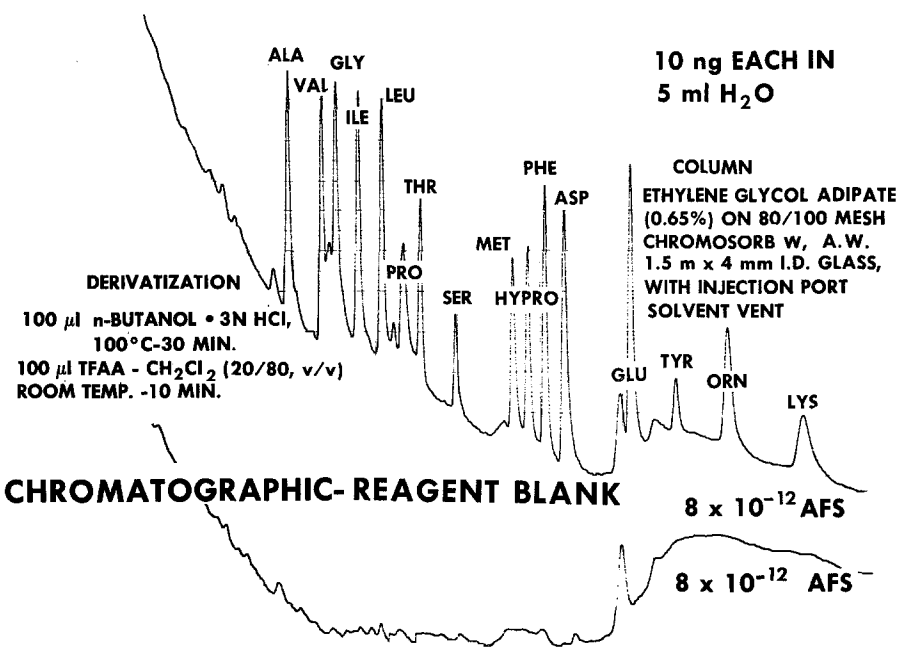

Fig. 2. Recovery of amino acids from water. 10 ng each in 5 ml H_2O.

chromatogram from the unhydrolyzed extract and the corresponding procedural blank. None of the protein amino acids were observed above background. Compare the corresponding positions on the two chromatograms. The arrows mark the elution positions for the amino acids and the retention temperatures were reproducible to better than 0.5 °C. The EGA chromatographic column gave a good background over most of the temperature scale, i.e., less than 500 pg to 180 °C, and 3 ng at a

