

Formation of Acetaldehyde from L-Ascorbic Acid and Related Compounds in Various Oxidation Systems

Takashi Miyake and Takayuki Shibamoto*

Department of Environmental Toxicology, University of California, Davis, California 95616

The quantities of acetaldehyde formed from L-ascorbic acid, L-threonic acid, and D-erythrose upon oxidation with Fenton's reagent and upon UV irradiation were determined by gas chromatography. When 10 μ mol of L-ascorbic acid was oxidized with Fenton's reagent, 316 nmol of acetaldehyde was recovered. When 10 μ mol of L-ascorbic acid was irradiated ($\lambda = 300$ nm) for 12 h, 297 nmol of acetaldehyde was produced. Upon oxidation with Fenton's reagent, L-threonic acid (10 μ mol) and D-erythrose (10 μ mol) produced 112 and 81.5 nmol of acetaldehyde, respectively. Acetaldehyde was formed from D-erythrose but not from L-threonic acid by photolysis, suggesting that acetaldehyde formation involved a Norrish type II reaction. Acetaldehyde was also formed from aqueous solutions of L-ascorbic acid (122 nmol/10 μ mol), D-erythrose (37.0 nmol/10 μ mol), or L-threonic acid (27.9 nmol/10 μ mol) with the presence of Fe^{2+} at 37 °C under an air stream. This study is the first to report formation of acetaldehyde from ascorbic acid.

Keywords: Antioxidant; acetaldehyde; ascorbic acid; low-density lipoprotein

INTRODUCTION

Recently, *in vivo* oxidations have received much attention as a process involved in the initiation and promotion of many diseases (Pryor, 1987) including cancer (Ames et al., 1993), cardiovascular disease (Belch, 1992), cataracts (Taylor, 1992), and atherosclerosis (Retsky et al., 1993). A strong relationship between atherosclerosis and amounts of lipid peroxidation products in the inside wall of arteries was suggested in the early 1950s (Glavind et al., 1952). Later, the progress of arteriosclerosis was found to correspond to an increase in thiobarbituric acid (TBA) reactive substances in the artery wall (Aoyama and Iwakami, 1965; Iwakami, 1965). Oxidation of low-density lipoprotein (LDL) in blood is also implicated in the development of human atherosclerosis (Retsky et al., 1993). Therefore, many studies have been conducted on inhibition of LDL oxidation using antioxidants such as vitamin C (Stocker et al., 1991). Several researchers have demonstrated that vitamin C inhibits lipid peroxidation in plasma and in LDL (Steinbrecher, 1988; Jialal and Grundy, 1991).

Monitoring the products of oxidative reactions in biological systems is difficult for several reasons. First, biological samples such as lipid peroxidation products often contain compounds that interfere with the detection of specific chemicals. Second, the concentrations of these products may be extremely low. Third, certain products of interest are very unstable. However, a newly developed, highly sensitive gas chromatographic method for measuring volatile carbonyl compounds, including acetaldehyde, has been satisfactorily applied to monitor lipid peroxidation *in vitro* (Yasuhara and Shibamoto, 1991; Niyati-Shirkhodae and Shibamoto, 1992). During a study of the role of antioxidants in LDL oxidation, using acetaldehyde as a marker for oxidation, formation of acetaldehyde from L-ascorbic acid was also observed in our laboratory. Because such formation has not been previously reported in the literature, the

present study was designed to investigate the formation of acetaldehyde on oxidation of L-ascorbic acid.

EXPERIMENTAL PROCEDURES

Chemicals. L-Ascorbic acid (reagent grade), butylated hydroxytoluene (BHT), Trizma hydrochloride, Trizma base, fatty-acid-free bovine serum albumin, and fat red 7B were purchased from Sigma Chemical Co. (St. Louis, MO). Cysteamine hydrochloride, 2,4,5-trimethylthiazole, L-threonic acid calcium salt, D-erythrose, and ferrous chloride were obtained from Aldrich Chemical Co. (Milwaukee, WI). Hydrogen peroxide was obtained from Fisher Scientific Co. Ltd. (Fair Lawn, NJ). The standard stock solution of 2,4,5-trimethylthiazole was prepared by adding 10 mg of 2,4,5-trimethylthiazole to 1 mL of dichloromethane and was stored at 5 °C. Authentic 2-methylthiazolidine was synthesized according to the method reported previously (Yasuhara and Shibamoto, 1989).

