

Acetonitrile: A Plausible Source of Amino Acids on the Primitive Earth.

¹Kavita Gururani, ²Chandra Kala Pant, ³H .D .Pathak .

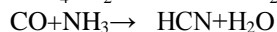
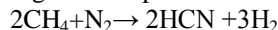
^{1,2,3} Chemical laboratory, Department of Chemistry , D .S B. Campus, Kumaun University, Nainital.

Abstract: Synthesis of amino acids from aqueous solution of acetonitrile has been carried out by the action of heat and revealed the formation of lysine, aspartic acid, serine, glycine, glutamic acid, alanine, valine and leucine. Amino acid synthesis was accelerated in the presence of copper exchanged montmorillonite clay followed by calcium and magnesium exchanged clays. Synthesis of important biomolecules from aqueous solution of acetonitrile may throw some light on the process of chemical evolution.

Keywords: Absorption, Chromatographic, Montmorillonite, Prebiotic, Sensitisers.

I. Introduction

The earth was formed some 4-5 million years ago.¹⁻⁴ Atmospheric components like H₂, NH₃, H₂O and CH₄ are believed to have been formed under the action of UV light, lightning, shock waves, heat and other forms of energy⁵⁻⁸. Among these compounds are the primordial biomolecules like amino acids, purine, pyrimidines, sugars (pentoses and hexoses) etc. These compounds or their precursors have been detected in fossils, meteorites and in the interstellar space. Hydrogen cyanide is considered to be a major precursor of nitrogenous organic molecules leading to the formation of cyanamide, cyanoacetylene and nitriles⁹⁻¹⁰. Abiotic synthesis precursors of specific life forming molecules has been investigated by earlier workers¹¹⁻¹³. Hydrogen cyanide, acetonitrile, cyanoacetylene and amino nitriles have been reported as reactive intermediates during discharge experiments¹⁴⁻¹⁶. Formation of acetonitrile by photolysis of acetylene and ammonia has been reported by Ferris and Ishikawa¹⁷. Most common reaction pathways leading to the abiotic formation of simple organic compounds containing N₂ is hydrogen cyanide, which is readily formed by reactions such as :



These reactions are promoted by heat, UV light or electric discharges. Under simulated primitive earth conditions HCN is in turn a precursor of other highly reactive compounds as cyanoacetylene, cyanamide, and nitriles which in turn are precursors of a variety of amino acids, purines and pyrimidines. UV initiated formation of amino acids has been reported from aqueous solution of hydrogen cyanide, acetonitrile and acrylonitrile by Ferris et al.¹⁸ and Pathak et al.¹⁹. However, heat induced synthesis of amino acids from aqueous solution of acetonitrile in presence and absence of montmorillonite clay with or without divalent cations has not been studied in detail. Therefore attempts have been made to synthesise biologically significant molecules from reaction system of acetonitrile – water vapor under conditions believed to have existed near hydrosphere – lithosphere boundaries of the primitive sea.

II. Experimental Procedure

2.1 PREPARATION OF SOLUTION:

All the investigations were carried out in aqueous medium. Sterilized double distilled water was used as the solvent in every experiment wherever necessary the vapors of the double distilled water was allowed to pass through the reaction vessels. Every care was taken to ensure the purity of the samples employed. Experimental solution (5 ml each) with pH 9.0 ± 0.5 were heated in borosil glass reaction vessels- Kjeldhal flasks (100 ml) fitted with air condensers on hot plates at a temperature of 90 ± 5°C in the presence and absence of montmorillonite clay with or without divalent cations (Mg²⁺, Cu²⁺, Ca²⁺).

Samples of reaction concentrates were analyzed for the possible formation of amino acids using chromatographic techniques on Whatman No- 01 paper both by uni- and two dimensional chromatography using butanol- acetic acid- water (4:1:1 v/v, 4:1:5 v/v upper layer), butanol- acetic acid- pyridine- water (15:3:10:12 v/v) and phenol- water (80:20 v/v). Amino acid spots were visualized with ninhydrin, identified with isatin and also by comparison of their R_f values with authentic amino acids as well as of their DNP derivatives. For the separation of DNP amino acids the solvent system used was n- butanol saturated with water. Colorimetric estimation of amino acids was carried out by comparison of color intensity of the unknown compound with that of a standard solution employing photochemical colorimeter MK

2.2:ULTRAVIOLET SPECTRA:

Ultraviolet absorption spectra of the various reaction mixtures or elutes of some products were determined in aqueous solution using Jasco V- series spectrophotometer.

