CHAPTER 6

Indigenous amino acids and chiral excess present in CR primitive meteorites

In this paper, we report on the first measurements of amino acids on Antarctic CR meteorites. CR meteorites are among the most primitive meteorites. EET92042, GRA95229 and GRO95577 were analysed for their amino acid content using high performance liquid chromatography with UV fluorescence detection (HPLC-FD) and gas chromatography-mass spectrometry (GC-MS). Our data show that EET92042 and GRA95229 are the most amino acid-rich carbonaceous chondrites ever analysed, with total amino acid concentrations ranging from 180 parts per million (ppm) to 249 ppm. GRO95577, however, is depleted in amino acids. The most abundant amino acids present in the EET92042 and GRA95229 meteorites are the α-amino acids glycine, isovaline, α-AIB, and alanine, with $\delta^{13}C$ values ranging from + 31.6‰ to +50.5‰, which were determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). The carbon isotope results together with racemic enantiomeric ratios determined for most amino acids suggest an extraterrestrial origin of these compounds. Additionally to the exceptionally high abundance of amino acids in EET92042 and GRA95229, these meteorites show the highest L-enantiomeric excess of isovaline (33.0% and 35.9%, respectively) ever measured.

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6.1 Introduction

Meteorites provide crucial insights into the chemical processes occurring in the early solar system. In particular, the carbonaceous chondrite meteorites have a carbon-rich matrix, with some of its classes, the CM and CI chondrites, containing up to 3 wt% of organic carbon (for review see e.g. Sephton 2002). Additionally, the CR chondrites are thought to contain the most primitive meteoritic insoluble organic material (see e.g. Cody and Alexander 2005). Meteorites have been investigated concerning their inventory of prebiotic molecules. Such compounds have properties (for example, chirality) that can be used to distinguish between terrestrial or extraterrestrial origins. Amino acids are therefore obvious candidates, and have been reported in several Antarctic and non-Antarctic meteorite samples (e.g. Cronin *et al.* 1979; Holzer and Oro 1979; Kotra *et al.* 1979; Shimoyama and Ponnamperuma 1979; Shimoyama and Harada 1984; Shimoyama *et al.* 1985; Botta and Bada 2002; Botta *et al.* 2002; Shimoyama and Ogasawara 2002; Glavin *et al.* 2006).

In previous work, the Antarctic Martian meteorites Elephant Moraine (EET) 79001 (McDonald and Bada 1995), Allan Hills (ALH) 84001 (Bada *et al.* 1998) and Miller Range (MIL) 03346 (Glavin *et al.* 2005) were analysed for their amino acid content. In all three samples, the meteoritic amino acid distribution was similar to the one in the Allan Hills ice, which suggested that the ice meltwater was the source of the amino acids in these meteorites.

Amino acids have also been reported in Antarctic carbonaceous chondrites showing different amino acid abundances. The CM2 ALH77306 (Cronin *et al.* 1979; Holzer and Oro 1979), Yamato (Y-) 74662 (Shimoyama and Ponnamperuma 1979) and Lewis Cliff (LEW) 90500 (Botta and Bada 2002; Glavin *et al.* 2006) show an amino acid distribution and abundance similar to other non-Antarctic CM2 chondrites. However, other Antarctic CM2 chondrites, ALH83100 (Glavin *et al.* 2006), Y79331 and Belgica (B-) 7904 (Shimoyama and Harada 1984), contain lower quantities of amino acids. Last, the CM2 meteorite Y791198 has the highest concentration of amino acids (71 parts per million (ppm)) previously reported for a carbonaceous chondrite (Shimoyama *et al.* 1985; Shimoyama and Ogasawara 2002).

Several non-Antarctic carbonaceous chondrite meteorites have also been analysed for amino acids (see e.g. Botta *et al.* 2002 and references in there), namely, the CM meteorites Murchison, Murray, Nogoya, Mighei and Essebi, which contain highly variable total amino acid abundances. Amino acid concentrations range from about 15 ppm for Murchison to about 6 ppm for Mighei. The CI chondrites Orgueil and Ivuna contain a much lower amino acid content, with total amino acid abundances of about 4.3 ppm (Botta *et al.* 2002). The CV3 Allende and the unclassified Tagish Lake meteorites are found to be essentially free of amino acids. The only detectable trace amounts of amino acids that were detected are thought to be terrestrial contamination (Botta *et al.* 2002). The CR2 Renazzo meteorite is the only reported fall in the CR group. To our knowledge this meteorite is also the only CR chondrite analysed for amino acids.

Renazzo has a total amino acid abundance similar to the CI chondrites Orgueil and Ivuna (Botta *et al.* 2002).

In the present paper we analysed the amino acid content of two aqueously altered Antarctic CR chondrites: EET92042 and Graves Nunataks (GRA) 95229 (Grossman and Score 1996; Grossman 1998). A third sample, Grosvenor Mountains (GRO) 95577, is even more aqueously altered than any other CR chondrite, and is classified as the first CR1 by Weisberg and Prinz (2000). We have measured the amino acid abundances of these three meteorites by high performance liquid chromatography with UV fluorescence detection (HPLC-FD) and gas chromatography-mass spectrometry (GC-MS). Additionally, the $\delta^{13}C$ values of individual amino acids from the EET92042 and GRA95229 meteorites were obtained by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

6.2 Materials and Methods

6.2.1 Tools and chemicals

All the tools, ceramics and glassware used for sample processing were cleaned for organic contaminants by heating in aluminium foil at 500°C for 3 h. All tips and Eppendorf tubes were supplied sterilised by Sigma-Aldrich. Amino acid standards were purchased from Sigma-Aldrich, except D- and L-isovaline, which were purchased from Acros Organics. Ammonium hydroxide (28-30 wt%) was also purchased from Acros Organics. Sodium acetate trihydrate, sodium borate decahydrate, HPLC-grade water, *o*-phthaldialdehyde (OPA), N-acetyl-L-cysteine (NAC), anhydrous ethyl acetate, methylene chloride, pyrene (99%), trifluoroacetic acid anhydride (TFAA, 99%, for derivatisation), acethylchloride and isopropanol (HPLC grade) were bought from Sigma-Aldrich. Methanol (absolute HPLC) was obtained from Biosolve Ltd. Sodium hydroxide and hydrochloric acid (37%) were acquired from Boom. AG® 50W-X8 cation exchange resin (100-200 mesh) was purchased from Bio-Rad.

