Marine Chemistry, 45 (1994) 243–256
0304-4203/94/$07.00 © 1994 – Elsevier Science B.V. All rights reserved

Can amino acids be synthesized by H_2S in anoxic lakes?

W. John Cole[a], Michael Kaschke[b,1], John A. Sherringham[b], Gordon B. Curry[b], Dugald Turner[b], Michael J. Russell[b]

[a]*Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK*
[b]*Department of Geology and Applied Geology, University of Glasgow, Glasgow G12 8QQ, UK*

(Received August 13, 1992; revision accepted September 8, 1993)

Abstract

The starting point of the current work is the hypothesis that amino acids are being synthesized abiotically in the hydrogen sulphide bearing anoxic Lake Nakanoumi (Kimoto and Fujinaga, 1988, 1990). Kimoto and Fujinaga claimed to have detected amino acids in an experiment modelling the lake. The amino acid analyser (HPLC), however, is considered an inappropriate analytical instrument in this case as it is dedicated primarily to the analyses of amino acids and not to other organic molecules. Analyses by HPLC confirmed the reported data, however gas chromatography and gas chromatography–mass spectrometry analyses, including selected ion monitoring, failed to verify the presence of amino acids. Some of the compounds identified (amines and carbon/sulphur- and carbon/sulphur/nitrogen ring systems) might account for false detection of amino acids by the amino acid analyser and can, unlike amino acids, be obtained from the starting materials by known chemical reactions. A number of the identified compounds are known to occur in sediments from hydrothermal vents, and so the Kimoto and Fujinaga experiment remains of importance to the origin of life debate.

1. Introduction

Recently Kimoto and Fujinaga (1988, 1990) published an hypothesis that in the bottom area of anoxic lakes amino acids are being synthesized owing to the abundance of H_2S. In attempting to model the limnological conditions in a laboratory experiment they claimed that about 10 different amino acids (HPLC) could be synthesized by reaction between ammonium formate, formaldehyde, hydrogen sulphide and magnesium chloride in aqueous solution. In these putative reactions H_2S would have to play two different roles: in the case of amino acids like glycine and serine which can in principle be synthesized just by condensation of the starting material, H_2S would have to act as a condensation catalyst (Fig. 1a), whereas in the synthesis of other amino acids, e.g. alanine and valine, where subsequent reduction is necessary, H_2S is also important as a redox reagent (Fig. 1b). We note that no Strecker-type condensation of the imine ($HNCH_2$) with formic acid would be possible. The reactions they describe seemed to us to be of such importance, not only from the chemical and limnological point of view, but also for the origin of life debate, that it served as a stimulus to attempt to reproduce their findings and substantiate them using other analytical techniques eg gas chromatography (GC), gas chromatography–mass spectrometry (GC-MS) and selected ion monitoring (SIM).

[1] *Present address*: Anorganisch-Chemisches Institut der Universität, Im Neuenheimer Feld 270, 6900 Heidelberg, Germany.

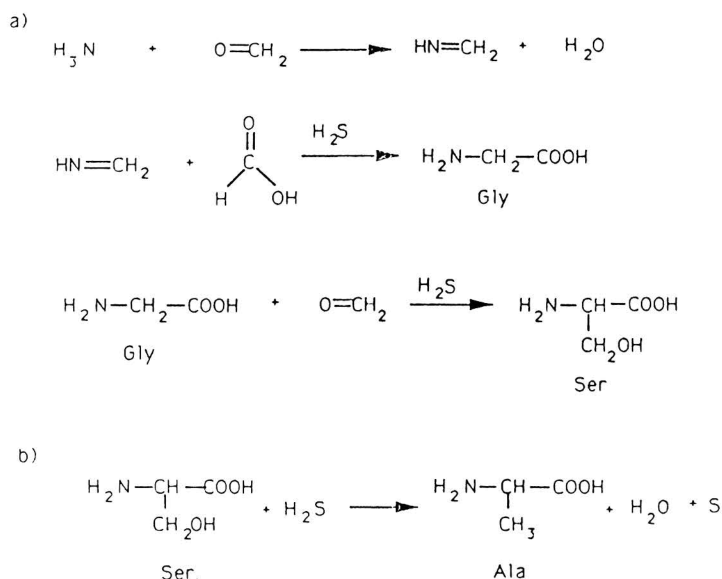


Fig. 1. Notional syntheses of amino acids from the starting materials of the Kimoto and Fujinaga experiment (a) by condensation, and (b) by condensation and subsequent reduction.

2. Experimental

2.1 Materials

Ammonium formate, $MgCl_2 \cdot 6H_2O$, formaldehyde, acetyl chloride, propan-2-ol and dichloromethane were AnalaR grade (BDH). Pentafluoropropionic acid anhydride, heptafluorobutyric acid anhydride and constant boiling HCl were purchased from Pierce. Nanograde ethyl acetate was obtained from Mallincroft and oxygen free water (milli^{TM} grade) was used throughout. Oxygen-free nitrogen was obtained from BOC and hydrogen sulphide from BDH.

2.2 Reevaluation of the first Kimoto and Fujinaga model experiment of 1988 (Fig. 2)

Ammonium formate (78.8 g), $MgCl_2 \cdot 6H2O$ (5.083 g) and HCHO (2.5 ml) were dissolved in water (250 ml) leading to a solution of 5 M ammonium formate, 0.1 M $MgCl_2$ and 0.1 M HCHO (pre-liquor **S1**). This mixture then was placed in a 500 ml round flask equipped with a sintered glass bubbler and a condenser. The bubbler was used for input of the hydrogen sulphide/nitrogen gas mixture (flow rate: 50 ml/min and 0.5 ml/min, respectively). Within 1 h the reaction mixture became milky; 24 h later a precipitate adhered to the glass wall of the flask. The solution was filtered (post-liquor **S2**) and the precipitate was washed several times with distilled water. Sometimes more of the product precipitated in the filtrate. The tacky product (**S3**) was dried for two days under reduced pressure over silica gel.

