

PHOTO-ALTERATION OF ISOVALINE AND ITS PRECURSOR 5-ETHYL-5-METHYLHYDANTOIN EXPOSED TO UV AND γ -RAY RADIATION

Palash K. Sarker^{1*}, Jun-ichi Takahashi², Yumiko Obayashi¹, Takeo Kaneko¹ and Kensei Kobayashi¹
¹Department of Chemistry and Biotechnology, Faculty of Engineering, Yokohama National University,
79-5 Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan

²NTT Microsystem Integration Laboratories, 3-1 Morinosato Wakamiya, Atsugi 243-0198, Japan

Fax: +81-45-339-3938, sarker-palash-nd@ynu.ac.jp

(Received February 6, 2012; Accepted June 22, 2012)

(Abstract)

We present laboratory studies examining the photolysis products of isovaline (Ival) and its precursor molecule, 5-ethyl-5-methylhydantoin (EM-Hyd) against continuous ultraviolet (UV), monochromatic UV, and γ -ray photons. The irradiation experiments were performed with Ival and EM-Hyd in aqueous state. In both UV and γ -ray irradiation experiments, Ival produced serine and alanine as common photolysis products. On the other hand, hydantoin and 5-methylhydantoin were detected as photolysis products of irradiated EM-Hyd. This experimental result may point out the potential importance of the prebiotic photochemistry of Ival and EM-Hyd in Solar System materials.

(Keywords)

isovaline, radiation, ultraviolet, γ -ray, photolysis products

1. Introduction

Since obtaining the first evidence of extraterrestrial amino acids in a carbonaceous chondrite (*e.g.*, Murchison meteorite) [1], a great number of laboratory experiments have been reported to study possible abiotic formation of bioorganic compounds including amino acids and their precursors from possible astrophysical media by irradiation with high-energy particles [2,3] or ultraviolet (UV) light [4-7]. It was suggested that amino acids among various other compounds were synthesized in quite cold environments such as in ice mantles of interstellar dusts (ISDs) in dense clouds by UV light, and they were then delivered to the primitive Earth by comets and meteorites [8,9]. Moreover, amino acids in several meteorites (*e.g.*, Murchison, Murray, Orgueil) have been found to have L-enantiomeric excess, the same handedness as observed in biological amino acids [10,11]. Therefore, a possible hypothesis can be advocated that life on Earth was seeded by the delivery of organic compounds from outer space during the intense bombardment period of the primitive Earth [12-14].

Among the non-proteinogenic amino acids in extraterrestrial meteorites, isovaline (Ival), a structural isomer of valine, was found in the Orgueil and Murchison meteorite showing a significant L-enantiomeric excess up to 18.5%. This observation suggests a possible prebiotic contribution to the origin of biological homochirality [15-16]. Moreover, many acid-labile amino acid precursors, for example, hydantoin (Hyd) and 5-substituted hydantoins were detected in carbonaceous chondrites. Among them, 5-ethyl-5-methylhydantoin (EM-Hyd), one of the

precursor molecules of Ival [17], was identified in the Yamato-791198 (24 pmol·g⁻¹) and Murchison (47 pmol·g⁻¹) meteorites [18]. But the origins of Ival and EM-Hyd in the chondrites still remain controversial, because amino acids in chondrites and in experimental simulations of ice irradiations are mostly detected after a strong acid hydrolysis of the residues formed. Therefore, it should be clarified whether amino acids and their precursors in carbonaceous chondrites have remained as organic monomer or generated from their relating compounds.

Organic molecules containing amino acid precursors formed in ice mantles of ISDs in dense clouds could be altered by cosmic rays and UV light before their incorporation in parent bodies of meteorites and comets. They might be altered again in the Solar System bodies. Further, organic compounds in interplanetary dusts particles (IDPs) are fully irradiated with strong solar UV light as well as high-energy particles near Earth orbit. Therefore, photo-alteration of organics against space radiations could be an important issue in prebiotic chemistry prior to the origin of life on Earth.

Accordingly, we analyzed UV- and γ -ray irradiated Ival and EM-Hyd to see their possible photolysis products. Here we report our detection of serine (Ser) and alanine (Ala) from irradiated Ival, and Hyd and 5-methylhydantoin (M-Hyd) from irradiated EM-Hyd.

