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Chemical Evolution of Simple Amino Acids to Asparagine under Discharge onto the Primitive Hydrosphere: Simulation Experiments Using Contact Glow Discharge

Toratane Munegumi

Department of Science Education, Naruto University of Education,
748 Nakashima, Takashima, Naruto-cho, Naruto, Tokushima 772-8502

E-mail: tmunegumi@naruto-u.ac.jp
Received: June 11, 2014; Accepted: July 17, 2014; Web Released: August 1, 2014

Asparagine is an important amino acid for abiotic polypeptide synthesis. In simulation experiments, it was obtained in 3.0% yield (based on the amount of consumed alanine) from alanine (100 mM) and formamide (200 mM) by contact glow discharge (Harada discharge) onto aqueous solutions. The present results suggest that asparagine could be abiotically synthesized from simple amino acids under possible primitive earth conditions.

Asparagine is a neutral proteinous amino acid that plays a number of important roles in metabolic pathways,[1] in particular in the synthesis of oxaloacetic acid via aspartic acid. It is formed by nucleophilic substitution of 2-aspartyladenosine monophosphate by ammonia from glutamine in a process catalyzed by asparagine synthetase.[1] Asparagine has a uniquely reactive methylcarboxamide side chain, the amide group of which serves as a leaving group to form polyaspartic acid in aqueous solutions[2–4] and to generate polypeptides containing aspartic residues in the solid phase.[5] Asparagine residues of some proteins cyclize to an imide structure,[6–10] which can accelerate epimerization.[11] These chemical features of asparagine seem to provide an important pathway of chemically consistent evolutionary polypeptide formation[2–5,12] under primitive earth conditions. However, the pathway for the abiotic formation of asparagine, as well as glutamine and basic amino acids, is still unclear, although many simulation experiments have been carried out and discussed.[13–18]

Sanchez et al.[19] reported asparagine formation from cyanoacetylene, which is a typical product in many Miller-type spark discharge experiments,[13,14] using gas mixtures containing methane and nitrogen. However, the existence of such a reducing atmosphere containing methane is disputed.[20–26] Nonreducing conditions in the primitive atmosphere make ammonia formation difficult, although ammonia, fumaric acid, aspartic acid, phosphoric acid, and asparagine can exist in equilibrium in aqueous solution.[27]

On the other hand, electric discharge (contact glow discharge[28,29]) into aqueous solutions makes it possible to produce ammonia from molecular nitrogen,[30] irrespective of whether a reducing or nonreducing atmosphere is present. Dissociation of water molecules to hydrogen (H) and hydroxyl (OH) radicals[31] is a trigger that initiates reducing reactions that yield ammonia[30] and other reducing molecules.[32] Contact glow discharge[29] (hereafter referred to as Harada discharge) was employed in the study described in this paper for simulative research on prebiotic chemistry occurring in the boundary between the atmosphere and hydrosphere. Harada discharge accelerates many types of reactions[30,32,33] including amination, carboxylation, reduction, oxidation, hydrogenation, etc. Although Harada and Iwasaki have reported the amination of succinamic acid to asparagine (1.1%),[33] very few examples of asparagine formation have been reported.[33,34]

After the publication of a previous letter,[34] amino acids were further investigated from the viewpoint of their extraterrestrial and terrestrial origins.[35] Glycine, alanine, and serine were found in interstellar medium ice analogs irradiated by ultraviolet light.[36–39] β-Alanine and γ-aminobutyric acid as well as glycine and alanine were obtained in the hydrolyzates after high energy proton irradiation to ice mixtures of carbon monoxide, ammonia, and water.[40,41] Bada et al.[42] reported on the use of the molar ratio between glycine, D-alanine, β-alanine, and 2-amino-2-methylpropanoic acid as a signature for parent body processes of carbonaceous chondrites. They applied Michael addition between ammonia and cyanoacetylene (or acrylonitrile) as a plausible pathway for explaining β-amino acid formation.[42] The significance of asparagine as a chiral-resolving agent of amino acids was also reported.[43] It was therefore considered meaningful to investigate the abiotic formation of asparagine in connection with other amino acids using simulation experimental methodology.

I now report on asparagine formation from simple amino acids with formamide or acetamide by using Harada discharge.[28,29] This is a detailed paper that expands on a previously published letter.[34] Asparagine is formed from alanine and formamide in aqueous solution under Harada discharge as shown in Figure 1. The three amino acids used for these studies (alanine, glycine, and β-alanine) are abundant in carbonaceous meteorites.[44–46] The two carboxylic acid amides (formamide and acetamide)[47] are the hydrolysis products of nitriles, which are thought to be important starting materials for prebiotic amino acid formation.[17]

