

Prebiotic Microreactors: A Synthesis of Purines and Dihydroxy Compounds in Aqueous Aerosol

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Received: 12 June 2006 / Accepted: 10 August 2006 /
Published online: 29 November 2006
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Abstract We report the synthesis of purine bases and other heterocycles and the formation of amino acids, hydroxy acids and dihydroxy compounds by the spark activation of an atmosphere of methane, nitrogen and hydrogen, in the presence of an aqueous aerosol. With the aid of the interface air–water, the organic material obtained shows greater amounts and diversity of molecules with biological interest than the products obtained in the absence of an aerosol. Our results support the suggestion that aerosols may have played a significant role in the prebiotic origin of molecular diversity and evolution.

Keywords prebiotic chemistry · prebiotic environments · aerosol chemistry ·
purine bases · amino acids

Introduction

According to modern ideas on the origin of life, organic compounds derived from chemical reactions in primitive Earth-like environments. These reactions took place in the transition period from abiotic, inorganic, or simple organic compounds to autonomous self-replicating molecules capable of evolving by natural selection, generally called the ‘prebiotic epoch’ (Bada 2004). Our vision of the prebiotic epoch is underpinned in Miller’s classic experiment (Miller 1953). There has been considerable discussion concerning the nature of the primitive atmosphere since Miller’s original publication. While arguments have been advanced that the primitive atmosphere would not have been as reducing as some have supposed (Delano 2001) there are no data available concerning the geochemistry of the period in question and the possibility of a methane-containing atmosphere has been left open. Consequently, syntheses have been modelled in a variety of atmospheres ranging

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from strongly reducing (Ring et al. 1972) through weakly reducing (Schlesinger and Miller 1983) to redox neutral (Civis et al. 2004).

In this work, we examine the possible effect of a greatly increased gas–water interface on the synthesis of organic compounds in a reducing atmosphere, by using aerosols as prebiotic microreactors.

The possible importance of aerosols in the origin of life on Archean Earth has recently been emphasized (Donaldson et al. 2004; Tuck 2002; Dobson et al. 2000; Lerman 1994). The first proposal of a possible role of aerosols as important reactors in prebiotic chemistry was made by Shah (1970). This suggestion is supported by physical–chemical observations which demonstrate that the formation of an aerosol depends only on a liquid water–air interface and a physical mechanism that ejects bubbles into the atmosphere, such as wind, sea waves or shock waves (Lerman 1996; Ellison et al. 1999).

On modern Earth, aerosols are ubiquitous in the troposphere and play an important role in climate and atmospheric chemistry (Ellison et al. 1999). Recent laboratory work (Donaldson and Anderson 1999) has shown that small organic molecules tend to concentrate at the water–air interface. Similarly, recent observations of the composition of natural aerosol particles have shown that at least 10% of the aerosol is organic matter, with more than 10,000 peaks in gas chromatography (Murphy et al. 1998; Murphy 2005) and organic coating of surfactants in marine aerosol particles (Tervahattu et al. 2002). Due to these facts, we could assume that the bubble–aerosol–droplet cycle (the bursting of bubbles that injects into the atmosphere the aerosol particles and subsequent condensation in droplets), was active in the Archean epoch. Therefore, it could be functionally relevant to chemical evolution on the early Earth or elsewhere. It is important to note that aerosol particles are different from cloud droplets and raindrops. The latter are larger hydrometeors with droplet radii greater by one to four orders of magnitude than aerosol droplets. These have a fractional organic content minuscule compared with aerosol particles (Tuck 2002).

The bubble–aerosol–droplet or ‘bubblesol’ cycle offers the possibility to overcome a number of problems with regard to prebiotically synthesized organic matter (Dobson et al. 2000; Lerman 1996). The goal of our work was to experimentally generate the bubble–aerosol–droplet cycle in putative prebiotic conditions and test the above hypothesis. Although the mechanism would presumably be effective in atmospheric models of a large range of compositions, we chose a mixture of CH₄, N₂, and H₂, since this composition is known to yield amino acids in sufficient amounts for comparative quantitative analysis (Schlesinger and Miller 1983).

Materials and Methods

Simulation of the prebiotic conditions

A 500 ml glass reactor is filled with 5 ml of ultrapure water (Millipore Milli-Q Q.Gard®). Additionally the ultrapure water was bubbled with dried N₂ for 1 h in order to remove the residual traces of oxygen) and a gas mixture containing CH₄:N₂:H₂ (40:30:30), purchased from Praxair SA. Prior to use, all glassware and the electrodes was heated in a high-temperature oven (Nabertherm L5) at 400°C for 2 h in order to eliminate any possible contaminants. Before the experiment the system is successively evacuated with a pump and purged with the reaction gas mixture for four times. The system working pressure is

1,200 mbar at 38°C. An ultrasonic aerosol generator (BONECO model 7035) was used for the generation of the bubble–aerosol–droplet cycle with the liquid water in reactor. Two tungsten electrodes attached to the reactor were used with a high voltage generator (Model BD-50E, Electrotechnic Products Inc. Illinois, USA) to produce the spark discharges (50 KV) in the aerosol. The system is maintained at constant temperature with active aerosol and electric discharge during 72 h. After this period, the yellow-brown liquid solution and the solid material were recovered for analytical study.

Another experiment was carried out as experimental control in the same conditions of pressure and temperature but without the aerosol.

Instrumental analysis

Elemental analysis. The elemental analysis of each fraction was made in Unidad de Instrumentación Científica (Universidad de Alcalá de Henares. 28801 Alcalá de Henares. Spain) using a ‘CHN-O-rapid’ (Heraeus) elemental analyzer.

Infrared spectroscopy. IR spectra were obtained using a Nexus Nicolet FTIR spectrometer. The spectra of water-soluble and water-insoluble fractions were obtained in CsI pellets. The assignment of adsorption bands was made using previous data reported in the literature, (McDonald et al. 1991, 1994; Nascimento et al. 1998; Khare et al. 2002; Imanaka et al. 2004).

Nuclear Magnetic Resonance (NMR). ^{13}C CP MAS-NMR spectra were obtained in a Bruker Advance 400 spectrometer, using a standard cross-polarization pulse sequence. Samples were spun at 10 kHz. Spectrometer frequencies were set to 100.62 and 400.13 MHz for ^{13}C and ^1H , respectively. A contact time of 1 ms and a period between successive accumulations of 5 s were used. The number of scans was 1,600. Chemical shift values were referenced to TMS. ^{13}C NMR spectra of water soluble fractions were obtained using a Mercury 400 Varian NMR spectrometer. The spectra were recorded in D_2O . The number of scans was 50,000. Chemical shift values were referenced to TMS.

Gas Chromatography–Mass Spectrometry (GC–MS). GC–MS analyses in the full-scan mode were carried out on an Autosystem XL-Turbo Mass Gold (Perkin Elmer) with an Elite-5 column (crossbond 5% diphenyl-95% dimethyl polisiloxane, 30 m×0.25 mm i.d.×0.25 μm film thickness) and using He as carrier gas.

High-Performance Liquid Chromatography (HPLC). HPLC analyses were carried out on a Surveyor (ThermoFinnigan) with a PDA detector using a Kromasil 100 C_{18} 5 μm 25×0.46 column.

Analytical procedure

In the two types of experiments (aqueous aerosol and control experiment without aerosol), we obtained one water soluble fraction and one water insoluble fraction. These fractions were separated by centrifugation and independently analyzed. They were freeze dried using a lyophilizer Cryodos 80 (Telstar). Each fraction was hydrolyzed with 6 M HCl at 110°C for 24 h, freeze dried again to remove water, HCl and any volatile

organics, and then analyzed as follows. The identification of the peaks of organic compounds were verified by comparing the retention times, mass spectra and UV absorbance spectrum with external standards, purchased from Sigma-Aldrich and Fluka.