6.2.2 Meteorite sample preparation and amino acid extraction procedure

The Antarctic CR EET92042 was collected in the 1992 Antarctic Search for Meteorites (ANSMET) expedition, and both Antarctic CRs GRA95229 and GRO95577 in the 1995 field season. Chips of EET92042, GRA95229 and GRO95577 were provided by the Antarctic meteorite curator at the NASA Johnson Space Center, Houston. Each meteorite sample was separately crushed and homogenised into powder in a glove box with a flow of ultra high purity argon, using a ceramic mortar and pestle, and stored in sterilized glass vials. A serpentine sample provided by the Natural History Museum in Bern was grounded into powder in the same glove box, heated to 500°C for 3 h prior to being subjected to the same processing procedure as the meteorite samples and was used as a control blank.

Two separate sets of approx. 100 mg of each powdered meteorite and serpentine control blank samples were analysed using the established procedure for extracting and

analysing amino acids in meteorites (Zhao and Bada 1995; Botta *et al.* 2002; Glavin *et al.* 2006). Set 1 contained the EET92042, GRA95229 and GRO95577 meteorites, plus a procedural blank, and set 2 contained the EET92042 and GRA95229 meteorites, and additionally a procedural blank. Each of the samples, together with 1 ml of water, were flame sealed inside a test tube and heated for 24 h in a heating block set at 100°C. One of two equal parts of the water supernatants was then dried under vacuum and subjected to 6 M acid vapour hydrolysis for 3 h at 150°C. The non-hydrolysed extracts of the meteorite samples were not analysed in this study. The acid hydrolysed extracts of the samples were each brought up in 3 ml of HPLC water and then desalted on a cation exchange resin. The amino acids were eluted from the resin with 5 ml of ammonium hydroxide and the eluates were dried under vacuum. The residues were dissolved in 100 µl of water prior analysis. Aliquots of sample set 1 were derivatised with *o*-phthaldialdehyde/N-acetyl-L-cysteine (OPA/NAC) and analysed by HPLC-FD (Zhao and Bada 1995) or derivatised with trifluoroacetic anhydride (TFAA)/isopropanol and analysed by GC-MS (based on the method by Pizzarello *et al.* (2004)). Sample set 2 was derivatised with (TFAA)/isopropanol and analysed by GC-C-IRMS (based on the method by Pizzarello et al. (2004)).

6.2.3 HPLC-FD analysis

Separation by HPLC-FD of the OPA/NAC-derivatised amino acids (Zhao and Bada 1995) was achieved in a C18 reverse phase (250 x 4.6 mm) Synergi 4µ Hydro-RP 80A column (from Phenomenex) kept at room temperature, elution at 1 ml min^{-1}, using 50 mM sodium acetate (4% methanol (v/v)) as buffer A, and methanol as buffer B. The gradient was 0 to 4 min, 0% buffer B; 4 to 5 min, 0 to 20% buffer B; 5 to 10 min, 20% buffer B; 10 to 17 min, 20 to 30% buffer B; 17 to 27 min, 30 to 50% buffer B; 27 to 37 min, 60% buffer B; 37 to 49 min, 60% buffer B; 49 to 50 min, 60 to 0% buffer B; 50 to 60 min, 0% buffer B. UV fluorescence detection was performed on a Shimadzu RF-$10A_{XL}$ (excitation wavelength at 340 nm and emission at 450 nm). Amino acids were identified by retention time comparison with known standards, and amino acid abundances (part per billion (ppb) by weight) calculated by comparison to the integrated peak area of each sample, corrected for the abundances in the serpentine blank sample, with the integrated peak area of known amino acid standards. The HPLC-FD detection limits were ~0.1 fmol.

6.2.4 GC-MS analysis

An aliquot of each meteorite extract was separately placed in 1 ml conical vials. The vials were placed under a stream of dry N_2 (60-80 ml min^{-1}) to evaporate water. For esterification, 100 µl of acetylchloride: isopropanol mixture (30:70 v/v) was added and the vials tightly capped with a Teflon-lined screw caps. Samples were placed in standard heating blocks for 1 h at 110°C. After cooling to room temperature the reagents excess was evaporated under the stream of dry N_2. 100 µl of methylene chloride and 50 µl of TFAA were added. The vials were tightly capped and heated at 100°C for 10 min. After the vials had cooled to room temperature, the excess of reagents was removed under the stream of dry N_2. Finally, the derivatised samples were dissolved in 55 µl of ethyl

acetate containing 18.3 ng μl^{-1} of pyrene, which was used as the external standard. 1 µl of sample was injected into the gas chromatography/flame ionisation detector/mass spectrometry (GC/FID/MS). GC-MS analyses were performed using a Varian Model GC-3800/FID/Ion-Trap Mass Spectrometer-Saturn 2000 equipped with an Electronic Pressure Control (EPC) system, and an autosampler Model 8200 (Varian). Injections of sample were performed using the autosampler programmed with a solvent flush sampling and a solvent plug of 0.2 µl, upper and lower air gaps, an injection rate of 0.2 $\mu l\ s^{-1}$ and a vial needle depth of 90%.

Separation of the D, L-amino acid enantiomers was achieved using a Helifex Chirasil-Val column (50 m x 0.25 mm ID x 16 µm film thickness) from Alltech. The end of the column was mounted into a Valco TEE connector, which splits the sample via transfer lines of 0.4 m x 0.1 mm ID and 1.6 m x 0.32 mm ID to the MS and FID, respectively. A very good alignment of corresponding peaks between the FID and the MS chromatograms, with a constant 0.08 min offset, was obtained. Helium was used as carrier gas with a flow of 2.3 ml min^{-1}. The injection port was set at 220ºC. The oven program was held for 5 min at 70ºC, increased by 2ºC min^{-1} to 100ºC, then increased to 200ºC by 4ºC min^{-1} and held for 30 min, and finally increased by 10ºC min^{-1} to 225ºC and hold for 5 min. Amino acids present in the meteorite samples were identified by comparison of the retention time and mass fragmentation pattern with known amino acid standard mixtures.

6.2.5 GC-C-IRMS analysis

The EET92042 and GRA 95229 meteorite extracts (set 2) were derivatised separately using TFAA/isopropanol, and generally carried through the same procedure as described for the GC-MS analysis. The only differences were in the volumes of reagent used, that is, in the esterification step 500 µl of acetylchloride: isopropanol mixture were added to the samples, and on the next step 500 µl of methylene chloride and 500 µl of TFAA were used. Additionally, the Chirasil-Val column had the dimensions of 50 m x 0.32 mm ID (0.2 µm film thickness), and helium was used at a constant pressure of 15 PSI. Carrier gas and temperature program were the same as the GC-MS analysis. Amino acids were separated by the GC column, and then oxidised to CO_2 through the oxidation oven maintained at 980ºC. A Thermo Finnigan MAT Delta Plus-XL GC-C-IRMS was used to perform the carbon isotope analyses. CO_2 reference gas ($\delta^{13}C$ value of -41.10‰ PDB) was injected via the interface to the IRMS for the computation of $\delta^{13}C$ values of samples. Mixtures of amino acid standards were subjected to the entire TFAA/isopropanol derivatisation procedure described before. The mixtures were run daily on the GC-C-IRMS, with typical standard deviation of ±0.99‰.

