

However, appropriate corrections were made from runs made without plants. Although most of the  $^{15}\text{N}$  derived from  $^{15}\text{NO}_2$  was found in the tops, 3 to 5 percent was translocated to the roots. Figure 1 shows a linear correlation between  $\text{NO}_2$  concentration and  $^{15}\text{N}$  accumulation in snap bean. This demonstrates the first-order nature of the  $\text{NO}_2$  sorptive process (that is, the first-order rate constant is independent of concentration). Previous studies in our laboratory (5) have shown that under constant environmental conditions the rate of  $\text{NO}_2$  uptake by several crop species is independent of concentration (that is, first order) over the range 0 to 58 ppm. Rates were found to increase with increasing light and to be linearly correlated with the reciprocal of total leaf diffusion resistance.

Fractionation results are given in Table 2. Approximately 97 percent of the absorbed  $^{15}\text{NO}_2$  was incorporated into reduced nitrogen compounds during the 3-hour exposure period. Nitrogen dioxide is known to react in aqueous solution to form nitrate and nitrite ions. The low levels of  $^{15}\text{N}$  observed as nitrate, a common storage form, suggested that most of the absorbed  $\text{NO}_2$  formed nitrite, which is rapidly assimilated via nitrite reductase. These data show conclusively that N derived from the air pollutant  $\text{NO}_2$  is metabolized by snap bean and incorporated into various plant nitrogen fractions. This conclusion is supported by several lines of indirect evidence. For example, Faller (9) recorded stimulation of plant biomass when  $\text{NO}_2$  was supplied as the sole source of fixed nitrogen. Similarly, Matsushima (10), using  $^{14}\text{C}$ , showed an increase in amino acid synthesis when citrus was exposed to  $\text{NO}_2$  and suggested that the N source was  $\text{NO}_2$ . Finally, Zeevaart (11) reported that nitrate reductase was induced by  $\text{NO}_2$  and that levels of nitrate, nitrite, and protein increased in  $\text{NO}_2$ -exposed plants.

We have demonstrated the sorption of  $\text{NO}_2$  by snap bean by a direct technique under usual plant growth conditions and at  $\text{NO}_2$  levels well within recorded ambient ranges. We have further shown that this  $\text{NO}_2$  is rapidly metabolized and incorporated into organic nitrogen compounds.

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### Amino Acids in an Antarctic Carbonaceous Chondrite

*Abstract. Amino acids have been found in aqueous extracts of a C2 carbonaceous chondrite recovered from Antarctica. The composition of the amino acids strongly suggests that they have a meteoritic origin. Comparison of these results with those obtained with other C2 chondrites supports the view that Antarctic meteorites have not been significantly altered by terrestrial processes since their fall.*

Since the Japanese discovery in 1969 of a concentration of meteorites near the Yamato Mountains, more than 1000 fragments from several hundred new meteorites have been recovered from Antarctica by Japanese and Japanese-American teams. The Antarctic is believed to have provided a clean, cold environment for these meteorites prior to their collection. It has been suggested that despite the fact that some are of great terrestrial age (1), they are pristine specimens, little changed since their fall (2). In view of this, meteorites found in the vicinity of the Allan Hills during the 1977-1978 field season were collected with considerable

care to avoid contamination and alteration by handling. Carbonaceous chondrites, because of their friable nature and content of organic compounds, are particularly susceptible to contamination, biological alteration, and leaching of soluble components by water. The meteorites collected during 1977-1978 have been found to include two carbonaceous chondrites: a C2 (ALHA 77306) and a C3 (ALHA 77307). Chondrites of type C2 contain among their organic constituents a diverse and characteristic suite of amino acids (3). To evaluate both the stability of meteoritic organic compounds in the Antarctic environment and the possibility of contamination or biological alteration, we conducted amino acid analyses on fragments of the Allan Hills C2 chondrite.