Amino acid analysis. The amino acids were characterized by GC-MS after esterification with 2-propanol and acylation with trifluoroacetic anhydride. The GC oven was programmed as follows: 60°C (initial temperature), heated to 130°C at 5°C/min with a hold time of 11 min and heated at 180°C at 10°C/min with a final hold time of 20 min. The temperature of injector was 220°C and the flow rate was 10 ml/min. Quantitative analysis was done by HPLC after derivatization with phenylisothiocyanate (PITC) and using the following conditions: Solvent A: 50 mM NH₄OAc buffer, pH 6.5. Solvent B: 100 mM NH₄OAc-CH₃CN (50:50), pH 6.5. 0 min 0% B (100% A), 45 min 70% B (30% A), 46 min 70% B (30% A), 48 min 100% B (0% A). The flow was 2 ml/min, the column was thermostated at 52°C and the chromatogram registered at 254 nm.

Purine base analysis: the purine bases (adenine and 2,6-diaminopurine) were characterized by GC-MS after treatment with N-methyl-N-trimethylsilyl-trifluoroacetamide containing 1% trimethylchlorosilane and anhydrous pyridine in a ratio 1:3 at 150°C for 30 min. The GC oven was programmed as follows: 60°C (initial temperature), heated to 130°C at 5°C/min with a hold time of 11 min and heated at 180 °C at 10 °C/min with a final time hold 80 min. The temperature of injector was 220°C and the flow rate of 2.5 ml/min. Quantitative analysis was done by HPLC using the following conditions: Solvent A: 50 mM KH₂PO₄ buffer, pH 3. Solvent B: MeOH. 0 min 1% B (99% A), 20 min 95% B (5% A), 40 min 30% B (70% A) at a flow of 1 ml/min. The column was thermostated at 30°C and the chromatogram was recorded at 260 nm.

Hydroxy- and carboxylic acids analysis: the hydroxy acids and carboxylic acids were identified by GC-MS by the same method using for the detection of purine bases. Quantitative analysis was done by GC-MS.

Other heterocycles analysis: parabanic acid and hydantoin were analyzed before acid hydrolysis. One portion of the water soluble fraction obtained in the aerosol experiment was extracted with chloroform and centrifuged. The pellet is a dark solid that was analyzed by GC-MS using the purine bases analysis protocol explained above.

Results and Discussion

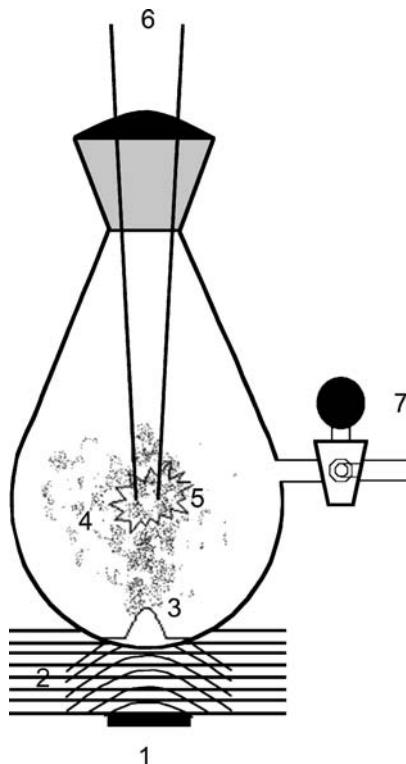
Our experimental set-up consists of a glass reactor filled with a gas mixture containing CH₄:N₂:H₂ 40:30:30 over a liquid water pool. The reactor is set up in an ultrasonic device that generates an aerosol, by focusing the ultrasound wave (1.8 MHz, 30 W) onto the liquid surface (Figure 1). The estimated aerosol droplet size of about 3 μm, was calculated accordingly with the equation deduced by Lang (1962):

$$d = \left(\frac{\pi \cdot \sigma}{4\rho \cdot f^2} \right)^{1/3}$$

Where σ is water surface tension at 20°C (72.75 erg/cm²): ρ is water density at 20°C (0.99821 g/cm³) and f is the frequency of the ultrasonic ceramic transducer (1.768 MHz).

Two tungsten electrodes attached to a high-voltage generator produce the spark discharge in the aerosol. The generation of aerosol by low power ultrasonic transducers

Figure 1 Experimental setting for bubble sol simulation: (1) piezoelectric transducer, (2) ultrasonic waves generated in the water cuvette, (3) aerosol fountain induced in the water surface inside the reaction recipient, (4) aerosol cloud, (5) spark discharge, (6) tungsten electrodes, (7) triple way valve.



is extensively used in simulations of aqueous aerosols (King et al. 2004) and in pharmaceutical field (Steckel and Eskandar 2003). This kind of ultrasonic device generates a continuous and stable bubble–aerosol–droplet cycle by capillary wave formation. The nebulizer does not generate cavitation bubbles in the bulk, the main mechanism for the sonochemical processes (Dalby et al. 1997; Singh et al. 1998).

With these conditions for the simulation, the spark discharge leads to the formation of organic material in different non volatile phases: a soluble fraction that remains in the liquid water pool as a yellow-brown solution of pH 8.6 (S fraction) and an insoluble fraction (I fraction) isolated by centrifugation. Both phases were analyzed separately.

Elemental analysis. Amount of C and N fixed in the aerosol experiment

The S fraction was freeze dried, giving a non-volatile water soluble residue with empirical formula $C_9H_{18}N_5O_5$ (Table I). This fraction represents the 16% of the total carbon introduced in the experiment (Table I). The I fraction, with an empirical formula $C_{30}H_{45}NO_3$, represents 10.48% of the total carbon. In the aerosol experiment the non volatile organic matter fixed was the 27.46% of C and 6.19% of N of the total C and N input in the system.

TABLE I C and N fixed and other data of the organic material obtained in aqueous aerosol experiment and control experiment

	S fraction (aerosol)	I fraction (aerosol)	S fraction (control)	I fraction (control)
pH (solution)	8.65	Insoluble	8.73	Insoluble
Amount obtained (mg)	60.7±4.4	17.8±1.9	43.3±3.1	25.8±2.8
C fixed (% control)	16.34±1.35	10.48±0.34	16.94±1.21	18.46±2.07
N fixed (% control)	5.98±0.52	0.25±0.03	10.93±0.78	0.99±0.11
C/N	1.8	43	1.8	21.2
C/O	1.8	5.4	1.8	7.6
Empirical formula	C ₉ H ₁₈ N ₅ O ₅	C ₃₀ H ₄₅ NO ₃	C ₉ H ₁₆ N ₅ O ₅	C ₁₀₆ H ₁₅₇ N ₅ O ₁₄

Values are expressed as mean±SEM of ten experiments performed in aerosol and control conditions.

The ratios C/N, C/O and C/H are lower in the S fraction than in I fraction. Moreover, the empirical formulas of these fractions suggest important structural differences. These differences are related to the presence of a higher number of functional groups in the S fraction than in the I fraction. In order to demonstrate this, the FTIR and ¹³C NMR spectra of each fraction were recorded.

Comparison among the organic materials obtained in aqueous aerosol experiment and control experiment

The control experiment carried out without aerosol yields a soluble fraction, (pH=8.7), and one insoluble fraction that is collected from the surface of liquid and from the wall of reaction vessel as a thin film. The two phases were analyzed separately in the same way indicated for the experiment with aerosol.

