DIPEPTIDES AND DIKETOPIPERAZINES IN THE YAMATO-791198 AND MURCHISON CARBONACEOUS CHONDRITES

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Dedicated to Kaoru Harada on the occasion of his 75th birthday

Abstract. The Yamato-791198 and Murchison carbonaceous chondrites were analyzed for dipeptides and diketopiperazines as well as amino acids and hydantoins by gas chromatography combined with mass spectrometry. Glycylglycine (gly-gly) and cyclo(gly-gly) were detected at the concentrations of 11 and 18 pmol g^{-1}, respectively, in Yamato-791198, and 4 and 23 pmol g^{-1}, respectively, in Murchison. No other dipeptide and diketopiperazine were detected. Five hydantoins were detected at 8 to 65 pmol g^{-1} in Yamato-791198 and seven in Murchison at 6 to 104 pmol g^{-1}. Total concentration of the glycine (gly) dimers is approximately four orders of magnitude less than the concentration of free gly in Yamato-791198, and three orders of magnitude less than that in Murchison. The absence of L- and LL-stereoisomers of dipeptides consisting of protein amino acids indicates that gly-gly and cyclo(gly-gly) detected are native to the chondrites and not from terrestrial contaminants. A possibility was discussed that the gly dimers might have been formed by condensation of gly monomers but not formed through N-carboxyanhydrides of gly.

Keywords: amino acids, carbonaceous chondrites, dipeptides, diketopiperazines, hydantoins

1. Introduction

Carbonaceous chondrites include organic compounds of extraterrestrial origin. The first persuasive evidence to indicate that they are extraterrestrial was presented by the analysis of amino acids and hydrocarbons in the Murchison carbonaceous chondrite (Kvenvolden *et al.*, 1970). Since then, amino acids among various other organic compounds in Murchison have been analyzed by several groups of investigators (Cronin and Moore, 1971; Kvenvolden *et al.*, 1971; Oro *et al.*, 1971; and other references sited in the review by Cronin and Chang, 1993). Other carbonaceous chondrites analyzed for amino acids are Allende (Cronin and Moore, 1971; Harada and Hare, 1980), Murray (Cronin and Moore, 1971; Lawless *et al.*, 1971), Orgueil (Lawless *et al.*, 1972), Allan Hills-77306 (Cronin *et al.*, 1979; Holzer and Oro, 1979; Kotra *et al.*, 1979), Yamato-74662 (Shimoyama *et al.*, 1979), and Yamato-791198 (Shimoyama *et al.*, 1985). Most recently an analysis of amino acids was reported for Orgueil, Ivuna, Murray, and Murchison (Ehrenfreund *et al.*, 2001).

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Seventy-four amino acids have been detected in Murchison and they range from glycine (gly) of C_2 to amino acids of C_7, including ones common and not common in proteins (Cronin and Pizzarello, 1986). Many of these have an asymmetric carbon and have been observed as racemic mixtures in Murchison (Kvenvolden *et al.*, 1970 and 1971), Yamato-74662 (Shimoyama *et al.*, 1979), and Yamato-791198 (Shimoyama *et al.*, 1985). However, recent studies suggested that some α-substituted α-amino acids are not racemic in Murchison (Cronin and Pizzarello, 1997) and Murray (Pizzarello and Cronin, 2000). Another characteristic of amino acids in carbonaceous chondrites is their increase by acid hydrolysis (Cronin and Moore, 1971; Shimoyama *et al.*, 1979 and 1985). Therefore, acid-labile amino acid precursors should exist in carbonaceous chondrites, and it was suspected that some of these might be peptides. However, it has been reported that the precursors are mainly amino acid derivatives of low molecular weight (Cronin, 1976a), and the presence of small peptides is not more than a few nmol g^{-1} in Murchison (Cronin, 1976b).

Organic compounds in carbonaceous chondrites give direct supporting evidence to the theory of chemical evolution. Therefore, it should be clarified whether amino acids in carbonaceous chondrites have remained as organic monomers or evolved to peptides. Total contents of those amino acids are no more than 1 μmol g^{-1}; e.g., 670 nmol g^{-1} in Yamato-791198 (Shimoyama *et al.*, 1985) and 230 nmol g^{-1} in Murchison (Cronin, 1976a). If peptides had been produced by condensation of amino acids rather than by some other processes such as the polymerization of amino nitriles (Hanafusa and Akabori, 1959), their contents are expected to be, at most, two or three orders of magnitudes less than those of amino acids. Gas chromatography combined with mass spectrometry (GC-MS) is one of the best methods to detect and confirm compounds in such a small abundance.

Accordingly, we analyzed for dipeptides (smallest peptides, and therefore, they may be the most abundant among peptides) and diketopiperazines (cyclic dimers of amino acids) as well as amino acids in Yamato-791198 and Murchison in this study. In addition, we analyzed for hydantoins which are another possible kind of acid-labile amino acid precursor. Here we report our detection of glycylglycine (gly-gly) and cyclo(gly-gly) together with amino acids and hydantoins in the two chondrites.