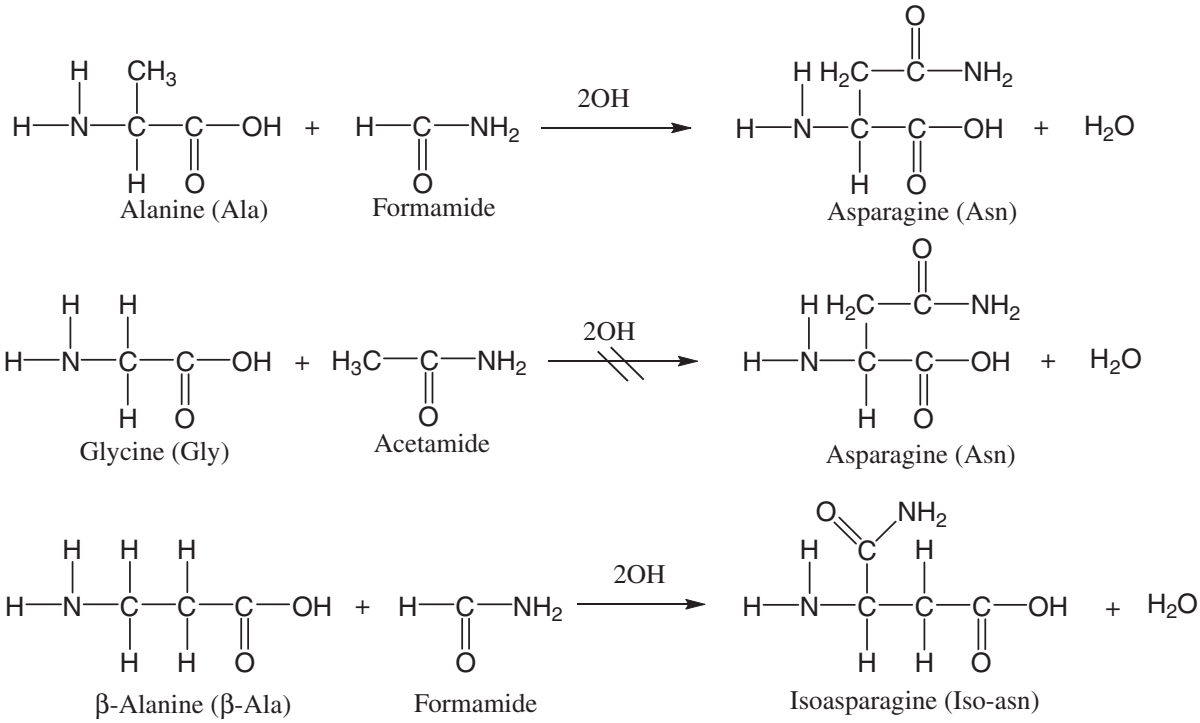

Figure 1. Asparagine formation from alanine and formamide by Harada discharge.

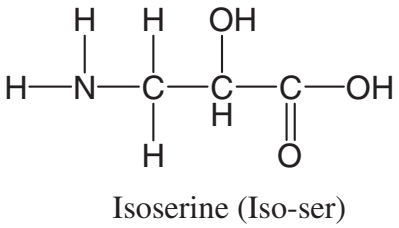

Figure 2. Structure of DL-isoserine.

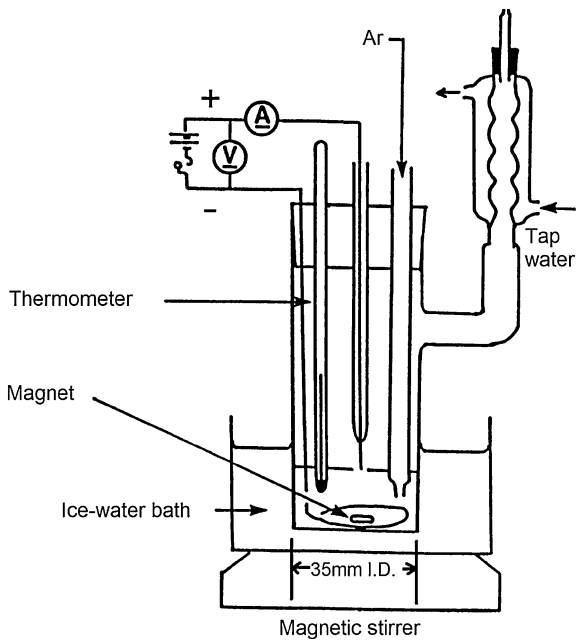

Figure 3. Apparatus used for Harada discharge.

Experimental

Chemicals. L-Alanine, glycine, β-alanine, L-asparagine, formamide, and acetamide were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). L-Isoasparagine (aspartic acid amide) and DL-isoserine (Figure 2) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). These compounds were used as substrates or as standard compounds for the quantitative analysis of reaction solutions.

Reaction Apparatus. The reaction apparatus is shown in Figure 3. The reaction vessel (114 × 35 mm i.d.) containing 20 mL reaction mixture was cooled in an ice–water bath to 0–30 °C. After bubbling argon gas through the mixture for 15 min, Harada discharge (440–520 V, 20–30 mA) was carried out between the platinum anode above the solution and the platinum cathode in the solution over bubbling argon gas. Electric power was supplied by a Model PS-1515 (Toyo Solid State) power supply. Aliquots of solution were removed from the reaction mixture at defined time intervals for analysis.

Analysis of Reaction Solution. The amino acid substrates (alanine, β-alanine, and glycine) and products (ammonia, aspartic acid, serine, asparagine, isoserine, and isoasparagine) were analyzed with a JEOL JLC-300 amino acid analyzer. The spectrophotometric detection at 440 and 570 nm was carried out after a post column derivatization with ninhydrin. Typical chromatograms are shown in Figure 4. The recovery and the yield were calculated from the absorption of peaks at 570 nm.

Asparagine, serine, and alanine were baseline separated, and their retention times were as follows: aspartic acid (13.28 min), serine (20.92 min), asparagine (23.28 min), glycine (39.56 min), isoserine (40.56 min), alanine (42.92 min), isoasparagine (50.28 min), β-alanine (68.40 min), and ammonia (84.48 min).