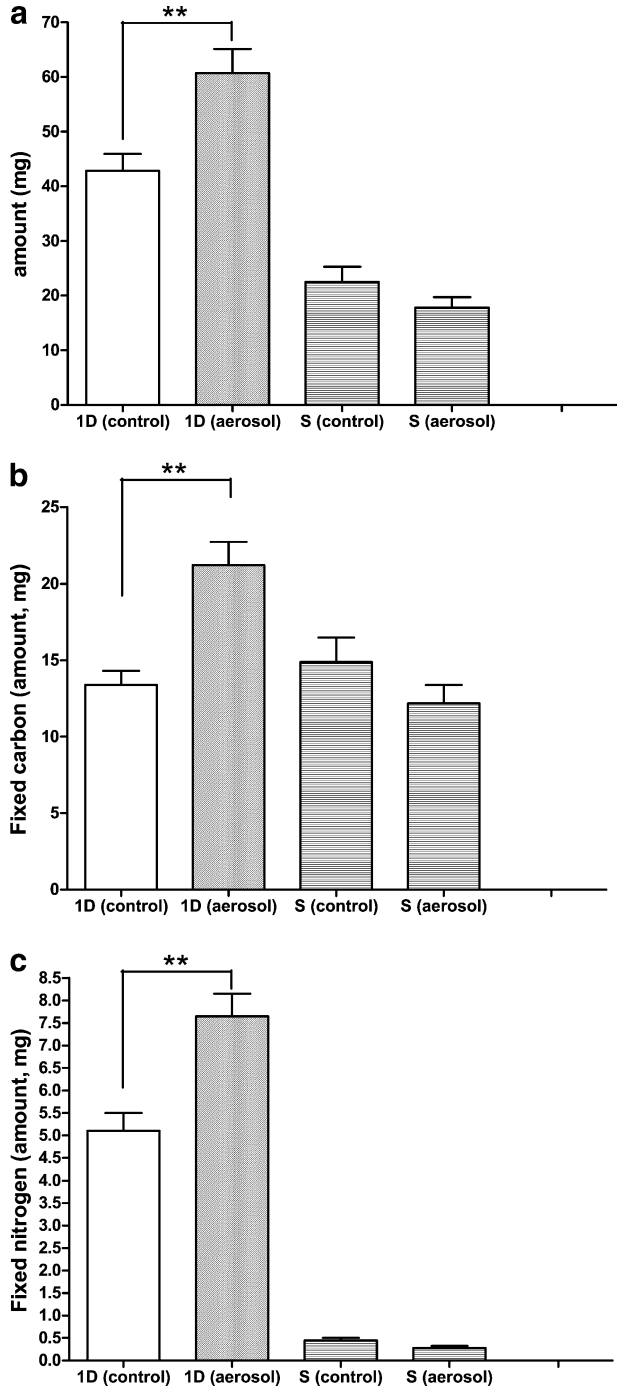
The results of the elemental analysis and the empirical formula for the fractions of control experiment are showed in Table I. The amount of S fraction obtained in the aqueous aerosol experiment is significantly higher than the S fraction in the control experiment (Figure 2). Analogous results were obtained if the amount is expressed as percentage of input C and N fixed in S fraction. This result suggests that the presence of aqueous aerosol increases significantly the amount of soluble organic material synthesized abiotically and the fixation of nitrogen and carbon from atmosphere in form of organic compounds.

On the other hand, the empirical formulas of both S fractions are very similar but it is possible to see clear differences amount their respective FTIR spectra and ¹³C NMR spectra (see Appendix). In both bulk water soluble fractions are observed the same functional groups but the ratio each other is different. The ¹³C chemical shift indicates different species with the same kind of functional groups. In particular the presence of aqueous aerosol seems to improve the formation of carboxylic acids.

Nevertheless, the differences among the I fraction in both experiments are not obvious. The aerosol does not seem to influence in the formation of insoluble solid under the conditions above explained. This could be related with the lack of recirculation of the insoluble solid fraction in the bubblesol cycle.

Significant differences were found in amount of atmospheric elements fixed and spectra among control and aerosol experiment. Taking into account these differences, we performed

Figure 2 Effect of aqueous aerosol in the production of solid organic material by spark discharge activation of a N_2 - CH_4 - H_2 atmosphere. (a) Amount obtained of each fraction. (b) Carbon fixed in each fraction (% total input carbon in experiment). (c) Nitrogen fixed in each fraction (% total input nitrogen). Values are mean \pm SEM from ten experiments performed in duplicate (aerosol vs. control without aerosol). Significant differences from control were statistically analyzed by *t*-Student method: $**P < 0.01$.



the chemical analysis of these fractions, to check the effect of aerosol in the synthesis of molecules of biological interest.

Analysis of molecules with biological interest

The acid hydrolysate of the non-volatile S fraction generates a complex mixture of compounds, essentially amino acids (7.5% respect to the total of non volatile organic material obtained), but also carboxylic acids and heterocyclic compounds (Table II, Figures 3 and 4). A significant number of the molecules obtained contain chiral centres, but in our synthesis we do not observe enantiomeric excess and we report the racemic mixture of asymmetric molecules.

Adenine is the most abundant heterocyclic compound with biological interest identified in our aerosol experiment. Adenine has been synthesized using spark discharges in CH_4 : C_2H_6 : NH_3 atmospheres (Yuasa et al. 1984), and with lower yield using proton irradiation of $\text{CO}:\text{N}_2:\text{H}_2\text{O}$ mixtures (Miyakawa et al. 2002a, b). We report a yield of 0.0521% in adenine, in respect to the total non volatile organic matter in a single 72 h spark discharge experiment (Figure 3). This corresponds to 0.015% yield in adenine respect to the total carbon introduced in the system. We show that presence of an aerosol increases the yield of adenine by two orders of magnitude, compared to previous works (Yuasa et al. 1984). Our yield is comparable to the yield obtained in NH_4CN solutions (Miyakawa et al. 2002a, b; Levy et al. 2000).

An important remark is that free adenine is present in the soluble fraction, not in the form of an unknown precursor, as stated in previous works. In such experiments adenine was only generated after acid hydrolysis. With respect to the mechanism of adenine formation, it was demonstrated that the key step to the adenine synthesis is the formation of HCN and the tetramer diaminomaleodinitrile, which isomerises when irradiated with UV light, to 4-amino-5-cyanoimidazole (Orgel 2004). Further addition of HCN to this compound yields adenine directly (Sanchez et al. 1968). The synthesis of the HCN tetramer and the formation of adenine compete with the reaction between HCN and formaldehyde, which would lead to the formation of glycolic acid or glycine, and possibly other reactions that imply HCN (Orgel 2004). The detection of glycolic acid and ammonium formate in our experiment supports the stated mechanism. Moreover, we detect 2,6-diaminopurine (Figure 5), consistent with the synthesis of both purine derivatives by polymerization of ammonium cyanide solutions (Borquez et al. 2005).

We interpret that the increased yield of adenine obtained in our experiments is due to the local enrichment of this compound or its immediate precursors in the liquid–gas interface (Donaldson et al. 2004; Dobson et al. 2000). This represents a reduction of importance of bulk-solution interfering reactions. A parallel argument was employed in the effect of montmorillonite on the increase purine yield through its selective adsorption on the clay surface (Cohn et al. 2001). Aerosols have the advantage of the ubiquitous presence in planetary environments with surface liquid pools (Lerman 1992).