2.3:INFRA RED SPECTROSCOPY:

IR spectra of reaction concentrates were recorded in Perkin Elmer 881 (4000-6000 cm^{-1}) spectrophotometer.

2.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

The reaction products were further identified by High Performance Liquid Chromatography which were ascertained by SHIMADZU SPD- 10 A UV visible detector with C_{18} column using triple distilled water: methanol (80:20 v/v) and 0.1% H_3PO_4 : acetonitrile (40:60 v/v) as mobile phase, flow rate 1.5 ml/min at pH 7.0, temperature 25°C and UV detector monitored at 197-210 nm for the detection of amino acids. Results were compared with retention times of the standard amino acids run in the same HPLC column under similar conditions.

In every experiment an identical solution heavily wrapped in several folds of black cloth and paper was kept along side of the reaction vessels in small pyrex flasks without heating. Such solutions were tested and analyzed in the same way as that of the experimental solutions.

Portions of heated samples were taken out with the help of sterilized measuring cylinders of different measurements. For the chromatographic analysis, the heated samples were concentrated in vacuum evaporator. The concentrates of experimental samples were analysed by paper chromatography on Whatman No. 1 filter paper chromatographically and also by chemical methods.

SOLVENT SYSTEM :

The solvent systems used for the development of papergram were prepared by employing high grade extra pure chemicals supplied by BDH/ E. Merck, Qualigens, exclusively meant for the chromatographic investigations of biologically significant molecules. The solvent is selected in such a way that the resolution of samples components is satisfactory.

Solvent system used in analyzing amino acids were:

- i. n- butanol-acetic acid-water [4:1:1 v/v]
- ii. n-butanol-acetic acid-water [4:1:5 v/v upper layer]

III. Results :

Formation of amino acids from reaction system of acetonitrile-water vapor have been carried out in presence and absence of Montmorillonite clay with or without divalent cations under the effect thermal energy simulating conditions believed to have existed near hydrosphere-lithosphere boundaries of the primitive sea. The presence of large amounts of solid surfaces such as ,clay minerals, metal ferrocyanides and metal oxides on the prebiotic earth crust have been reported. The possibility that weather these solid surface play a role in the origin and early evolution of the life on earth? This possibility has received considerable attention in the past few decades.

The effect of heat on reaction system comprised of acetonitrile and water vapour in presence and absence of montmorillonite clay with or without cations has been investigated for the possible formation of amino acids in round bottom flasks kept on hot plates at $90\pm 5^\circ\text{C}$ under wetting and drying conditions. The pH was maintained at 8.5 ± 0.5 .

Heating was continued till the last drop of reaction concentrate was left. After completion of each cycle fresh double distilled water was added to the flask for the next cycle to start. Heated concentrate of acetonitrile and water vapour drawn out periodically after 10 hrs, 25 hrs, 50 hrs, and 100 hrs were subsequently analyzed by paper chromatography and HPLC for the formation of amino acids .The resulting products were further characterized by various physico-chemical methods and UV/IR spectral studies.

Paper chromatographic analysis of reaction concentrate heated for short period up to 10 hrs showed no ninhydrin positive spots on the papergram .Prolonging the duration of heating for 25 hrs showed four chromatographically separable ninhydrin positive products glycine(II) and leucine(IX) were formed in appreciable amount. (fig.1a).Heating the reaction system up to 50 hrs enhanced the amount of all these products along with the formation of three new products (fig.1 b). On extending the heating period up to 75 hrs,in all nine products appeared on the papergram. Lysine was formed in moderate amount while other products in good amount(fig 1 c). On further prolonging the duration of heating for 100 hrs almost identical range of products were formed. Glycine(II)was formed in good amount while products lysine, glutamic acid, α -alanine valine and leucine were formed in moderate amount Fig (1d).However, identity of products V, VIand VIII could not be ascertained Fig (1d).