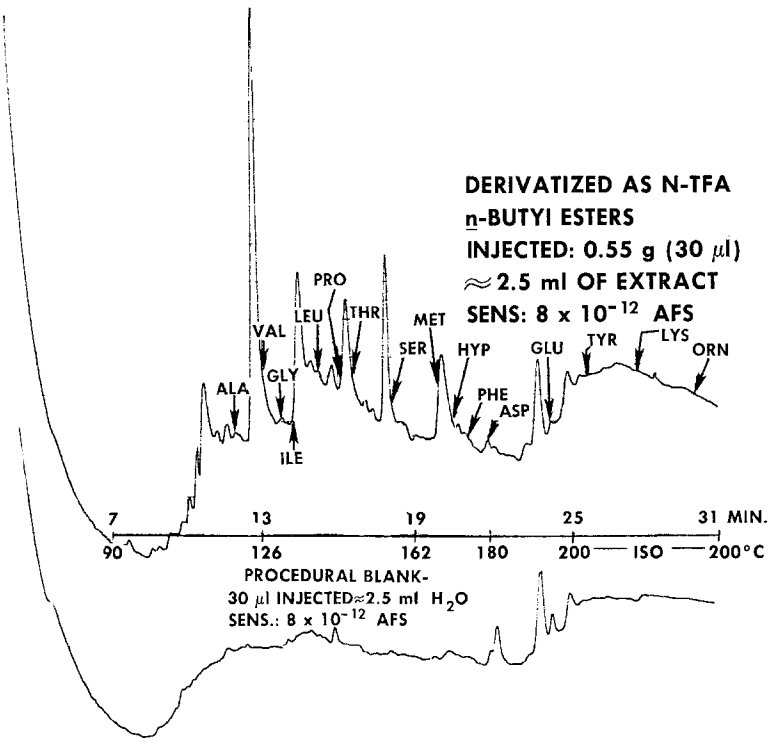

Fig. 3. GLC of water extract of Apollo 14 (ARC 14240, SESC) – derivatized as N-TFA *n*-butyl esters.

temperature of 195 °C. However, this background did not interfere with the detection of glutamic acid as it eluted at 197 °C and was completely resolved from the interference. Although unidentified chromatographic peaks could have partially obscured valine, isoleucine, threonine, serine, methionine, and hydroxyproline, the positions of the other amino acids were free from interferences with a background of <500 pg. This is especially noteworthy in the case of alanine and glycine; amino acids of primary interest because they had been reported to occur in relatively large amounts in lunar fines by Harada *et al.* (1971) and Nagy *et al.* (1970). The chromatographic peaks for the hydrolyzed portion of the extract were of the following magnitudes: alanine, ca. 1 ng; glycine, ca. 3 ng; serine, ca. 1 ng; aspartic acid, ca. 2 ng; and

glutamic acid, ca. 2 ng; and the same amino acids were present at comparable levels in the hydrolyzed water blank.

A further series of analyses for amino acids were conducted on a separate 0.5 g Apollo 14 sample (ARC 14298). Alanine, glycine, serine, aspartic acid, and glutamic acid were observed at concentrations ranging from 6 to 12 ng g^{-1} and were identified by co-chromatography of a standard amino acid mixture with the remainder of the sample. Since the 0.5 g suggested the presence of traces of amino acids, a second 1.0 g sample was analyzed as a 'scale-up' study. No increase in the size of the amino acid peaks was noted on comparison of the chromatograms for the 0.5 and 1.0 g samples. After subtraction of amino acids present in the hydrolyzed procedural blank, it was concluded that these amino acids were not present at levels >1 ng g^{-1} of lunar fines.

A. JOINT COLLABORATIVE EXPERIMENTS AT AMES

In an effort to resolve the divergent results which have originated from the analyses of the lunar fines with regard to amino acids, a special collaborative investigation was conducted on the Apollo 14 fines (14240 SESC) at the Ames Research Center, Moffett Field, California, during October, 1971. The major objective was to resolve the question of whether amino acids were or were not present in hydrolyzed water extracts of the lunar fines and whether the different techniques of ion-exchange chromatography and gas-liquid chromatography would yield the same results.

The two participating teams were composed of the following: Ponnamperuma and his associates, Gehrke, Zumwalt, Kuo, and Kvenvolden on the one hand; and Fox and co-workers, Hare, and Harada on the other. Gas-liquid chromatographic methods were used in the analyses by Ponnamperuma and co-workers, and Fox and associates used ion-exchange chromatography in their studies. The samples analyzed were: (a) a 6 g sample of Apollo 14 SESC assigned to Ponnamperuma, (b) a 6 g sample of Apollo 14 SESC assigned to Fox, and (c) another portion of the Apollo SESC sample extracted and hydrolyzed in Miami and brought to the Ames Research Center. In all of the experiments the two teams worked together in the extraction, preparation, and hydrolysis of the extracts. The final analysis for amino acids was made by GLC and IEC on the divided hydrolyzed lunar extracts by the two participating teams.

All the glassware used for the extraction, evaporation, and hydrolysis was cleaned by washing with Alconox in hot water, and thorough rinsing with hot water and distilled H_2O. The glassware was then placed in a furnace at 520 to 550 °C for 3 to 15 h to remove organic contaminants.