Carbon isotopic values were obtained by mass balance by measuring a set of standards (O'Brien *et al.* 2002): $\delta^{13}C$ amino acid standard derivatised = (% of carbon amino acid) (EA amino acid standard) + (% of carbon TFAA/isopropanol) ($\delta^{13}C$ TFAA/isopropanol), where the EA amino acid standard value is the $\delta^{13}C$ value of the amino acid standard established by a Carlo Erba elemental analyser (EA)-IRMS. Finally, the $\delta^{13}C$ values of

the amino acids present in the meteorite samples were obtained by correcting for carbon added from the TFAA/isopropanol, and were calculated by mass balance: $\delta^{13}C$ amino acid in sample derivatised = (% of carbon in amino acid) ($\delta^{13}C$ amino acid in sample) + (% of carbon in TFAA/isopropanol) ($\delta^{13}C$ TFAA/isopropanol).

6.3 Results and Discussion

Fig. 6.1 displays typical HPLC-FD chromatograms of the acid hydrolysed, hot-water extracts of the Antarctic CR meteorites plus a serpentine blank. The most abundant amino acids in the EET92042 meteorite are α-aminosobutyric acid (α-AIB), glycine, D-alanine, L-alanine, L-isovaline, and D-isovaline, whereas in the GRA95229 meteorite the most abundant amino acids are glycine, D-alanine, L-alanine, α-AIB, L-isovaline, and D-isovaline. Lower levels of valine, glutamic acid, β-amino-*n*-butyric acid (β-ABA), β-alanine, γ-amino-*n*-butyric acid (γ-ABA), β–aminosobutyric acid (β-AIB) and aspartic acid were also present in both meteorites (Table 6.1).

The GRO95577 meteorite had the lowest concentration of amino acids, with values ranging from 8 ppb to 136 ppb (Table 6.1). A possible reason for the low amino acid concentration might be leaching of amino acids from GRO95577 during its residence time in Antarctica. Alternatively, during the more extensive aqueous alteration experienced by GRO95577 on its parent body decomposition of the amino acids might have taken place. A third possibility is that GRO95577 originated on a parent body in which amino acid formation was not active, or where amino acid precursors were depleted.

We further analysed the three Antarctic CRs for amino acids using GC-MS in order to detect amino acids by their characteristic mass fragmentation patterns. The amino acid contents of the GRO95577 meteorite were below the GC-MS detection limits (~1 pmol). Fig. 6.2 shows a typical ion chromatogram of the acid hydrolysed, hot-water extracts of the EET92042 and GRA95229 meteorites. All the detected amino acids and corresponding abundances are given in Table 6.2. The GC-MS analysis confirmed the results obtained by HPLC-FD, with values generally agreeing within the associated errors, or at least in the same order of magnitude. The most abundant amino acids for both CR2 chondrites matched those determined by HPLC-FD. EET92042 and GRA95229 meteorites have relatively high amino acid contents in comparison with other meteorites (Table 6.1 and 6.2). They are in fact the most amino acid-rich carbonaceous chondrites ever reported. The total amino acid abundances of these CR2 chondrites, 180 ppm and 249 ppm, respectively for EET92042 and GRA95229 (Table 6.1) are at least a factor 10 higher than other primitive chondrites, such as the CM2s Murchison and Murray (e.g. Ehrenfreund *et al.* 2001).

Renazzo, a non-Antarctic CR2 and the only CR chondrite previously analysed for amino acids, has a total amino acid concentration of only 4.8 ppm (Botta *et al.* 2002), much lower than the Antarctic CR2s analysed here. Additionally, the CR2 Renazzo has a distinct amino acid distribution, with γ-ABA (1092 ppb), glycine (875 ppb) and L-glutamic acid (856 ppb) as the most abundant amino acids (Botta *et al.* 2002). Only

upper limits for alanine and α-AIB concentrations were reported for this meteorite, while isovaline was tentatively identified. The Renazzo meteorite was quickly recovered after its fall. Therefore it is highly unlikely that this meteorite lost its amino acid content as a result of weathering.

The differences observed between the CR2 Renazzo and the Antarctic CR2s analysed in this paper indicate different parent bodies for these meteorites. Amino acid synthesis may have been less active on the Renazzo parent body due to low pH and a lack of α-amino acid precursor compounds (namely, low ammonia concentration), leading predominantly to the formation of hydroxycarboxylic acids. Degradation or removal of the amino acids as a result of a higher degree of aqueous alteration might be another explanation for the differences among CR meteorites. Renazzo was shown to be generally more aqueously altered when compared to Antarctic CR meteorites (Weisberg *et al.* 1993). As referred by Glavin *et al.* (2006) the relative abundance of β-alanine (relative to glycine) appears to be generally higher in meteorites that have experienced more extensive aqueous alteration, while the relative abundance of α-AIB in these meteorites is lower than in the less aqueous altered meteorites. In Renazzo (Botta *et al.* 2002) the relative abundance of β-alanine (0.25) is higher than in EET92042 and GRA95229 (respectively 0.11 and 0.05; Table 6.1). Also, the relative abundance of α-AIB (Botta *et al.* 2002) in Renazzo is lower (<0.08) than in EET92042 and GRA95229 (respectively 2.15 and 0.48; Table 6.1). These results support the aqueous alteration hypothesis.

The amino acid concentrations of EET92042 and GRA95229 are only comparable to the total amino acid abundance of the Antarctic CM2 Y791198, but are still at least a factor of 2.5 higher (Shimoyama and Ogasawara 2002). If the amino acids detected in EET92042 and GRA95229 (or their precursors) were formed prior to accretion of the meteorite parent body (or bodies), they would not survive chondrule and CAI formation. Amino acids must therefore have entered the meteorite matrix at a later stage. If this was the case, then the appropriate comparison should be between amino acid matrix-normalised abundances. As CMs contain more than 50 vol% matrix (McSween 1979) and CRs about 30 vol% matrix (Weisberg *et al.* 1993), this would imply that the amino acid matrix-normalised concentrations would be even higher in EET92042 and GRA95229 than in CMs. Fig. 6.3 displays the relative amino acid abundances (glycine = 1) for Y791198 (Shimoyama *et al.* 1985; Shimoyama and Ogasawara 2002), EET92042 and GRA95229. Except for alanine the amino acid distribution in Y791198 looks similar to the two Antarctic CRs. This may indicate that these three meteorites had similar amino acid precursor material available.

We used three approaches to determine whether the amino acids, present in the Antarctic CRs EET92042, GRA95229 and GRO95577 are terrestrial or extraterrestrial.