2. Experimental Section

2.1. Chemicals

As starting material for the radiation experiment, EM-Hyd (equimolar mixture of D- and L-EM-Hyd) and Ival (equimolar mixture of D- and L-Ival) were abiotically synthesized in the Mita Laboratory at the Fukuoka Institute of Technology, Fukuoka, Japan. Ethylmethylketone was condensed with ammonium carbonate in the presence of potassium cyanide to produce EM-Hyd according to the Strecker synthesis. Then, Ival was synthesized by hydrolyzing EM-Hyd with barium hydroxide. Purity of the synthesized Ival and EM-Hyd was over 99%.

2.2. Continuous Spectral UV Irradiation Experiment

Aqueous solution of Ival and EM-Hyd were placed separately in synthesized quartz cells for UV irradiation with continuous spectra (190 to 400 nm) using a deuterium lamp (L1835; HAMAMATSU Photonics), as shown in Figure 1. Every sample (2 mL, 10 mM, pH = 7) was irradiated with a power

density of $66 \mu\text{W}/\text{cm}^2$ for 8 and 16 h at a 30-cm distance from the photon source of the lamp. The beam spot was 6 cm in diameter.

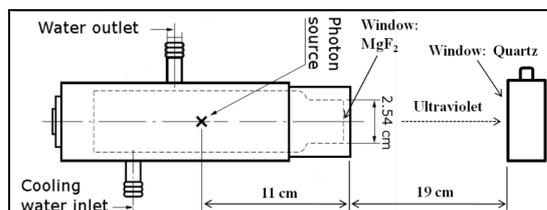


Fig. 1. Schematic diagram of the experimental setup for UV irradiation (continuous, 190–400 nm) from a deuterium lamp (L1835; HAMAMATSU Photonics). The lamp is positioned horizontally and its two nozzles (water inlet and outlet) are aligned vertically. The target cell (window: quartz) is set up at a 30-cm distance from the photon source of the lamp.

2.3. Monochromatic UV Irradiation Experiment

Ival and EM-Hyd were taken individually in synthesized quartz cells for UV irradiation with monochromatic spectra (215 nm). Each sample (2 mL, 10 mM, pH = 7) was irradiated with the monochromatic UV light generated from free electron laser (FEL) of UVSOR-II facility (Institute for Molecular Science, Okazaki, Japan) at the beam line BL5U. The total irradiation doses were 100 and 150 mWh (360 and 540 joule) and the beam spot size at the sample surface was 5 mm in diameter.

2.4. γ -Ray Irradiation Experiment

Ival and EM-Hyd were sealed in borosilicate glass tubes in air. Every sample (2 mL, 10 mM, pH = 7) was irradiated with γ -rays from a ^{60}Co source (1.25-MeV-photon energy) at the Japan Atomic Energy Agency, Takasaki, Japan. The total irradiation doses were 10 and 20 KGy (20 and 40 J) as absorbed by the solvent.

2.5. Analysis of Ival and EM-Hyd

The photolysis products of irradiated Ival and EM-Hyd were analyzed by ion-exchange high-performance liquid chromatography (IE-HPLC) using post-column derivatization with *o*-phthalaldehyde and *N*-acetyl-L-cysteine. The IE-HPLC system was equipped with two HPLC pumps (Shimadzu LC-10AT), a cation-exchange column (4 mm i.d. \times 150 mm; Shimpak ISC-07/S1504), a post column derivatization system, and a Shimadzu RF-535 fluorometric detector (358-nm excitation wavelength; 450-nm emission wavelength). The temperature of the column was maintained at 55 °C. Gradient elution was performed using eluents A (0.07 M trisodium citrate perchloric acid with 7% ethanol, pH 3.20) and B (0.2 M trisodium citrate boric acid with NaOH, pH 10). The flow rate of the carrier was 0.3 mL/min. A Wako amino acid mixture (type AN-II and type B) was used as the amino acid standard for IE-HPLC analysis.

In addition, the photolysis products (other than amino acids) of irradiated EM-Hyd were analyzed by

reversed-phase high-performance liquid chromatography (RP-HPLC). The RP-HPLC system was composed of two pumps (SHIMADZU LC-20AD), a column (Capcell Pak C₁₈ UG120 S-5 μm , 6.0 mm i.d. \times 250 mm), and a detector (SHIMADZU SPD-20AV, 210-nm detection wavelength). The temperature of the column was maintained at 37 °C. Phosphate buffer (25 mM, pH = 3.5, flow rate 0.90 mL/min) and acetonitrile (100%, flow rate 0.10 mL/min) were used as the mobile phase under isocratic conditions. Hydantoin and 5-substituted hydantoin standards were purchased from Tokyo Chemical Industry Co., Ltd. for RP-HPLC analysis.