Prior to the extraction of the fines, blanks of the extraction system, distilled water, and reagents were prepared and analyzed both by GLC and IEC.

The water extracts were prepared by refluxing 6 g of Apollo 14 fines (14240) with ca. 22 ml of water for 12 h. At the end of this period, the samples were allowed to cool, the water decanted into pyrex glass centrifuge tubes, and centrifuged at 3500 rpm for 15 min. The supernatants were then decanted into small beakers, which were

placed in a dessicator over NaOH, and a partial vacuum applied (<25 mm). In most instances, the aqueous extracts became frozen during this step and sublimation occurred.

After vacuum drying of the aqueous extracts, the samples were dissolved in 3 ml of 6 N HCl and transferred to a pyrex glass hydrolysis tube. A vacuum was applied to the samples for a few minutes to remove air and the tubes were sealed with a propane-oxygen torch. The hydrolysis tubes were wrapped in aluminum foil to ensure even heat distribution throughout the tube, then placed in 110 °C oven for 20 h. After removal of the tubes from the oven, they were placed in a dessicator over NaOH pellets with partial vacuum for removal of the 6 N HCl. After drying, the residue was taken up in 200 μl of 0.01 N HCl, then one-half (100 μl) was taken for analysis by GLC, and the other one-half for analysis by IEC.

Studies were first made to optimize the derivatization and GLC systems of analysis, and to establish the integrity of the derivatization reagents. Analysis of the procedural blanks by both GLC and IEC established that amino acid contamination of the total system and all reagents was less than 1 ng g^{-1}.

The GLC analyses of three consecutive water extracts of the sample (a) (Ames Research Center), are presented in Figure 4. In extract 1, only 3 ng g^{-1} of glycine were observed, less than 0.5 ng g^{-1} of alanine, ca. 1 ng g^{-1} of serine, and ca. 0.5 ng g^{-1}

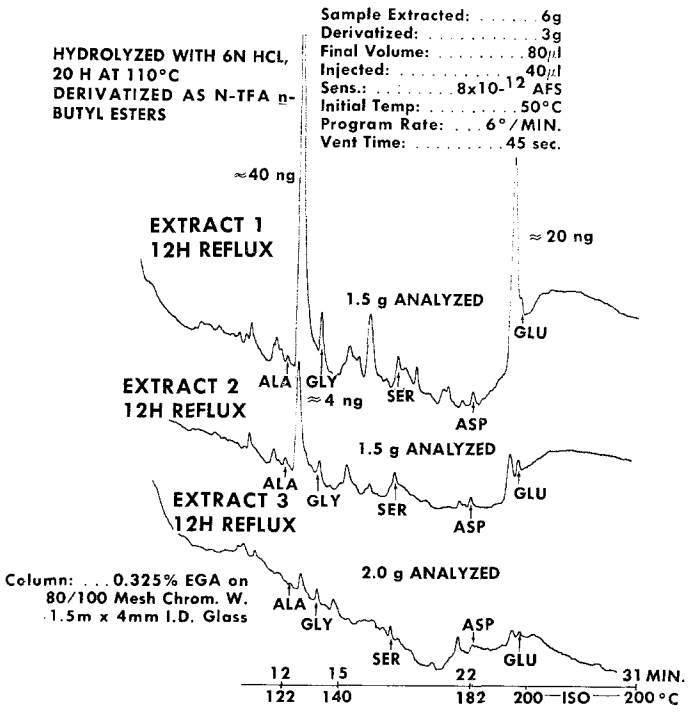

Fig. 4. GLC of water extract of Apollo 14 (14240 SESC) ames sample joint study.

of both aspartic and glutamic acids. Extract 2 contained a similar amount of alanine, ca. 1 ng g^{-1} of glycine, and lesser amounts of serine, aspartic acid, and glutamic acid. In extract 3, the amount of glycine was similar to extract 2 (1 ng g^{-1}), alanine was well below the 0.5 ng g^{-1} level, serine at ca. 0.5 ng g^{-1} or less, and smaller quantities of aspartic and glutamic acids.