Since the presence of polymeric material was suspected, the dried solid S3 (300 ± 100 mg) was hydrolysed using HCl vapour. The latter avoided possible contamination by impurities in aqueous HCl. The product was placed in the base of a pyrex glass tube (50 × 6 mm) and the tube introduced into a glass hydrolysis bottle containing constant boiling HCl (0.5 ml). After filling with nitrogen, the bottle was heated in an oven at 165°C for 1 h. The bottle was then opened whilst hot in order to avoid condensation of HCl in the tubes (Walton, 1992). Approximately 30 mg of the solid (now red/brown) were then placed in a tube and heated in 1 ml of water at 100°C for 1 h in order to dissolve any amino acids. An aliquot (20 μl) of the water extract (**S4**]) was analysed

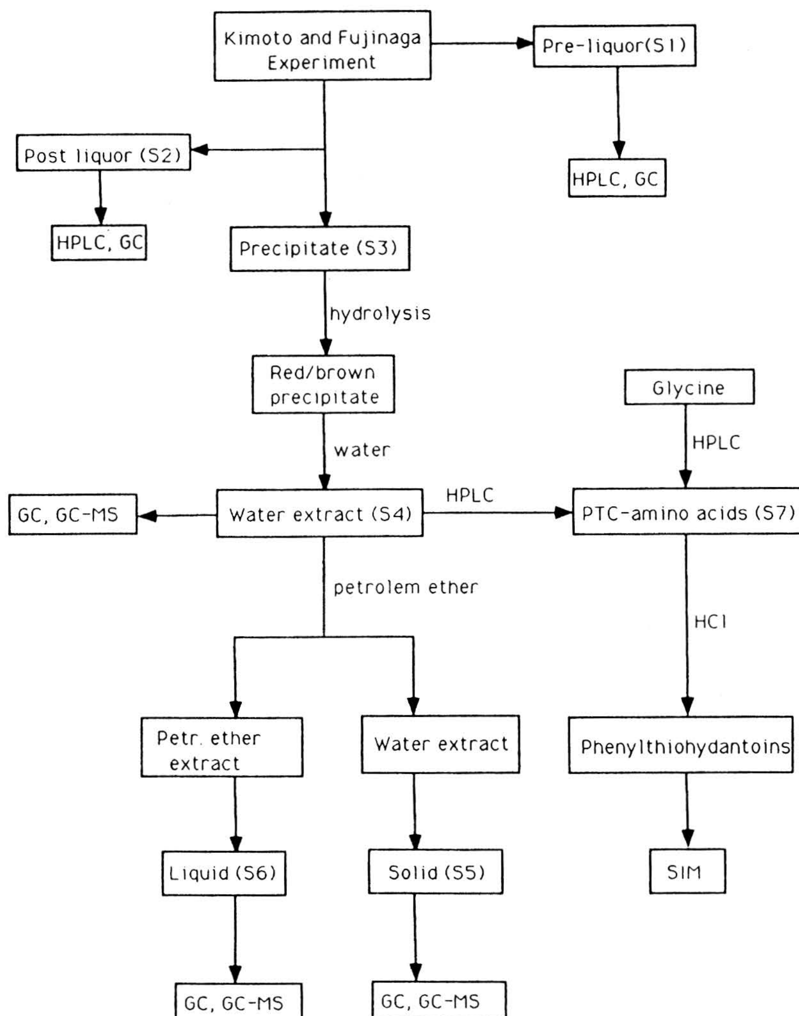


Fig. 2. Flow diagram of the extraction procedure.

using the amino acid analyser. In other analyses, further treatment of S4 was carried out. After refluxing S4 (full yield of the experiment, 300 ± 100 mg) for 2 h in 50 ml of water, the insoluble solid was removed by filtration. The filtrate was then extracted with 50 ml of petroleum ether (b.p. 40–60°C) in an attempt to effect partial subfractionation. After removal of the petroleum ether by distillation, the remaining product (**S6**) was analysed by GC and GC-MS. Lyophilisation of the water extract produced a brown solid (**S5**) which was derivatised and analysed as described above. As the whole amount of S4 was used for analyses, the detection limit was about 2 ng amino acids/mg precipitate.

2.3 Amino acid analyser

Analyses were carried using an ABI 420H instrument. Samples were automatically derivatised with phenylisothiocyanate to yield the phenylthiocarbamyl (PTC) amino acids (**S7**) (Heinrikson and Meredith, 1984). Chromatographic separation was achieved using a reverse phase HPLC column (C-18, 220 × 2.1 mm I.D.×0.5 μm; Brownlee).

2.4 Gas chromatography

Gas chromatographic separations were achieved with an Hewlett-Packard 5880A instru-

ment equipped with CP Sil 5 CB (Chrompack) and CP Sil 19 CB (Chrompack) fused silica capillary columns (25 m×0.32 mm I.D.×0.12 μm and 25 m×0.32 mm I.D.×0.18 μm, respectively) and flame ionisation detectors (FID). The Grob-type injectors were operated in split mode (50:1) and the helium carrier-and make-up gas flow rates were 2 ml/min and 25 ml/min, respectively. The column temperature was programmed from 80°C (2 min) to 275°C (5 min) at 4°C/min. The injection port and detector temperatures were 255 and 260°C, respectively.

2.5 Gas chromatography–mass spectrometry

Analyses were carried out with an Hewlett-Packard 5971 mass selective detector interfaced to a 5890 series II gas chromatograph and computer (Vectra QS/16S). Separations were effected with HP1 fused silica capillary column (12.5 m×0.2 mm I.D.×0.33 μm). Injection and temperature programming conditions were identical to those for GC above. Retention times from the total ion current (TIC) traces practically matched those of the FID chromatograms. Mass spectra (70 eV) were recorded in continuous scanning mode.

2.6 Comparison of amino acid analyser and mass spectrometry methodology

Authentic glycine samples were run through the AAA, collecting only the fractions eluting at the predetermined elution time of glycine. The total quantity isolated amounted to 332 pmol of glycine. The material, now as the phenylthiocarbamyl amino acid, was cyclised to the corresponding 3-phenyl-2-thiohydantoin as described in the synthetic section. Following conversion to its pentafluoropropionate (PFP) derivative, the material was analysed by SIM.

Aliquots (ca. 25) of S4 were put through the AAA. The combined fractions collected at the elution time of glycine amounted to 8740 pmol of glycine-like material. Following cyclisation as above, the product was divided into two equal portions, and analysed (1) as the free material, and (2) as its PFP derivative by SIM.