Two samples of the meteorite were obtained from the curatorial facility at the Johnson Space Center: a small exterior chip (specimen 77306.8) weighing 235 mg and two interior pieces (specimen 77306.16) with a combined weight of 208 mg. The former was broken and a small fragment plus the resulting fines (a total of 68 mg) were used for analysis. The larger of the interior pieces was broken and a 62-mg fragment was used for analysis. Each sample was ground to a fine powder with an agate mortar and pestle and extracted with 5 ml of water in an evacuated Pyrex vial at 110°C for 24 hours. The aqueous extract was separated from the insoluble residue by vacuum filtration through a fine-porosity (4 to 5.5  $\mu\text{m}$ ) glass frit filter funnel. The extract was

Table 1. Amino acids in the meteorite extracts. Data are expressed as nanomoles per gram.

| Amino acid                       | Allan Hills        |                     | Murchison |
|----------------------------------|--------------------|---------------------|-----------|
|                                  | Exterior (77306.8) | Interior (77306.16) |           |
| Asp                              | 3.2                | 1.0                 | 5.1       |
| Glu                              | 1.6                | 1.8                 | 23.5      |
| Gly                              | 26.3               | 18.3                | 96.0      |
| Ala                              | 5.4                | 3.8                 | 43.3      |
| Aib                              | 2.8                | 2.6                 | 100.2     |
| Abu                              | 1.3                | 1.4                 | 16.1      |
| Val                              | 0.4                | 0.6                 | 11.3      |
| Iva                              | 0.6                | 1.2                 | 23.2      |
| Aeb                              | 0.3                | 0.8                 | 11.1      |
| Ple                              | 0.2                | 0.1                 | 3.6       |
| $\alpha\beta\text{M}_2\text{ab}$ | 0.4                | 1.6                 | 16.7      |
| $\beta\text{Abu}$                | 1.4                | 1.1                 | 5.2       |
| $\beta\text{Ala}$                | 5.1                | 3.3                 | 15.2      |
| $\beta\text{Aib}$                | 0.9                | 0.6                 | 3.8       |
| $\gamma\text{-Abu}$              | 1.7                | 1.8                 | 23.1      |
| Total                            | 51.6               | 40.0                | 397.4     |

dried on a rotary evaporator, dissolved in 2 ml of 6*N* (constant boiling) HCl, and hydrolyzed in an evacuated Pyrex vial at 110°C for 24 hours. The hydrolyzed extract was dried in a vacuum desiccator over NaOH pellets. The dry extract was dissolved in 200  $\mu$ l of sodium citrate buffer, 0.2*M* Na<sup>+</sup>, pH 2.2, for amino acid analysis. As a blank, Monterey sand that had been heated to 550°C was carried through the same extraction and hydrolysis procedures. Water and 6*N* HCl were prepared by distillation from a quartz subboiling distillation apparatus. All glassware was thoroughly washed and heated to 550°C for 20 minutes before use. Amino acid analyses were conducted with a 2 by 250 mm ion-exchange column and fluorimetric detection of the amino acid-*o*-phthalaldehyde-methanethiol reaction product (4).

Figure 1 shows chromatograms of hydrolyzed extracts of the exterior and interior samples from the Allan Hills meteorite. The two traces are qualitatively similar. Amino acids were identified by coincidence of elution times with those of standards; the predominant components are indicated in the chromatogram of the exterior sample (Fig. 1a). The amount of each amino acid detected in the meteorite extracts is given in Table 1. The main quantitative differences are small increments of aspartic acid (Asp), glycine (Gly), and alanine (Ala) in the exterior sample.