Furthermore, the photolysis products (organic acids) of UV irradiated (monochromatic) Ival were analyzed by a capillary electrophoresis system (Photal CAPI-3300, Otsuka Electronics Co., Ltd). Analyses were performed in a pH 6.1 carrier electrolyte containing 20 mM 2,3-pyridinedicarboxylic acid, 0.5 mM cetyltrimethylammonium bromide. The samples were run at 30 °C with applied voltage from -15~30 kV. The indirect UV detection wavelength was set at 205 nm.

3. Results and Discussion

3.1. Photolysis Products of Irradiated Ival

A recent study investigated that serine (Ser) and alanine (Ala) were detected as common photolysis products of Ival during continuous UV and γ -ray irradiation. Moreover, two other amino acids (*i.e.*, aspartic acid, glutamic acid) were formed from Ival by γ -ray irradiation [19]. In the present study, Ival was additionally subjected to monochromatic UV irradiation, and we detected the same photolysis products as Ser and Ala (Figure 2). The chromatograms for control samples (*i.e.*, trace c, d and e in Figure 2 for Ival without γ -ray irradiation, Ival without UV-continuous irradiation and Ival without UV-monochromatic irradiation respectively) assured that Ser and Ala were formed as photolysis products of Ival. It is assumed that the formation of Ser needs an additional OH group, which might come from COOH groups during UV and γ -ray irradiation, or the solvent (*e.g.*, H₂O) might be another possible source of OH group as H₂O could be attacked by newly formed ions during γ -ray irradiation. Other than amino acids, formic acid, acetic acid and isovaleric acid were formed from the monochromatic UV irradiated Ival (Figure 3). The whole photo-alteration process by the energetic photons might involve the release of methyl (-CH₃), ethyl (-CH₂CH₃), carboxyl (-COOH) and amino (-NH₂) groups from Ival in the transition period, which would lead to the formation of the photolysis products (Figure 4a and 4b). But extensive sets of future experiments are required to clarify the precise formation mechanism of the photolysis products generated from Ival during UV and γ -ray irradiation.

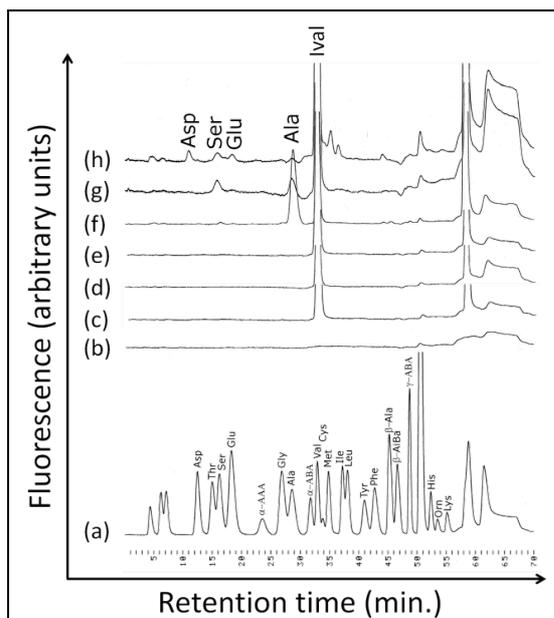


Fig. 2. Ion exchange chromatograms of amino acids: (a) Wako amino acid standard, (b) Blank (milli-Q water), (c) Ival without γ -ray irradiation, (d) Ival without UV (continuous) irradiation, (e) Ival without UV (monochromatic) irradiation (f) UV (monochromatic) irradiated Ival, (g) UV (continuous) irradiated Ival, (h) γ -ray irradiated Ival. Abbreviations: Asp: Aspartic acid, Thr: Threonine, Ser: Serine, Glu: Glutamic acid, α -AAA: α -Amino adipic acid, Gly: Glycine, Ala: Alanine, α -ABA: α -Aminobutyric acid, Val: Valine, Cys: Cystine, Met: Methionine, Ile: Isoleucine, Leu: Leucine, Tyr: Tyrosine, Phe: Phenylalanine, β -ala: β -Alanine, β -AiBa: β -Aminoisobutyric acid, γ -ABA: γ -Aminobutyric acid, His: Histidine, Orn: Ornithine, Lys: Lysine. The IE-HPLC system gives identical retention time for Val and Ival.