2. Experimental

2.1. SAMPLES

The samples we analyzed were a piece of the Yamato-791198 carbonaceous chondrites (CM2) from the National Institute of Polar Research, Tokyo, and of the Murchison carbonaceous chondrites (CM2) from the Field Museum of Natural History, Chicago. These samples were taken out from a teflon (Yamato-791198)

and a plastic (Murchison) bag on a clean bench set inside of a clean room and pulverized with great care.

2.2. Extractions and fractionations

2.2.1. *Amino Acids*

About 50 mg for each chondrite sample were extracted with 1 mL of water in a screw-cap (teflon-lined) vial at 100 °C for 24 h. The extracted solution was recovered by centrifugation, and two water rinses (0.5 mL each) of the precipitate were combined to the solution. The combined solution was divided into two aliquots, and they were dried. One aliquot was saved without any chemical treatment for the analysis of amino acids (free amino acids). The other was hydrolyzed with 1 mL of 6 M HCl at 110 °C for 24 h. After evaporation of the HCl solution, the residue was saved for the analysis of amino acids (hydrolyzed amino acids).

2.2.2. *Dipeptides*

About 300 mg of another portion of the chondrite sample was extracted with 1 mL of methanol in a screw-cap (teflon-lined) vial at 50 °C for 6 h. The extracted solution was recovered by centrifugation, and two methanol rinses (0.5 mL each) of the precipitate were combined with the solution. To the combined solution 5 ml of water was added and acidified to pH 1.8 with 3 M HCl. White precipitate formed by the water addition was removed by centrifugation and the supernatant was applied into a Dowex 50W-X8 (H^+) cation exchange column (10 cm $\times$ 1 cm I.D.). Compounds were eluted from the column with 25 mL of water, followed by 25 mL of 2 M NH_4OH. The eluted water solution was saved for the extraction of diketopiperazines and hydantoins described in the next section (*2.2.3. Diketopiperazines and Hydantoins*). The eluted NH_4OH solution was saved for dipeptide analysis.

The sample after the methanol extraction and rinse was then extracted with 4 mL of water at 100 °C for 24 h. The extracted solution was recovered by centrifugation, and two water rinses (0.5 mL each) of the precipitate were combined with the solution. The combined solution was acidified to pH 1.8 with 3 M HCl and applied into a Dowex 50W-X8 (H^+) cation exchange column. Compounds were eluted from the column with 25 mL of water, followed by 25 mL of 2 M NH_4OH. The eluted NH_4OH solution was combined with the saved NH_4OH solution. After evaporation of the combined NH_4OH solutions, the dried residue was saved for the analysis of dipeptides.

2.2.3. *Diketopiperazines and Hydantoins*

The saved water solution described in the former section (*2.2.2. Dipeptides*) was dried. To the dried residue 2 ml of dichloromethane and 2 ml of 1 M acetic acid were added and diketopiperazines and hydantoins were extracted with acetic acid three times (2 mL each). The three extracted solutions were combined. After evap-

TABLE I

Concentrations (nmol g^{-1}) of free, hydrolyzed, and acid-produced[a] amino acids in hot water extracts of Yamato-791198 and Murchison

Compounds	Yamaato-791198			Murchison		
	Free	Hydro-lyzed	Acid-produced[a]	Free	Hydro-lyzed	Acid-produced[a]
Glycine	139.7	188.2	48.5	25.3	77.5	52.2
D-Alanine	25.4	32.5	7.1	6.4	12.7	6.3
L-Alanine	25.4	32.7	7.3	6.8	18.8	12.0
P-Alanine	39.8	47.0	7.2	6.6	17.6	11.0
Sarcosine	23.5	29.5	6.0	6.0	11.0	5.0
D-α-Amino-n-butyric acid	6.9	8.8	1.9	1.8	3.1	1.3
L-α-Amino-n-butyric acid	6.8	8.8	2.0	1.9	3.1	1.2
α-Aminoisobutyric acid	193.6	219.5	25.9	26.0	30.7	4.7
D-β-Amino-n-butyric acid	< 14	< 17	< 3.0	< 2.6	< 4.3	< 1.7
L-β-Amino-n-butyrie acid	12.4	14.5	2.1	2.2	3.6	1.4
D-β-Aminoisobutyric acid	6.2	8.9	2.7	1.1	2.6	1.5
L-β-Aminoisobutyric acid	6.3	8.9	2.6	1.1	2.6	1.5
γ-Aminobutyric acid	1.1	4.8	3.7	1.3	11.9	10.6
D,L-N-Methylalanine	5.2	8.3	3.1	1.9	2.8	0.9
D-Valine	3.0	4.2	1.2	1.4	2.6	1.2
L-Valine	3.1	4.4	1.3	1.7	7.8	6.1
D,L-lsovaline	29.1	34.8	5.7	8.2	10.5	2.3
D-Norvaline	0.7	1.7	1.0	0.2	0.4	0.2
L-Norvaline	0.7	1.7	1.0	0.2	0.5	0.3
D-3-Amino-2-ethylpropanoic acid	0.4	0.9	0.5	0.1	0.4	0.3
L-3-Amino-2-ethylpropanoic acid	0.4	0.9	0.5	0.1	0.4	0.3
D-4-Aminopentanoic acid	1.0	1.8	0.8	0.7	1.3	0.6
L-4-Aminopentanoic acid	1.0	1.8	0.8	0.6	1.3	0.7
D-4-Amino-2-methylbutanoic acid	0.4	0.5	0.1	0.3	0.7	0.4
L-4-Amino-2-methylbutanoic acid	< 1.0	< 1.4	< 0.4	< 0.6	< 1.4	< 0.8
5-Aminopentanoic acid	0.4	2.2	1.8	0.5	6.6	6.1
D,L-Proline	3.7	4.1	0.4	3.6	9.3	5.7
D-Leucine	0.2	0.3	0.1	0.1	0.3	0.2
L-Leucine	< 0.4	< 0.8	< 0.4	< 0.3	< 4.3	< 4.0
D-isoleucine	1.4	1.9	0.5	< 1.4	< 2.1	< 0.7
L-Isoleucine	1.4	2.1	0.7	0.9	4.7	3.8
D-Alloisoleueine	0.1	0.6	0.5	0.3	0.6	0.3
L-Alloisoleucine	< 0.8	< 2.3	< 1.5	< 0.5	< 1.0	< 0.5