The amino acid compositions of several C2 chondrites have been found to be qualitatively similar and, although the total amounts of amino acids vary among

these meteorites, certain characteristic quantitative relationships are seen. The Murchison meteorite is prototypical. A chromatogram of a hydrolyzed aqueous extract of this meteorite, obtained in the same manner as those of the Allan Hills samples, is shown in Fig. 2a. The pattern is dominated by a suite of saturated aliphatic monoamino-monocarboxylic acids such as Gly, Ala, and Aib (5) and includes several  $\beta$ - and  $\gamma$ -amino members. Several of these amino acids are relatively rare in biological materials (for example, Aib and  $\beta$ Aib) or have never been found to occur biologically [for example, Iva, Aeb, Ple, and  $\alpha\beta$ M<sub>2</sub>ab (5)]. In addition, dicarboxylic amino acids such as Asp and Glu have been found. It can be shown that within the homologous series Gly, Ala, Aib, Nva, and Nle

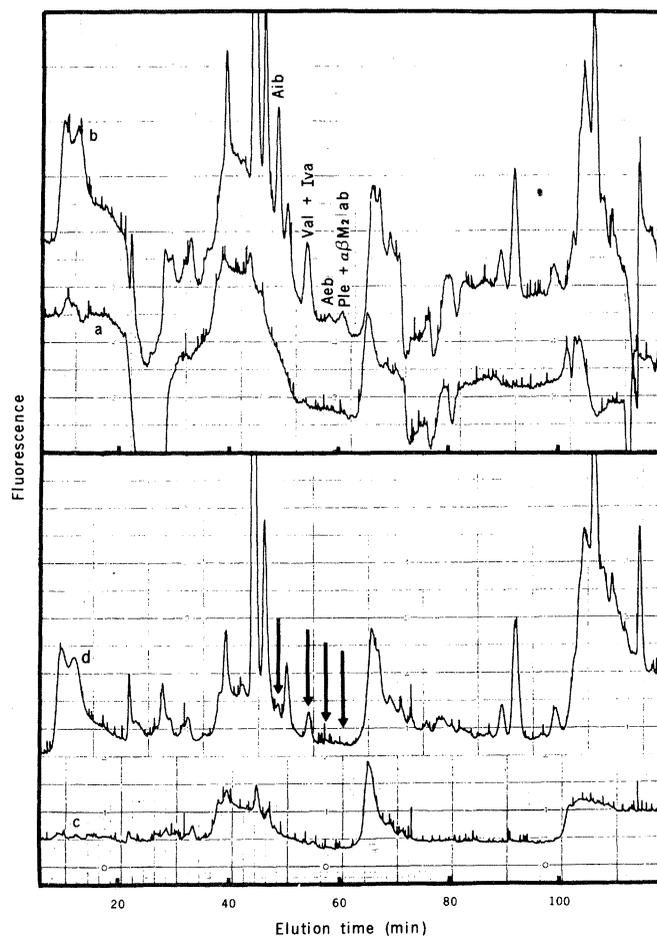
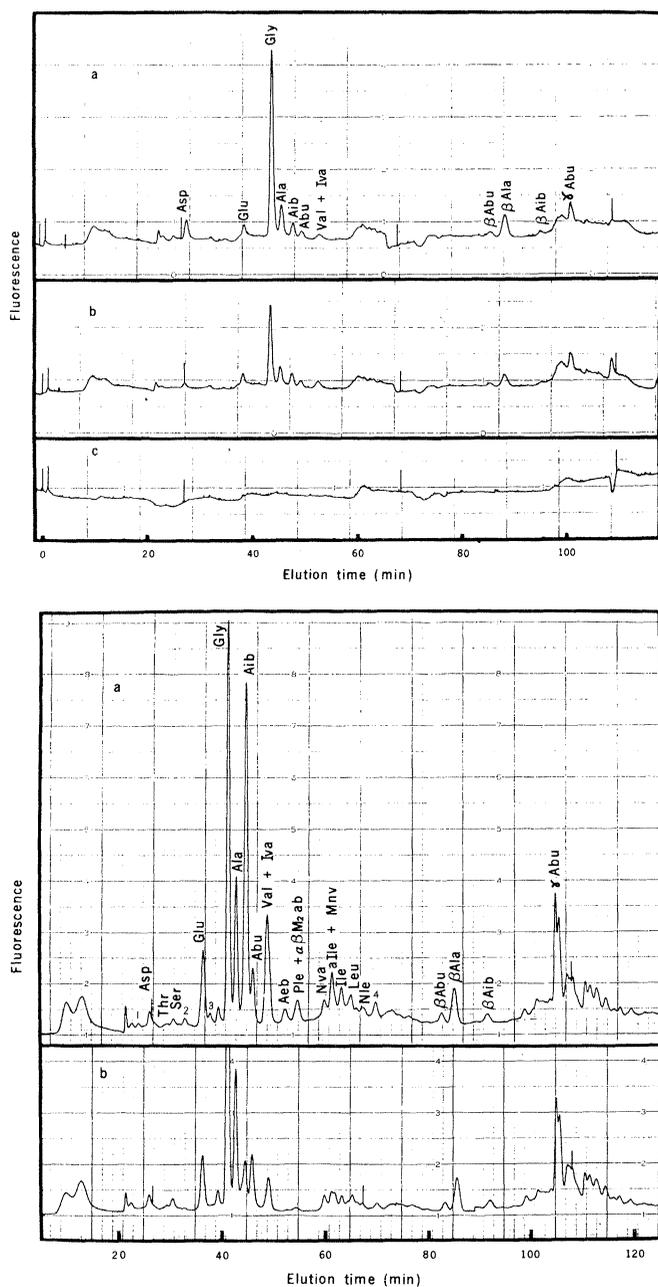