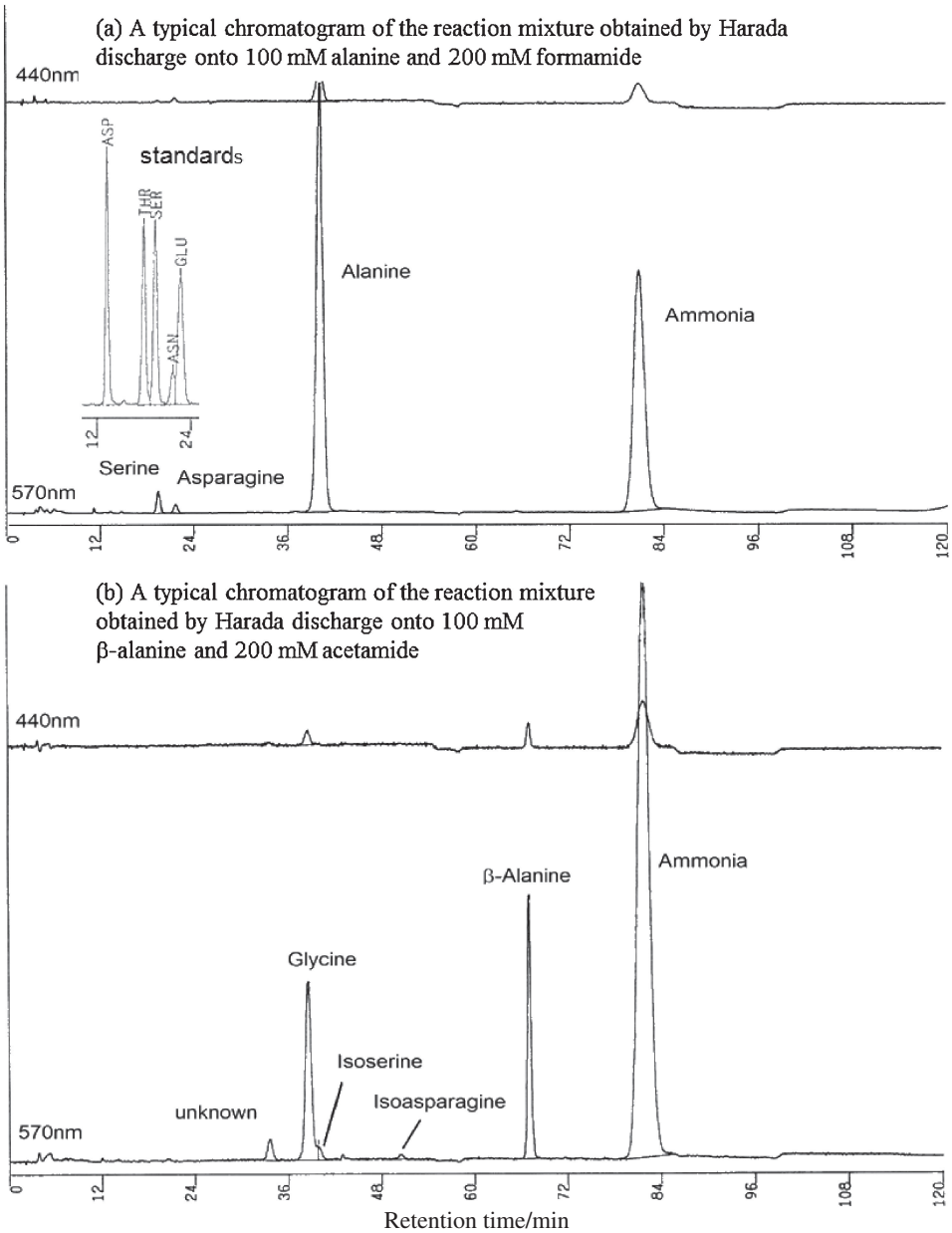

Figure 4. Typical amino acid chromatograms of reaction mixtures. JLC-300 amino acid analyzer was used for analysis. Eluted compounds were reacted with ninhydrin to enable detection by absorption at 440 and 570 nm.

The identification limit and the linearity of analysis are usually several tens pmol and between 1 to 100 nmol, respectively.

Results and Discussion

Harada discharge experiments[32] were carried out on aqueous solutions containing substrate materials in the concentration rage of 1 mM to 2.5 M. Concentrations less or equal to 10 mM were adopted for the self-reactions of substrate molecules, and concentrations higher than 10 mM were adopted for the reactions between substrate molecules. On the other hand, irradiation of γ-rays[48] as a typical hydroxyl radical source for amino acid solutions leads to the degradation of amino acids in concentrations less or equal to 10 mM and the polymerization of amino acids at higher concentration (1 M). Amination of carboxylic acid in 100 mM succeeded under Harada discharge electrolysis. As the focus of this research is not on the polymerization but on the asparagine formation from amino acids and carboxamide, 10 and 100 mM were mainly used as the concentration settings. In order to facilitate the coupling reaction between simple amino acids and carboxamide, a higher concentration of carboxamide than of simple amino acids was used for the experiments.

From Alanine and Formamide to Asparagine. Harada discharge was carried out between the anode and an aqueous solution containing alanine (10 mM) and formamide (20 mM) in 13 mM sulfuric acid. Figure 5 shows the change in concentration of compounds containing amino groups in the solution over time. The concentration of alanine progressively decreased to about 50% (5.0 mM) of the initial concentration after 180 min, while the concentration of ammonia increased to

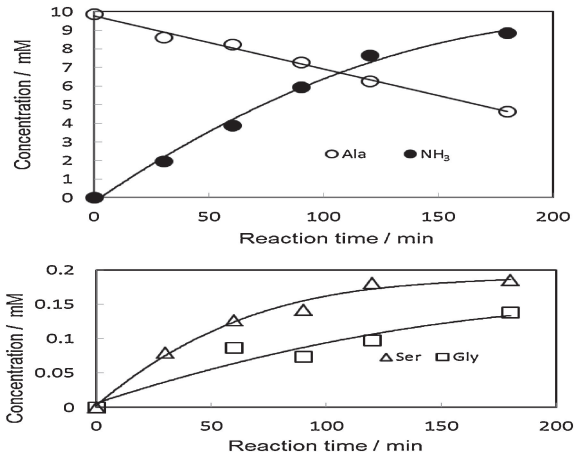

Figure 5. Time course of the reaction induced by Harada discharge (440–480 V, 20 mA) in 13 mM sulfuric acid containing 10 mM alanine and 20 mM formamide: alanine (○), ammonia (●), serine (△), and glycine (□).

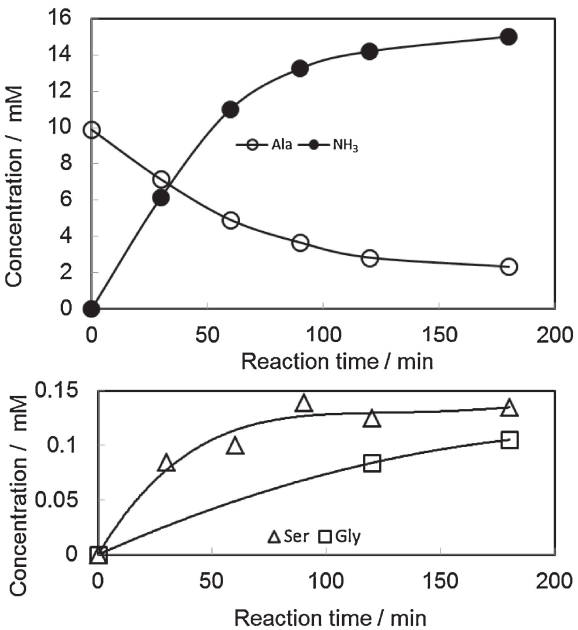

Figure 6. Time course of the reaction induced by Harada discharge (440–480 V, 20 mA) in 9.4 mM sodium sulfate containing 10 mM alanine and 10 mM formamide: alanine (○), ammonia (●), serine (△), and glycine (□).