2.7 Selected ion monitoring

The mass selective detector was set up using its mid-mass tune facility. Analyses were effected using a dwell time of 75 ms and fractionally increased sensitivity could be achieved by decreasing the AMU setting by 10 units. The HPI column temperature was programmed from 170 to 230°C at 10°C/min for rapid elution of components. Ethyl acetate solutions of 3-phenyl-2-thiohydantoin pentafluoropropionate (2.84 min) were analysed by monitoring the ion at m/z 337.80 (M^+ and base peak) while methanol solutions of free 3-phenyl-2-thiohydantoin (4.20 min) were analysed by monitoring the ion at m/z 191.90 (M^+ and base peak).

2.8 Preparation of derivatives

Iso-propyl esters–heptafluorobutyrates (IPR/HFB)

These derivatives were prepared by a modification of the method of Kirkman (1974). Samples (S1, S2, S4, S5 and S6, ca. 40 mg) were placed in a Reactivial (Pierce) and treated with propan-2-ol (400 μl) and acetyl chloride (125 μl). The vial was sealed and heated in a hot block, at 100°C, agitating the liquid every 15 min on a Whirlimixer (Fisons). After 1 h the vial was cooled, opened and heated again at 80°C, evaporating off the reagents with a stream of nitrogen. Methylene dichloride (80 μl) and heptafluorobutyric acid anhydride (40 μl) were then added to the vial and heated at 100°C for 30 min with occasional shaking. After removal of solvent as before, the products were redissolved in ethyl acetate (50 μl) and analysed by GC and GC-MS. This procedure allowed detection down to 10 ng amino acid/mg precipitate.

Pentafluoropropionates

Synthetic 3-phenyl-2-thiohydantoin and fractions derived from the AAA from the ana-

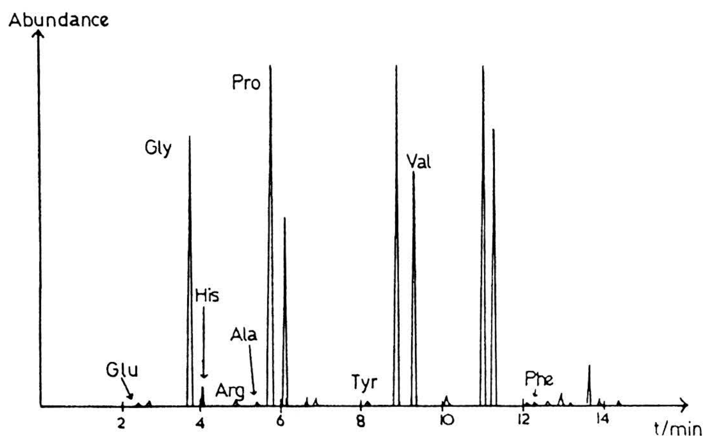


Fig. 3. Amino acid analyser trace of S4.

lyses of both glycine itself and the water extract S4 (collecting only those materials eluting at the retention time of glycine) were converted (following the cyclisation procedure outlined in section 2.9) to their corresponding penta-fluoro-propionate derivatives analogous to the method of HFB formation using pentafluoropropionic acid anhydride. Aliquots of these products were analysed by SIM.

2.9 Syntheses

3-phenyl-2-thiohydantoin (PTH)

This material was prepared by heating a mixture of glycine and phenylisothiocyanate in the presence of base according to the method of Edman (1950). The resulting phenylthiocarbamyl amino acid was cyclised to the required product by boiling under reflux in 0.1 M HCl for 2 h. After evaporation to dryness, the PTH was recrystallised from a glacial acetic acid–water mixture.

1,2,3,5,6-pentathiepane (Lenthionine)

Acetic acid was added with vigorous stirring to a mixture of $Na_2S_{2.5}$, 37% formaldehyde and chloroform, over a period of 5 h, according to the method of Morita and Kobayashi (1966). The resulting oil was purified by silica gel chromatography to give the required product [nmr: found, δ 4.32 $CDCl_3$; lit, δ 4.33 CS_2 (Moriarty et al., 1969)].

1,3,5-trithiane

This was prepared by bubbling H_2S through a solution of HCl and 37% formaldehyde according to the method of Bost and Constable (1943). The precipitate obtained was recrystallised from toluene [nmr: found, δ 4.14 $CDCl_3$; lit, δ 4.18 CS_2 (Campaign et al., 1962)].

5,6-dihydro-1,3,5-dithiazine

This was prepared from formaldehyde, ammonia and H_2S after the procedure of Levi (1929). To a solution of aqueous ammonia (5.1 g of a 33% solution, 0.1 mol) was added 37% formaldehyde (1.5 ml, 0.02 mol). H_2S was bubbled through the solution for 30 min. A white cloudy solution was obtained which also contained an insoluble oil. The solution was decanted off to yield 300 mg of 5,6-dihydro-1,3,5-dithiazine. This material was further purified by preparative thin layer chromatography (1:1 ether:petroleum ether). The partially purified compound was converted to the HFB derivative by standard methodology. δ_H (200 MHz, $CDCl_3$) 4.18 (2H,s), 4.67 (2H,s), 4.95 (2H,s).

Dihydro-5-acetyl-1,3,5-dithiazine

In order to further establish its structure, the partially purified dithiazine above was converted

Table 1
Comparison of the amounts of amino acids obtained from current work with those of Kimoto and Fujinaga

Amino acid	pmol/20 μl water extract[a] (ab intra)	pmol amino acid/mg precipitate[a] (ab intra)	pmol amino acid/mg precipitate (Kimoto and Fujinaga)
Glycine	3552 ± 1700	5914 ± 2824	2971
Serine	25 ± 15	38 ± 29	1085
Glutamic acid	43 ± 18	68 ± 27	1033
Aspartic acid	16 ± 7	30 ± 11	691
Leucine	bdl	bdl	572
Lysine	bdl	bdl	424
Valine	2850 ± 166	4738 ± 282	401
Cysteine	bdl	bdl	305
Isoleucine	17 ± 4	30 ± 8	252
Threonine	bdl	bdl	277
Alanine	51 ± 19	90 ± 34	224
Proline	4730 ± 230	12942 ± 651	bdl
Arginine	57 ± 31	98 ± 52	bdl
Histidine	200 ± 50	354 ± 90	bdl
Tyrosine	10 ± 5	17 ± 8	bdl
Phenylalanine	11 ± 4	18 ± 6	bdl

bdl =below detection limit.
[a]Mean of five analyses.

to its acetate by treatment with acetic anhydride and pyridine (2:1 *v/v*). The mixture was heated at 70°C for 1 h and allowed to cool. The reagents were evaporated off with a stream of nitrogen and the crude product was easily purified by column chromatography (alumina, dichloromethane). δ_H (200 MHz,$CDCl_3$) 2.22 (3H,s), 4.18 (2H,s), 4.78 (2H,s), 4.82 (2H,s). δ_C(50 MHz,$CDCl_3$) 21.66 (CH_3), 33.54 (CH_2), 47.64 (CH_2), 51.66 (CH_2), 169.48 (CO).