Preparation of Low-Density Lipoprotein (LDL). Blood samples (45 mL) obtained by heart puncture from male Simonsen guinea pigs were collected in test tubes containing ethylenediaminetetraacetic acid (EDTA, 1 mg/mL), mixed by inversion, and kept at 25 °C for 1 h and then at 4 °C for 2 h. Plasma was separated by centrifugation at 5000 rpm for 30 min at 4 °C. The plasma solvent density was adjusted to 1.019 with a high-density salt solution (0.29 g/mL) containing sodium chloride and potassium bromide. After centrifugation for 16 h at 60 000 rpm and 4 °C in a Beckman L-70 ultracentrifuge using a 70 Ti rotor, the infranatant was collected. The pooled infranatant was adjusted to density 1.063 and recentrifuged as before, and then the low-density fraction ($d = 1.019\text{--}1.063$ g/mL) was collected. This is the fraction referred to as LDL. Prior to the oxidation experiments, the LDL was exhaustively dialyzed at 4 °C for 24 h against four changes of at least 50 volumes of an isotonic saline solution (0.15 M sodium chloride) with 0.01% EDTA, adjusted to pH 7.4.

After filtration sterilization (0.45 μ m; Nalge Stbron) of the LDL, protein concentration was determined by the Coomassie Blue dye-binding assay (Bradford, 1976). A 10- μ L aliquot of appropriately diluted LDL was added to the dye reagent, the solution mixed, and absorbance at 594 nm measured versus a reagent blank, using a Hewlett-Packard 8452A diode array UV Spectrophotometer. A standard curve using bovine serum albumin was used to calculate the LDL concentration.

Gel Electrophoresis of LDL. Electrophoresis of LDL was performed using 1% (w/v) agarose dissolved in 50 mM Tris-HCl, pH 8.4, and in barbital buffer, pH 8.6, at 90 V for 50

* Author to whom correspondence should be addressed [telephone (916) 752-4523; fax (916) 752-3394; e-mail Internet:tshibamoto@ucdavis.edu].

min. The 2- μ L samples were placed in 5-mm-long slits. The plate was dried in an oven at 60 °C for 30 min. The gel was lipid-stained with fat red 7B for 4–5 min, rinsed in a methanol–water solution (2:1, v/v), followed by distilled water, and dried. The relative electrophoretic mobility of each sample was evaluated by measuring the distance (mm) from the origin to the middle of the fat-stained band. The purity of the LDL was assessed by the finding of only one band.

Oxidation of LDL with Fenton's Reagent with or without L-Ascorbic Acid. A 5-mL aqueous solution containing either 10.85 or 54.25 μ g of LDL, 0.25 mmol of Trizma buffer (pH 7.4), and 0.75 mmol of potassium chloride was incubated with 1 mmol of ferrous chloride and 0.5 mmol of hydrogen peroxide at 37 °C for 15 h with and without 10 μ mol of L-ascorbic acid. While the incubation continued, the mixture was covered with parafilm. A 5-mL solution containing exactly the same materials except for the ferrous chloride and the hydrogen peroxide was incubated in the same manner as a control sample. Oxidation of the samples was stopped by adding 50 μ L of a 4% BHT–ethanol solution.