1) The presence of amino acids that are atypical in terrestrial proteins - The amino acids α-AIB, isovaline, β-ABA and β-AIB were detected in the EET92042 and GRA95229 meteorites using HPLC-FD (Table 6.1) and GC-MS (Table 6.2). The GRO95577 meteorite also contained α-AIB, isovaline, β-ABA and β-AIB, but in low abundances

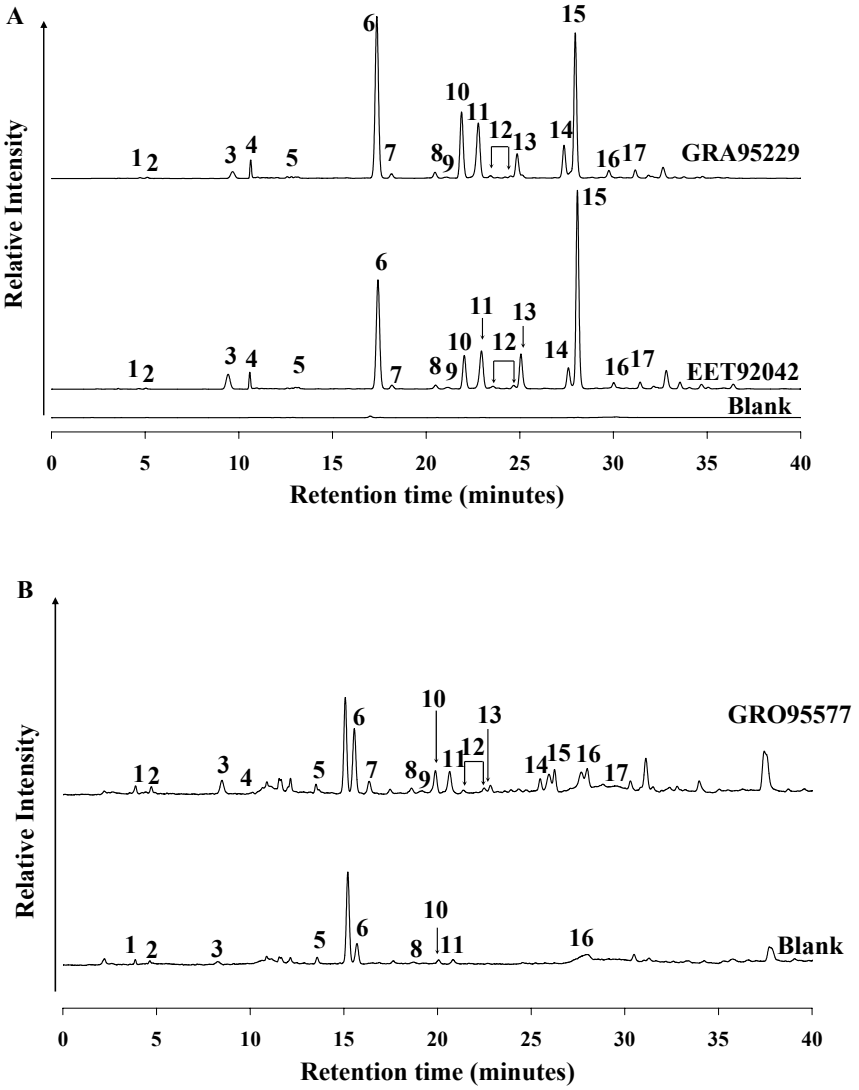

Fig. 6.1 - The 0 to 40 min region (no peaks were observed outside this region) of the HPLC-FD chromatograms. OPA/NAC derivatisation of amino acids in the 6 M HCl-hydrolysed hot-water extracts from (A) the CR2 carbonaceous chondrite EET92042 and GRA95229, and the serpentine blank; (B) the CR1 carbonaceous chondrite GRO95577 and corresponding serpentine blank. HPLC-FD chromatograms (A) and (B) are not on the same scale. Peaks were identified by comparison of the retention time to those in the amino acid standard run on the same day: 1. D-aspartic acid; 2. L-aspartic acid; 3. L-glutamic acid; 4. D-glutamic acid; 5. D, L-serine; 6. glycine; 7. β-Alanine; 8. γ-ABA; 9. D, L-β-AIB; 10. D-alanine; 11. L-alanine; 12. D, L-β-ABA; 13. α-AIB; 14. D-isovaline; 15. L-isovaline; 16. L-valine; 17. D-valine.

Table 6.1 - Summary of the average total amino acid abundances (in ppb) in the 6 M HCl acid hydrolysed hot-water extracts of the EET92042, GRA95229 and GRO95577 meteorites measured by HPLC-FD*.

Amino Acid	CR2 EET92042	CR2 GRA95229	CR1 GRO95577
D-Aspartic Acid	467 ± 71	669 ± 7	13 ± 2
L-Aspartic Acid	524 ± 76	696 ± 9	19 ± 4
L-Glutamic Acid	3989 ± 97	3668 ± 319	40 ± 3
D-Glutamic Acid	2309 ± 339	3005 ± 86	16 ± 6
D,L-Serine†	742 ± 42	1807 ± 84	50 ± 11
Glycine	26875 ± 1176	57796 ± 358	136 ± 14
β-Alanine	3005 ± 95	2910 ± 277	122 ± 6
γ-ABA	1975 ± 176	2848 ± 146	54 ± 6
DL-β-AIB†‡	1526 ± 88	1645 ± 61	30 ± 2
D-Alanine	23862 ± 324	50722 ± 419	74 ± 22
L-Alanine	23215 ± 609	50681 ± 2884	96 ± 20
DL-β-ABA‡	3094 ± 149	5986 ± 83	49 ± 5
α-AIB	57856 ± 2030	27679 ± 1113	48 ± 3
D-Isovaline	7640 ± 37	8924 ± 605	58 ± 15
L-Isovaline	15158 ± 977	18920 ± 2408	73 ± 8
L-Valine	3632 ± 60	6053 ± 150	13 ± 4
D-Valine	3665 ± 92	5736 ± 205	8 ± 3
Total	**180000**	**249000**	**900**

*Quantification of the amino acids included background level correction using a serpentine blank. The associated errors are based on the standard deviation of the average value between five separate measurements (N) with a standard error, $\delta x = \sigma_x \cdot N^{-1/2}$.

†Enantiomers could not be separated under the chromatographic conditions.

‡Optically pure standard not available for enantiomeric identification.