9.0 mM over the same time. The products serine and glycine were obtained in 1.9 and 1.3 mM, respectively, at 180 min. The formation of these amino acids can be explained by the oxidation of alanine by hydroxyl radicals generated during Harada discharge. The presence of hydroxyl radicals in such aqueous solutions has previously been confirmed by electron spin resonance spectroscopic analysis.[31]

The time course of the reaction of 10 mM alanine and 10 mM formamide in 9.4 mM sodium sulfate is shown in Figure 6. The concentration of alanine decreased faster in 9.4 mM sodium sulfate (Figure 6) than in 13 mM sulfuric acid (Figure 5), and the rate of ammonia formation (11.5 mM at 60 min) in neutral sodium sulfate was faster than in sulfuric

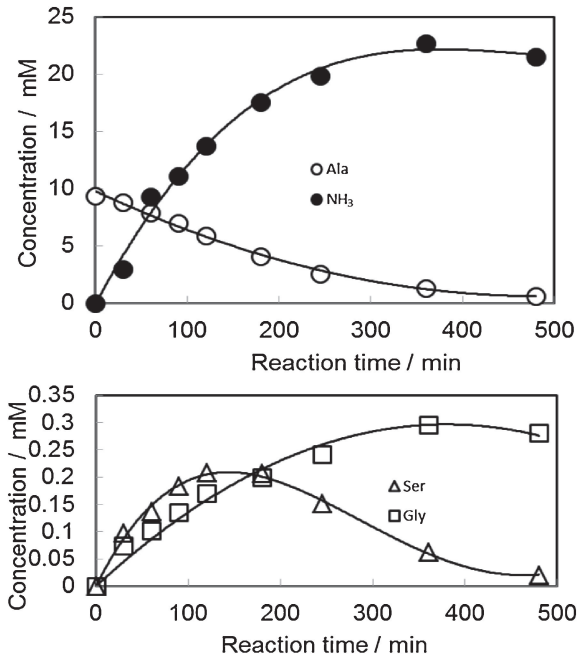

Figure 7. Time course of the reaction induced by Harada discharge (440–480 V, 20 mA) in 13 mM sulfuric acid containing 10 mM alanine and 100 mM formamide: alanine (○), ammonia (●), serine (△), and glycine (□).

acid (4 mM at 60 min; Figure 4). Similar maximum yields of serine and glycine were obtained in sulfuric acid and sodium sulfate (Figures 5 and 6).

Figure 7 shows the time course of the reaction using 10 mM alanine and a higher concentration of formamide (100 mM) in 13 mM sulfuric acid. The rate of decrease in the concentration of alanine was similar to the rate of decrease observed with 20 mM formamide in sulfuric acid (Figure 5) but slower than that observed in sodium sulfate (Figure 6). The maximum yields of serine and glycine were similar to those obtained with 10 mM formamide (Figures 5 and 6). The lower concentration (10 mM) of amino acids did not produce asparagine. Under the lower concentration of alanine and formamide, the self-decomposition of these substrates may prevail over the coupling between substrates. The similar yield (0.18 mM in Figure 5; 0.20 mM in Figure 7) of serine from 10 mM alanine even in the different concentration of formamide (20 mM and 100 mM) suggests that the degradation of alanine may prevail over the radical coupling between alanine and formamide.

Figure 8 shows the results obtained when using 100 mM alanine and 200 mM formamide in 13 mM sulfuric acid. The higher concentrations of substrate compared with the amounts used in previous reactions resulted in more intermolecular reactions; these conditions led to the formation of asparagine in addition to serine and glycine. The maximum yield of asparagine was 1.5% (1.5 mM), which corresponds to a yield of 3.0% based on the amount of consumed alanine.

Further increases in the concentrations of starting materials also led to the formation of asparagine, as shown in Figure 9. Under these conditions, the yield of asparagine was 0.64% at 120 min based on the initial concentration of alanine, which corresponds to about 27% yield relative to the consumed

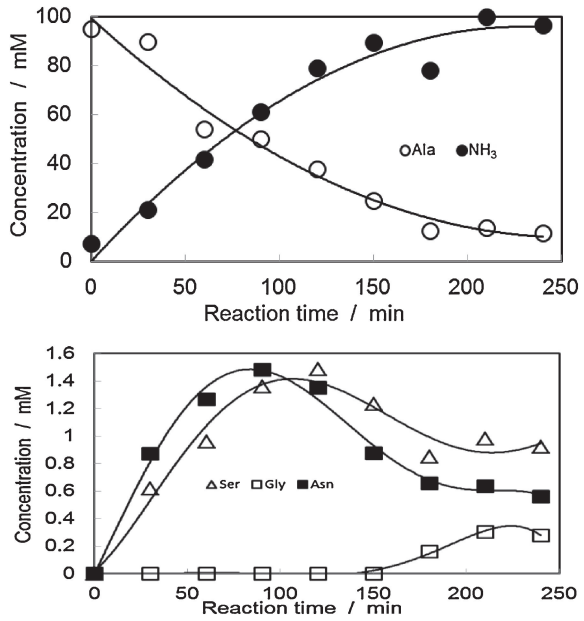

Figure 8. Time course of the reaction induced by Harada discharge (520 V, 20 mA) in 13 mM sulfuric acid containing 100 mM alanine and 200 mM formamide: alanine (○), ammonia (●), serine (△), glycine (□), and asparagine (■).

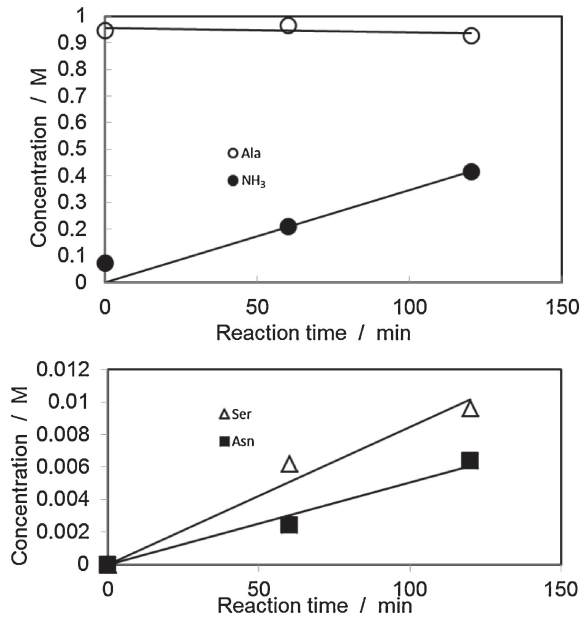

Figure 9. Time course of the reaction induced by Harada discharge (500 V, 20 mA) in 13 mM sulfuric acid containing 1 M alanine and 2 M formamide: alanine (○), ammonia (●), serine (△), and asparagine (■).