TABLE I
Continued

Compounds	Yamaato-791198			Murchison		
	Free	Hydro-lyzed	Acid-produced[a]	Free	Hydro-lyzed	Acid-produced[a]
D-Norleucine	< 0.2	< 0.8	< 0.6	< 0.1	< 0.4	< 0.3
L-Norleucine	0.1	0.3	0.2	0.1	0.1	0.0
6-Aminohexanoic acid	1.3	1.6	0.3	0.1	0.9	0.8
D-Serine	0.3	0.8	0.5	0.6	1.4	0.8
L-Serine	0.3	1.2	0.9	1.1	6.8	5.7
D-Threonine	0.1	0.3	0.2	0.1	0.1	0.0
L-Threonine	0.1	0.6	0.5	0.7	5.4	4.7
D-Aspartic acid	0.1	1.7	1.6	0.2	2.0	1.8
L-Aspartic acid	0.1	2.1	2.0	1.0	6.2	5.2
D-Gutamic acid	0.3	3.6	3.3	0.2	4.0	3.8
L-Gutamic acid	0.3	4.0	3.7	0.4	13.3	12.9
Total	558.7	714.8	156.1	117.3	299.1	181.8

[a] Differences in concentration between free and hydrolyzed amino acids.

oration of the combined solution, the dried residue was saved for the analysis of diketopiperazines and hydantoins.

2.3. Analyses by GC-MS

2.3.1. *Amino Acids*

The saved residues for analyses of free and hydrolyzed amino acids were treated with 1 mL of 1.5 M HCl-isopropanol at 110 °C for 1 h to make isopropyl esters of amino acids. After evaporation of the isopropanol solution, the isopropyl esters were treated with 1 mL of trifluoroacetic anhydride (TFAA) at room temperature for 30 min to make N-TFA-isopropyl esters of amino acids. After evaporation of TFAA, 1 mL of water and 1 mL of ethyl acetate were added to the residue. The N-TFA-isopropyl esters were extracted with ethyl acetate three times (1 mL each). The three extracted solutions were combined and concentrated to 10 μL under a N_2 flow. One μL of the solution was analyzed by GC-MS (Hewlett Packard, HP MSD). The GC was equipped with a Chirasil-L-Val capillary column (25 m $\times$ 0.25 mm I.D., Alltech) and operated with a ramp rate of 0.5 °C min^{-1} from 70 to 90 °C, 1 °C min^{-1} from 90 to 110 °C, 4 °C min^{-1} from 110 to 200 °C. The GC injector was maintained at 200 °C. A chemical ionization mode was used with isobutane as reacting gas. Mass fragmentograms were obtained for the ions $(M\text{-}41)^+$ and $(M\text{+}1)^+$, where M stands for the molecular weight of the esters of amino acids. These characteristic ions were revealed by mass spectra of the standard amino

acid derivatives. Identification and quantification of amino acids were made by comparison of peak retention times and areas on the mass fragmentograms with those of standard compounds listed in Table I.