(Table 6.1). These amino acids have also been detected in the CM2 Antarctic meteorites ALH77306 (Cronin *et al.* 1979; Kotra *et al.* 1979), Y74662 (Shimoyama and Ponnamperuma 1979), LEW90500 (Botta and Bada 2002; Glavin *et al.* 2006), ALH83100 (Glavin *et al.* 2006) and Y791198 (Shimoyama *et al.* 1985; Shimoyama and Ogasawara 2002). Except for Y791198, all these amino acids were present in much lower abundances than in EET92042 and GRA95229, with concentrations usually on the order of ~100 ppb (Cronin *et al.* 1979; Kotra *et al.* 1979; Botta and Bada 2002; Glavin *et al.* 2006). LEW90500 (Glavin *et al.* 2006) contained a slightly higher abundance of α-AIB (2706 ppb) and isovaline (1306 ppb). Y791198 (Shimoyama and Ogasawara 2002) contained similar abundances of α-AIB (22630 ppb), β-ABA (< 3250 ppb) and β-AIB (1835 ppb) as EET92042 and GRA95229, but lower abundance of isovaline (4075 ppb).

The potential for contamination from the surrounding environment includes ice and microbial biomass, thus it is important for us to consider these sources. To our knowledge, ice from the Elephant Moraine (EET), Graves Nunataks (GRA) or Grosvenor Mountains (GRO) Antarctic regions was not analysed for amino acids. However, amino acid analyses of Allan Hills (McDonald and Bada 1995; Bada *et al.* 1998) and La Paz Antarctic ices (Glavin *et al.* 2006) showed similar distributions, with

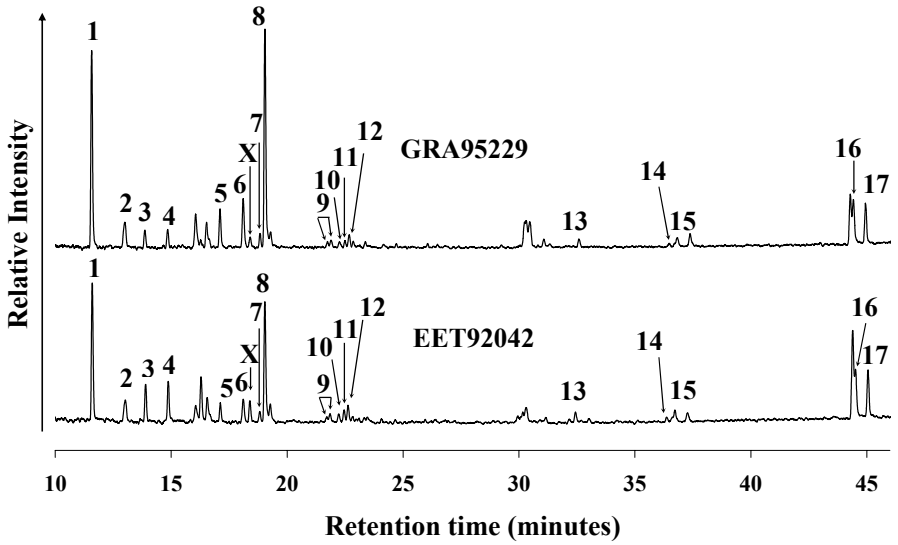

Fig. 6.2 - GC-MS analysis of the derivatised (*N*-TFA, *O*-isopropyl) EET92042 and GRA95229 HCl-hydrolysed hot-water extracts, *m/z* 69, 126, 138, 140, 154, 168, 180, 182, and 184 single ion trace. The peaks were identified by comparison of the retention time and mass fragmentation pattern to those in the amino acid standard run on the same day: 1. α-AIB; 2. isovaline; 3. D-alanine; 4. L-alanine; 5. D-α-ABA; 6. L-α-ABA+D-Valine; X. N-methylated acid; 7. L-valine; 8.glycine; 9. β-AIB; 10. β-Alanine; 11. D-β-ABA; 12. L-β-ABA; 13. γ-ABA; 14. D-aspartic acid; 15. L-aspartic acid; 16. D-glutamic acid; 17. L-glutamic acid.

trace levels of aspartic acid, serine, glycine and alanine (1 ppb of total amino acid concentration). No isovaline, β-ABA or β-AIB was detected above detection limits. Only an upper limit of α-AIB (<2 parts-per-trillion (ppt)) was detected in the Allan Hills ice (Bada *et al.* 1998), while a relatively high abundance (46 ppt) of α-AIB was detected in a La Paz Antarctic ice sample (Glavin *et al.* 2006). However, these concentrations are 10^6 times lower than the α-AIB values found in the EET92042 and GRA95229 meteorites, and 10^3 times lower than values measured for GRO95577. Most likely the Antarctic ice was not the source of α-AIB, isovaline, β-ABA and β-AIB detected in EET92042, GRA95229 and GRO95577.

2) D/L enantiomeric ratios - The amino acid enantiomeric ratios (Table 6.3) for both protein and non-protein amino acids in EET92042 and GRA95229 are nearly racemic (D/L ~ 1), indicating either an abiotic synthetic origin or an advanced age or thermal history. The only exceptions are isovaline and glutamic acid, which showed L-enantiomeric excesses (see detailed discussion later). In the case of glutamic acid, the D/L in EET92042 (0.58 measured by HPLC-FD and 0.69 by GC-MS) and in GRA95229 (0.82 measured by HPLC-FD and 0.83 by GC-MS) can be explained by terrestrial L-glutamic acid contamination of the meteorites during their residence time on Earth. Biologically derived glutamic acid is principally in the L-form therefore any addition of terrestrial glutamic acid would decrease the D/L. The amino acid enantiomeric ratios for the GRO95577 meteorite are all smaller than 0.8 (Table 6.3), which is an indication of significant terrestrial contamination.

Table 6.2 - Summary of the average total amino acid abundances (in ppb) in the 6 M HCl acid hydrolysed hot-water extracts of the EET92042, GRA95229 and GRO95577 meteorites measured by GC-MS*.

Amino Acid	EET92042	GRA95229
D-Aspartic Acid	409 ± 41	551 ± 75
L-Aspartic Acid	465 ± 24	576 ± 51
L-Glutamic Acid	4468 ± 503	4209 ± 415
D-Glutamic Acid	3090 ± 422	3489 ± 389
Glycine	24975 ± 608	40496 ± 1028
β-Alanine	3046 ± 50	3143 ± 495
γ-ABA	1512 ± 66	1914 ± 398
DL-β-AIB‡	1429 ± 333	2091 ± 405
D-Alanine	21664 ± 1009	52465 ± 6860
L-Alanine	22297 ± 1583	51141 ± 6272
D-β-ABA	1327 ± 33	3903 ± 377
L-β-ABA	1458 ± 99	4239 ± 494
α-AIB	50210 ± 870	30257 ± 1226
D,L-Isovaline†	22806 ± 459	29245 ± 2229
L-Valine	2084 ± 129	6996 ± 700
D-Valine	1969 ± 255	7154 ± 788
D-α-ABA	1123 ± 54	2956 ± 125
L-α-ABA	1244 ± 28	2955 ± 120
Total	**165000**	**247300**

*Quantification of the amino acids included background level correction using a serpentine blank.
†Enantiomers could not be separated under the chromatographic conditions.
‡Optically pure standard not available for enantiomeric identification.