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The quantitative estimation and physico-chemical properties of the resulting thermal products have been recorded in table 01 and shown in figure01.

Thus, time lapse studies have shown that formation of amino acids depends upon the duration of heating.

The UV-absorption spectra of the reaction concentrate heated for 100 hrs. showed a band at 203nm. The band corresponding to 203 nm consist of amino acids because their absorption band lies in this region 193-220nm (Fig 02).

The results were further confirmed by high performance liquid chromatography. 10µl sample of reaction concentrate of acetonitrile and water vapour heated up to 100 hrs was injected in SHIMADZU SPD 10A UV-visible detector with C18 column monitored at 210 nm, mobile phase 1 % H₃PO₄ (pH 2.8), temperature 24⁰C, flow rate 1.0 ml/min showed peaks corresponding to glutamic acid (3.840 min.), glycine (4.023 min), α-alanine (4.421 min.), valine (5.088 min.), leucine (56.965min.) and lysine (10.001min.) matched with standard amino acids run under identical conditions. (Fig 03)

Heating the reaction system of acetonitrile and water vapour with montmorillonite clay with or without divalent cations (Ca²⁺, Cu²⁺, Mg²⁺) for a period of 100 hrs under wetting and drying condition and on subsequent chromatographic analysis showed the formation of eight ninhydrin positive products on the papergram. Out of these products lysine, aspartic acid and leucine were formed in trace amount whereas serine, glycine, α-alanine and valine in moderate amount. (Fig. 4a)

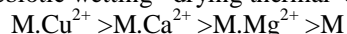
Results are recorded in table 2 and illustrated in Fig04.

In presence of Cu²⁺ exchanged montmorillonite clay serine, glycine, α-alanine and valine were formed in appreciable amount while other products in lesser amount. (Fig. 4b)

The effect of heat on same reaction system in presence of Ca²⁺ clay showed eight products on the papergram. (Fig. 4c)

In presence of Mg²⁺ exchanged clay identical range of amino acids were formed. Serine, glycine and valine were formed in appreciable amount as compared to other products. (Fig. 4d)

On the basis of quantitative analysis of amino acids formed from reaction system of acetylene-ammonia and water vapour under prebiotic wetting-drying thermal conditions was found in the following order –



Where, M=montmorillonite clay.

IV. Conclusion:

From the results discussed above, it may be concluded that acetonitrile formed by photolysis of acetylene and ammonia gases in the atmosphere, might have transported to the earth's surface along with rain water as the primitive earth cooled and would have contributed in the formation of amino acids by the action of heat energy. The clay minerals available near sea-shores might have catalysed the formation of biomonomers by the process of adsorption and desorption and also stabilized them from decomposition via electrostatic forces of attraction.

TABLE- 01

Composition of reaction system	Duration of heating	No. of products formed in (Quantity in mg/lit.)								Fig.Ref.		
		I	II	III	IV	V	VI	VII	VIII		IX	
CH ₃ CN H ₂ O(V)	25 hrs	T	0.65	-	0.39	T	T	-	-	0.52	1a	
	50 hr	T	0.34	T	0.32	-	-	-	-	0.36	1b	
	75 hrs.	0.22	0.40	0.19	0.41	-	-	-	0.44	-	0.40	1c
	100 hrs.	0.30	0.51	0.26	0.45	-	-	-	0.51	-	0.49	1d
2. R _f in:		12	17	25	28	36	40	43	50			
B:A:W(4:1:1) V/V	52	V	V	V	V	V	Y	V	V			
3. Colour with i. Ninhydrin ii. Isatin	V	PBr	dB	P	BP	BV	B	BP	RV			
4. Solubility in	BP	Ins	ins	ins	ins	ins	ins	ins	ins			
	ins											

a. Ether	S	S	S	S	S	S	S	S	S
b. Water	S								
5. UV-Fluorescence	WB	WB	WB	WB	WB	WB	WB	WB	WB
6. R _f of standard amino acids in B:A:W(4:1:1) V/V	52.2	12.1	17	24.9	28	36	40	43	50
7. Amino acids overlapped in co-Chromatography	leu	Lys	gly	glu	α-alanine	-	-	val	-
8. Amino acids identified	leu	Lys	gly	glu	α-alanine	-	-	val	-