We identified other heterocyclic compounds in the sample (Figure 3b). One of the most interesting, from a biological point of view, is the imidazoline-2,4-dione or hydantoin. Recently, hydantoin has been suggested as a precursor for the emergence of prebiotic peptides and amino acids, under similar conditions of pH and temperature to those of our experiment (Taillades et al. 1998). Moreover, it was hypothesised that primitive microorganism on Earth may be able to use hydantoins as C or N sources. This idea is

TABLE II Identification and quantification of molecules of biochemical interest obtained in spark discharge activation of CH₄-N₂-H₂ atmosphere with or without aqueous aerosol

		Aqueous aerosol experiment Amount (μmol)	Control experiment Amount (μmol)
AMINO ACIDS			
Glycine	C ₂ H ₅ NO ₂	3.93	2.89
Aminomalonic acid	C ₃ H ₅ NO ₄	1.06	0.001
Alanine	C ₃ H ₇ NO ₂	12.98	8.68
β-Alanine	C ₃ H ₇ NO ₂	1.16	0.21
Sarcosine	C ₃ H ₇ NO ₂	0.45	1.01
Isoserine	C ₃ H ₇ NO ₃	0.34	0.12
Serine	C ₃ H ₇ NO ₃	0.14	0.07
Aspartic acid	C ₄ H ₇ NO ₄	0.34	0.10
Iminodiacetic acid	C ₄ H ₇ NO ₄	0.03	d
2-Aminobutyric acid	C ₄ H ₉ NO ₂	0.18	0.40
3-Aminobutyric acid	C ₄ H ₉ NO ₂	0.68	1.02
3-Aminoisobutyric acid	C ₄ H ₉ NO ₂	1.65	1.34
N-Methylalanine	C ₄ H ₉ NO ₂	d	0.09
Glutamic acid	C ₅ H ₉ NO ₄	0.08	0.16
Ornithine	C ₅ H ₁₂ N ₂ O ₂	0.43	0.24
Histidine	C ₆ H ₉ N ₃ O ₂	d	–
t-Leucine	C ₆ H ₁₃ NO ₂	0.19	–
TOTAL ^a		1.63 ^a	1.19 ^a
HYDROXY ACIDS			
Glycolic acid	C ₂ H ₄ O ₃	0.17	–
2,3-Hydroxypropanoic acid	C ₃ H ₆ O ₄	0.02	d
Malic acid	C ₄ H ₆ O ₅	0.03	d
Tartaric acid	C ₄ H ₆ O ₆	0.01	–
2-Hydroxypentanodioic acid	C ₅ H ₈ O ₅	0.01	–
6-Hydroxycaproic acid	C ₆ H ₁₂ O ₃	0.02	–
TOTAL ^a		0.018 ^a	d
CARBOXYLIC ACIDS			
2-Methylbutanodioic acid	C ₅ H ₈ O ₄	0.02	d
Succinic acid	C ₄ H ₆ O ₄	0.05	0.01
Glutaric acid	C ₅ H ₈ O ₄	d	–
Tricarballic acid	C ₆ H ₈ O ₆	0.01	–
TOTAL ^a		0.007 ^a	8 × 10 ⁻⁵
HETEROCYCLES			
Parabanic acid	C ₃ H ₂ N ₂ O ₃	0.02	–
Hydantoin	C ₃ H ₄ N ₂ O ₂	0.06	–
Adenine	C ₅ H ₅ N ₅	0.11	d
2,6-diaminopurine	C ₅ H ₆ N ₆	0.02	–
TOTAL ^a		0.022 ^a	d

Values are expressed as mean of μmol obtained in one experiment (ten experiments performed). Amino acids were identified and confirmed using authentic standards and two techniques (HPLC and GC-MS). Ornithine and histidine are tentatively assigned by HPLC only, using authentic standards.

d detected (Noise/Signal>0.1).

^ayield (%) respect to total carbon in the system.

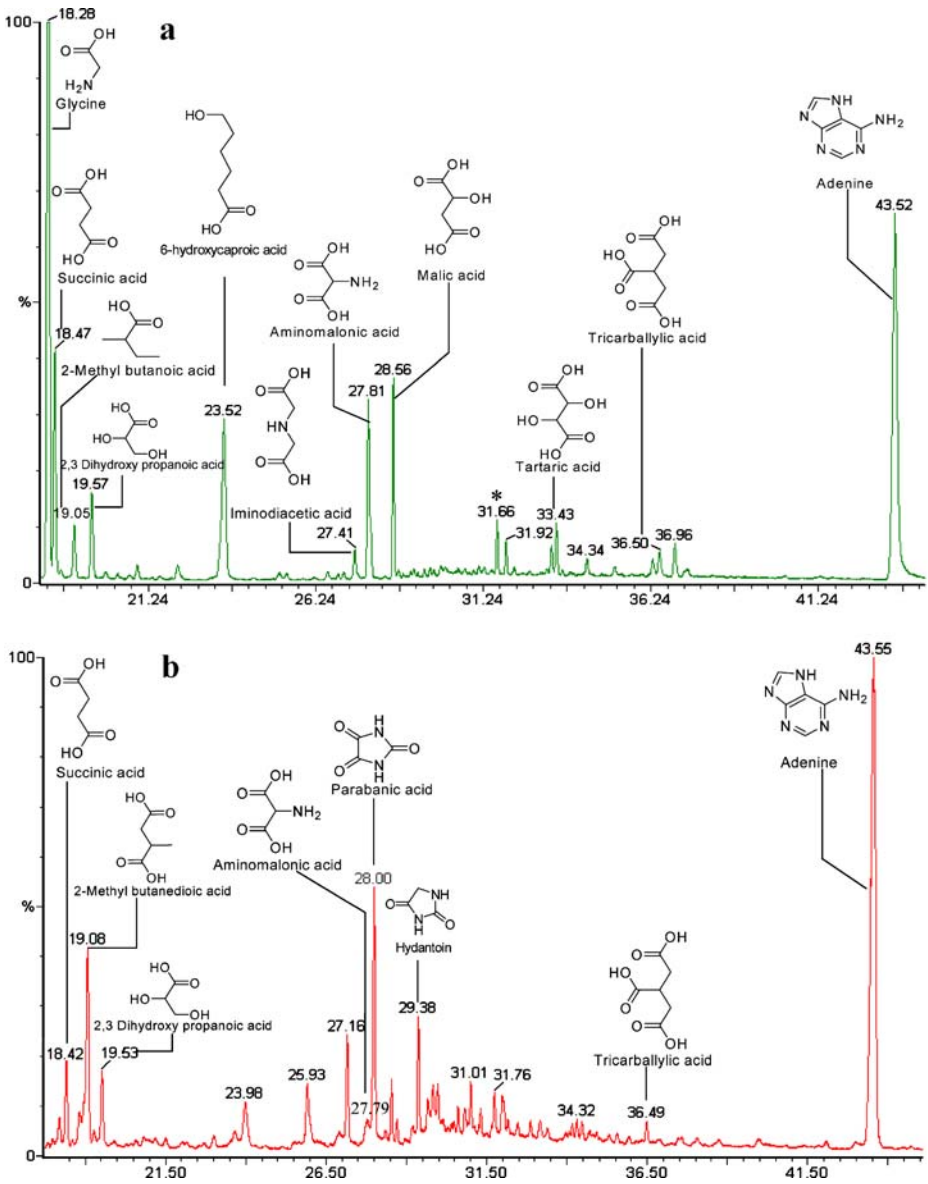


Figure 3 Gas chromatograms showing the carboxylic acids and heterocyclic compounds obtained in a CH_4 : N_2 : H_2 atmosphere with water aerosol. (a) Analysis of the residue of the water soluble organic fraction after hydrolysis with HCl 6 M and derivatization as stated in the methods section. In min 83 appears the 2,4-diaminopurine peak, not shown in the figure. *: this peak corresponds to a possible isomer of tartaric acid. Both molecules have the same mass spectra. (b) Analysis of the water soluble organic fraction after CHCl_3 extraction and before acid hydrolysis.

based on studies on the hydantoinases group, enzymes that catalysed the hydrolysis of hydantoins, (Syldatk et al. 1999). We demonstrate that the availability of hydantoins is a possibility that could occur on the early stages of the biochemical or biological evolution.

