Proc. Nat. Acad. Sci. USA
Vol. 69, No. 4, pp. 809–811, April 1972

Nonprotein Amino Acids from Spark Discharges and Their Comparison with the Murchison Meteorite Amino Acids

(gas chromatography–mass spectrometry/prebiotic synthesis/amino-acid analyzer)

YECHESKEL WOLMAN*, WILLIAM J. HAVERLAND, AND STANLEY L. MILLER†

Department of Chemistry, University of California, San Diego, La Jolla, Calif. 92037

Communicated by Harold C. Urey, December 20, 1971

ABSTRACT **All the nonprotein amino acids found in the Murchison meteorite are products of the action of electric discharge on a mixture of methane, nitrogen, and water with traces of ammonia. These amino acids include α-amino-*n*-butyric acid, α-aminoisobutyric acid, norvaline, isovaline, pipecolic acid, β-alanine, β-amino-n-butyric acid, β-aminoisobutyric acid, γ-aminobutyric acid, sarcosine, *N*-ethylglycine, and *N*-methylalanine. In addition, norleucine, alloisoleucine, *N*-propylglycine, *N*-isopropylglycine, *N*-methyl-β-alanine, *N*-ethyl-β-alanine α,β-diaminopropionic acid, isoserine, α,γ-diaminobutyric acid, and α-hydroxy-γ-aminobutyric acid are produced by the electric discharge, but have not been found in the meteorite.**

A number of amino acids have been found recently in the Murchison meteorite (1–4). The evidence is strong that the meteorite was not contaminated with amino acids after it fell on the earth, since the amino acids are racemic and since many of them do not occur or are rare in terrestrial biology. Several of these meteorite amino acids have been properly demonstrated to be synthesized by the action of electric discharges on CH_4, NH_3, H_2O, and H_2 (5–7). These include glycine, alanine, sarcosine, β-alanine, *N*-methylalanine, α-amino-*n*-butyric acid, and α-aminoisobutyric acid. We have recently repeated the electric discharge experiment (8) and have demonstrated, by modern techniques, the presence of aspartic acid, glutamic acid, valine, isovaline, norvaline, and proline, all of which are found in the meteorite. We have also demonstrated the electric discharge synthesis of leucine, isoleucine, alloisoleucine, norleucine, serine, threonine, allothreonine, α-hydroxy-γ-aminobutyric acid, and α,γ-diaminobutyric acid, which have not yet been found in the meteorite, although some of them may be present [e.g., the leucines (3)].

The strong resemblance between the amino acids from the electric discharge and in the meteorite induced us to look for the other nonprotein amino acids found in the meteorite. We have found in the electric discharge reaction all the amino acids so far reported to be present in the meteorite.

MATERIALS AND METHODS

The authentic samples of the *N*-alkylated amino acids were prepared by reaction of the amine with the corresponding haloacid, and recrystallization from alcohol.

The same sample of amino acids produced by electric discharge that was used for the identification of hydrophobic amino acids (8) was used in the present analysis. The 18 fractions from the Dowex 50(H^+) chromatography were quantitated with an amino-acid analyzer. The amino acids were sufficiently separated to quantitate the β-alanine, γ-aminobutyric acid, isoserine, and α,β-diaminopropionic acid. Sarcosine and *N*-ethylglycine were quantitated on the amino-acid analyzer with a pH 2.80 buffer; the column eluent and ninhydrin were heated for 30 min instead of the usual 8-min heating period (9). The other amino acids were either present in amounts too small or the color yield was too low to allow quantitation on the amino-acid analyzer. They were, therefore, estimated by comparison of the area of the peaks of the unknown on gas chromatography with the area of an amino acid, on the same gas chromatogram that had been quantitated on the amino-acid analyzer.

The unknown amino acids were identified by gas chromatography–mass spectrometry (8). α,β-Diaminopropionic acid was separated on an OV-1 column, while all the other amino acids were separated on a capillary column of OV-225. Of the compounds in Table 1, only the diastereoisomers of β-aminoisobutyric acid were resolved on these columns.