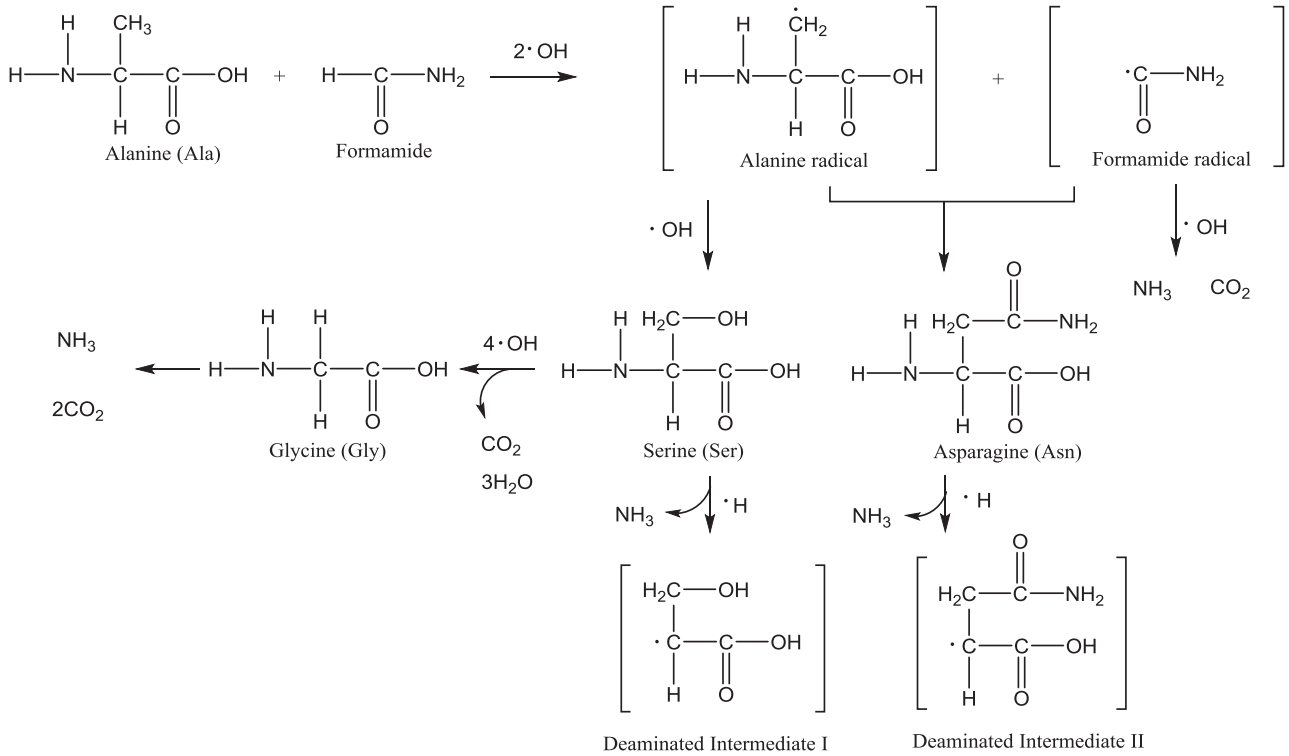

Figure 10. Plausible reaction pathway: from alanine and formamide to glycine, serine, and asparagine.

alanine (0.024 mM). However, as the reaction solution yielded a suspension containing oxamide,[49,50] the reaction, and sampling of the solution, could not be continued after 120 min. A higher asparagine yield, which was calculated from the consumed alanine, was obtained from the higher formamide and alanine. On the other hand, the yield of serine was 1.0%, which corresponds to about 42% yield relative to the consumed alanine (0.024 mM). The slightly lower yield of asparagine compared with that of serine may be explained by the decrease of formamide radical caused by oxamide formation.

Figure 10 summarizes the reaction pathway from alanine and formamide. Two hydroxyl radicals produced by Harada

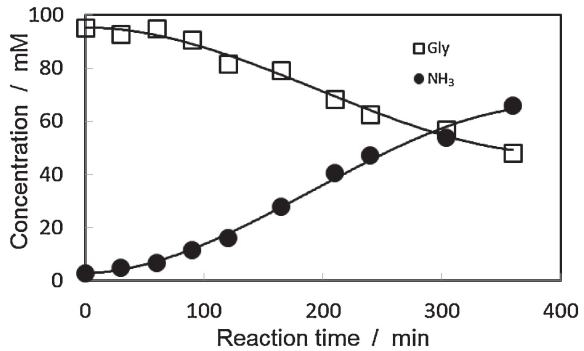

Figure 11. Time course of the reaction induced by Harada discharge (460 V, 20 mA) in 13 mM sulfuric acid containing 100 mM glycine and 200 mM acetamide: ammonia (●) and glycine (□).

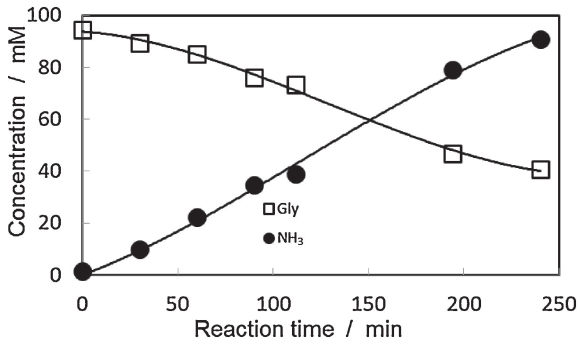

Figure 12. Time course of the reaction induced by Harada discharge (500 V, 30 mA) in 13 mM sulfuric acid containing 100 mM glycine and 200 mM malonamide: ammonia (●) and glycine (□).