Heat induced (90 ±5⁰C) synthesis of amino acids from reaction system of acetonitrile -water vapor under primitive earth conditions.

n-BAW, n-butanol: acetic acid: water 4:1:1 V/V; V- violet; RV-Reddish Violet; Violet; PBr-Pink brown; dB-Dull blue; P-Pink; BV-Blue violet; B-Blue; BP-Blue pink; RV-Red violet; ins- insoluble; s- soluble; lys- lysine; gly-glycine; α-ala- α-alanine; R val- valine; leu- leucine. R_f values were calculated at 25 0 C.



Fig.1: Chromatogram of acetonitrile-water vapour showing formation of amino acids heated up to 100 hrs. under pre biotic wetting drying conditions.

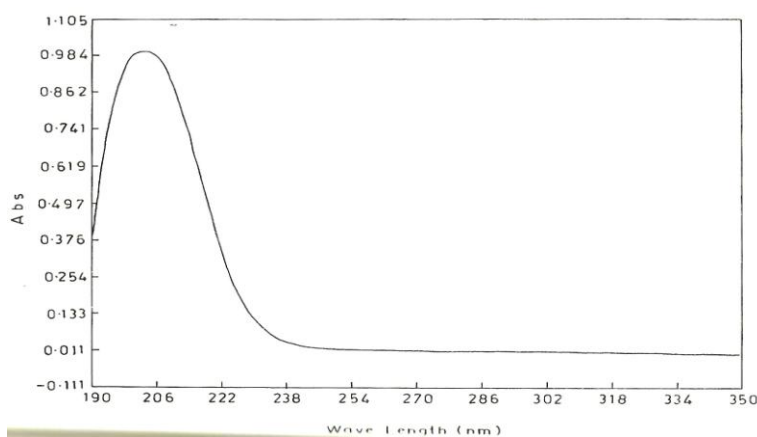


Fig. 2:UV absorption spectra of reaction concentrate of acetonitrile- water vapour heated upto 100 hrs.

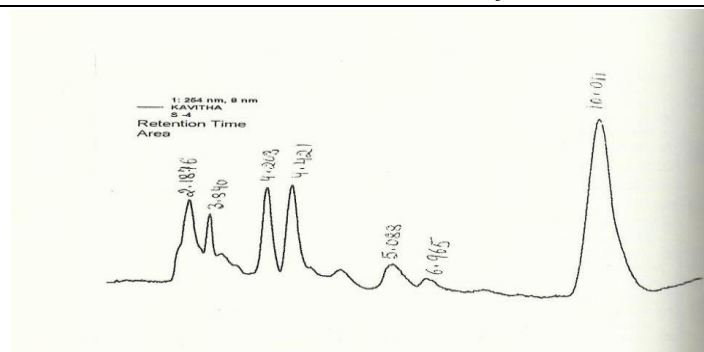


Fig. 3:HPLC of reaction concentrate of acetonitrile- water vapour heated upto 100 hrs

Table 02.

Quantity (mg/lit) and physico-chemical characteristics of products formed from reaction system of acetonitrile -water vapour heated up to 100 hrs with montmorillonite clay with or without divalent cations under wetting-drying conditions.

Composition of Reaction system	No. of products formed								Fig ref.
	I	II	III	IV	V	VI	VII	VIII	
CH3CN-H ₂ O(V) -M	T	T	0.2	0.3	T	0.6	23.	T	02
CH3CN -H ₂ O(V) - M.Cu ²⁺	T	T	0.7	0.5	0.46	T	0.7	T	02
CH3CN -H ₂ O(V) - M.Ca ²⁺	T	T	0.4	0.3	0.41	T	0.6	T	02
CH3CN -H ₂ O(V)- M.Mg ²⁺	T	T	0.3	0.2	0.35	T	0.5	T	02
1 R _f (%) using B:A:W 4:1:1 V/V	25	31	35	39	46	54	58	62	
2 Colour with a. Ninhydrin	V	BV	V	RV	BV	V	V	V	
b. Isatin	PBr	Db	BP	P	BP	BP	P	BP	
3 Solubility a. Ether	ins	ins	ins	ins	ins	ins	Ins	ins	
b. Water	S	S	S	S	S	S	S	S	
4 UV Fluorescence	WB	WB	WB	WB	WB	WB	WB	WB	
5 Amino Acid overlapped in co-chromatography	lys	Asp	Ser	Gly	α-ala	-	Val	leu	
6 Amino acid identified	lys	Asp	Ser	Gly	α-ala	-	Val	leu	