In addition, we have found dihydroxy acids in the aerosol experiments (absent in the control experiments without aerosols). We report the prebiotic synthesis of 2,3-dihydroxybutanedioic acid (tartaric acid) and 2,3-dihydroxypropanoic acid (glyceric acid). These compounds have not yet been identified in experiments in prebiotic synthesis.

Other biologically relevant molecules identified in the products of spark discharge in the aerosol environment are the propane-1,2,3-tricarboxylic acid (tricarballic acid, Figure 3a) and succinic and malic acids. The succinic and malic acids are members of the Krebs cycle and the tricarballic acid is structurally related to citric and isocitric acid that are also members of the Krebs cycle, as well. It has been suggested the possibility that these carboxylic acids, including tricarballic acid, could take part in a primordial variant of the Krebs cycle (Meléndez-Hevia et al. 1996). However, the hypothesis on self-organizing protometabolic cycles is a controversial issue (Orgel 2000). Anyway, our experiment demonstrates that aerosol chemistry could have contributed to the production of the raw materials for this suggested primordial metabolism, in addition to previous work in synthesis of dicarboxylic acids by irradiation of acetic acid solutions (Negron-Mendoza and Ponnamperna 1976). Further work must be performed to explore and optimise the potential of the aerosol as a prebiotic reactor. In fact, our future prospects include testing the

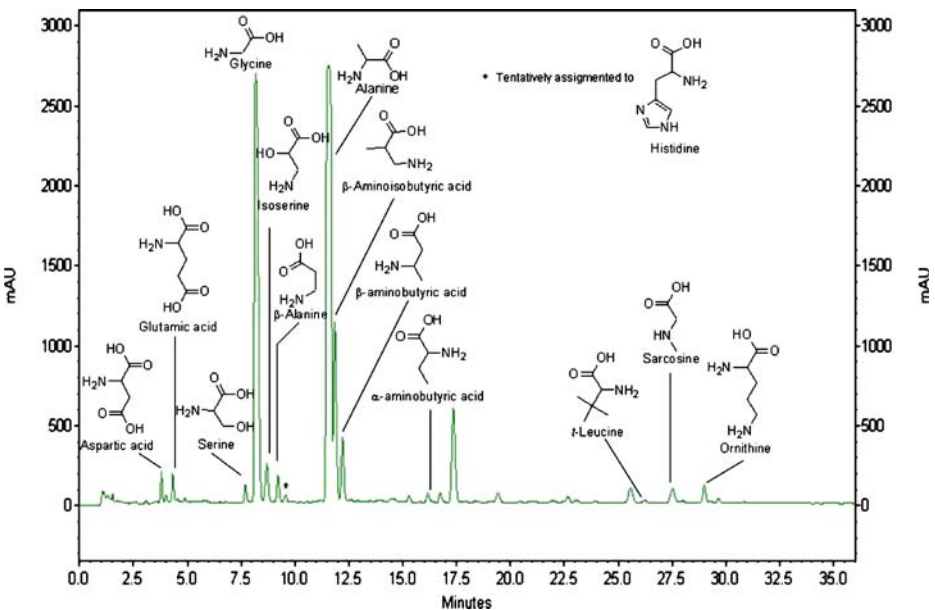


Figure 4 HPLC chromatogram showing the amino acid profile obtained in a $\text{CH}_4:\text{N}_2:\text{H}_2$ atmosphere with water aerosol. The analysis of the residue of the water soluble organic fraction after hydrolysis with HCl 6 M and derivatization with PITC as stated in the methods section. All amino acid were identified by two different methods (HPLC and GC–MS) against authentic standards, with the exception of histidine and ornithine, identified tentatively. We confirm the synthesis of t-leucine, but we can not assign any peak to Leu, Ile or Val.

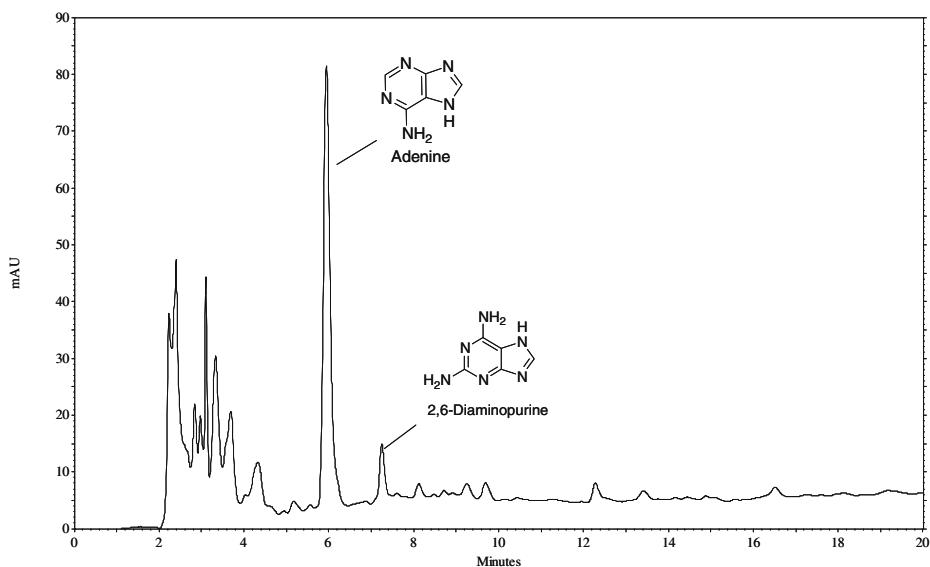


Figure 5 HPLC chromatogram showing the purine bases profile obtained in a $\text{CH}_4:\text{N}_2:\text{H}_2$ atmosphere with water aerosol. The analysis of the residue of the water soluble organic fraction after hydrolysis with HCl 6 M was performed in C18 RP column after previous separation of heterocyclic fraction by 2D-TLC.

effects of a solid, organic or inorganic, core in the aerosol (formed by partial evaporation of aerosol solution droplets), because the core could improve the catalytic properties of the droplet (Lerman and Teng 2004).