The amount of acetaldehyde was determined using a method reported previously (Miyake and Shibamoto, 1993). Cysteamine hydrochloride (1 mL of 1.2 M) was added to the solution, and the pH was immediately adjusted to 8 with 6 N sodium hydroxide. After stirring for 30 min at room temperature, the reaction mixture was extracted for 3 h with 10 mL of dichloromethane using a liquid–liquid continuous extractor; the extract was dried over anhydrous sodium sulfate for 10 h. After removal of the sodium sulfate, the volume of the extract was adjusted to exactly 10 mL with dichloromethane. A standard solution of 2,4,5-trimethylthiazole (40 μ L) was added as an internal standard prior to analysis by a gas chromatograph (GC). The incubation system was covered with aluminum foil to avoid any influence of light on the LDL peroxidation system. The experiment was replicated three times.

Recovery Efficiency from LDL Solutions for Acetaldehyde. A 5-mL aqueous solution containing 0, 2.2, or 10.9 μ g of LDL was spiked with 300 μ L of an aqueous acetaldehyde standard (5 μ mol/mL). Samples were prepared for acetaldehyde analysis as described above and analyzed by GC. The experiment was replicated three times.

Oxidation of L-Ascorbic Acid, L-Threonic Acid, and D-Erythrose with Fenton's Reagent. A 5-mL aqueous solution containing L-ascorbic acid (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, or 10.0 μ mol), L-threonic acid (10 μ mol), or D-erythrose (10 μ mol), 0.25 mmol of Trizma buffer (pH 7.4), and 0.75 mmol of potassium chloride was incubated with and without 1 mmol of ferrous chloride and 0.5 mmol of hydrogen peroxide at 37 °C for 16 h in a 20-mL test tube. The test tube was sealed with parafilm. The oxidation of the samples was stopped by adding 50 μ L of a 4% BHT–methanol solution. The incubation system was covered with aluminum foil to avoid the influence of light. The samples were prepared for acetaldehyde analysis by the method described above and analyzed by GC. The experiment was replicated three times.

Photooxidation of L-Ascorbic Acid, L-Threonic Acid, and D-Erythrose. A 4-mL aqueous solution containing L-ascorbic acid (10 μ mol), L-threonic acid (10 μ mol), or D-erythrose (10 μ mol), 0.25 mmol of Trizma buffer (pH 7.4), and 0.75 mmol of potassium chloride was irradiated with a Rayonet photochemical reactor Model RPR 100 (Southern New England Ultraviolet Co., Hamden, CT) equipped with six UV lamps ($\lambda = 300$ nm) in a 10-mL Pyrex test tube for various periods of time. The UV intensity was measured near the center of the reactor with a Spectroline UVB meter (Spectronic Corp., Westbury, NY) at 1.02 ± 0.3 mW/cm² (mean \pm SD, $n = 3$). One sample tube was wrapped with aluminum foil throughout the experiment as a control. The samples were prepared for acetaldehyde analysis by the method described above and analyzed by GC. The experiment was replicated three times.

Oxidation of L-Ascorbic Acid, L-Threonic acid, and D-Erythrose with Fe²⁺ under an Air Stream. A 100-mL aqueous solution containing 5 g of L-ascorbic acid, L-threonic acid, or D-erythrose and 0.35 g of ferrous chloride was placed in a 500-mL, two-neck, round-bottom flask, and the pH of the solution was adjusted to 6.8 with 6 N sodium hydroxide. The

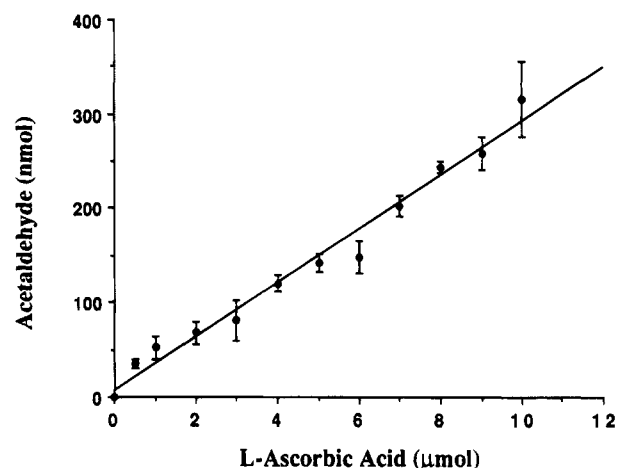


Figure 1. Quantity of acetaldehyde formed from L-ascorbic acid oxidized with Fenton's reagent.