3. Results

3.1 Results of analysis by amino acid analyser

This methodology essentially confirmed the work of Kimoto and Fujinaga. The results are displayed in Table 1 and an amino acid analyser trace is illustrated in Fig. 3. The published data have been transformed to amount of picamoles of the respective amino acid per mg of precipitate for comparison with the calculated amounts of amino acids (picamoles) found in the current work. Leucine, lysine, cysteine and threonine were not detected, whereas proline, arginine, phenylalanine, histidine and tyrosine were abundant in the current work. Apart from glycine, valine and proline the amounts of amino acids found by Kimoto and Fujinaga are higher. However, altogether about 2600 ± 360 ng amino acids per mg precipitate were detected which was three times that reported.

In the current work, peaks for small amounts of aromatic amino acids were detected. Furthermore, analyses of the pre-liquor (S1), consisting essentially of AnalaR reagents, also showed trace evidence of amino acids. Even if one considers that the elution times of some non-amino acid material can be changed by subsequent treatment with HCl in the instrument (Curry, 1991), doubts arise concerning the validity of the amino acid analyser results.

Analysing the synthesized compounds by GC-MS, we identified products containing secondary amine groups that are indistinguishable in behaviour from amino acids as far as the amino acid analyser is concerned, in that they equally react with phenylisothiocyanate (March, 1985, fig. 4b) and withstand HCl treatment. The results for the GC-MS analyses, of S4 in particular, are presented in detail.

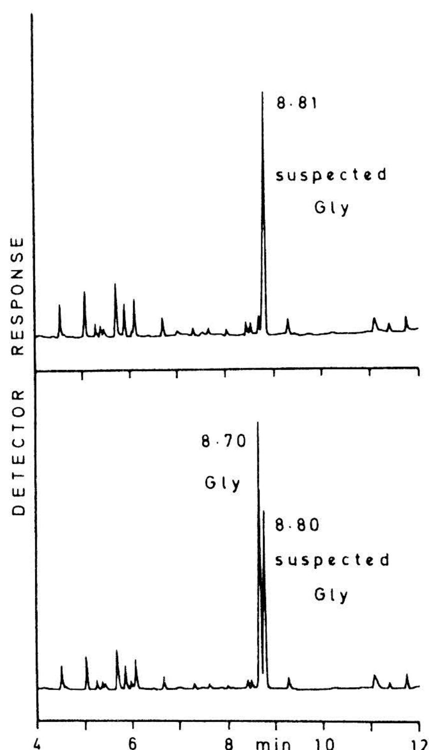


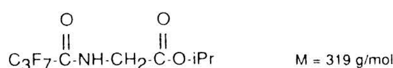
Fig. 4. Suspected glycine peak (top) and sample co-injected with authentic glycine (bottom) on a slightly polar column.

3.2 Results of analysis by gas chromatography and gas chromatography–mass spectrometry

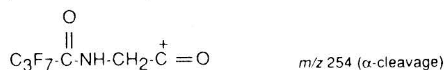
Analyses of S1 and S2 gave no evidence for the existence of amino acids. In fact, the absence of peaks in the GC trace of the pre-liquor S1 serves as an effective blank for derivatisation procedures. Of the limited number of components observed for the post liquor S2 neither their GC retention times nor the MS fragmentation patterns matched those of authentic amino acid derivatives. The water extract (S4) on the other hand exhibited a peak at exactly the retention time of glycine when the non-polar column (CP Sil 5 CB) was used. The MS fragmentation pattern also closely matched that of the glycine standard indicating the existence of glycine, but when the sample was chromatographed using a more polar column (CP Sil 19 CB) the authentic glycine and the suspected glycine peak differed slightly in retention time indicating that the peak of interest was not glycine (Fig. 4). Examination of the MS fragmentation patterns showed that although many ions were identical, only three of them are derived from the actual compounds, whereas the others resulted from the fragmentation of the heptafluorobutyryl (HFB) moiety (m/z 169, 150, 119, 100 and 69). The molecular ions were not detected in either mass spectra. The explanation for the three crucial ions for glycine are illustrated in Fig. 5a, and a postulated compound (N-ethylaminomethanethiol, no standard available) which is likely to fragment in a similar manner to glycine is depicted in Fig. 5b. The ions at m/z 254 and 226 in the glycine derivative can be explained by α-cleavage. In the case of N-ethylaminomethanethiol the ion at m/z 254 can be interpreted as a result of α-cleavage whereas that at m/z 226 can be derived from m/z 254 by Onium reaction. The ions at m/z 206 are a consequence of abstraction of HF in both cases.

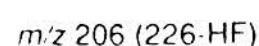
A comparison of AAA and GC-MS methodologies was sought using the technique of SIM. Samples of glycine were analysed by the AAA and fractions collected at the predetermined elution time of glycine. The combined material from the eluants was cyclised by refluxing with HCl (Edman, 1950) to give 3-phenyl-2-thiohydantoin (PTH). After PFP derivative formation, the material was analysed by SIM, monitoring its molecular ion and base peak at m/z 337.80. A signal was readily observed for this ion at the required retention time (2.84 min). From analyses of serial dilutions of authentic PTH, PFP the total quantity of glycine present in the elutes amounted to 376 pmol, which compared well to the value derived from the AAA (332 pmol). The fraction S4 derived from the model experiment was analysed in the same manner. The AAA indicated the presence of 4,370 pmol of glycine-like material. When SIM was performed on this sample, no signal for the ion m/z 337.80 was observed. A second sample of 4,370 pmol of glycine-like material was analysed by monitoring the ion at m/z 191.90, the molecular ion and base peak of free PTH. A weak signal for

a) Fragmentation of the glycine derivative

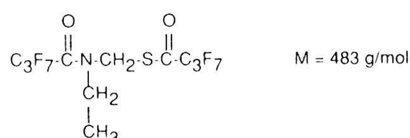


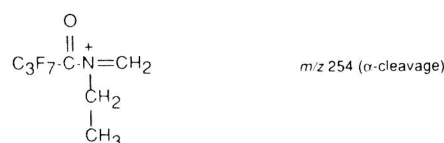
Interpretation of the MS peaks:





b) Suggested fragmentation of the N-Ethylaminomethanethiol HFB, a possible compound that could elute with the retention time of glycine