2.3.2. *Dipeptides*

The saved residue for dipeptide analysis was treated with 3 mL of 1.5 M HCl-isopropanol at 40 °C for 12 h. The resulting isopropyl esters were treated with 1 mL of TFAA at room temperature for 30 min to make N-TFA-isopropyl esters of dipeptides. After further treatments as used for amino acids, 1 μL of the 10 μL ethyl acetate solution was analyzed by GC-MS. The GC was equipped with a Chirasil-L-Val capillary column (25 m $\times$ 0.25 mm I.D., Chrompack not Alltech) and operated with a ramp rate of 4 °C min^{-1} from 80 to 120 °C (10 min hold), and 2 °C min^{-1} from 120 to 200 °C. The GC injector was maintained at 230 °C. A chemical ionization mode was used with methane as reacting gas. Mass fragmentograms were obtained for the ions $(M\text{-}59)^+$ and $(M\text{-}41)^+$ for neutral dipeptides, $(M\text{-}83)^+$ and $(M\text{-}41)^+$ for acidic ones, where M stands for the molecular weight of the esters of dipeptides. These characteristic ions were revealed by mass spectra of the standard dipeptide derivatives. Identification and quantification of dipeptides were made by comparison of peak retention times and areas on the mass fragmentograms with those of standard compounds listed in Table II.

2.3.3. *Diketopiperazines and Hydantoins*

The saved residue for analysis of diketopiperazines and hydantoins was treated with 20 μL of N-methyl-N-(*tert*-butyldimethylsilyl)trifluoroacetamide and 100 μL of acetonitrile at 100 °C for 3 h to prepare their *tert*-butyldimethylsilyl derivatives. After cooling to room temperature, the solution was concentrated to 10 μL under a N_2 flow. One μL of the solution was analyzed by GC-MS. The GC was equipped with a DB-5ht capillary column (30 m $\times$ 0.25 mm I.D., GL Sciences). The GC column was heated with a ramp rate of 5 °C min^{-1} from 100 to 140 °C (4 min hold), 1 °C min^{-1} from 140 to 180 °C, and 10 °C min^{-1} from 180 to 300 °C. The GC injector was maintained at 280 °C. A chemical ionization mode was used with isobutane as reacting gas. Mass fragmentograms were obtained for the ion $(M + 1)^+$, where M stands for the molecular weight of the derivatives. Identification and quantification of diketopiperazines and hydantoins were made by comparison of peak retention times and areas on the mass fragmentograms with those of standard compounds listed in Table III and IV, respectively.

2.4. CARE AGAINST CONTAMINATION

All glassware was heated at 500 °C for at least 3 h prior to use in order to eliminate organic contaminants. Water was obtained by a successive treatment of distillation, deionization, and additional double-distillation. Organic solvents and HCl (6 M) were double-distilled. The 1.5 M HCl-isopropanol was prepared by bubbling

TABLE II
Concentration (pmol g^{-1}) of dipeptides in Yamato-791198 and Murchison

No.	Compounds	m/z ($(M-41)^+$)	Yamato-791198	Murchison
1	Glycylglycine	229	11	4
2	Glycyl-D-alanine	243	n.d.	n.d.
3	Glycyl-L-alanine	243	n.d.	n.d.
4	D-Alanylglycine	243	n.d.	n.d.
5	L-Alanylglycine	243	n.d.	n.d.
6	Glycyl-β-alanine	243	n.d.	n.d.
7	β-Alanylglycine	243	n.d.	n.d.
8	Glycyl-D-α-amino-n-butyric acid	257	n.d.	n.d.
9	Glycyl-L-α-amino-n-butyric acid	257	n.d.	n.d.
10	L-α-Amino-n-butyrylglycine	257	n.d.	n.d.
11	D-Alanyl-D-alanine	257	n.d.	n.d.
12	D-Alanyl-L-alanine	257	n.d.	n.d.
13	L-Alanyl-D-alanine	257	n.d.	n.d.
14	L-Alanyl-L-alanine	257	n.d.	n.d.
15	β-Alanyl-L-alanine	257	n.d.	n.d.
16	Glycyl-L-proline	269	n.d.	n.d.
17	L-Prolylglycine	269	n.d.	n.d.
18	Glycyl-D-valine	271	n.d.	n.d.
19	Glycyl-L-valine	271	n.d.	n.d.
20	L-Alanyl-L-α-amino-n-butyric acid	271	n.d.	n.d.
21	L-α-Amino-n-butyryl-L-alanine	271	n.d.	n.d.
22	Glycyl-D-leucine	285	n.d.	n.d.
23	Glycyl-L-leucine	285	n.d.	n.d.
24	D-Leucylglycine	285	n.d.	n.d.
25	L-Leucylglycine	285	n.d.	n.d.
26	Glycyl-D-aspartic acid	329	n.d.	n.d.
27	Glycyi-L-aspartic acid	329	n.d.	n.d.
28	L-Aspartylglycine	329	n.d.	n.d.
29	L-Alanyl-L-aspartic acid	343	n.d.	n.d.
30	L-Aspartyl-L-alanine	343	n.d.	n.d.
31	L-Valyl-L-aspartic acid	371	n.d.	n.d.
32	L-Aspartyl-L-valine	371	n.d.	n.d.

n.d.: Not detected above 1 pmol g^{-1}.

HCl gas into isopropanol. Cation exchange resins were washed several times with methanol, 2 M NH_4OH, 3 M HCl and water prior to use.

