Analyses of Amino Acids and Benzene Extracts in the Condensed Water Collected from Inside of the MIR Space Station

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Amino acids were found in the condensed water samples collected from the inside of MIR indicating the presence of microorganisms probably grown in orbit. Aromatic alcohols and aldehydes were found in the benzene extracts of the water samples and they were likely from the sterilants and fragrances used in MIR. Present analysis of the benzene extracts failed to find a signature of the fire on February 23, 1997 that took place inside MIR.

Three samples of condensed water at different sites inside the Russian space station MIR were collected by the crew on February 27, 1997. The collection was a part of the investigation of the microflora developed inside the station since its operation in orbit in 1986. The three samples are named as C1 collected at the Panel no. 4 of transfer-docking compartment in the Crystal Module, C2 at the heat pipe of the transfer component in the Core Module, and C3 at the life support system "VIKA" in the Kvant-2 Module. Three samples were obtained by National Space Development Agency of Japan.

We analyzed for amino acids in C1 and C3 (the C2 portion was lost during analysis), and benzene-extractable organic compounds in the three samples. Each sample (1089 mg of C1, 876 mg of C2, and 961 mg of C3) was extracted with benzene (2 mL). The extracts were carefully concentrated under nitrogen flow, and analyzed by a gas chromatograph (GC) combined to a mass spectrometer (MS) (Hewlett Packard GCD). The GC was equipped with a DB-5ht capillary column. The mass spectra were acquired every 0.5 s over *m/z* 45 to 450 in the electron impact ionization mode at 70 eV. Chromatographic peaks were identified by comparison of mass spectra with those from the library data of the National Institute of Standards and Technology, U. S. A. (1992).

The aqueous portion left after the benzene extraction was hydrolyzed with 6 mol L^{-1} HCl and a half of the hydrolyzed solution was analyzed for amino acids quantitatively by an amino acid analyzer (JEOL JLC-300). The rest was dried and treated first with HCl–2-propanol and then with trifluoroacetic anhydride to make the derivatives of amino acids (*N*-trifluoroacetyl amino acid isopropyl esters). The amino acid derivatives were examined by GC–MS (Shimadzu QP-5000) for the separation of D- and L-enantiomers by a Chirasil-L-Val capillary column and identified by comparison of GC retention times and mass spectra of standard compounds. Mass spectra were acquired every 0.1 s for selective ions in the chemical ionization mode at 200 eV with isobutane as reacting gas.

Analytical grade hexane (Wako Pure Chemicals), twice distilled 6 mol L^{-1} HCl were used. Water used as solvent was distilled, deionized, and twice distilled. All glassware was heated at 500 °C for 3 h prior to use to remove contaminants.

Table 1. Concentrations and ratios of amino acids in the condensed water samples (C1 and C3) collected from the inside of MIR

	Concentration/nmol ml^{-1}			Ratio (gly=1)	
	C1	C3	DW^a	C1	C3
Glycine	35.4	1.97	0.41	1.00	1.00
Alanine	41.4	1.90	n.d.	1.17	0.97
Valine	18.4	0.82	n.d.	0.52	0.42
Leucine	22.7	1.59	n.d.	0.64	0.81
Isoleucine	13.6	0.72	n.d.	0.39	0.37
Aspartic acid	25.1	1.27	n.d.	0.71	0.65
Glutamic acid	29.0	1.81	n.d.	0.82	0.92
Methionine	1.34	n.d.	n.d.	0.04	0.00
Serine	23.2	1.49	n.d.	0.66	0.76
Threonine	24.9	1.17	n.d.	0.70	0.59
Phenylalanine	10.1	0.51	n.d.	0.29	0.26
Tyrosine	4.56	n.d.	n.d.	0.13	
Histidine	2.78	0.15	n.d.	0.08	0.07
Lysine	9.18	0.46	n.d.	0.26	0.23
Arginine	11.0	0.49	n.d.	0.31	0.25
Cysteine	2.57	n.d.	n.d.	0.07	
4-Aminobutyric acid	0.98	n.d.	n.d.	0.03	
Ornithine	1.72	n.d.	n.d.	0.05	

[a]Distilled water. n.d. not detected.

Eighteen amino acids found in C1 and 13 amino acids in C3 were quantified, and their concentrations were listed in Table 1. The concentrations in C1 were generally one order of magnitude higher than those in C3. However, the amino acid compositions in the two samples were rather similar to each other as seen by the ratio of each to glycine (Table 1). Only two amino acids (4-aminobutanoic acid and ornithine) were non-protein amino acids and the rests were protein ones. In addition, very small amounts of *N*-methylglycine, 2-amino-2-methylpropanoic acid (AIBA), and 2-amino-2-methylbutanoic acid (isovaline) were found by GC-MS analysis. We failed to detect any amino acid in a control analysis using our distilled water sample. Therefore, the results of the C1 and C3 samples were not due to artifact. D/L ratios of abundant 9 amino acids in C1 and C3 were estimated and shown in Table 2. All of the ratios are small indicating the L-enantiomer predominance.