BAW 4:1:1 V/V n-butanol: acetic acid: water 4:1:1V/V; -,not detected; T, trace ; V, violet; B, blue; P, pink; W, white; R, red; ins, insoluble; s, soluble; lys , lysine; asp, aspartic acid; gly, glycine; α-ala, α-alanine; val , valine ; leu, leucine.

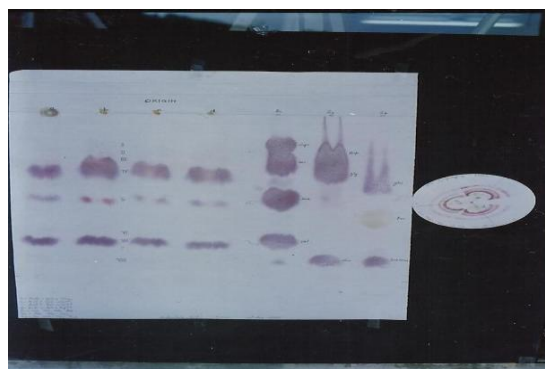


Fig. 4: Chromatogram showing the formation of amino acids heated upto 100 hrs. with montmorillonite clay with or without divalent cations.

References:

- [1] Trimble, V.: (1997) *Origins Life Evol. Biosphere* **27**, 3-21
- [2] Lemmon, R.M.: (1970) *Chemical evolution, Chem.Rev.* **70**, 95-109
- [3] Urey, H.C.: (1952) *The Planets: The Origin and Development* Yale Univ. Press, New Haven.
- [4] Kuhn, W. : (1956) *Chem. Ber.* **89**, 303.
- [5] Sanchez,R.A.,Ferris,J.P.,Orgel,L.E : (1967) *J.Mol.Evol.* **30**,223-253
- [6] Bar-Nun,A.,Tauber,M.E.: (1972) *Space Life Sciences* **3**, 254-259 .
- [7] Hubbard,G.E.,Hobby,G.L.,Ferris,J.P Williams,E.A.Nicoderm,D.E : (1975) *J.Mol.Evol.* **5** 223-241 J.S.,Voecks,
- [8] Ferris,J.P.,Chen,C.T. (1975) *Nature*,**258**,587-588.
- [9] Oro,J.,Kimball,A.P. (1962).*Arch.Biochem.Biophys.* **96**,293-313.
- [10] Oro,J.,Kimball,A.P. (1962).*Arch.Biochem.Biophys.* **94**,217-226.
- [11] Miller,S.L. : (1955) *J.Am.Chem.Soc.*, **77**:2351-61.
- [12] Oro,J. (1963) *Nature* ,**197**,862-67 .
- [13] Perti,O.N.and Pathak. H.D., (1966) *Poc.Natl.Acad.Sci.(India)*,Section-A,Vol.XXXVI ,p.495.V V
- [14] Sidle,A.S.: (1967) *Nature*,**216**:408 .
- [15] Ponnampuruma, C. : (1972) *Exobiology (North –Holland,Amsterdam and London)*
- [16] Oro,J. : (1965) in the *Origin of Prebiological systems and of their molecular Materials* (ed by S.W.Fox) Academic Press, N.Y.,p, 137-71.s
- [17] Ferris,J.P.;Ishikawa,Y. : (1988) *J.Amer.Chem.Soc.* **110**,784-785
- [18] . Sanchez, R. A., Ferris, J.P. and : (1967) *J. Molec. Biol.* **30**, 223-253. Orgel, L.E. et al (1967)
- [19] . Pathak, C.K. Pant, J.N. and Pathak, H.D. : (1978) *J. British interplanetary Soc. London*, **33**, 103-106.

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