3. Results and Discussion

3.1. Identification and concentration

3.1.1. *Amino Acids*

Concentrations of the free and hydrolyzed amino acids in Yamato-791198 and Murchison are shown in Table I, together with those of 'acid-produced' amino acids which are the concentration differences between the free and hydrolyzed amino acids. The concentrations of several amino acids could not be determined, because their peaks on mass fragmentograms did not separate enough from those of unknown compounds. Therefore, their concentrations are shown with a sign of inequality.

Gly and α-aminoisobutyric acid were the two most abundant amino acids in the two chondrites. Their concentrations in hydrolyzed fraction were 188 and 220 nmol g^{-1}, respectively, in Yamato-791198, which were about twice and two-thirds, respectively, of those reported previously using an amino acid analyzer by fluorescent detection and also ninhydrin detection (Shimoyama *et al.*, 1985). The two amino acids were 78 and 31 nmol g^{-1}, respectively, in Murchison, which were close to those reported using an amino acid analyzer by the two detections (Cronin, 1976a).

Total concentrations of free and hydrolyzed amino acids were 559 and 715 nmol g^{-1}, respectively, in Yamato-791198, and 117 and 299 nmol g^{-1}, respectively, in Murchison. These values were close to those reported in the two previous studies, i.e., 550 and 670 nmol g^{-1} in Yamato-791198, and 110 and 230 nmol g^{-1} in Murchison. However, these similarities did not come from similar individual concentrations of amino acids in this work and the cited publications (as can be observed by the different concentrations of gly and α-aminoisobutyric acid). Instead, the similarities seem to come from totaling individual concentrations. The difference in individual concentrations between this work and the reported ones was probably caused by the different detection methods (GC-MS vs. amino acid analyzer), in addition to heterogeneity of the two chondrites and terrestrial contamination. Nevertheless, it is useful to use the concentrations in Table I because very precise concentrations are not necessary for the purpose of this work.

Both protein and non-protein amino acids in Yamato-791198 were observed to be nearly racemic (Table I). This indicates that the detected amino acids in Yamato-791198 are almost entirely indiginous to the chondrite and included only a small content of terrestrial amino acids (ca. 3 nmol g^{-1} by subtraction of D- from L-enantiomers). On the other hand, protein amino acids in Murchison showed a clear L-enantiomer predominance, especially in the hydrolyzed fraction (Table I). This indicates that the Murchison sample we analyzed was contaminated and included a considerable content of terrestrial amino acids (ca. 50 nmol g^{-1}). A similar result was also reported for Murchison (Ehrenfreund *et al.*, 2001). Total concentration of acid-produced amino acids was 156 nmol g^{-1} in Yamato-791198 and 182 nmol g^{-1} in Murchison including terrestrial contaminants. Accordingly, the total concentra-