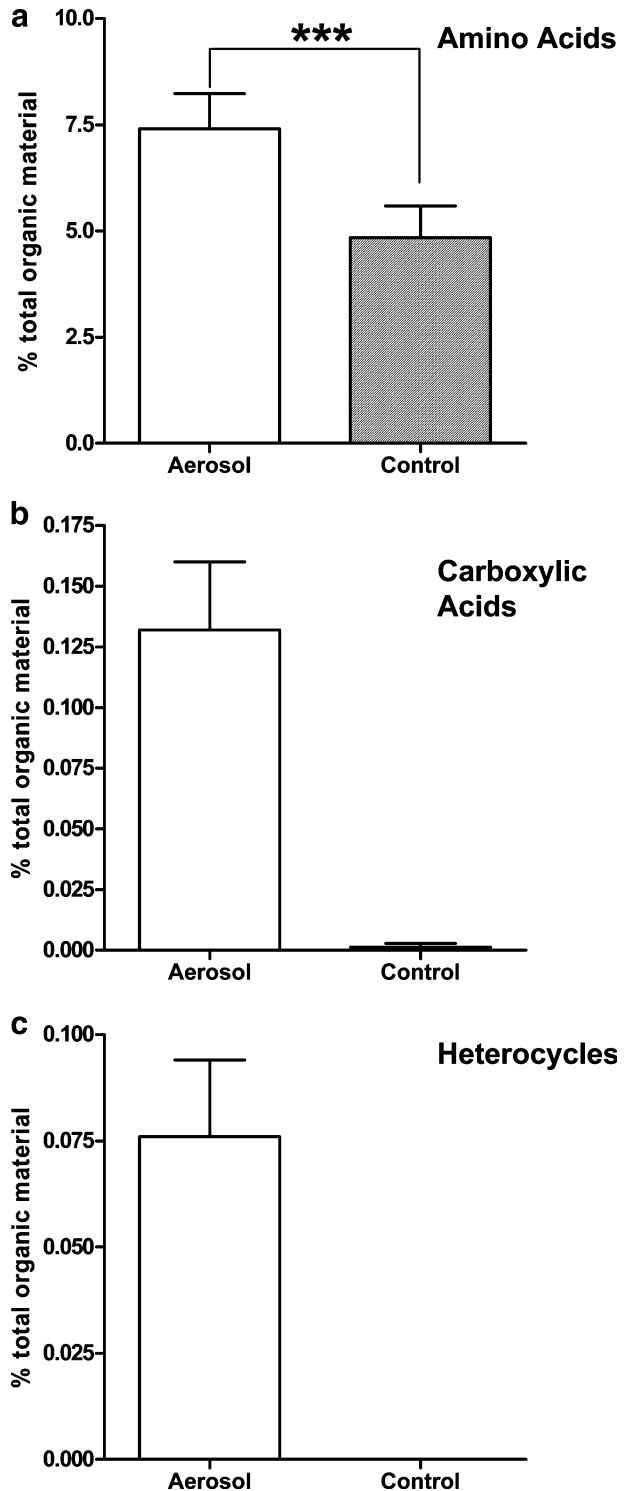
In summary, the chemical analysis of the S fractions of aerosol and control experiments are consistent with the structural data. The formation of polar compounds with biological interest (i.e., amino acids, purine bases and carboxylic acids) is improved in the presence of aqueous aerosols.

Conclusions

We can conclude that organic molecules of potential biochemical interest obtained in the presence of an aqueous aerosol show greater amounts and diversity than the material obtained using the same conditions without aerosol (see Figure 6). The experimental evidence obtained in this work support the hypothesis made by Shah and developed by Lerman, in which the aerosol droplet behaves as a microscopic chemical reactor that offers a number of potential advantages for prebiotic synthesis.

Acknowledgments The authors acknowledge the Centro de Astrobiología (CAB) for the research facilities and the grants from Instituto Nacional de Técnica Aeroespacial ‘Esteban Terradas’ (INTA). We especially thank Juan Pérez Mercader for his continuous efforts for the development of Prebiotic Chemistry in the CAB. We acknowledge the thorough and constructive revision and comments of Alan W. Schwartz, James H. Cleaves, Antonio Lazcano, Adrian Tuck, Louis Lerman, Guillermo Muñoz Caro and M. Paz Zorzano.

Figure 6 Effect of aerosol in the production of chemical families with astrobiological interest. (a) Yield of amino acids in the total amount of solid organic material. (b) Yield of carboxylic acids in the total amount of S fraction. (c) Yield of heterocycles obtained in the total amount of S fraction. The control value for heterocycles is zero because the detected compounds in the experiment without aerosol are under quantification limit of the technique. Values are expressed as mean \pm SEM of ten experiments. Significant differences were determined by Student *t*-test (** $P < 0.001$).



Appendix

Spectroscopic analysis of the organic material in aerosol simulation experiments

FTIR Spectra

The IR spectrum of S fraction (Figure 7a) shows features at 3,321 and at 3,207 cm^{-1} which are assigned to O-H stretching mode and N-H stretching mode, respectively (Table III). At 1,671 cm^{-1} a very strong band is observed, which is associated to the C=O bending mode. At 2,250 cm^{-1} the spectrum shows a weak band assigned to R – C \equiv N stretching. The bands characteristic of the asymmetric and symmetric stretching and bending modes, respectively, for $-\text{CH}_3$ and $-\text{CH}_2$ groups are observed like weak bands at 2,977, 2,933, 2,880 and 1,452, 1,379 cm^{-1} , respectively.

The IR spectra of the I fraction also show (Figure 7c) bands that could be assigned to N-H or O-H bonds (3,435 cm^{-1}). Indeed, the IR spectrum of I fraction show a feature at 1,707 cm^{-1} associated with C=O bonds, a band at 1,536 cm^{-1} that may be correlated with amide II bonds or N-H bending mode (also present in the S fraction as a weak band at 1,541 cm^{-1}) and bands that probably are due to alkenes, alkynes, nitriles and imines groups. In the spectra of I fractions a band at 1,955 cm^{-1} can be seen that may be due to C=C=C (cumulene system) or C=C=CHCONH₂. Finally, it is important to indicate that the features related to $-\text{CH}_3$ and $-\text{CH}_2$ groups are actually enhanced in the I fraction with respect to the S fraction. For a complete assignment of all bands in S and I fractions, see Table III.

NMR Spectra

The solid-state ¹³C NMR spectrum of the S fraction shows four groups of broad resonances about 166, 126, 78 and 47 ppm (Figure 8a) In order to resolve these bands, the spectra were recorded in solution of deuterium oxide, and in this case the previous broad resonances could be resolved. As a result in the ¹³C NMR (D₂O, 100 MHz) of S fraction a great number of resonances are observed in a very complex spectrum (Figure 9a). Nevertheless, as a first approximation, it is possible to group all resonances in six main types of carbons. The resonances about 180–160 ppm indicate the presence of amides and/or carboxylic groups (carboxylic acid or esters). It is remarkable that resonances at 210–190 ppm related with ketones and aldehydes were absent. The resonances about 120–110 ppm can be assigned to nitriles groups and in the region 80–65 ppm are observed resonances that may be due to C-OH groups. The possible amine groups are observed about 65–40 ppm. The resonances in the 37–15 ppm range indicate likely $-\text{CH}$ and $-\text{CH}_2$ groups and finally the resonances about 14–9 ppm may be assignment to $-\text{CH}_3$. All these resonances are consistent with the observed features in the IR spectrum; in particular, the assignment of the continuous signal among 3,350 and 1,700 cm^{-1} (Figure 7a) to the presence of carboxylic acid is supported by the high number of resonances about 180–160 ppm.

In the solid-state ¹³C NMR spectra of I fraction, broad resonances are observed (Figure 8b). The signals that could be due to nitriles ($-\text{C}\equiv\text{N}$) and alkenes (C=C) are overlapped (centred resonances around 129 and 115 ppm). The resonances near 75 ppm can be assigned to hydroxylic carbon (C-OH) but also it is possible an alkyne group (C \equiv C) contribution. A very broad resonance is observed in the range 60–10 ppm. In these signal is