Identification of the unknown amino acids was based on the elution time from Dowex 50 (H^+), retention time on the gas chromatography column, and identity with the mass spectrum

TABLE 1. *Yields and mole ratios from sparking CH_4 (336 mmol), N_2, and H_2O with traces of NH_3*

Amino acid	μmol	Mole ratio
Sarcosine	55	12.5
N-Ethylglycine	30	6.8
N-Propylglycine	∼2	∼0.5
N-Isopropylglycine	∼2	∼0.5
N-Methylalanine	∼15	∼3.4
N-Ethylalanine	<0.2	—
β-Alanine	18.8	4.3
β-Amino-*n*-butyric acid	∼0.3	∼0.1
β-Aminoisobutyric acid	∼0.3	∼0.1
γ-Aminobutyric acid	2.4	0.5
N-Methyl-β-alanine	∼5	∼1.0
N-Ethyl-β-alanine	∼2	∼0.5
Pipecolic acid	∼0.05	∼0.01
α,β-Diaminopropionic acid	6.4	1.5
Isoserine	5.5	1.2

The mole ratios are relative to glycine as 100 (8). The amino acids in this table account for 0.35% of the carbon added as methane.

---

* Present address: Department of Organic Chemistry, The Hebrew University, Jerusalem.

† To whom reprint requests should be addressed.

of the authentic compound. The mass spectra of β-aminoisobutyric acid and β-amino-*n*-butyric acid contained a few extraneous peaks; therefore, the identification of these compounds is tentative.

RESULTS

The yields of the amino acids are shown in Table 1. Several other amino acids were found in the gas chromatography–mass spectrometry analysis, among them no less than eight compounds with a molecular mass number (M^+) of 269. These are isomers of valine, but we were unable to demonstrate their identity with any of the known compounds in our possession. There were also two compounds of M^+ 281 (isomers of pipecolic acid). Two unidentified compounds of M^+ 269 and two of M^+ 281 were found in the meteorite (2).

DISCUSSION

There is a striking similarity between the products and relative abundances of the amino acids produced by electric discharge and the meteorite amino acids. Unfortunately, there are only a few quantitative values (4) for the meteorite amino acids, but we have estimated their relative abundances from the published gas chromatography data (2). Table 2 shows a comparison, including the aliphatic amino acids previously reported from the same sample (8).

The most notable difference between the meteorite and the electric-discharge amino acids is the pipecolic acid, the yield being extremely low in the electric discharge. Proline is also present in relatively low yield from the electric discharge. The amount of α-aminoisobutyric acid is greater than α-amino-*n*-butyric acid in the meteorite, but the reverse is the case in the electric discharge. The amounts of aspartic and glutamic acids in the meteorite are comparable, but there is five times as much aspartic acid as glutamic acid in the electric discharge.

TABLE 2. *Relative abundances of amino acids in the Murchison meteorite and an electric-discharge synthesis*

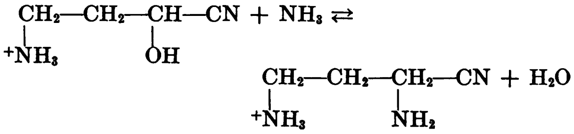
Amino acid	Murchison meteorite	Electric discharge
Glycine	4	4
Alanine	4	4
α-Amino-*n*-butyric acid	3	4
α-Aminoisobutyric acid	4	2
Valine	3	2
Norvaline	3	3
Isovaline	2	2
Proline	3	1
Pipecolic acid	1	<1
Aspartic acid	3	3
Glutamic acid	3	2
β-Alanine	2	2
β-Amino-*n*-butyric acid	1	1
β-Aminoisobutyric acid	1	1
γ-Aminobutyric acid	1	2
Sarcosine	2	3
N-Ethylglycine	2	3
N-Methylalanine	2	2

Mole ratio to glycine (= 100): 0.05–0.5, 1; 0.5–5, 2; 5–50, 3; >50, 4. The meteorite abundances are estimated from the published gas chromatogram (2); these estimates are approximate.

We do not believe that reasonable differences in ratios of amino acids detract from the overall picture. Indeed, the ratio of α-aminoisobutyric acid to glycine is quite different in two meteorites of the same type, being 0.4 in Murchison and 3.8 in Murray (4).