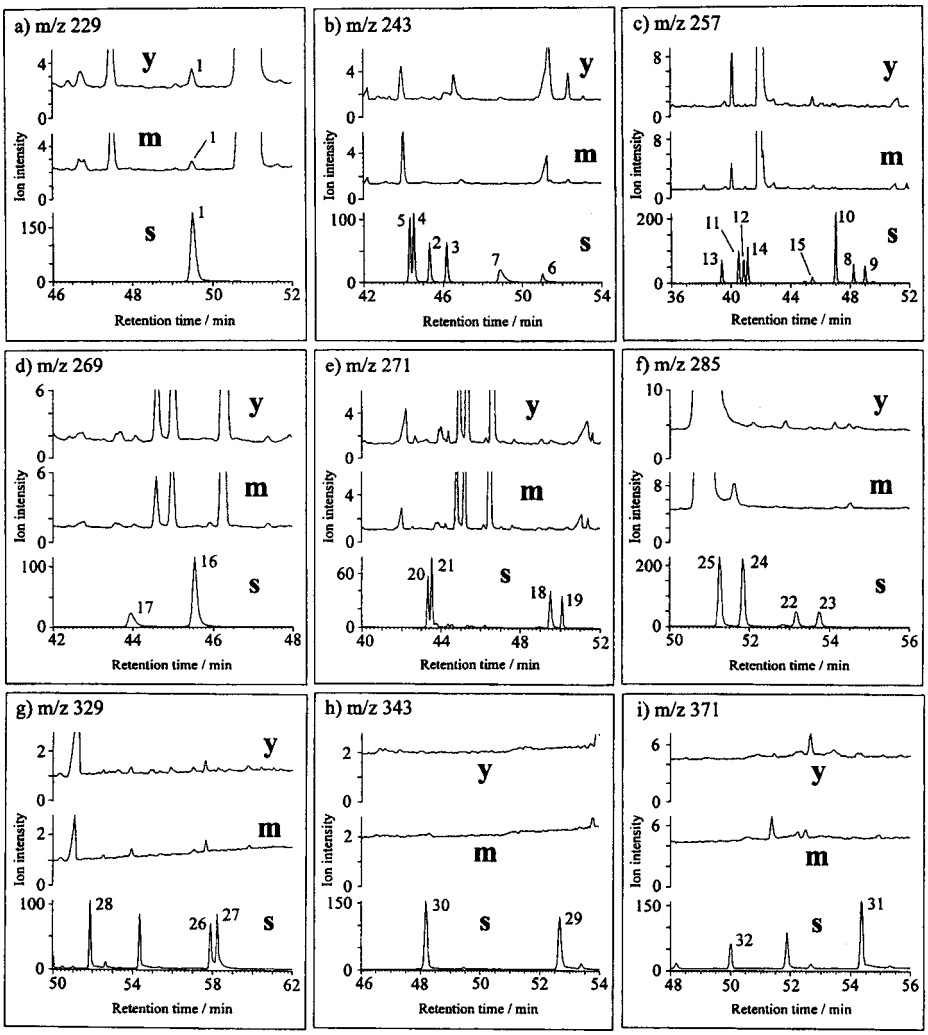

Figure 1. Mass fragmentograms of N-trifluoroacetyl isopropyl esters of dipeptides recovered from y) Yamato-791198, m) Murchison, and s) derivatives of standard compounds. Peak numbers correspond to those for compounds in Table II.

tions of acid-produced amino acids native to the chondrites were about 153 nmol g^{-1} in Yamato-791198 and about 132 nmol g^{-1} in Murchison.

3.1.2. *Dipeptides*

The most intense mass fragment ion was observed as $(M\text{-}41)^+$ for each standard dipeptide derivative, and the second intense one as $(M\text{-}59)^+$ for the neutral dipeptides and as $(M\text{-}83)^+$ for the acidic ones. Therefore, the mass fragmentograms of $(M\text{-}41)^+$ were used for preliminary identification as well as quantification, and

TABLE III
Concentration (pmol g^{-1}) of diketopiperazines in Yamato-791198 and Murchison

No.	Compounds	m/z ($(M+1)^+$)	Yamato-791198	Murchison
33	Cyclo(glycylglycine)	343	18	23
34	Cyclo(glycylalanine)	357	n.d.	n.d.
35	Cyclo(alanylalanine)	371	n.d.	n.d.
36	Cyclo(glycylleucine)	399	n.d.	n.d.
37	Cyclo(glycylaspartic acid)	515	n.d.*	n.d.*

n.d.: Not detected above 1 pmol g^{-1}.
n.d.*: Not detected above 5 pmol g^{-1}.

those of $(M\text{-}59)^+$ and $(M\text{-}83)^+$ for confirmation of neutral and acidic dipeptides, respectively. Figure 1 shows mass fragmentograms of $(M\text{-}41)^+$ for dipeptides from Yamato-791198 and Murchison. The peak numbers and their identifications are shown in Table II together with the concentrations in the two chondrites. Gly-gly was detected (m/z 229), and its concentration was 11 pmol g^{-1} in Yamato-791198 and 4 pmol g^{-1} in Murchison. Other 31 dipeptides (including stereoisomers) were searched for but not detected above 1 pmol g^{-1} (Figure 1). Since gly-gly has no asymmetric carbon, there is no stereoisomeric evidence to support that the detected gly-gly is extraterrestrial in origin. However, it was concluded that the gly-gly was proper to the chondrites and included little terrestrial one, because terrestrial dipeptides in recent sediments include not only gly-gly but also L- and LL-stereoisomers of dipeptides consisting of protein amino acids at the same order of magnitude (Ogasawara *et al.*, 2001). The abundance of gly-gly was approximately four orders of magnitude less than that of free gly in the two chondrites. Gly-gly accounted for 0.05% of acid-produced gly in Yamato-791198 and 0.02% in Murchison.

3.1.3. *Diketopiperazines*

Mass fragmentograms of $(M+1)^+$ for diketopiperazines in Yamato-791198 and Murchison are shown in Figure 2. The peak numbers and their identifications as well as concentrations in the two chondrites are shown in Table III. Only cyclo(gly-gly) was detected (m/z 343), and its concentration was 18 pmol g^{-1} in Yamato-791198 and 23 pmol g^{-1} in Murchison. Cyclo(glycylalanine), cyclo(alanylalanine), and cyclo(glycylleucine) were not detected above 1 pmol g^{-1}, and cyclo(glycylaspartic acid) was not detected above 5 pmol g^{-1}. The abundance of cyclo(gly-gly) is approximately four orders of magnitude less than that of free gly in Yamato-791198 and three orders of magnitude less than that in Murchison. Cyclo(gly-gly) accounted for 0.07% of acid-produced gly in Yamato-791198 and 0.09% in Murchison.