flask was connected to a simultaneous purging and extraction apparatus described previously (Umano and Shibamoto, 1987). A purified air stream was bubbled into the solution at a flow rate of 8 mL/min. The temperature of the solution was maintained at 37 °C. The headspace of the solution was purged into 250 mL of water containing 18 g of cysteamine hydrochloride. The cysteamine solution was simultaneously and continuously extracted with 70 mL of dichloromethane for 6 h. Acetaldehyde in the extracts was analyzed as 2-methylthiazolidine by GC.

Analysis of Acetaldehyde as 2-Methylthiazolidine. A Hewlett-Packard Model 5890 GC equipped with a nitrogen phosphorus detector (NPD) and a 30 m \times 0.25 mm i.d. ($d_f = 1$ μ m) DB-1 bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA) was used for quantitative analysis of 2-methylthiazolidine derivatized from acetaldehyde with cysteamine. The detector and injector temperatures were 250 °C. The linear velocity of the helium carrier gas was 30 cm/s with a split ratio of 21:1. The oven temperature was programmed from 60 to 180 °C at 4 °C/min and held for 10 min. GC peak areas were integrated with a Tsp SP 4400 series integrator. An HP Model 5890 series II GC interfaced to an HP 5971 mass spectrometer was used to confirm the identity of the thiazolidine derivative of acetaldehyde, 2-methylthiazolidine, in the sample. The GC conditions were the same as those for the GC with NPD. The mass spectra were obtained by electron impact ionization at 70 eV with an ion source temperature of 250 °C.

RESULTS AND DISCUSSION

The NPD detection limit for acetaldehyde as 2-methylthiazolidine was 7.1 pg. Acetaldehyde (1.5 μ mol) recoveries from a 5-mL aqueous solution containing 0, 2.2, or 10.9 μ g of LDL were $93.2\% \pm 5.33$, $92.6\% \pm 3.83$, or $92.3\% \pm 4.98$, respectively.

When 10.9 and 54.3 μ g of LDL were incubated with Fenton's reagent, 163 ± 18.1 and 281 ± 32.7 nmol of acetaldehyde were formed, respectively. The control samples containing 10.9 and 54.3 μ g of LDL also contained 0.95 ± 0.13 and 5.4 ± 0.7 nmol of acetaldehyde, respectively, indicating that a certain amount of acetaldehyde was present in the LDL prior to oxidation. When 50 μ g of LDL was oxidized in the presence of 10 μ mol of L-ascorbic acid with Fenton's reagent, 891 ± 83.8 nmol of acetaldehyde was formed. Obviously, formation of acetaldehyde was increased by the presence of L-ascorbic acid. These values are given as the mean \pm standard deviation ($n = 3$).

Figure 1 shows the quantity of acetaldehyde formed from L-ascorbic acid oxidized with Fenton's reagent. Formation of acetaldehyde and the amount of L-ascorbic

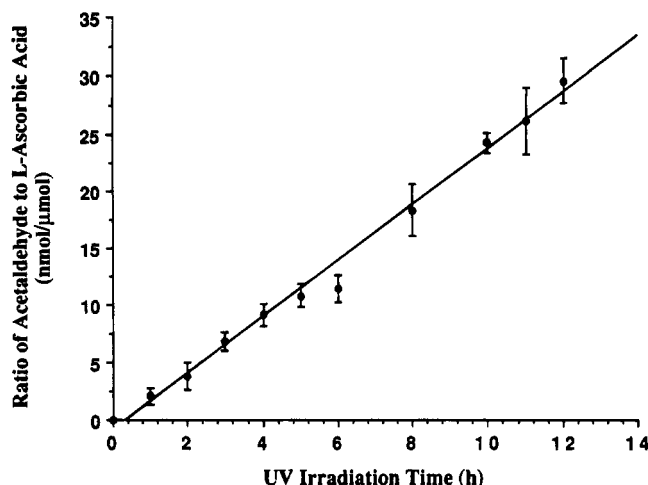


Figure 2. Relative quantity of acetaldehyde formed from L-ascorbic acid upon UV irradiation.