The compositions and the enantiomeric feature strongly suggest that those amino acids in C1 and C3 were mainly from proteins. It is rather difficult to consider that protein molecules were floated in the space of MIR and dissolved in the condensed water, but reasonable to attribute them to microorganisms in the water. The concentration difference between the two water samples likely reflect the difference in population of the organisms. The finding of AIBA and non-racemic isovaline in C1 supports this interpretation. The two amino acids are

Table 2. D/L ratios of amino acids in the condensed water samples (C1 and C3) collected from the inside of MIR

	C1	C3
Alanine[a]	0.10	0.06
Valine	0.01	0.03
Leucine	0.01	0.03
Aspartic acid	0.03	0.04
Glutamic acid	0.05	0.06
Serine	0.01	0.00
Threonine	0.01	0.00
Lysine	0.03	0.06
Phenylalanine	0.01	0.03

[a] D/L ratios of alanine are probably over estimated due to partial overlap of D-alanine and *N*-methylglycine peaks on their mass fragmentogrm.

very rare in organisms except that they are unique amino acids in fungous peptide antibiotics.[1] Consequently, fungi were likely present in C1.

Total ion chromatogram of benzene extract in the C3 sample is shown in Figure 1. A total of 22 major peaks were identified and their compound names are listed in Table 3 together with those found in C1 and C2. Many of these are aromatic alcohols and aldehydes whose concentrations are roughly at the level of sub-nmol mL^{-1}. Since the presence of microorganisms in the water samples was indicated, cultivated *Escherichia coli* was extracted with benzene and the extract was analyzed by the GC–MS method. The major compounds found were indole, alkanes, alkanoic acids and alkanoic aldehydes. Those compounds are different kinds from those found in the water samples. Aromatic alcohols and aldehydes are common chemicals used in the sterilants and fragrances.[2] Therefore, it is likely that these alcohol and aldehyde molecules were in the space of MIR and accumulated in the condensed water having been dissolved during in orbit. It is worthy to note that these compounds were present more abundantly in C3 than C1, which may account for the difference in population of microorganisms between the two water samples.

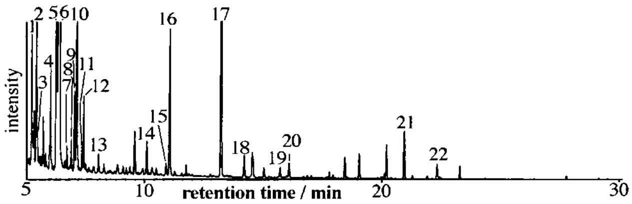

Figure 1. Total ion chromatogram of benzene extract in the condensed water sample C3 collected from the inside of MIR. Peak numbers correspond those in Table 3.

Relatively large peaks of siloxanes were found in the early retention time region on the total ion chromatogram in Figure 1 (those peaks are not marked) in addition to the phthalate (peak no. 22). They are known to be constituents of rubbers and plastics, and therefore, considered to be contaminants during the sampling and storage of the water samples. No special care had been taken to avoid organic contamination prior to the organic analyses, because the analyses were not planed initially.

Table 3. Compounds detected in the benzene extracts from the condensed water samples collected from the inside of MIR

Peak no.	Compound	C1	C2	C3
1	Benzaldehyde			+
2	Phenol			+
3	3-Methylbutanal			+
4	Dichlorobenzene	+	+	+
5	2-Ethyl-1-hexanol		+	+
6	Benzylalcohol		+	+
7	Methylphenol[a]		+	+
8	α-Methylbenzenemethanol		+	+
9	Acetophenone		+	+
10	Methylphenol[a]		+	+
11	4-Methylbenzaldehyde		+	+
12	α-,α-Dimethylbenzenemethanol	+	+	+
13	Phenylethylalchohol		+	+
14	3-Cyclohexane-1-methanol			+
15	Phenoxyethanol			+
16	Benzothiazole	+	+	+
	Caprolactam		+	
17	*p*-*t*-Butylphenol	+	+	+
18	Dimethyloctanone	+	+	+
19	2-Butyl-1,3-cyclopentanedione	+	+	+
20	Biphenyl	+	+	+
21	1,6-Dioxacyclodecane-7,12-dione	+	+	+
22	Diethylphthalate	+	+	+

Peak nos. correspond those shown in Fig. 1. +, detected. [a] Positions of substituents are not determined.

Aromatic hydrocarbons such as fluoranthene, pyrene, chrysene, benzopyrene, and coronene were not detected in the benzene extracts of the three water samples. These compounds can be produced under combustion of plants and woods.[3] Apparently, no signature was found from the water analysis to show an evidence of the fire on February 23, 1997 that took place inside MIR, although those aromatics do not easily dissolve in water.

In conclusion, the organic analyses of the condensed water samples collected inside MIR indicate the growth of microorganisms during the stay in orbit, and the presence of aromatic alcohol and aldehyde molecules in its space. However, it was failed to find a signature of the fire by the present analyses.

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