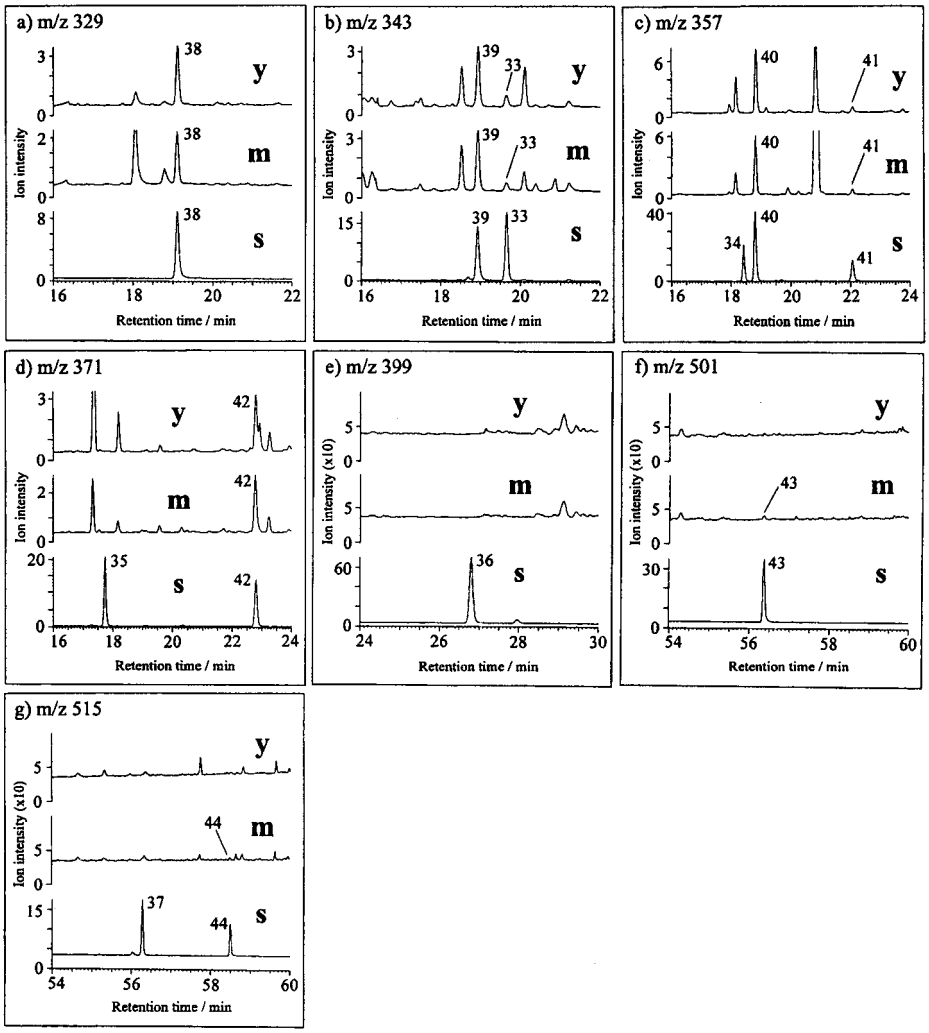

Figure 2. Mass fragmentograms of *tert*-butyldimethylsilyl derivatives of diketopiperazines and hydantoins recovered from y) Yamato-791198, m) Murchison, and s) derivatives of standard compounds. Peak numbers correspond to those for compounds in Table III and IV.

3.1.4. *Hydantoins*

Mass fragmentograms of $(M + 1)^+$ for hydantoins recovered from Yamato-791198 and Murchison are also shown in Figure 2. The peak numbers and their identifications as well as concentrations in the two chondrites are shown in Table IV. Five hydantoins were detected in Yamato-791198 at a concentration ranged from 8 to 65 pmol g^{-1} with a total of 189 pmol g^{-1}. Seven hydantoins were detected in Murchison at a concentration ranged from 6 to 104 pmol g^{-1} with a total of 320 pmol g^{-1}. Of these, hydantoin, 5-methylhydantoin, 5,5-dimethylhydantoin, and 5-

TABLE IV
Concentration (pmol g^{-1}) of hydantoins in Yamato-791198 and Murchison

No.	Compounds	m/z $(M+1)^+$	Yamato-791198	Murchison
38	Hydantoin	329	65	73
39	5-Methylhydantoin	343	48	104
40	5,5-Dimethylhydantoin	357	44	70
41	5-Ethylhydantoin	357	8	12
42	5-Ethyl-5-methylhydantoin	371	24	47
43	5-Carboxymethylhydantoin	501	n.d.	6
44	5-(2-Carboxyethyl)hydantoin	515	n.d.	8
	Total		189	320

n.d.: Not detected above 5 pmol g^{-1}.

(2-carboxyethyl)hydantoin have been detected in Murchison (Cooper and Cronin, 1995), but their concentrations have not been known. The abundances of the total hydantoins in the two chondrites were one order of magnitude more than those of the total amino acid dimers. The individual hydantoins accounted for 0.13 – 0.42% of the corresponding acid-produced amino acids in Yamato-791198 and 0.05 – 2.0% in Murchison.