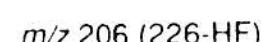
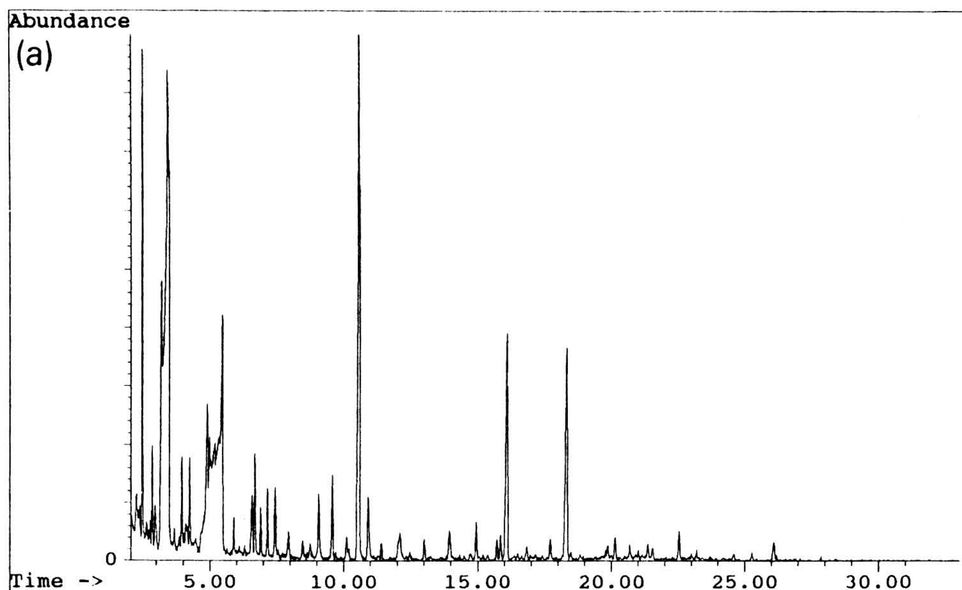


Fig. 5. MS-fragmentations of derivatives of glycine (a) and the N-ethylaminomethanethiol (b).

the ion m/z 191.90 was observed at the correct retention time (4.20 min). From analyses of serial dilutions of authentic PTH, the total amount of glycine that could be present in the sample was 66.5 pmol. The preferred method of analysis is undoubtedly that of using the PTH, PFP derivative. Elution times are fast (2.84 min), the peak shape is Gaussian, and the ion monitored has a high atomic mass (m/z 337.80). Analyses of free PTH are less reliable. The elution times are longer (4.20 min), the peak shapes show considerable tailing, and monitoring at a lower mass value (m/z 191.90) could result in trace amounts of interfering material giving spurious results. The latter fact is believed to be responsible for the observation of trace amounts of glycine-like material (66.5 pmol) in the second SIM analysis. In effect, the SIM experiments demonstrate the degree of difficulty in confirming the presence or absence of trace amounts of materials, and that little reliance can be placed on the results obtained by one method of analysis.

The hydrolysed solid (S4) was shown to be a multicomponent mixture by GC and GC-MS. The total ion current chromatogram is illustrated in Fig. 6; proposed structures of compounds identified are listed underneath. Identification of a number of constituents was possible from their mass spectral fragmentation patterns. All the cyclic sulphur compounds



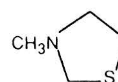
(b)

CH_3NHCH_2SH

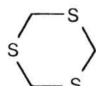
1 (3.4 min)

$C_2H_5NHCH_2SH$

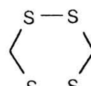
2 (3.9 min)



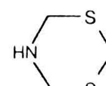
3 (6.5 min)



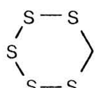
4 (7.4 min)



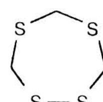
5 (9.0 min)



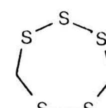
6 (10.5 min)



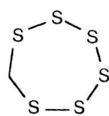
7 (10.9 min)



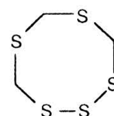
8 (12.9 min)



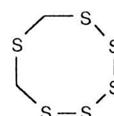
9 (16.0 min)



10 (18.3 min)



11 (20.1 min)



12 (22.4 min)

Fig. 6. Total ion current (TIC) trace of S4 and the proposed structures of compounds indentified.

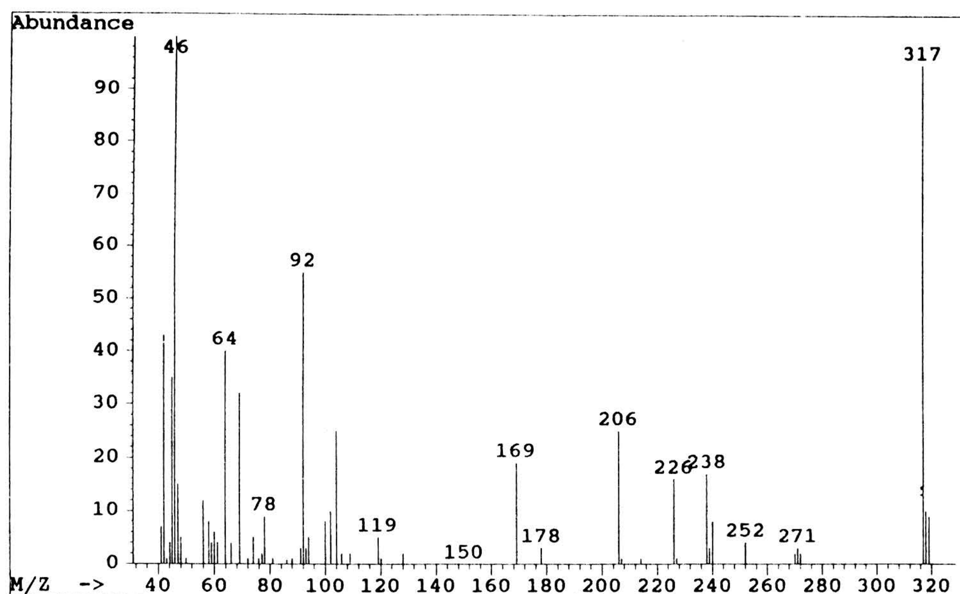


Fig. 7. Mass spectrum of the principal component of S4 [compound (6)].