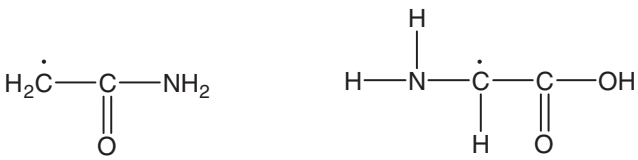

Figure 13. Unstable radical intermediates in the reactions between glycine and acetamide.

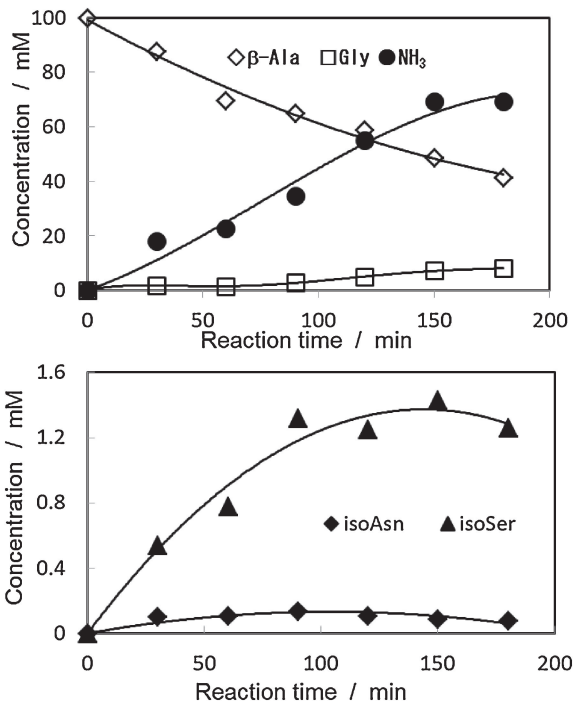

Figure 14. Time course of the reaction induced by Harada discharge (520 V, 30 mA) in 13 mM sulfuric acid containing 100 mM alanine and 200 mM formamide: β-alanine (◇), ammonia (●), glycine (□), isoasparagine (▲), and isoserine (◆).

discharge subtract hydrogen atoms from alanine and formamide to afford the corresponding alanine and formamide radicals; the coupling reaction between these radicals affords asparagine. Alternatively, the alanine radical can react with a hydroxyl radical to afford serine, which undergoes oxidation and decarboxylation to glycine. This reaction pathway was investigated previously.[32] The lower yield of glycine than that of asparagine and serine suggests the formation of the deaminated radical intermediates I and II (Figure 10).[51,52] These intermediates may react with hydrogen (H), hydroxyl (OH), and formamide radicals to afford corresponding non-amino compounds, which cannot be detected with ninhydrin. No main products on the amino acid chromatograms besides serine, asparagine, glycine, ammonia, and alanine support the deamination paths. The high yield of ammonia in every reaction also supports the deamination paths, which may explain why asparagine is not a main product in these reactions.

The involvement of formamide radicals may be inferred from the observed formation of oxamide from formamide.[49,50] Although exposing solutions containing lower concentrations of alanine (10 mM) and formamide (20 mM) to Harada discharge did not generate any coupling product, exposing solutions containing higher concentrations of alanine (100 mM, 1.0 M) and formamide (200 mM, 2.0 M) to the same conditions gave higher levels of asparagine. These results suggest that a higher concentration of substrate accelerates the intermolecular coupling between alanine and formamide radicals.

From Glycine and Acetaldehyde to Asparagine. Figures 11 and 12 show the time course of the reaction in aqueous solutions of glycine (100 mM) plus acetamide (200 mM) and glycine (100 mM) plus malonamic acid (200 mM), respectively. These reactions did not yield observable amounts of any amino compounds besides ammonia; no asparagine was observed.

These results indicate that radical formation from acetamide is more difficult than from formamide. The yield of succinic acid amide from acetamide was lower than that of oxamide from formamide under plasma jet blowing conditions.[50] Plasma jet blowing is a technique that has given results consistent with many types of reactions with Harada discharge. The acetamide radical and glycine radical (Figure 13) may therefore be more difficult to form under these conditions.

From β-Alanine and Formamide to Isoasparagine. Figure 14 shows the time course of the reaction from β-alanine (100 mM) and formamide (200 mM) that takes place during

Table 1. Summary of the Maximum Yield of Products under Harada Discharge Conditions

Substrate (initial concentration)		Maximum yield[a] of products/%				
Amino acid	Amide	Gly	Ser	Iso-Ser	Asn	Iso-Asn
Ala (10 mM)	formamide (20 mM)	2.6	3.6	0.0	0.0	0.0
Ala (10 mM)	formamide (100 mM)	3.3	4.0	0.0	0.0	0.0
Ala (100 mM)	formamide (200 mM)	2.0	2.6	0.0	3.0	0.0
Ala (1.0 M)	formamide (2.0 M)	0.0	42[b]	0.0	27[b]	0.0
Gly (100 mM)	acetamide (200 mM)	—	0.0	0.0	0.0	0.0
β-Ala (100 mM)	formamide (200 mM)	14	0.0	3.8	0.0	0.4

a) Calculated from the amino acid substrate consumed. b) Maximum yield for 120 min reaction.