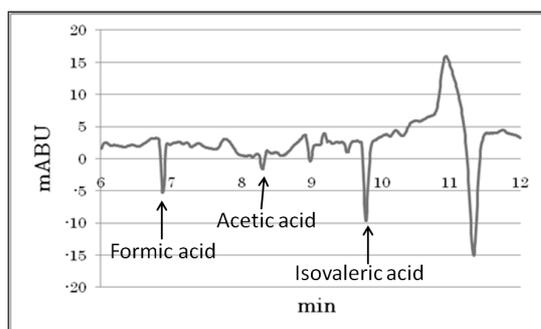
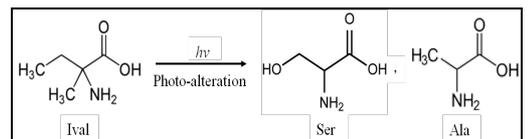


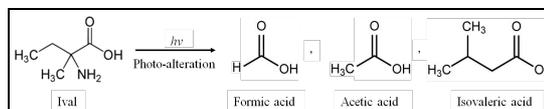
Fig. 3. Electropherogram of UV (monochromatic) irradiated Ival.

3.2. Photolysis Products of Irradiated EM-Hyd

As the photolysis products of EM-Hyd during UV (continuous) irradiation, Hyd was only detected (Figure 5a), whereas Hyd and M-Hyd were formed during UV (monochromatic) irradiation, as shown in Figure 5b. On the other hand, M-Hyd and trace amount of 5,5-dimethylhydantoin (DM-Hyd) and 5-ethylhydantoin (E-Hyd) were formed during γ -ray irradiation (Figure 5c). Additionally, an aliquot of UV (monochromatic) irradiated EM-Hyd was subjected to acid hydrolysis to convert its photolysis products into amino acids. The hydrolyzed fraction

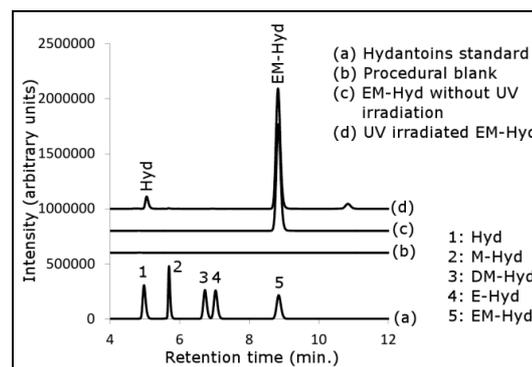


(a)

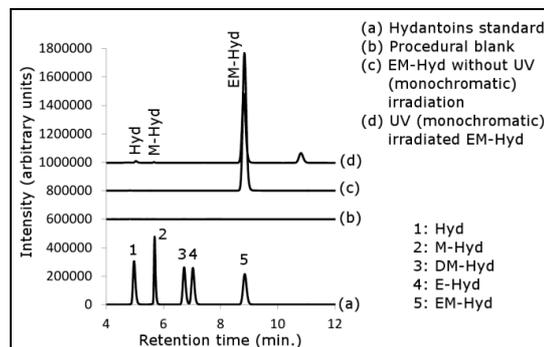


(b)

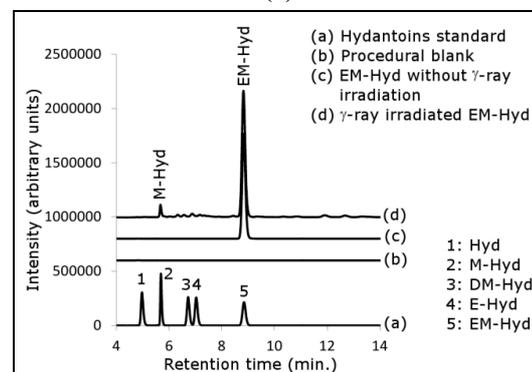
Fig. 4. The photo-alteration process of Ival by the energetic photons forms dominantly (a) Ser and Ala, (b) formic acid, acetic acid and isovaleric acid.