exhibited strong molecular ions and in most cases possessed characteristic isotopic m+2 ions. Fragment ions of *m/z* 60 were indicative of sulphur atoms separated by methylene groups (-CH_2–S–CH_2). Two of the compounds, 1,3,5-trithiane (4) and 1,2,3,5,6-pentathiepane (9) possessed identical retention data and mass spectra to those of authentic synthetic material, and this lends credence to the proposed structures of the other carbon/sulphur ring compounds. In addition, some of these compounds could be compared directly with the fragmentation patterns of those existing in the computer's NBS library database. Table 2 lists the principal ions observed for each compound, together with their abundances relative to their respective base peaks.

The results obtained for the analyses of S4, S5

Table 2
Main MS ions of compounds (1)–(12)

Compound	M^+ (%)	Base peak	Other principal ions *m/z* with relative abundances in parentheses
(1) Methylaminomethanethiol	–	226	274(15), 240(25), 241(18), 227(10), 214(10), 197(5), 194(10), 169(40), 150(10), 119(25), 100(25), 69(30), 43(10)
(2) Ethylaminomethanethiol	–	226	254(15), 240(10), 227(70), 206(10), 169(40), 150(15), 119(20), 100(20), 69(50),43(85)
(3) N-methylthiazolidine	103(35)	43	69(10), 61(5)
(4) 1,3,5-trithiane	138(100)	138	140(14), 92(30), 73(12), 64(15), 60(15), 46(86), 45(78)
(5) 1,2,4,5-tetrathiane	156(100)	156	158(18), 119(8), 110(49), 91(12), 78(14), 69(10), 64(22), 46(56), 45(78)
(6) 5,6-dihydro-1,3,5-dithiazine	317(80)	46	319(6), 272(5), 252(5), 240(10), 238(15), 226(15), 206(20), 169(15), 119(10), 92(50), 69(35), 64(45), 60(5), 45(37)
(7) Pentathiane	174(100)	174	176(22), 128(10), 110(40), 78(15), 64(45), 46(30), 45(45)
(8) 1,2,4,6-tetrathiepane	170(85)	78	172(5), 124(55), 78(100), 69(20), 60(30), 46(45), 45(85)
(9) 1,2,3,5,6-pentathiepane	188(45)	142	190(12), 124(54), 110(12), 96(10), 78(95),64(22),46(40),45 (55)
(10) Hexathiepane	206(60)	142	208(15), 160(15), 110(8), 96(18), 78(60), 64(50), 46(24), 45 (38)
(11) 1,2,3,5,7-pentathiocane	202(8)	45	138(65), 137(68), 110(58), 91(6), 78(25), 64(14), 60(12), 46(65)
(12) 1,2,3,4,5,7-hexathiocane	220(25)	124	174(12), 142(12), 110(65), 78(55), 64(35), 46(45), 45(90)

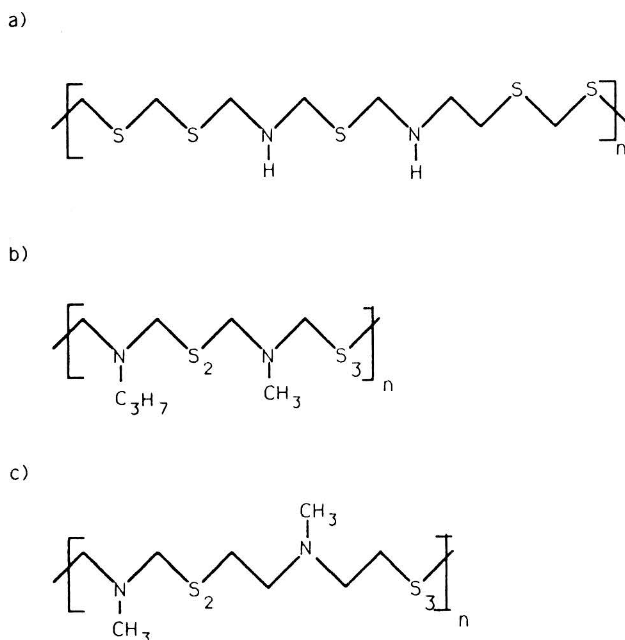


Fig. 8. Possible polymeric structures of the insoluble precipitate S3.

and S6 were very similar. Petroleum ether subfractionation of S4 reduced the number of compounds eluting in the first 5 min of the chromatogram. This allowed the identification in S5 and S6 of two other five-membered carbon/sulphur cyclic compounds (1,2,4-trithiolane and tetrathiolane). Also another eight-membered ring (1,3,5,7-tetrathiocane) and even a ten-membered ring (1,3,5,7, 9-pentathiacyclodecane) were found in the petroleum ether extract S6. It is interesting to note that compounds 7, 9, 10 and tetrathiolane are significant components of samples from the Guaymas basin and are interpreted as being synthesized from formaldehyde and S or HS_x in the hydrothermal fluids (Kawka and Simoneit, 1987).

The mass spectrum of the main component (R_t 10.5 min) of S4 is depicted in Fig. 7. It possessed an intense molecular ion at m/z 317. Detailed inspection of the mass spectral fragmentation pattern indicated that its structure was the HFB derivative of 5,6-dihydro-1,3,5-dithiazine (6). Subsequent GC and GC-MS data of the HFB derivative of the authentic dithiazine proved to be identical in all respects to that derived from S4. Levi (1929) synthesized compound 6 from formaldehyde, H_2S and ammonium ions and depicted the overall reaction as

$$NH_3 + 3CH_2O + 2H_2S \rightarrow (6) + 3H_2O$$

The reactants and experimental conditions are very similar to those used in the model experiments, thus the preponderance of 5,6-dihydro-1,3,5-dithiazine is not unexpected. Indeed it was also the main component of S5.