One would expect quantitative differences between the meteorite composition and the electric-discharge products, even if the mechanism of formation in the two cases were identical. Thus cyanoacetylene, which is synthesized by sparking CH_4 and N_2, is probably the major precursor of aspartic acid, but the yield of cyanoacetylene is decreased by the addition of small amounts of NH_3 to the $CH_4 + N_2$ mixture (10). Therefore, local differences in the NH_3 partial pressures on the parent body of the carbonaceous chondrites would result in substantial differences in the aspartic acid concentration if the amino acids were not completely mixed on the parent body. Temperature differences can also affect the yields. For example, the stability of α-aminoisobutyronitrile is quite sensitive to temperature and cyanide concentration, while α-amino-*n*-butyronitrile, being more stable, is not particularly sensitive to these factors.

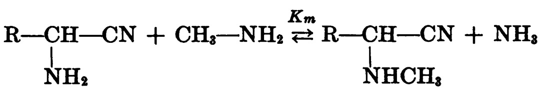
The electric-discharge amino acids reported here appear to have been synthesized through the aminonitriles for three reasons: there was considerable hydrogen cyanide produced by the electric discharge, the HCN concentration in the aqueous phase being 0.03 M. Secondly, previous electric-discharge experiments (7) have shown that most, if not all, of the amino acids were produced from nitriles. Thirdly, the presence of α-hydroxy-γ-aminobutyric acid and α,γ-diaminobutyric acid, as well as isoserine and α,β-diaminopropionic acid, is consistent with nitrile precursors. Under the conditions of the aqueous phase (pH 8.7, 0.05 M NH_4Cl), the following equilibrium is established rapidly.



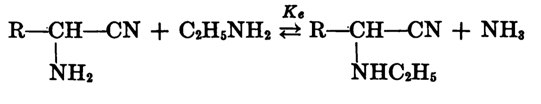
$$\underset{^{+}NH_3}{CH_2}-CH_2-\underset{OH}{CH}-CN + NH_3 \rightleftarrows \underset{^{+}NH_3}{CH_2}-CH_2-\underset{NH_2}{CH_2}-CN + H_2O \quad [1]$$

On acid hydrolysis in these experiments, or slower hydrolysis under primitive earth or meteorite conditions, both α-hydroxy-γ-aminobutyric acid and α,γ-diaminobutyric acid would be obtained. A similar equation can be written for isoserine and α,β-diaminopropionic acid.

Although we were able to find substantial yields of sarcosine, *N*-ethyl-, *N*-propyl-, and *N*-isopropylglycine, we could find *N*-methylalanine, but not *N*-ethylalanine. This result can be understood if aminonitriles are the precursors of these amino acids. The following equilibria would have been attained under the conditions of the experiment:



$$R-\underset{NH_2}{CH}-CN + CH_3-NH_2 \overset{K_m}{\rightleftarrows} R-\underset{NHCH_3}{CH}-CN + NH_3 \quad [2]$$



$$R-\underset{NH_2}{CH}-CN + C_2H_5NH_2 \overset{K_e}{\rightleftarrows} R-\underset{NHC_2H_5}{CH}-CN + NH_3 \quad [3]$$

and similar equilibria for propylamine and isopropylamine. There is also a similar equilibrium for the aminonitrile and hydroxynitrile (Eq. [1]).

The values of K_m and K_e are not known, but one would expect K_m and K_e for formaldehyde to be about the same. However, in the case of acetaldehyde, steric hindrance between the alkyl group on the nitrogen and the R group on the α-carbon could make K_e substantially smaller than K_m.

These considerations are valid whether the aminonitriles are formed from the aldehyde, ammonia, or amine, and HCN, or whether the nitrile is formed directly in the electric discharge (11). The equilibria [**2**] and [**3**] are rapidly reversible in solution under the pH conditions of the experiment.

The substantial and comparable yields of β-alanine, *N*-methyl-β-alanine, and *N*-ethyl-β-alanine can be accounted for by the addition of ammonia, methylamine, and ethylamine to acrylonitrile, which has been postulated (7) to be a product of an electric discharge in $CH_4 + NH_3$. The rate constants for the addition of NH_3 and the amines would be expected to be comparable (12); the concentrations of the amines, by Eq. [**2**] and [**3**] and the yields of sarcosine and *N*-ethylglycine, should also be comparable. The synthesis of β-alanine and the *N*-alkylated derivatives could also be accounted for by the direct synthesis of the nitriles in an electric discharge (11).