3) Compound-specific carbon isotopic measurements - We have focused our carbon isotope measurements on the most abundant amino acids present in the EET92042 and GRA95229 meteorites, which were the α-amino acids including glycine, alanine, α-AIB, and isovaline. We also analysed common biological amino acids, glutamic and aspartic acids, because these could be terrestrial contaminants. Carbon isotopic compositions for GRO95577 amino acids were below the detection limits (~ 1 pmol).

Stable carbon isotope values of α-amino acids present in the EET92042 meteorite ranged from +31.8‰ for glycine to +49.9‰ for L-alanine, while in the GRA95229 meteorite values ranged from +31.6‰ for α-AIB to +50.5‰ for isovaline (Fig. 6.4 and Table 6.4). These $\delta^{13}C$ values are clearly outside the terrestrial range (from -70.47‰ to +11.25‰) (Scott *et al.* 2006) and agree with the $\delta^{13}C$ values of the same α-amino acids (glycine, alanine, α-AIB, and isovaline) measured by other authors in the CM2 chondrite Murchison (Pizzarello *et al.* 2004). The similarity in $\delta^{13}C$ values may indicate a common

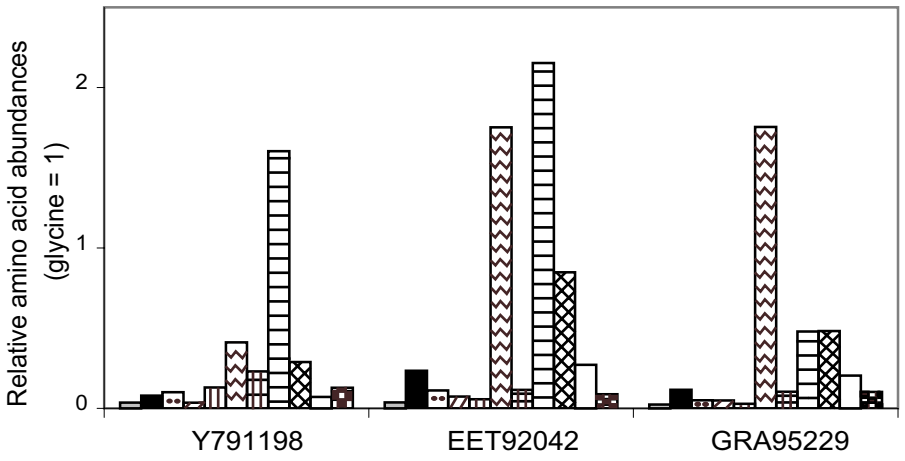

Fig. 6.3 - Relative (glycine = 1) amino acid abundances for the amino acids aspartic acid (grey), glutamic acid (black), β-alanine (black dots), γ-ABA (diagonal lines), β-AIB (vertical lines), alanine (waves), β-ABA (squares), α-AIB (horizontal lines), isovaline (diamonds), valine (white), α-ABA (black and white squares) in the CM2 Y791198 (data taken from Shimoyama *et al.* 1985; Shimoyama and Ogasawara 2002), and Antarctic CR2s (data taken from Table 6.1 and 6.2).

reservoir (interstellar and/or protosolar) for the amino acid precursors in the CR2 and CM2 meteorites.

EET92042 shows $\delta^{13}C$ values for the L- and D-enantiomers of alanine that are similar (+49.9‰ and +44.5±2.0‰, respectively), which is in agreement with the D/L alanine ratio of ~1 seen before (Table 6.3). In the case of GRA95229, L-alanine and D-alanine have also high and identical $\delta^{13}C$ values (+40.9 ± 6.2‰ and +41.7 ± 2.4‰, respectively) within the associated errors, indicating that unless terrestrial contamination was limited to very specific peptides with equal amounts of D- and L-alanine, terrestrial contamination was minimal.

The carbon isotopic analysis of the glutamic acid showed that both meteorites have substantially lower $\delta^{13}C$ values for the L-enantiomer, even falling into the negative range (-19.5 ± 1.7‰ and -17.6 ± 1.9‰, respectively for EET92042 and GRA95229), while the D-enantiomer is rich in ^{13}C (+46.1 ± 2.1‰ and +47.2‰, respectively for EET92042 and GRA95229). This is consistent with the L-enantiomeric excess being due to terrestrial contamination described previously.

EET92042 has a $\delta^{13}C$ value for aspartic acid that is slightly lower for the L-enantiomer, while GRA95229 has $\delta^{13}C$ values for the aspartic acid enantiomers that are equivalent within the associated errors (Table 6.4). The stable carbon isotopic analysis showed that, except for L-glutamic acid, all the amino acids analysed in this study and present in the EET92042 and GRA95229 meteorites are highly enriched in ^{13}C, suggesting an extraterrestrial origin for the carbon in these compounds.

Table 6.3 - Amino acid enantiomeric ratios (D/L) in the CR carbonaceous chondrite EET92042, GRA95229 and GRO95577*.

Amino Acids	CR2		CR2		CR1
	EET92042†	EET92042‡	GRA95229†	GRA95229‡	GRO95577†
Aspartic acid	0.89 ± 0.19	0.88 ± 0.10	0.96 ± 0.02	0.96 ± 0.16	0.68 ± 0.18
Glutamic acid	0.58 ± 0.09	0.69 ± 0.12	0.82 ± 0.08	0.83 ± 0.12	0.40 ± 0.15
Alanine	1.03 ± 0.03	0.97 ± 0.08	1.00 ± 0.06	1.03 ± 0.18	0.77 ± 0.28
β-ABA§	¶	0.91 ± 0.07	¶	0.92 ± 0.14	¶
Isovaline§	0.50 ± 0.03	¶	0.47 ± 0.07	¶	0.79 ± 0.22
Valine	1.01 ± 0.03	0.94 ± 0.14	0.95 ± 0.04	1.02 ± 0.15	0.62 ± 0.30
α-ABA	¶	0.90 ± 0.05	¶	1.00 ± 0.06	¶

*The uncertainties are based on the absolute errors shown respectively in Table 6.1 and Table 6.2, and are obtained by standard propagation calculation.
†D/L ratios calculated from the concentrations reported in Table 6.1, measured by HPLC-FD.
‡D/L ratios calculated from the concentrations reported in Table 6.2, measured by GC-MS.
§Non-proteic amino acid.
¶Not determined, because enantiomeric separation was not possible or amino acid abundance was not determined.