Thus, the amino acid found by GLC in the largest concentration was glycine, at 3 ng g^{-1} in the Apollo 14 SESC lunar fines (Ames). The GLC data obtained for this sample (extract 1) were in excellent agreement with IEC analysis on the other half of the sample.

Also of interest in Figure 4 are the chromatographic peaks which do not correspond to the common protein amino acids. Analysis of extract 1 resulted in a large peak (30 to 40 ng g^{-1}) between the alanine and glycine elution positions, a second large peak near glutamic acid, and numerous smaller peaks throughout the chromatogram. Essentially all of these peaks decreased in intensity as the sample was re-extracted.

Figure 5 presents the chromatogram obtained on GLC analysis of the hydrolyzed water extract of Sample (b) (Miami). The top GLC chromatogram shows ca. 2 to 3 ng g^{-1} of glycine present, and less than 0.5 ng g^{-1} of alanine, serine, aspartic acid, and glutamic acid. Again, these results were in excellent agreement with IEC

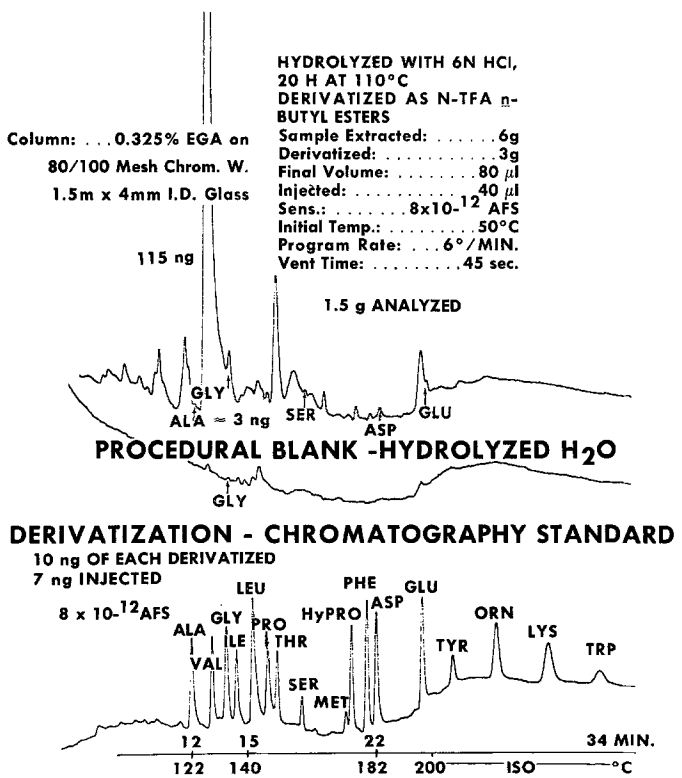

Fig. 5. GLC of water extract of Apollo 14 (14240 SESC) Miami sample joint study.

data on the remaining half of the extract. Also, these results are in agreement with those obtained from the Ames sample, with the most abundant amino acids, glycine, being present at the 3 to 4 ng g^{-1} level.

Figure 6 shows the chromatogram which results from the analysis of a hydrolyzed water extract of Apollo 12 sample (ARC 12023). Again, although small amounts of amino acid peaks were observed (3 to 4 ng of gly, ser, asp) similar quantities were observed in the corresponding procedural blank. The large chromatographic peak in the valine region of the chromatogram was identified as derivatized oxalic acid by GC-MS.