A plausible explanation for the synthesis of the major product (6) is presented above (condensation of formaldehyde, hydrogen sulphide and ammonia). 1,3,5-trithiane (4), 1,3,5,7-tetrathiocane and 1,3,5,7,9-pentathiacyclodecane can be considered as trimer, tetramer and pentamer of thioformaldehyde, respectively. They can only be synthesized by condensations of formaldehyde and hydrogen sulphide. As the products were detected after hydrolysis of the precipitate (S3) it is not clear to what extent the hydrolysis is responsible for the generation of the products. In the synthesis of 5,6-dihydro-1,3,5-dithiazine (6), Levi (1929) reported that a major by-product of

the reaction was 1,3,5-trithiane and although ill-defined, the presence of 3,5-dihydro-1,3,5-thiadiazine was intimated. Furthermore, treatment of the dithiazine (6) with HCl also led to the production of 1,3,5-trithiane. Thus, the presence of the latter compound in S4 is not surprising. However, precursors must also have existed. Even after hydrolysis only about 5% of the precipitate was soluble. This is the portion analysed by GC and by GC-MS. The bulk of the precipitate appears to consist of polymeric compounds. Assuming that the same type of chemical reactions needed for explanation of the products (1)–(12) are valid for the synthesis of the polymers, and considering the result of the microanalysis of total precipitate S3 (C: 33%, H: 5.5%, N: 10% and S: 50%, which leads to the empirical formula: $C_8H_{16}S_5N_2$), some structural possibilities for the polymers can be entertained. Fig. 8 illustrates three examples for linear polymers.

4. Discussion

The aim of Kimoto and Fujinaga's experiment was to prove the synthesis of amino acids in an anoxic lake rich in H_2S. Apart from the importance to the explanation of their field observations, they argue that the formation of amino acids may be of significance for the origin of life. This they surmise because the environment in which life originated could have been similar to the environment of their anoxic lake.

Recently, abiotic synthesis of amino acids under hydrothermal conditions has also been attempted by Hennet et al. (1992). They used different substrates chosen on the basis of present-day submarine hydrothermal chemistry. These were the redox buffers of the oceanic crust, pyrite (FeS_2), pyrrhotite (FeS) and magnetite (Fe_3O_4). A second set of reactions used illite in place of the iron minerals. Platinum powder provided the sink for free oxygen and reactions took place in titanium oxide autoclave liners. NaHS was used as the sulphur source. Starting materials were formaldehyde, ammonia and cyanide. CO_2 and H_2 were introduced and the total pressure in the autoclave was about ten bars and the temperature was 150°C. An extraordinary ten millimoles of glycine were identified by HPLC in the first set of experiments and twenty in the second, results supported by GLC. But one other large N-containing peak (ca. 38 min) remains uninterpreted. Trace racemic concentrations of alanine, aspartic acid and glutamic acid were also recorded along with evidence for phenylalanine and histidine, the latter surprises considering the starting materials. None of the work outlined in the discussion above, however, has been supported by molecular evidence for amino acid production.

It is recognised that in the current work, amino acids are detected as their phenylthiocarbamyl derivatives (as compared to ninhydrin and o-phthalaldehyde reagents used for the reported data) by the AAA. However, conversion of the PTC derivative of glycine to 3-phenyl-2-thiohydantoin (PTH) and analysis of PTH as its PFP derivative by SIM demonstrated a good correlation between our AAA (332 pmol) and MS (376 pmol) methodologies for the analysis of glycine. Subsequent analyses of the model experiment extracts by GC-MS and additional SIM measurements, failed to give any unequivocal evidence for the existence of amino acids. A number of compounds that were detected have been tentatively identified, while the structural identity of compounds 4, 6 and 9 have been confirmed. Some of these compounds (e.g. compound 6), contain nitrogen. Thus the field observations can be explained by synthesis of nitrogen compounds other than amino acids. Many of the sulphur/carbon cyclic compounds identified in the current work are found in nature. For example, compounds (8) and (9), which exhibit antibacterial behaviour (Holzmann et al., 1982), have been isolated from the red alga *Chondria californica* (Wratten and Faulkner, 1976). The seven membered rings 1,2,4,6-tetrathiepane (8), 1,2,3,5,6-pentathiepane (9) and hexathiepane (10) can be isolated from mush-

rooms (Morita and Kobayashi, 1966; Oae, 1977). The eight membered ring 1,2,3,5,7-pentathiocane (11) has been found in the bean *Parkia speciosa* (Gmelin et al., 1981). So, although amino acids could not be detected, we did discover compounds of potential biological significance. The Kimoto and Fujinaga experiment therefore remains interesting to the origin of life debate in that it prompts us to consider possible biological functions of these compounds. These results could have a bearing on recent hypotheses that the origin of life could have taken place in an H_2S-rich environment (Russell et al., 1988, 1989; Wächtershäuser, 1988a–c, 1990a,b; Russell and Daniel, 1992).

5. Conclusions

(1) Whilst peaks were observed at the retention times of amino acids in the amino acid analyser, no amino acids were detected by GC-MS in our reproduction of the Kimoto and Fujinaga experiment.

(2) Some of the synthesized compounds have been detected by GC and analysed by GC-MS and their syntheses from the starting materials of the experiment have been explained. The compounds are mainly amines and organic sulphides and polysulphides. Some of these have been isolated from a variety of organisms. The amines are probably responsible for the misidentification in the amino acid analyser.

(3) As we do not know with what materials life on Earth would have started (Cairns-Smith, 1982) the Kimoto/Fujinaga experiment remains of significance to the origin of life debate in that we identify some new materials to consider for possible protobiological roles.

Acknowledgements

The GC-MS instrument was purchased from, and the research work supported by, a NERC grant, number GR3/7779. We thank D. Walton and Maggie Cusack for experimental help, D.D. MacNicol for support in the interpretation of the results, and A.G Cairns-Smith and the anonymous referees for critical reading of the manuscript.

References

Bost, R.W. and Constable, E.W., 1943. *sym*-Trithiane. In: A.H. Blatt (Editor), Org. Synth., Coll. Vol. II. Wiley, New York, NY, pp. 610–611.

Buck, R.H and Krummen, K., 1984. Resolution of amino acid enantiomers by high performance liquid chromatography using automated pre-column derivatisation with a chiral reagent. J. Chromatogr., 315: 279–285.