(a)



(b)



(c)

Fig. 5. RP-HPLC chromatograms of (a) UV (continuous) irradiated EM-Hyd, (b) UV (monochromatic) irradiated EM-Hyd and (c) γ -ray irradiated EM-Hyd

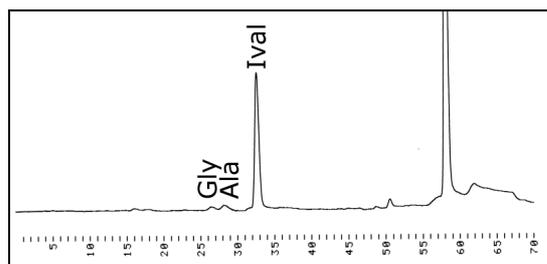


Fig. 6. Ion exchange chromatogram of hydrolyzed UV (monochromatic) irradiated EM-Hyd.

was analyzed using IE-HPLC; as a result, glycine (Gly) and Ala were identified, which were produced from Hyd and M-Hyd respectively, and Ival was detected as a hydrolysis product of EM-Hyd (Figure 6). These results may carry significant implications for the photo-alteration process of irradiated EM-Hyd against UV and γ -ray radiation. Firstly, EM-Hyd in meteoritic parent bodies might play as a possible precursor of Hyd and 5-substituted hydantoins (*e.g.*, M-Hyd, DM-Hyd and E-Hyd) and secondly, the photo-altered products (*e.g.*, Hyd, M-Hyd) and EM-Hyd might produce Gly, Ala and Ival respectively in the early stage of their hydrolysis (Figure 7). The photo-alteration process by the energetic photons might involve the release of methyl ($-\text{CH}_3$) and ethyl ($-\text{CH}_2\text{CH}_3$) groups from EM-Hyd in the transition period, which would lead to the formation of Hyd and 5-substituted hydantoins (*e.g.*, M-Hyd, DM-Hyd and E-Hyd). On the other hand, hydrolysis of Hyd, M-Hyd and E-Hyd involved the cleavage of N1-C2 and N3-C4 bonds inside the heterocyclic ring and produced Gly, Ala and Ival respectively. Therefore, it is assumed that hydrolysis of DM-Hyd and E-Hyd may predominantly produce α -aminoisobutyric acid (AIB) and α -aminobutyric acid (AABA) respectively.

4. Conclusions

We have described here the ways in which photoreactions initiated by UV-photons and γ -rays are capable of generating amino acids and amino acid precursors from their relating compounds. The detection of Ser and Ala produced from Ival, and Hyd, M-Hyd, DM-Hyd and E-Hyd produced from EM-Hyd by UV and γ -ray photo-alteration process may facilitate to point out the chemical evolution of these prebiotic compounds on the chondrite parent bodies.

Acknowledgements

The authors convey their gratitude to Masahiro Katoh, UVSOR-II, Institute for Molecular Science (IMS), Okazaki, Japan, for his kind help and guidance in UV (monochromatic) irradiation experiments; Issay Narumi, Japan Atomic Energy Agency, Takasaki, Japan, for his kind help in ^{60}Co experiments; and Hajime Mita, Fukuoka Institute of Technology, Japan, for providing racemic Ival and EM-Hyd.

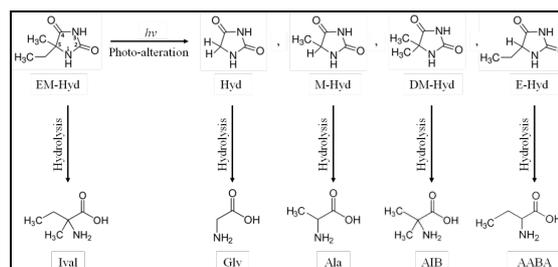


Fig. 7. The photo-alteration process of EM-Hyd by the energetic photons forms dominantly Hyd, M-Hyd, DM-Hyd and E-Hyd. Hydrolysis of EM-Hyd, Hyd, M-Hyd, DM-Hyd and E-Hyd involves the cleavage of N1-C2 and N3-C4 bonds inside the heterocyclic ring and produces Ival, Gly, Ala, AIB and AABA respectively.