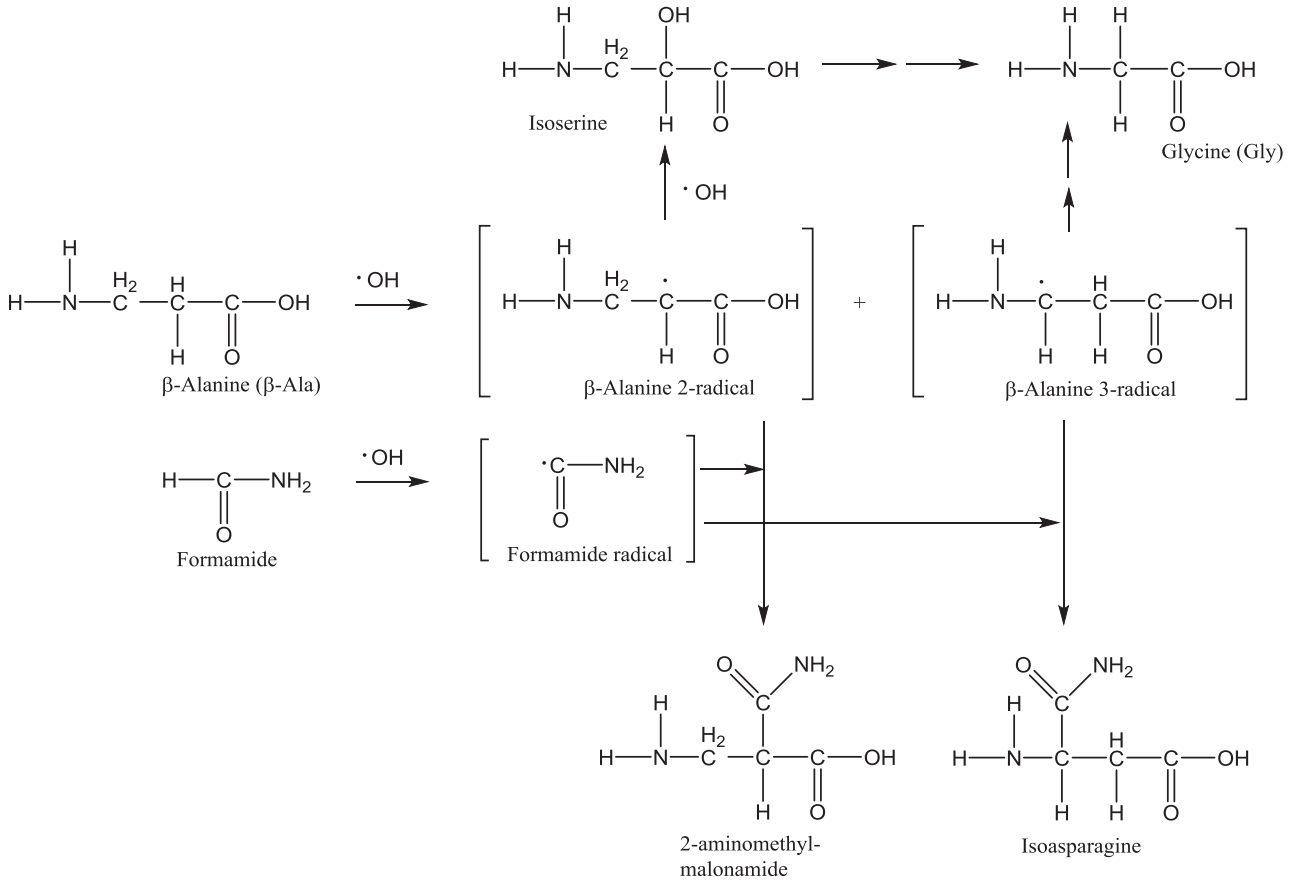

Figure 15. Plausible pathway for the reaction from β-alanine and formamide.

Harada discharge. Ammonia formed concomitantly with a decrease in the amount of β-alanine, and the yield of glycine (10% at 180 min) was higher than that observed in the reactions described above (Figures 5–9). Isoserine and isoasparagine formed in yields of up to 1.3% at 150 min and 0.1% at 90 min, respectively. The yield of isoasparagine from β-alanine was lower than the yield of asparagine (1.5%) from alanine. Table 1 summarizes the maximum yields of products calculated from the consumed amino acid substrates.

A plausible pathway for the reaction from β-alanine and formamide is presented in Figure 15. The formation of isoserine suggests the formation of a β-alanine 2-radical. This radical may also yield a methylamidation compound, 2-(aminomethyl)malonamide. An unknown peak was observed at 33.44 min on the chromatogram (Figure 4); the identity of this compound was not established.

Conclusion

Harada discharge was carried out onto a solution containing 100 mM alanine and 200 mM formamide to afford 1.5% asparagine; the calculated yield based on consumed alanine was 3.0%. Moreover, 1 M alanine and 2 M formamide afforded 27% (the calculated yield based on consumed alanine). The combination of glycine and acetamide in the solution did not afford asparagine under Harada discharge, whereas the combination of β-alanine and formamide afforded isoasparagine (0.14%) and isoserine (1.3%); the calculated yields based on consumed β-alanine were 0.4 and 3.8%, respectively. The presence of isoserine suggested the formation of 2-aminomethylmalonamide, which is an aminomethylated derivative of β-alanine.

The results described here demonstrate that if the primitive hydrosphere contained more than about 100 mM alanine and

200 mM formamide, asparagine, and related compounds might have formed as a result of lightning strikes onto the hydrosphere. Although higher concentration of alanine and formamide than 100 mM could not be obtained all over the primitive ocean, regional higher concentration might form by the evaporation of water near high temperature sources. Harada discharge represents a simulative experimental system that can be used to mimic lightning strikes onto such a primitive hydrosphere. The formation of asparagine is explained by radical coupling between an alanine radical and a formamide radical.

I thank Emeritus Professors Akira Shimoyama and the late Kaoru Harada of the University of Tsukuba for fruitful discussions and assistance with amino acid analysis.