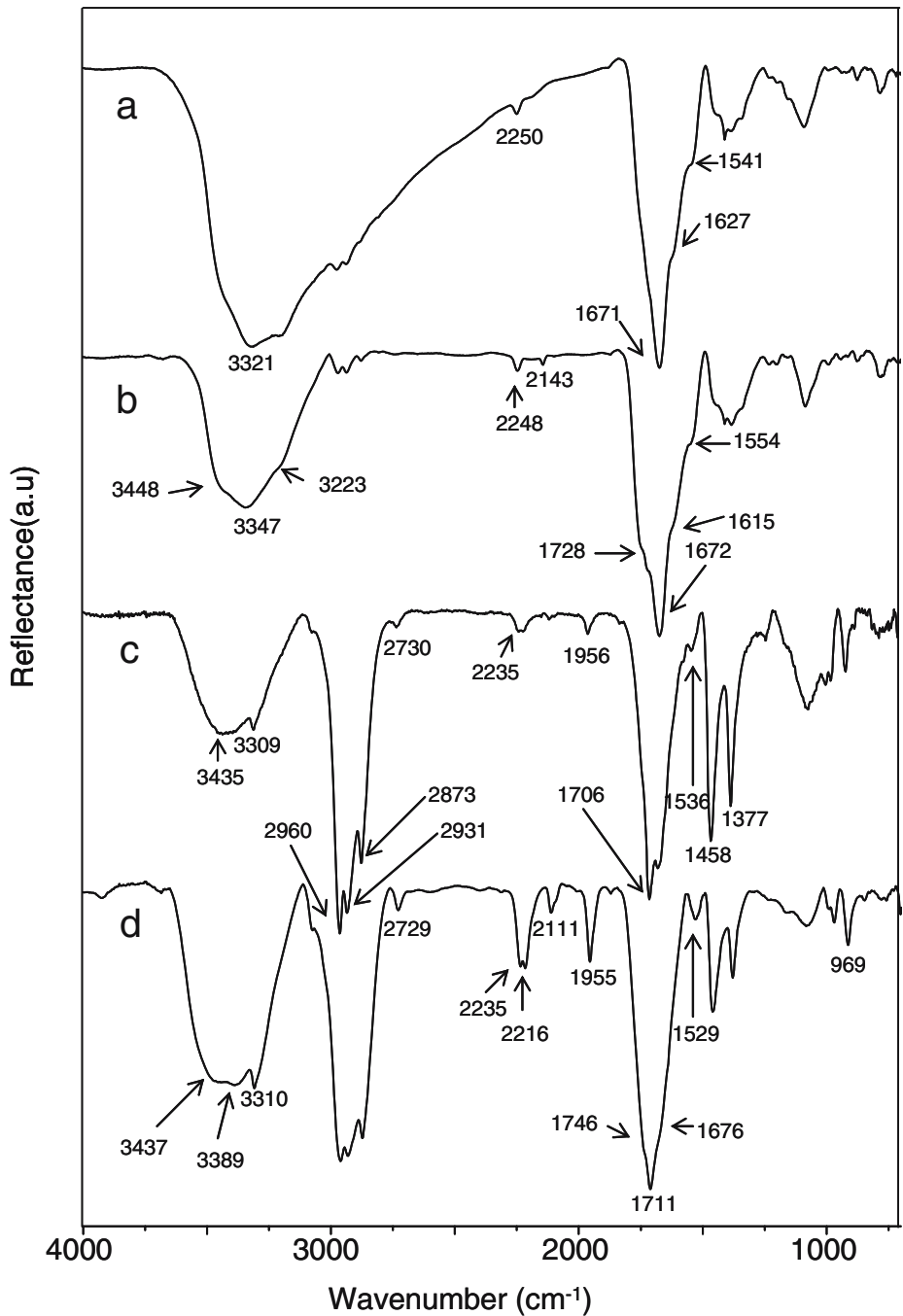


Figure 7 Transmission FTIR spectrum obtained from water soluble and insoluble fractions of CH₄-N₂-H₂ aqueous aerosol spark experiments and CH₄-N₂-H₂ control experiments. (a) Water soluble fraction, *S*, from aqueous aerosol experiment; (b) Water soluble fraction, *S*, from control experiment; (c) Insoluble fraction, *I*, from aqueous aerosol experiment; (d) Insoluble fraction, *I*, from control experiment.

TABLE III Characteristic frequency of IR absorption spectra obtained from water soluble and insoluble fractions of CH₄-N₂-H₂ aqueous aerosol spark experiments and CH₄-N₂-H₂ control spark experiment

D fraction (aerosol)	D fraction (control)	S fraction (aerosol)	S fraction (control)	Vibrational groups identity
	3448 (sh)	3435 (b, m)	3437 (b, m)	N-H stretching (single bond) or NH ₂ asym stretching or O-H stretching
			3389 (b, m)	NH ₂ asym stretching or O-H stretching
3321 (b, s)	3347 (b, s)			O-H stretching
		3309 (m)	3310 (m)	C≡CH
3207 (b, s)	3223 (sh)			N-H stretching or overtone NH ₂ bending
		3075 (sh)	3078 (v w)	C=CH ₂ stretching
2977 (v w)	2971 (w)	2960 (v s)	2961 (v s)	C-H stretching (CH ₃ asym)
2933 (v w)	2936 (w)	2931 (v s)	2932 (v s)	C-H stretching (CH ₂)
2880 (sh)	2878 (w)	2873 (v s)	2873 (v s)	C-H stretching (CH ₃ sym)
		2730 (w)	2729 (w)	N-CH ₃ or N-CH ₂ -
2250 (w)	2248 (w)	2235 (w)	2235 (m)	R-CN stretching or R-C≡C-R
		2209 (w)	2216 (m)	C≡C or conjugated nitriles
	2143 (w)			
		2112 (v w)	2111 (w)	N=C=O or N=C=N
		1956 (w)	1955 (m)	C=C=C or C=C=CHCONH ₂
			1746 (sh)	
	1728 (sh)	1706 (v s)	1711 (v s)	C=O stretching
1671 (v s)	1672 (v s)	1671 (v s)	1676 (sh)	C=O bending or N-C=C, O-C=C or C=C (stretching) or CH=N-N=CH
1627(s)	1615 (sh)			N-H bending
1541 (sh)	1554 (sh)	1536 (v w)	1529 (w)	N-H bending (possible amide) or aromatics (quadrant stretch) or C=C (+ contribution C=N, N=N)
1452 (w)	1452 (sh)	1458 (v s)	1460 (m)	CH ₂ asym bending
	1410 (m)			
1379 (b, m)	1382 (m)	1377 (v s)	1379 (m)	CH ₃ sym bending
1234 (w)	1234 (w)			C-O st (arc-OH) or
1207 (v w)	1199 (w)			C-C / C-N stretching
1096 (w)	1083 (b, m)		1082 (b, w)	C-O vibrational mode
		1073 (b, m)		C-O vibrational mode or C=C=st si
998 (sh)	994 (w)			Vinyl C-H bending or C-N-C (saturated heterocycles)
		970 (w)	969 (w)	Vynil C-H bending
776 (w)	782 (w)			Aromatics/N-H bending

The frequency is expressed in cm⁻¹. Intensity code: (b) = broad, (m) = medium, (s) = strong, (v s) = very strong, (sh) = shoulder, (w) = weak, (v w) = very weak.