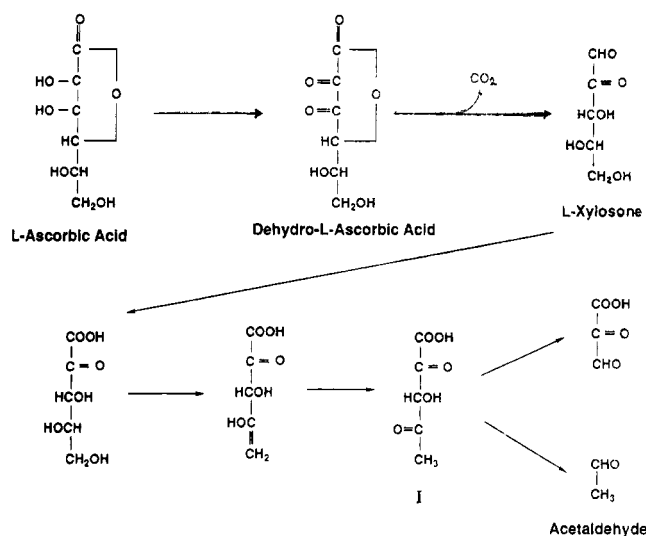


Figure 3. Proposed mechanism of formation of acetaldehyde from L-ascorbic acid upon oxidation with Fenton's reagent.

acid used exhibited a linear relationship ($r^2 = 0.978$). Figure 2 shows the quantity of acetaldehyde formed relative to the available L-ascorbic acid over the time of UV irradiation in nmol/mg. Acetaldehyde formed linearly with irradiation time ($r^2 = 0.991$). It has been previously hypothesized that L-ascorbic acid is first oxidized to dehydro-L-ascorbic acid and then is oxidized to diketogluconic acid at a neutral pH. This diketogluconic acid further breaks down to form threonic acid and oxalic acid upon hydrolysis (Terada and Ohmura, 1966). Also, threose (or erythrose) was reportedly formed from L-ascorbic acid in either the presence or the absence of oxygen at pH 7 and 37 °C (Lopez and Feather, 1992). Therefore, L-threonic acid and D-erythrose could be expected to be oxidized by Fenton's reagent in the present study. Upon oxidation with Fenton's reagent, L-threonic acid (10 μmol) and D-erythrose (10 μmol) produced 112.2 and 81.5 nmol of acetaldehyde, respectively.

In the present study, acetaldehyde was also formed from aqueous solutions of L-ascorbic acid (694.8 ± 63.4 nmol/g), D-erythrose (308.3 nmol/g), and L-threonic acid (204.7 nmol/g) in the presence of Fe²⁺ at 37 °C under an air stream, suggesting that acetaldehyde can be formed from L-ascorbic acid under mildly oxidizing conditions. Ascorbic acid is known to undergo degradation in solution under various conditions (Finholt et al.,