References

1 D. Voet, J. G. Voet, *Biochemistry*, 4th ed., Wiley, **2011**.
2 J. Kovács, H. Nagy, *Nature* **1961**, *190*, 531.
3 K. Harada, M. Matsuyama, E. Kokufuta, *Polym. Bull.* **1978**, *1*, 177.
4 T. Munegumi, N. Tanikawa, H. Mita, K. Harada, *Viva Origino* **1994**, *22*, 109.
5 T. Munegumi, K. Akao, T. Yamada, K. Harada, *Asian J. Chem.* **2014**, *26*, in press.
6 M. P. DeHart, B. D. Anderson, *J. Pharm. Sci.* **2007**, *96*, 2667.
7 T. Geiger, S. Clarke, *J. Biol. Chem.* **1987**, *262*, 785.
8 R. C. Stephenson, S. Clarke, *J. Biol. Chem.* **1989**, *264*, 6164.
9 H. Tonie Wright, D. W. Urry, *Crit. Rev. Biochem. Mol. Biol.* **1991**, *26*, 1.
10 N. D. Clarke, *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11084.
11 M. Zhao, J. L. Bada, T. J. Ahern, *Bioorg. Chem.* **1989**, *17*, 36.
12 T. Munegumi, N. Suzuki, N. Tanikawa, K. Harada, *Chem. Lett.* **1992**, 1679.
13 S. L. Miller, *Science* **1953**, *117*, 528.
14 S. L. Miller, *J. Am. Chem. Soc.* **1955**, *77*, 2351.
15 S. L. Miller, L. E. Orgel, *The Origins of Life on the Earth*, Prentice Hall Inc., New York, U.S.A., **1974**.
16 A. L. Weber, S. L. Miller, *J. Mol. Evol.* **1981**, *17*, 273.
17 A. Brack, in *The Chemistry of Life's Origin* in *NATO ASI Series*, ed. by J. M. Greenberg, C. X. Mendoza-Gomez, V. Pirronello, Kluwer Academic Publishers, **1993**, Vol. 416, p. 357. doi:10.1007/978-94-011-1936-8_14.
18 J. Oró, C. L. Guidry, *Arch. Biochem. Biophys.* **1961**, *93*, 166.
19 R. A. Sanchez, J. P. Ferris, L. E. Orgel, *Science* **1966**, *154*, 784.
20 W. W. Rubey, *Geol. Soc. Am. Bull.* **1951**, *62*, 1111.
21 P. H. Abelson, *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 1365.
22 A. Henderson-Sellers, A. J. Meadows, *Nature* **1977**, *270*, 589.
23 T. Owen, R. D. Cess, V. Ramanathan, *Nature* **1979**, *277*, 640.
24 R. A. Kerr, *Science* **1980**, *210*, 42.
25 F. C. Chyba, *Science* **2005**, *308*, 962.
26 F. Tian, O. B. Toon, A. A. Pavlov, H. De Sterck, *Science* **2005**, *308*, 1014.
27 E. A. Talley, T. J. Fitzpatrick, W. L. Porter, *J. Am. Chem. Soc.* **1959**, *81*, 174.
28 Y. Ito, T. Munegumi, K. Harada, *Res. J. Pharm., Biol. Chem. Sci.* **2013**, *4*, 1811.
29 Harada discharge is an experimental methodology that involves the use of contact glow discharge originally developed by Hickling et al. (A. Hickling, J. K. Linacre, *J. Chem. Soc.* **1954**, 711) to simulate electric discharge onto the prebiotic hydrosphere, or the use of contact glow discharge to examine possible prebiotic chemistry at the boundary between the primitive atmosphere and hydrosphere.
30 K. Harada, S. Igari, T. Munegumi, M. Takasaki, A. Shimoyama, *Bull. Chem. Soc. Jpn.* **1991**, *64*, 1776.
31 H. Hase, K. Harada, *Viva Origino* **2001**, *29*, 61.
32 K. Harada, *J. Synth. Org. Chem., Jpn.* **1990**, *48*, 522.
33 K. Harada, T. Iwasaki, *Nature* **1974**, *250*, 426.
34 T. Munegumi, A. Shimoyama, K. Harada, *Chem. Lett.* **1997**, 393.
35 Z. Matins, M. A. Sephton, in *Amino Acids, Peptides and Proteins in Organic Chemistry: Origins and Synthesis of Amino Acid*, ed. by A. B. Hughes, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2009**, Vol. 1, pp. 3–42. doi:10.1002/9783527631766.ch1.
36 M. P. Bernstein, J. P. Dworkin, S. A. Sandford, G. W. Cooper, L. J. Allamandola, *Nature* **2002**, *416*, 401.
37 G. M. Muñoz Caro, U. J. Meierhenrich, W. A. Schutte, B. Barbier, A. Arcones Segovia, H. Rosenbauer, W. H.-P. Thiemann, A. Brack, J. M. Greenberg, *Nature* **2002**, *416*, 403.
38 M. Nuevo, U. J. Meierhenrich, G. M. Muñoz Caro, E. Dartois, L. d'Hendecourt, D. Deboffle, G. Auger, D. Blanot, J.-H. Bredehöft, L. Nahon, *Astron. Astrophys.* **2006**, *457*, 741.
39 M. Nuevo, U. J. Meierhenrich, L. d'Hendecourt, G. M. Muñoz Caro, E. Dartois, D. Deboffle, W. H.-P. Thiemann, J.-H. Bredehöft, L. Nahon, *Adv. Space Res.* **2007**, *39*, 400.
40 K. Kobayashi, T. Kasamatsu, T. Kaneko, J. Koike, T. Oshima, T. Saito, T. Yamamoto, H. Yanagawa, *Adv. Space Res.* **1995**, *16*, 21.
41 T. Kasamatsu, T. Kaneko, T. Saito, K. Kobayashi, *Bull. Chem. Soc. Jpn.* **1997**, *70*, 1021.
42 O. Botta, D. P. Glavin, G. Kminek, J. L. Bada, *Origins Life Evol. Biospheres* **2002**, *32*, 143.
43 K. Kvenvolden, J. G. Lawless, K. Perking, E. Peterson, J. Flores, C. Ponnamperuma, I. R. Kaplan, C. Moore, *Nature* **1970**, *228*, 923.
44 S. Kojo, H. Uchino, M. Yoshimura, K. Tanaka, *Chem. Commun.* **2004**, 2146.
45 A. Shimoyama, C. Ponnamperuma, K. Yanai, *Nature* **1979**, *282*, 394.
46 A. Shimoyama, K. Harada, K. Yanai, *Chem. Lett.* **1985**, 1183.
47 J. M. Hollis, F. J. Lovas, A. J. Remijan, P. R. Jewell, V. V. Ilyushin, I. Kleiner, *Astrophys. J., Lett.* **2006**, *643*, L25.
48 Z. D. Draganić, V. Niketić, S. I. Vujošević, *J. Mol. Evol.* **1985**, *22*, 82.
49 E. H. Brown, W. D. Wilhide, K. L. Elmore, *J. Org. Chem.* **1962**, *27*, 3698.
50 K. Harada, Y. Ito, T. Wada, T. Munegumi, *Bull. Chem. Soc. Jpn.* **2001**, *74*, 2175.
51 W. M. Garrison, *Chem. Rev.* **1987**, *87*, 381.
52 P. Neta, M. Simic, E. Hayon, *J. Phys. Chem.* **1970**, *74*, 1214.