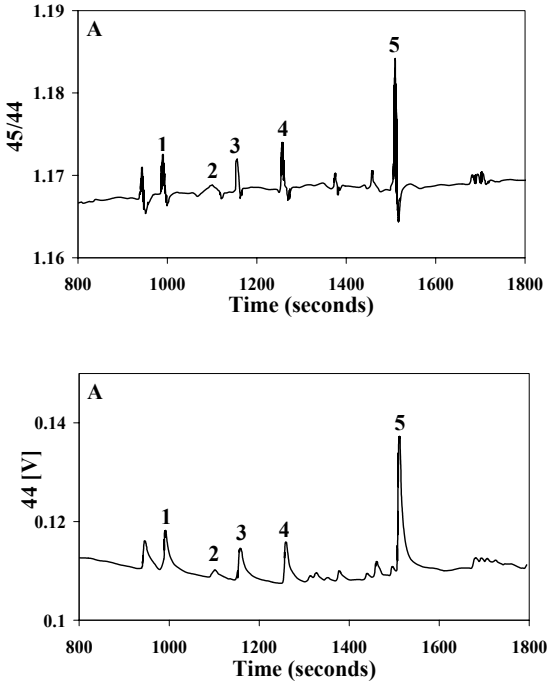

Fig. 6.4 - (A) Typical GC-C-IRMS chromatogram obtained in this study. *m/z* 44 trace (bottom) and ratio between the *m/z* 45 and *m/z* 44 trace (top) for the GC-C-IRMS analysis of a portion of the GRA95229 HCl-hydrolysed hot-water extract containing the α-amino acids 1. α-AIB; 2. isovaline; 3. D-alanine; 4. L-alanine; 5. glycine.

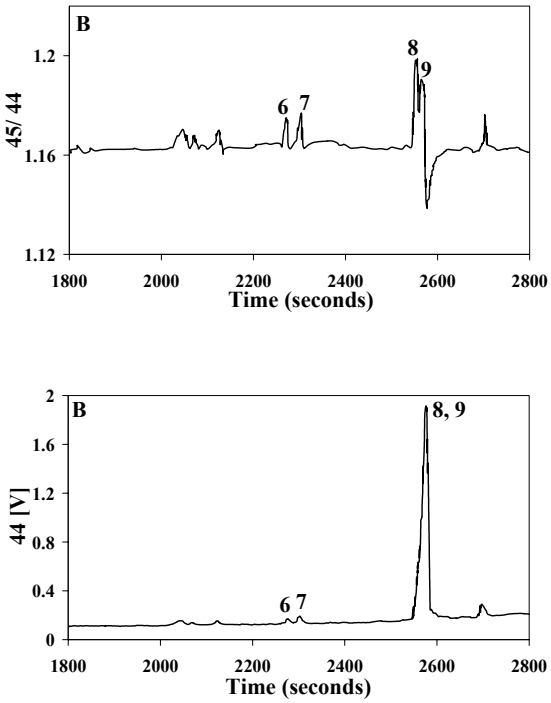

Fig. 6.4 - (B) Typical GC-C-IRMS chromatogram obtained in this study. *m/z* 44 trace (bottom) and ratio between the *m/z* 45 and *m/z* 44 trace (top) for the GC-C-IRMS analysis of a portion of the GRA95229 HCl-hydrolysed hot-water extract containing the following amino acids: 6. D-aspartic acid; 7. L-aspartic acid; 8. D-glutamic acid; 9. L-glutamic acid.

Table 6.4 - Summary of the $\delta^{13}C$ values (‰) of amino acids in the EET92041 and GRA95229 meteorites*.

Amino Acid	EET92042	GRA95229
D-Aspartic Acid	+34.4 ± 4.1	+34.9 ± 0.5
L-Aspartic Acid	+23.4 ± 0.7	+33.0 ± 3.1
L-Glutamic Acid	-19.5 ± 1.7	-17.6 ± 1.9
D-Glutamic Acid	+46.1 ± 2.1	+47.2‡
Glycine	+31.8 ± 2.0	+33.8 ± 1.6
D-Alanine	+44.5 ± 2.0	+41.7 ± 2.4
L-Alanine	+49.9‡	+40.9 ± 6.2
α-AIB	§	+31.6 ± 6.1
Isovaline†	§	+50.5‡

*The associated errors are based on the standard deviation of the average value between three and five separate measurements (N) with a standard error, $\delta x = \sigma_x \cdot N^{-1/2}$.
†Enantiomers could not be separated under the chromatographic conditions.
‡Average of two repeated analyses.
§Not determined.

6.3.1 Formation of α-meteoritic amino acids

The EET92042 and GRA95229 meteorites are the most amino acid-rich carbonaceous chondrites reported to date. Racemic enantiomeric ratios, as well as the highly enriched $\delta^{13}C$ values, indicate primitive indigenous organic matter. These findings are supported by Busemann *et al.* (2006), who reported D and ^{15}N hotspots in EET92042 insoluble macromolecular organic matter, showing that primitive organic matter was preserved in this meteorite. Both meteorites have amino acid distributions, total amino concentrations, D/L enantiomeric ratios, and also carbon isotope values for individual amino acids that are very similar, suggesting that both meteorites originated from the same, or similar, parent body. Furthermore, these meteorites may have formed by the same mechanism from similar starting materials in the protoplanetary nebula.

The high α-amino acid content (Table 6.1 and Table 6.2) is suggestive of a two-step formation process for these amino acids (Cronin *et al.* 1995), in which the amino acid precursors (aldehydes, ketones, ammonia and HCN) were present (or formed) in the protosolar nebula, and later incorporated into the asteroidal parent body. During aqueous alteration on the parent body, Strecker-cyanohydrin synthesis would have taken place to form the α-amino acids (Peltzer *et al.* 1984; Lerner *et al.* 1993; Ehrenfreund *et al.* 2001). Since the carbonyl precursors (aldehydes and ketones) are thought to be synthesised by addition of a one-carbon donor to the growing alkane chain, a decrease of the α-amino acid abundances (e.g. glycine > alanine > α-ABA) with increasing chain length would be expected (see e.g. Cronin and Pizzarello 1983). This trend was observed for example in the CM2 Murchison (see e.g. Cronin and Pizzarello 1983).

In the case of the EET92042 and GRA95229 meteorites, an exceptionally high alanine concentration is found, which does not follow the excepted trend. The high alanine abundances suggest a high abundance of the precursor acetaldehyde on the parent body (or bodies) of these meteorites. Also, synthesis of branched carbon chain analogues is thought to be favoured over straight carbon chain analogues (see e.g. Cronin and Pizzarello 1983). This trend is observed in the EET92042 and GRA95229 meteorites. For example, the abundance of α-AIB in both these meteorites is higher than the analogue α-ABA (Table 6.1 and Table 6.2).