Cairns-Smith, A.G., 1966. The origin of life and the nature of primitive genes. J. Theor. Biol., 10: 53–88.

Cairns-Smith, A.G., 1982. Genetic Takeover and the Mineral Origin of Life. Cambridge Univ. Press, Cambridge, 477 pp.

Cairns-Smith, A.G., 1985. Seven Clues. A Scientific Detective Story. Cambridge Univ. Press, Cambridge, 131 pp.

Campaign, E., Chamberlain, N.F. and Edwards, B.E., 1962. Nuclear magnetic resonance spectra of 1,3,5-trithiane and 2,4,6-trisubstituted-1,3,5-trithianes. J. Org. Chem., 27: 135–138.

Curry, G.B., 1991. Experimental synthesis of abiotic amino acids in hydrogen sulphide rich systems. In: The Inorganic–Organic Interface. Geological, Chemical and Biological Potential. Ross Priory, 7–10 May 1991, Glasgow Univ., Geology and Applied Geology.

Edman, P., 1950. Preparation of phenyl thiohydantoins of some natural amino acids. Acta. Chem. Scand., 4: 277–282.

Forster, M.O. and Rao, K.A.N., 1926. Isomeric phenylserines. J. Chem. Soc., 1943–1951.

Fukunaga, K. and Yamaguchie, H., 1981. Debromination of *vic*-dibromides with sodium sulphide in dimethylformamide. Synthesis: 879–881.

Gmelin, R., Susilio, R. and Fenwick, G.R., 1981. Cyclic polysulphides from *Parkia speciosa*. Phytochemistry, 20: 2521–2523.

Hartman, W.W. and Silloway, H.C., 1955. 2-Amino-4-nitrophenol. Org. Synth., Coll. Vol. 3: 82–84.

Heinrikson, R.L. and Meredith, S.C., 1984. Amino acid analysis by reverse-phase high performance liquid chromatography: pre-column derivatisation with phenylisothiocyanate. Anal. Biochem., 136: 65–74.

Hennet, R.J.-C., Holm, N.G. and Engel, M.H., 1992. Abiotic synthesis of amino acids under hydrothermal conditions and the origin of life: a perpetual phenomenon? Naturwissenschaften, 79: 361–365.

Holzmann, G., Susilo, R. and Gmelin, R., 1982. Collisional activation of cyclic polysulphides. Org. Mass Spectrom., 17: 165–172.

Kawka, O.E. and Simoneit, B.R.T., 1987. Survey of hydrothermally-generated petroleums from the Guaymas Basin spreading centre. Org. Geochem., 11: 311–328.

Kimoto, T. and Fujinaga, T., 1988. Non-biotic synthesis of cellular organic polymers in the presence of hydrogen sulphide. Chem. Express, 2: 123–126.

Kimoto, T. and Fujinaga, T., 1990. Non-biotic synthesis of organic polymers in H_2S-rich sea-floor: a possible reaction for the origin of life. Mar. Chem., 30: 179–192.

Kirkman, M.A., 1974. Comparative determination of protein amino acids in plant materials by automated cation exchange and gas liquid chromatography of the amino acid N-heptafluorobutyryl, *n*-propyl esters. J. Chromatogr., 97: 175–191.

Levi, T.G., 1929. La ditioazina-1,3,5 (Formotialdina). Atti Accad. Lincei, 9: 424–427.

Mango, F.D., 1983. The diagenesis of carbohydrates by hydrogen sulphide. Geochim. Cosmochim. Acta, 47: 1433–1441.

March, J., 1985. Advanced Organic Chemistry. Wiley, New York, NY, 1346 pp.

Moore, S. and Stein, W.H., 1963. Chromatographic determination of amino acids by the use of automatic recording equipment. Methods Enzymol., 6: 819–831.

Moriarty, R.M., Ishibe, N., Kayser, M., Ramsey, K.C. and Grisler, H.J., Jr., 1969. Conformational studies on seven-membered rings containing the disulfide and trisulfide groups. Tetrahedron Lett., 55: 4883–4888.

Morita, K. and Kobayaski, S., 1966. Isolation and synthesis of lenthionine, an oderous substance of *shiitake*, an edible mushroom. Tetrahedron Lett., 6: 573–577.

Nakayama, S., Machida, H. and Hoshino, M., 1983. A new reduction system: combination of sodium sulfide (sodium hydrosulfide) with phase transfer agent in a two phase mixture. Reductive debromination of *vic*-dibromides to olefins. Tetrahedron Lett., 24: 3001–3004.

Oae, S., 1977. Organic Chemistry of Sulfur. Plenum, New York, NY, 713 pp.

Russell, M.J. and Daniel, R.M., 1992. Emergence of life via catalytic hydrothermal colloidal iron sulphide membranes. Ann. Geophys., Suppl. III, 10: 506.

Russell, M.J., Hall, A.J., Cairns-Smith, A.G. and Braterman, P.S., 1988. Submarine hot springs and the origin of life. Nature, 336: 117.

Russell, M.J., Hall, A.J. and Turner, D., 1989. *In vitro* growth of iron sulphide chimneys: possible culture chambers for origin-of-life experiments. Terra Nova, 1: 238–241.

Wächtershäuser, G., 1988a. Pyrite formation, the first energy source for life: a hypothesis. Syst. Appl. Microbiol., 10: 207–210.

Wächtershäuser, G., 1988b. Before enzymes and templates: theory of surface metabolisms. Microbiol. Rev., 52: 452–484.

Wächtershäuser, G., 1988c. Deutsches Patentamt, Offenlegungsschrift DE 3812158A1.

Wächtershäuser, G., 1990a. The case for the chemoautotrofic origin of life in an iron-sulfur world. Origins Life Evol. Biosphere, 20: 173–176.

Wächtershäuser, G., 1990b. Evolution of the first metabolic cycles, Proc. Nat. Acad. Sci. USA, 87: 200–204.

Walton, D., 1992. Biochemistry of Brachiopod Intercrystalline Proteins and Amino Acids. Ph.D. Thesis. Glasgow Univ., Glasgow (unpubl.).

Wratten, S.J. and Faulkner, D.J., 1976. Cyclic polysulfides from the red alga *Chondria californica*. J. Org. Chem., 14: 2465–2467.