The yields of amino acids from the electric-discharge experiments allow a simple calculation, similar to Urey's (13, 14), of the concentration of amino acids in the oceans of the primitive earth. If we assume that all the surface carbon on the earth (3000 g/cm^2) passed through the atmosphere as CH_4, and that decomposition of amino acids after synthesis was minimal, then a 1.9% yield (0.35% from Table 1 + 1.55% in Table 1, ref. 8) would give 57 g of carbon in amino acids per cm^2, or about 0.6 mol/cm^2 of amino acids. If the primitive oceans were the present size (300 liters/cm^2), the concentration of amino acids would have been about 2 mM.

The yields from these experiments can also be used to calculate the expected amino-acid concentration in the carbonaceous chondrites. The Murchison meteorite contains a total of 2% organic carbon (1) and about 30 μg/gram of amino acids, so the amino acids are 0.15% of the organic carbon. If all the methane on the parent body was converted to organic compounds, the 0.15% of amino acids is a 10-times less than the yields from this electric-discharge experiment. These yields are in reasonable agreement, if we consider that synthesis on the parent body may not have been as efficient as the spark discharge in the laboratory and that various losses of amino acids would have occurred on the parent body.

The results of these experiments with an electric discharge do not exclude other sources of energy, such as ultraviolet light, high-energy radiation, shock waves, etc. from having contributed to the amino acids and other organic compounds in the carbonaceous chondrites. Indeed, these other sources of energy must have played some role, even if small. It is not possible to make a realistic calculation of the relative importance of the various sources of energy, since the conditions on the parent body are not known. These considerations also apply to the recent synthesis of amino acids and other organic compounds from carbon monoxide, hydrogen, and ammonia by a Fischer–Tropsch type of synthesis (15, 16). The yields of amino acids are 10- to 100-times lower than the electric-discharge yields. This synthesis, which is asserted to have been the sole source of organic compounds in the carbonaceous chondrites, presents several problems, such as the source of the CO and the complicated temperature sequence. In any case, the low yields from the Fischer–Tropsch type of synthesis make this hypothesis less attractive from the standpoint of amino-acid production than electric discharges, although the Fischer–Tropsch process might have been the major source of other organic compounds, such as straight-chain hydrocarbons.

We thank Mrs. Rosiland Smith and Mr. Fred Castillo for running the samples on the amino-acid analyzer. This research was supported by National Science Foundation Grant GB 25048. The gas chromatograph–mass spectrometer was obtained, in part, with NSF Grant GP 18245.

1. Kvenvolden, K., Lawless, J., Pering, K., Peterson, E., Flores, J., Ponnamperuma, C., Kaplan, I. R. & Moore, C. (1970) *Nature* **228**, 923–926.
2. Kvenvolden, K. A., Lawless, J. G. & Ponnamperuma, C. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 486–490.
3. Oró, J., Gibert, J., Lichtenstein, H., Wickstrom, S. & Flory, D. A. (1971) *Nature* **230**, 105–106.
4. Cronin, J. R. & Moore, C. B. (1971) *Science* **172**, 1327–1329.
5. Miller, S. L. (1953) *Science* **117**, 528–529.
6. Miller, S. L. (1955) *J. Amer. Chem. Soc.* **77**, 2351–2361.
7. Miller, S. L. (1957) *Biochim. Biophys. Acta* **23**, 480–489.
8. Ring, D., Wolman, Y., Friedmann, N. & Miller, S. L. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 765–768.
9. Coggins, J. R. & Benoiton, N. L. (1970) *J. Chromatogr.* **52**, 251–256.
10. Sanchez, R. A., Ferris, J. P. & Orgel, L. E. (1966) *Science* **154**, 784–785.
11. Ponnamperuma, C. & Woeller, F. (1967) *Curr. Mod. Biol.* **1**, 156–158.
12. Friedman, M. & Wall, J. S. (1964) *J. Amer. Chem. Soc.* **86**, 3735–3741.
13. Urey, H. C. (1952) *Proc. Nat. Acad. Sci. USA* **38**, 351–363.
14. Urey, H. C. (1952) in *The Planets* (Yale Univ. Press, New Haven), pp. 152–153.
15. Yoshino, D., Hayatsu, R. & Anders, E. (1971) *Geochim. Cosmochim. Acta* **35**, 927–938.
16. Hayatsu, R., Studier M. H. & Anders, E. (1971) *Geochim. Cosmochim. Acta* **35**, 939–951.