A series of experiments was then conducted to gain information on the recovery of amino acids added to lunar fines. Ten (10) ng of each amino acid were added to 50 mg of lunar fines. The amino acids were added to the lunar fines in the following manner. Five microliters (5 μl) of an aqueous standard solution (2 ng μl^{-1} of each amino acid) were added to the sample, dried for a few minutes under the IR lamp, and one ml of water added. After water extraction and derivatization, the recoveries were low (10 to 20%). To this extracted sample an additional 50 ng of each amino acid were added, followed by re-extraction and derivatization. This experiment yielded much higher recoveries of 60 to 80%. Another experiment was then conducted in which 70 ng of each amino acid were added to 70 mg of Apollo 14 fines. The resultant chromatogram is seen in Figure 7, with recovery of the amino acids varying greatly. The aliphatic amino acids gave the highest recoveries (40 to 50%), while phenylalanine yielded only 19% and ornithine and lysine were completely lost. These recovery

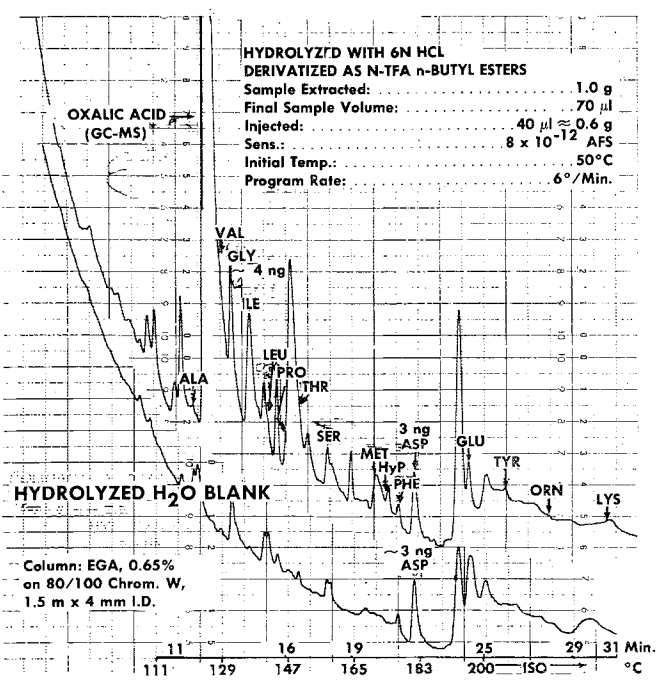

Fig. 6. GLC of water extract of Apollo 12 (ARC 12023, 22).

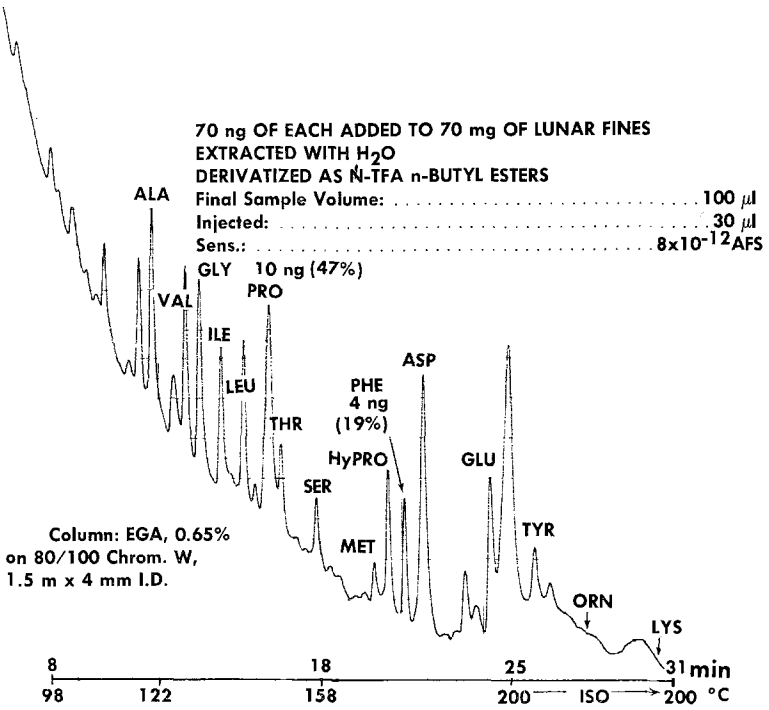

Fig. 7. Recovery of amino acids added to lunar fines (ARC 14240, 1.01 SECS).

experiments are of considerable significance in the interpretation of the extractability of free amino acids at pH 4.6 from lunar fines. Further studies on the extraction of amino acids and peptides must be conducted.