The large L-enantiomeric excess observed for isovaline measured by HPLC-FD (Table 6.1 and Table 6.3), 33.0% and 35.9% for EET92042 and GRA95229 respectively, cannot be explained by this two-step formation process. Therefore, it is important to rule out any potential sources of terrestrial contamination. Isovaline is atypical in the terrestrial biosphere, but it may occur in bacteria and fungal peptides in the D-configuration (e.g. Keller *et al.* 1990). Therefore, microbes present in the EET92042 and GRA95229 meteorites would reduce the isovaline L-excess and subsequently the carbon isotope value. Fungi have been detected in Antarctica; however, compound specific isotopic analyses of endolithic communities did not contain appreciable quantities of atypical amino acid compositions (Scott *et al.* 2006). As seen before, GRA95229 has a $\delta^{13}C$ value of +50.5‰ for isovaline (Table 6.4), which is well outside the terrestrial range. The D- and L-isovaline enantiomers were not separated with the GC-MS column used in

this study, and therefore we do not have $\delta^{13}C$ values for each of the enantiomers. However, using HPLC-FD we are able to separate D- and L-isovaline (Table 6.1; Fig. 6.1). The total isovaline abundance obtained by HPLC-FD for the GRA95229 meteorite (27844 ± 2482 ppb) is perfectly consistent at >99% confidence with the isovaline abundance obtained by GC-MS (29245 ± 2229 ppb). This, together with the high $\delta^{13}C$ value (+50.5‰) of isovaline and the fact that only contamination of the D-configuration could occur (e.g. Keller *et al.* 1990) provides extremely compelling evidence of an L-isovaline enantiomeric excess.

A possible reason for the observed enantiomeric excess of isovaline (Table 6.1 and Table 6.3) could be chiral selection due to the adsorption of the amino acids on mineral surfaces. Hazen *et al.* (2001) showed that the non-chiral crystal calcite exhibited significant enantiomeric selection when immersed in an aqueous solution of racemic amino acids. The results of Hazen *et al.* (2001) may indicate that the enantiomeric excess in meteoritic amino acids could reflect the association of amino acids with the minerals present in the matrix of the meteorites. Pizzarello *et al.* (2003) showed that there was a good relationship between the abundances of isovaline and of the hydrous silicate mineral serpentine present in the same powder samples of the CM2 Murchison (~0.5 g each sample). Their results suggest that the meteoritic amino acid L-excess may have been caused by interaction with the meteorite minerals. The matrix of EET92042 is dominated by phyllosilicates (serpentine and saponite) rather than anhydrous minerals (Abreu and Brearley 2005). Therefore, L-isovaline excess would increase with increasing aqueous alteration. However, our results show (Table 6.3) that the L-isovaline excess is higher on the least aqueously altered CRs (EET92042 and GRA95229), than in the more aqueously altered CR (GRO95577).

It is interesting to note that isovaline is the only α-amino acid that shows an L-enantiomer excess, and that all other indigenous amino acids present in the EET92042 and GRA95229 meteorites are racemic (Table 6.3), except for a small terrestrial contamination from L-glutamic acid. The meteoritic enantiomeric excess of α-methyl-α-amino acids (such as isovaline) and the absence for the α-H-α-amino acids (such as valine) could be explained by the resistance to racemisation of α-methyl-α-amino acids during aqueous alteration due to their lack of an α-hydrogen (Pollock *et al.* 1975; Cronin and Pizzarello 1999; Pizzarello and Cronin 2000). Considering that the CRs are most primitive carbonaceous chondrites (see e.g. Cody and Alexander 2005), and that isovaline cannot undergo secondary racemisation (Pollock *et al.* 1975), the isovaline D/L ratio in the EET92042 and GRA95229 meteorites might represent the original D/L ratio for primitive carbonaceous chondrites. Another explanation could be a different amino acid formation process, namely presolar formation of the α-methyl-α-amino acids and subsequent incorporation into the parent body, followed by parent body formation of the α-H-α-amino acids (Cronin and Pizzarello 1999; Pizzarello and Cronin 2000).

Previous analyses (Cronin and Pizzarello 1997; Cronin and Pizzarello 1999; Pizzarello and Cronin 2000; Pizzarello *et al.* 2003) have also shown an L-enantiomeric excess of isovaline. In samples of the CM2 Murchison an isovaline L-enantiomeric excess of from 0 to 15.2% (Pizzarello *et al.* 2003) was determined, with significant variations between

meteorite stones and even within the same meteorite stone. A comparison of these values to the ones obtained in this paper by HPLC-FD, clearly show that EET92042 (33.0%) and GRA95229 (35.9%) have a significantly higher L-enantiomeric excess of isovaline. If we take into account the L-enantiomeric excess of isovaline in the GRO95577 meteorite (11.4%; Table 6.1), then the data suggest a possible correlation between aqueous alteration and isovaline L-enantiomeric excess. Therefore, a high L-enantiomeric excess could reflect a lower degree of aqueous alteration (e.g. EET92042 and GRA95229).

6.4 Conclusion

We have analysed the amino acid content of three Antarctic CR meteorites, EET92042, GRA95229 and GRO95577. The total amino acid abundances in the CR2 chondrites EET92042 and GRA95229 were found to be the highest ever detected in any meteorite. This could be the result of CR chondrites being the most primitive and least aqueously altered meteorites. Compared to these two meteorites, the CR1 GRO95577 is depleted in amino acids. The CR2 meteorites EET92042 and GRA95229 have similar amino acid distribution to the CM2 Y791198. This fact, together with similar carbon isotope values for the amino acids present in the Antarctic CR2s and the CM2 Murchison may indicate a common reservoir in the interstellar medium and/or protosolar nebula for the amino acid precursors in both CR2s and CM2s.

The racemic enantiomeric ratios and the high $\delta^{13}C$ values determined for nearly all the amino acids present in the EET92042 and GRA95229 meteorites indicate that the compounds have a primarily extraterrestrial origin. Isovaline and glutamic acid, however, showed L-enantiomeric excess. In the case of glutamic acid, terrestrial contamination is indicated by the much lighter $\delta^{13}C$ values of the L-enantiomers. For isovaline, the origin of the large L-enantiomeric excess is puzzling and intriguing. An L-enantiomeric excess for isovaline of more than 30% has never been measured before in a meteorite. According to our results this L-enantiomeric excess seems to be anti-correlated with aqueous alteration. The rich amino acid content observed in the EET92042 and GRA95229 meteorites, as well as the L-enantiomeric excess of isovaline make these Antarctic CR chondrites the most scientifically valuable of the carbonaceous meteorites. Further investigation of their carbonaceous inventory may help to reveal the processes which occurred in the early solar system that formed abundant organic prebiotic material.

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