3.2. Possible processes to amino acid dimers

3.2.1. *Condensation of Amino Acids*

It is possible to assume that the detected amino acid dimers were formed from amino acids in aqueous solution on the chondrite parent bodies. The reactions to form gly-gly and cyclo(gly-gly) are

$$\text{glycine} + \text{glycine} = \text{glycylglycine} + H_2O \quad (1)$$

$$\text{glycine} + \text{glycine} = \text{cyclo(glycylglycine)} + 2H_2O \quad (2)$$

Free energy (ΔG°) of reaction (1) in aqueous solution at 300 K is 3.5 kcal mol^{-1} and reaction (2) is 6.5 kcal mol^{-1} (Shock, 1992). At 300 K, the molar ratio of gly-gly/gly at equilibrium is 4.7×10^{-8} in dilute solution (1 mM gly) and 1.4×10^{-4} in nearly saturated one (3 M gly). The ratio of gly-gly/gly is 7.9×10^{-5} in Yamato-791198 and 1.6×10^{-4} in Murchison, which are about the same order of magnitude as that in nearly gly-saturated solution at equilibrium. The molar ratio of cyclo(gly-gly)/gly at equilibrium is 2.8×10^{-10} in dilute solution (1 mM gly) and 8.5×10^{-7} in nearly saturated one (3 M gly). The ratio of cyclo(gly-gly)/gly is 1.3×10^{-4} in Yamato-791198 and 9.1×10^{-4} in Murchison, which are three orders of magnitude larger than that in nearly gly-saturated solution at equilibrium. Accordingly, it is

possible thermodynamically that the detected gly-gly was formed in the nearly gly-saturated aqueous solution, and cyclo(gly-gly) was formed and precipitated in the solution at 300 K which may be a possible temperature of an aqueous environment on the parent bodies (Zolensky and Browning, 1994). However, it is not appropriate to assume that a gly-saturated aqueous condition at equilibrium existed on the parent bodies.

It has been reported that the reaction under drying-wetting cycles in the presence of inorganic materials, especially clay minerals, promotes the oligomerization of amino acids (Bujdak and Rode, 1997, 1999; Lahav *et al.*, 1978; Lawless and Levi, 1979). When amino acids oligomerize on the surface of a clay mineral under the cycles, gly is more reactive than other amino acids to form dipeptides; e.g., gly-gly was formed from gly about 19 times more than alanylalanine from alanine in the presence of hectorite under the cycles (Bujdak and Rode, 1999). Such a selected formation of gly-gly might be observed as the gly-gly predominance among dipeptides in the two chondrites, i.e., 11 pmol g^{-1} in Yamato-791198 and 4 pmol g^{-1} in Murchison, whereas, the second most abundant dipeptide, if present, is less than 1 pmol g^{-1} in the two chondrites. The cycles can provide a hypo-hydrous condition which aids to concentrate amino acids and remove water formed in the reactions of dimer formations. In addition, the hectorite surface contributes to promote dimerization probably by lowering the activation energy. Such a drying-wetting condition probably existed on the parent bodies. This, together with the predominance of gly may explain why only gly-gly and cyclo(gly-gly) pair were detected at 1 pmol g^{-1} level but not other pairs in the two chondrites.

3.2.2. *Condensation of Activated Species of Amino Acids*

Intramolecular dehydration of N-carbamyl amino acids which were formed by the reaction of cyanates with amino acids was proposed for the formation process of hydantoins in Murchison (Cooper and Cronin, 1995). The presence of hydantoins suggests the presence of N-carbamyl amino acids in the chondrites. It was reported that N-carbamyl amino acids have the potential to afford N-carboxyanhydrides (NCA) of amino acids quantitatively in mild conditions (Collet *et al.*, 1996). Therefore, oligomerization of NCA of amino acids was proposed as one of the possible processes of peptide formation on the primitive earth (Taillades *et al.*, 1998), which might have also taken place on the parent bodies. The concentrations of hydantoin and 5-methylhydantoin were roughly equal in the two chondrites. Gly-gly, glycylalanine, alanylglycine, and alanylalanine can be formed at the same order of magnitude from equal amounts of N-carbamyl gly and N-carbamyl alanine through NCA of the two amino acids. However, only gly-gly was detected in the two chondrites, which indicated that gly-gly had not been formed by the process.

4. Conclusions

Gly-gly and cyclo(gly-gly) were detected in the Yamato-791198 and Murchison carbonaceous chondrites. Their concentrations were 11 and 18 pmol g^{-1}, respectively, in Yamato-791198, and 4 and 23 pmol g^{-1}, respectively, in Murchison. No other dipeptide and diketopiperazine was detected. The gly dimers accounted for 0.12% of acid-produced gly in Yamato-791198 and 0.11% in Murchison. Hydantoins were also detected in concentrations ranging from 8 to 65 pmol g^{-1} with a total of 189 pmol g^{-1} in Yamato-791198, and from 6 to 104 pmol g^{-1} with a total of 320 pmol g^{-1} in Murchison. The individual hydantoins accounted for 0.13 – 0.42% of the corresponding acid-produced amino acids in Yamato-791198 and 0.05 – 2.0% in Murchison. The abundances of total amino acid dimers were one order of magnitude less than those of hydantoins and accounted for only a small portion of the acid-produced amino acids. However, the finding of amino acid dimers in the two carbonaceous chondrites suggests that chemical evolution of biologically important compounds had proceeded possibly to the oligomer level on the chondrite parent bodies and therefore also possibly on the primitive Earth.

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