Fig. 1 (top left). Chromatograms of hydrolyzed aqueous extracts from the Allan Hills meteorite. Fluorogen development, 100°C; fluorimeter attenuation, 100 times. (a) Exterior sample (77306.8), 6.8 mg; (b) interior sample (77306.16), 6.2 mg; (c) sand blank. Fig. 2 (bottom left). Chromatograms of hydrolyzed aqueous extracts (4.4 mg) from the Murchison meteorite. Fluorogen development: (a) 100°C; (b) 25°C; fluorimeter attenuation, 100 times. Fig. 3 (right). Chromatograms of hydrolyzed aqueous extracts from the Allan Hills meteorite, interior sample. Fluorimeter attenuation, 10 times. (a) Sand blank; fluorogen development, 100°C; (b) 6.2-mg meteorite; fluorogen development, 100°C; (c) sand blank; fluorogen development, 25°C; (d) 6.2-mg meteorite; fluorogen development, 25°C.

there is an exponential decline in amount with increasing carbon number.

In distinguishing between indigenous and contaminant amino acids, it is useful to look for this distinctive composition as well as for the presence or absence of certain species that are of common biological occurrence, but which are either absent in carbonaceous meteorites or present in such small amounts as to have escaped detection: lysine, histidine, arginine, phenylalanine, tyrosine, methionine, and cysteine. The hydroxyamino acids threonine (Thr) and serine (Ser) should perhaps be included in this category, although it is possible that the small amounts measured in some C2 chondrites are indigenous. In any case, large amounts of Ser strongly suggest contamination, particularly by handling, because of the prominence of Ser among the "finger" amino acids (6).