4. Summary and Conclusions

Analyses of both unhydrolyzed and hydrolyzed water extracts of Apollo 12 and 14 lunar fines were conducted by gas-liquid chromatography. The Apollo 14 sample 14240 SESC returned by the astronauts from Fra Mauro to the Space Sciences Laboratory, Berkeley, California, had been specially designated for amino acid studies. This SESC sample had been exposed to a minimum of manipulative steps prior to analysis and was considered to be of primary importance. On examination of reflux aqueous extracts of sample 14240 SESC, the common protein amino acids were not extracted or observed in concentrations above background in either the unhydrolyzed or hydrolyzed extracts. Another Apollo 14 sample (14298) was also studied and again extractable indigenous amino acids were not found above the procedural blank values.

A hydrolyzed water extract of an Apollo 12 sample (12023) presented no indication of indigenous protein amino acids. Oxalic acid dibutyl ester was identified by GC-MS as the compound responsible for a large peak observed on GLC analysis of this sample.

To resolve the divergent data which have been reported for amino acids in lunar fines, a special collaborative study was conducted on Apollo 14 fines (14240 SESC) at the Ames Research Center, Moffett Field, California, during October, 1971. The objective was to determine whether amino acids were or were not present in hydrolyzed water extracts of the lunar fines and whether the different methods of gas-liquid and ion-exchange chromatography would yield the same results. The two teams of scientist were as follows: Ponnamperuma and co-investigators, Dr Gehrke, Zumwalt, Kuo, and Kvenvolden as one team; and Fox and co-workers, Hare, and Harada as the other. GLC methods were used by Ponnamperuma and co-workers, while Fox and associates used IEC. The complementary GLC and IEC methods were found to give closely similar and therefore confirmatory analytical results, when applied to samples which had been subjected to identical processing and handling. Both GLC and IEC, glycine was observed at 2 to 4 ng g^{-1}; ser at 1 ng g^{-1}; and ala, and asp and glu acids at less than 0.5 ng g^{-1}. Of interest are the GLC chromatographic peaks which do not correspond to the common protein amino acids. A large unknown peak (30 to 40 ng g^{-1}) eluted between the ala and gly peaks. As the quantities of the amino acids are so minute, GLC-MS data were not obtained. The source of the amino acids may be: synthesis during extraction and hydrolysis, rocket exhaust, low-level contamination, or indigenous.

In studies on the recovery of amino acids added to lunar fines, low recoveries were obtained (10 to 20%) when 10 ng of each amino acid were added to 50 mg of virgin fines, but the subsequent addition of 50 ng of each to the previously extracted sample resulted in much higher recoveries (60 to 80%). These results show that there is a need for an indepth study of the extractability of amino acids and peptides from lunar material.

In our experiments we have not found amino acids in the water extracts or hydrolyzed water extracts of Apollo 11, 12, and 14 samples above the procedural blank values (0.5 ng to 180° R.T.).

In the joint collaborative studies at Ames (Gehrke-Zumwalt-Kuo-Missouri) and (Hare and Harada-Miami) larger samples were used and background levels were <0.5 ng. By both GLC and IEC glycine was indicated as present at 2–4 ng g^{-1} and serine at 1 ng g^{-1}. The others were <0.5 ng g^{-1}. There is insufficient evidence to ascertain their source or origin.

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