References

1. Kvenvolden, K. A., Lawless, J. G., Pering, K., Peterson, E., Flores, J., Ponnampertuma, C., Kaplan, I. R. and Moore, C. B. Evidence for Extraterrestrial Amino Acids and Hydrocarbons in the Murchison Meteorite, *Nature* 228, 923-926 (1970).
2. Kobayashi, K., Kasamatsu, T., Kaneko, T., Koike, J., Oshima, T., Saito, T., Yamamoto, T. and Yanagawa, H. Formation of amino acid precursors in cometary ice environments by cosmic radiation, *Adv. Space Res.* 16, 21-26 (1995).
3. Takano, Y., Takahashi, J., Kaneko, T., Marumo, K. and Kobayashi, K. Asymmetric synthesis of amino acid precursors in interstellar complex organics by circularly polarized light, *Earth Planet. Sci. Lett.* 254, 106-114 (2007).
4. Bernstein, M. P., Dworkin, J. P., Sandford, S. A., Cooper, G. W. and Allamandola, L. J. Racemic amino acids from the ultraviolet photolysis of interstellar ice analogues, *Nature* 416, 401-403 (2002).
5. Muñoz Caro, G. M., Meierhenrich, U. J., Schutte, W. A., Barbier, B., Arcones, S. A., Rosenbauer, H., Thiemann, W. H. -P., Brack, A. and Greenberg, J. M. Amino acids from ultraviolet irradiation of interstellar ice analogues, *Nature* 416, 403-406 (2002).
6. Nuevo, M., Bredehöft, J. H., Meierhenrich, U. J., d'Hendecourt, L., and Thiemann, W. H. Urea, glycolic acid and glycerol in an organic residue produced by ultraviolet irradiation of interstellar/pre-cometary ice analogs, *Astrobiology* 10, 245-256 (2010).
7. de Marcellus, P., Bertrand, M., Nuevo, M., Westall, F. and d'Hendecourt, L. Prebiotic significance of extraterrestrial ice photochemistry: Detection of hydantoin in organic residues, *Astrobiology* 11, 847-854 (2011).
8. Nakamura-Messenger, K., Messenger, S., Keller, L. P., Clemett, S. J. and Zolensky, M. E. Organic Globules in the Targish Lake Meteorite: Remnants of Protosolar Disk, *Science* 314, 1439-1442 (2006).
9. Greenberg, J. M., Kouchi, A., Niessen, W., Irth, H., Paradijs, J., van de Groot, M. and Hermsen, W. Interstellar Dust, chirality, Comets and the Origins of life: Life from Dead Stars? *J. Biol. Phys.* 20, 61-70 (1994).
10. Cronin, J. R. and Pizzarello, S. Amino acid enantiomer excesses in meteorites: Origin and significance, *Adv. Space Res.* 23, 293-299 (1999).
11. Sephton, M. A. Organic compounds in carbonaceous meteorites, *Nat. Prod. Rep.* 19, 292-311 (2002).
12. Bailey, J., Chrysostomou, A., Hough, J. H., Gledhill, T. M., McCall, A., Clark, S., Menard, F. and Tamura, M. Circular polarization in star-formation regions: Implications for biomolecular Homochirality, *Science* 281, 672-674 (1998).
13. Bailey, J. Astronomical sources of circularly polarized light and the origin of Homochirality, *Orig. Life Evol. Biosph.* 31, 167-183 (2001).
14. Buschermöhle, M., Whittet, D. C. B., Chrysostomou, A., Hough, J. H., Lucas, P. W., Adamson, A. J., Whitney, B. A. and Wolff, M. J. An extended search for circularly polarized infrared radiation from the OMC-1 region of Orion, *Astrophys. J.* 624, 821-826 (2005).
15. Pizzarello, S., Zolensky, M. and Turk, K. A. Nonracemic

- isovaline in the Murchison meteorite: Chiral distribution and mineral association, *Geochim. Cosmochim. Acta* 67, 1589-1595 (2003).
16. Glavin, D. P. and Dworkin, J. P. Enrichment of the amino acid L-isovaline by aqueous alteration on CI and CM meteorite parent bodies, *Proc. Natl. Acad. Sci. USA* 106, 5487-5492 (2009).
 17. Olson, E. S. Amino acids from coal gasification? *Nature* 357, 202 (1992).
 18. Shimoyama, A. and Ogasawara, R. Dipeptides and diketopiperazines in the Yamato-791198 and Murchison carbonaceous chondrites, *Orig. Life Evol. Biosph.* 32, 165-179 (2002).
 19. Sarker, P. K., Takahashi, J. -I., Kawamoto, Y., Obayashi, Y., Kaneko, T. and Kobayashi, K. Photostability of Isovaline and its Precursor 5-Ethyl-5-methylhydantoin Exposed to Simulated Space Radiations, *Int. J. Mol. Sci.* 13, 1006-1017 (2012).