Evaluation of the Allan Hills results in terms of the preceding criteria leads to the conclusion that the meteorite contains a suite of indigenous amino acids that is essentially free of contaminants. Serine amounts are vanishingly small, and the nonmeteoritic biological amino acids are absent. Many of the characteristic meteoritic constituents are present, and the expected declining content is seen in the series Gly, Ala, and Aib. Several of the unique meteoritic amino acids cannot be seen at the level of detection sensitivity used to obtain the chromatograms in Fig. 1. However, when the analyses were repeated with a tenfold increase in detection sensitivity, several additional components became apparent. Figure 3a shows the sand blank run at ten times the sensitivity used to obtain the trace shown in Fig. 1c. Baseline irregularities and random noise are greatly magnified under these conditions. When the hydrolyzed extract of the Allan Hills interior sample was repeated at this sensitivity, peaks corresponding to Aeb and Ple plus  $\alpha\beta M_2$ ab were seen. These amino acids, which, except for Ple, have a fully substituted  $\alpha$  carbon, show a unique temperature dependence for their reaction with *o*-phthalaldehyde (4). Although they give the usual fluorescent response (that is, the response given by amino acids with at least one  $\alpha$  hydrogen) when the reaction occurs at 100°C, the fluorescence decreases by 90 percent or more when the reaction occurs at 25°C. This is illustrated for the Murchison analysis by comparing Fig. 2a (100°C reaction) with Fig. 2b (25°C reaction). Traces b and d of Fig. 3 show the analogous comparison for the Allan Hills interior extract. The diminution or disappearance of the marked peaks confirms the presence of

Aib, Iva, Aeb, and Ple and/or  $\alpha\beta M_2$ ab.

There is a significant difference between the Allan Hills hydrolyzed extract and that of Murchison in the overall amount of amino acids present: Allan Hills has only about 10 percent of the total amino acid content of Murchison. With respect to individual amino acids, depletions by as much as 40-fold relative to Murchison are seen (compare Aib). However, these differences are not necessarily an indication of amino acid loss due to terrestrial processes such as leaching. Several C2 chondrites have been analyzed and found to have amino acid contents substantially lower than that of Murchison. In the case of the Nogoya chondrite (7), the amino acid content is quite similar to that reported here for Allan Hills. There are also pronounced textural differences between Nogoya and Murchison; Nogoya is comparatively homogeneous and lacking in inclusions. Both the lower amino acid content and the distinctive morphology of Nogoya very likely reflect fundamental differences with respect to Murchison in their formation and subsequent history. The Allan Hills chondrite also lacks well-defined chondrules and in an alteration sequence of C2 chondrites approaches Nogoya much more closely than it does Murchison (8). The amino acid data for the Allan Hills C2 chondrite are thus consistent with what might be expected for a specimen from a recent fall of a meteorite of this type.

In summary, amino acid analyses of the Allan Hills C2 chondrite support the assertion that the Antarctic meteorite finds are pristine specimens—even in the case of types as susceptible to alteration as C2 chondrites. Therefore, continued care in the collection, transport, curation, and sampling of these important extraterrestrial materials is highly recommended.

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## Comparison of Total Sequence of a Cloned Rabbit $\beta$ -Globin Gene and Its Flanking Regions with a Homologous Mouse Sequence

**Abstract.** *The nucleotide sequence of a cloned rabbit chromosomal DNA segment of 1620 nucleotides length which contains a  $\beta$ -globin gene is presented. The coding regions are separated into three blocks by two intervening sequences of 126 and 573 base pairs, respectively. The rabbit sequence was compared with a homologous mouse sequence. The segments flanking the rabbit gene, as well as the coding regions, the 5' noncoding and part of the 3' noncoding messenger RNA sequences are similar to those of the mouse gene; the homologous introns, despite identical location, are distinctly dissimilar except for the junction regions. Homologous introns may be derived from common ancestral introns by large insertions and deletions rather than by multiple point mutations.*

We have recently described the cloning and characterization of a 5100-base pair (bp) Kpn I fragment of rabbit DNA containing a  $\beta$ -globin gene (1). The coding sequences were arranged in three blocks, separated by two intervening sequences or introns, a smaller one of 126 and a larger one of 573 base pairs. The positions of both introns relative to the

coding sequences were identical to those found in a mouse  $\beta$ -globin major gene cloned by Tilghman *et al.* (2). Although the corresponding mouse and rabbit  $\beta$ -globin introns had very similar sequences in the vicinity of the junctions to the coding sequences, the similarities within the introns diminished rapidly with increasing distance from the junc-