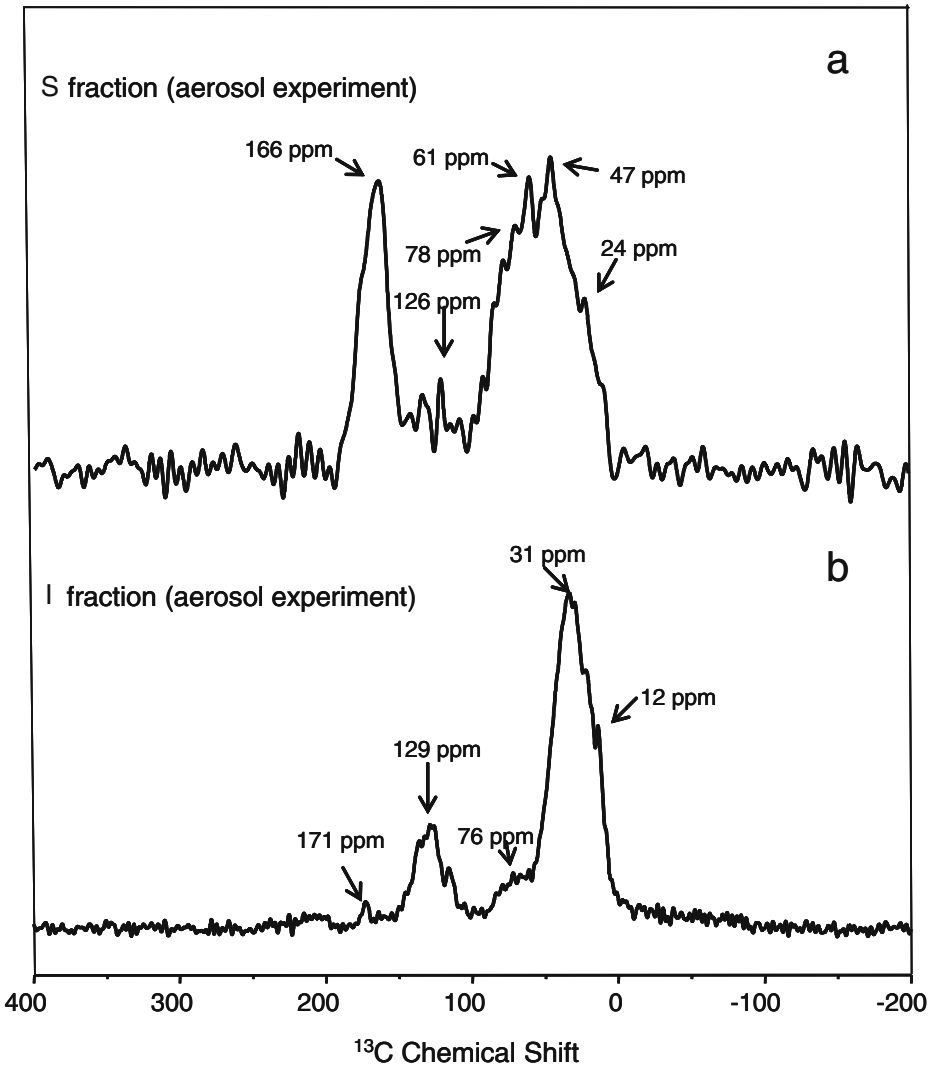


Figure 8 Solid-state ^{13}C NMR spectra from water soluble (a) and insoluble fractions (b) of $\text{CH}_4\text{-N}_2\text{-H}_2$ aqueous aerosol spark discharge experiments.

likely that they are overlapping the resonances corresponded to amine ($-\text{CNH-R}$) and $-\text{CH}$, $-\text{CH}_2$ and $-\text{CH}_3$ groups. An additional resonance at 171 ppm is observed in the I fraction (Figure 8) that could be assigned to $\text{C}=\text{O}$ of either carboxylic acids or esters.

With the structural information present, we could infer that the water soluble (S) fraction is a complex mixture, where there are carboxylic acids, amines, alcohols, and nitriles and it is possible the presence of amides and esters. In this raw mixture we could not observe other functional groups like ketones, aldehydes or $\text{C}=\text{C}$ from alkenes or aromatics. Therefore, in any case the S fraction obtained from aqueous aerosol is constituted by different polar units. By the contrary, insoluble (I) fractions are apolar solids, insoluble in water and in the common organic solvents. The fraction I seem to consist in large and rigid

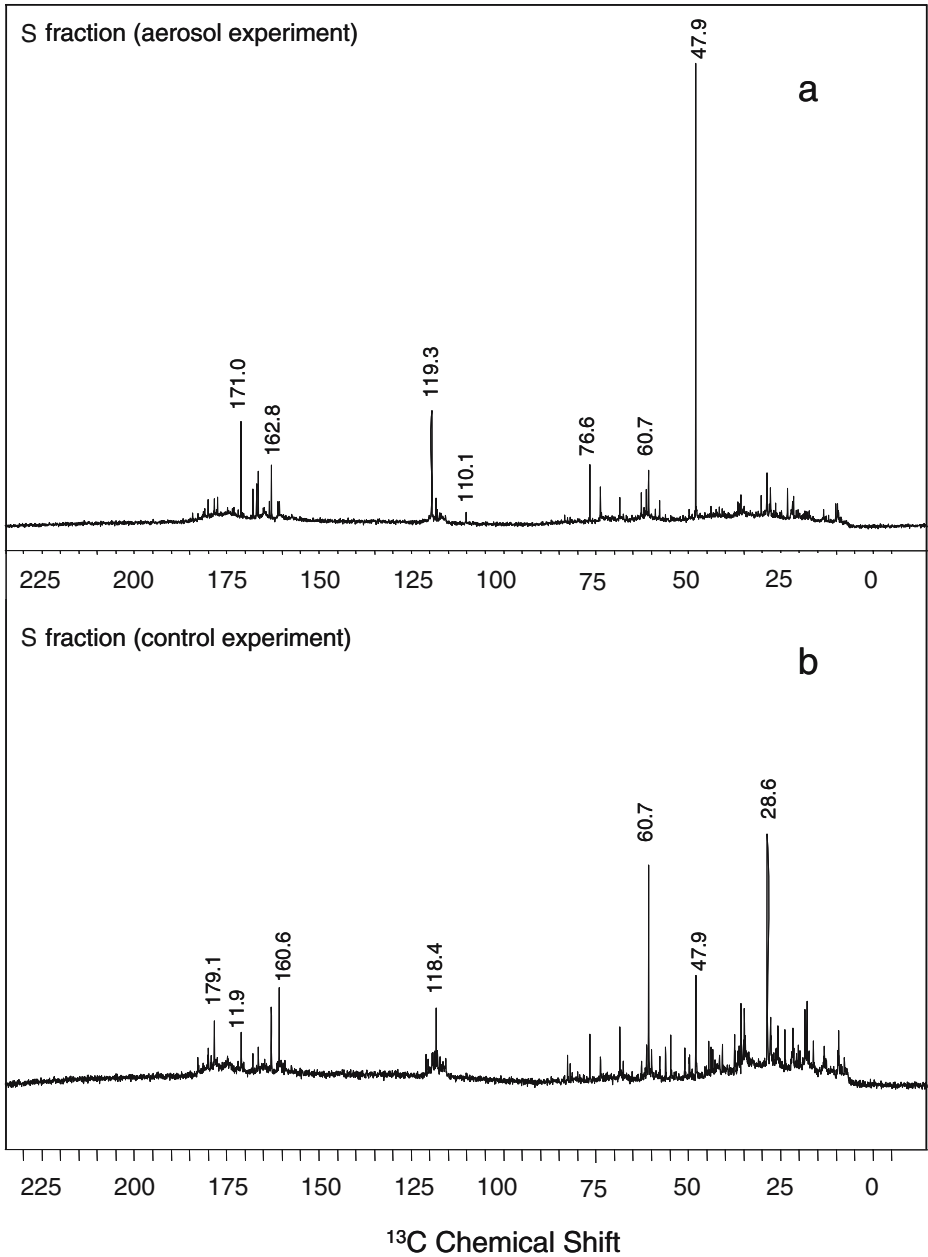


Figure 9 ^{13}C NMR (D_2O) spectra from water soluble fractions (S) from $\text{CH}_4\text{-N}_2\text{-H}_2$ aqueous aerosol spark experiments and from the control experiment. (a) Water soluble fraction from aqueous aerosol experiment; (b) Water soluble fraction from control experiment.

hydrocarbon chains. The occasional presence of unsaturated bond ($\text{C}=\text{C}$, $\text{C}\equiv\text{C}$) increase the rigidity of the chains and the amine and hydroxyl groups could favour the hydrogen bond interactions among them. Both factors contribute to the low solubility of I fraction.

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