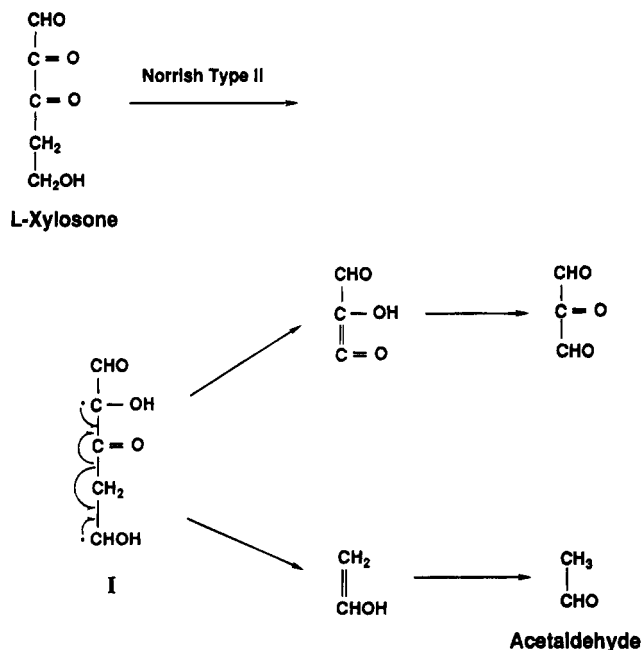


Figure 4. Proposed mechanism of formation of acetaldehyde from L-xylosone upon photoirradiation.

1963; Niemela, 1987). The degradation products include many carboxylic acids such as threonic acid, oxalic acid, glyceric acid, and glyoxylic acid (Shin and Feather, 1990; Niemela, 1987). However, formation of acetaldehyde from ascorbic acid has never before been reported.

Figure 3 shows a proposed mechanism of formation of acetaldehyde from L-ascorbic acid upon oxidation. Formation of L-xylosone from L-ascorbic acid has been hypothesized previously (Kurata and Sakurai, 1967; Shin and Feather, 1990). L-Xylosone would undergo dehydration and keto-enol reactions to form 2,4-diketo-3-hydroxypentanoic acid (I in Figure 3), which would then break down into 1,2-diketopropanoic acid and acetaldehyde. For either D-erythrose or L-threonic acid, glyoxylic acid and acetaldehyde would be formed through 2-hydroxy-3-ketobutanoic acid.

For the effect of UV irradiation, we hypothesized that a Norrish type II reaction formed acetaldehyde from L-ascorbic acid via L-xylosone as shown in Figure 4. Norrish type II photocleavage involves intramolecular abstraction of the γ -hydrogen followed by cleavage of the resulting 1,4-biradical [I in Figure 4 (Lissi and Encina, 1979)] to give an enol that tautomerizes to an aldehyde or a ketone (Yang and Yang, 1958). In fact, L-threonic acid did not produce acetaldehyde because the carboxylic group does not abstract γ -hydrogen upon UV irradiation. In contrast, the aldehyde group of D-erythrose abstracted γ -hydrogen to form the 1,4-biradical which then gave acetaldehyde and glyoxal via a Norrish type II cleavage according to our hypothesis.

It is well-known that a hydroxy radical also causes lipid peroxidation *in vivo*. For example, hemoglobin promotes formation of a hydroxyl radical (Sadrzadeh et al., 1984) which subsequently implicates DNA degradation and lipid peroxidation (Gutteridge, 1986). L-Ascorbic acid (vitamin C) has begun to receive much attention as an antioxidant that inhibits the atherosclerosis associated with LDL oxidation (Retsky et al., 1993) and is used for certain disease treatment. On the other hand, acetaldehyde which we have demonstrated to form from ascorbic acid is extremely reactive. Due to its strong electrophilic properties, acetaldehyde binds

readily to proteins, to the peptide glutathione, to individual amino acids, and to DNA (Donohue et al., 1983; Tuma and Sorrell, 1985). Lam et al. (1986) reported that acetaldehyde has the ability to cross-link to proteins in rat nasal respiratory mucosa both *in vitro* and *in vivo*, suggesting that acetaldehyde may react with DNA. This could cause further biological changes, including mutagenesis and carcinogenesis. Therefore, further study of the formation of acetaldehyde from ascorbic acid in biological systems and of the potential genotoxic effects of the acetaldehyde seems warranted by the results of the present study.

ACKNOWLEDGMENT

We thank Dr. Essam Enan for technical support throughout this project and Dr. Mark Kurth for expert assistance on photochemistry.

LITERATURE CITED

- Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. Oxidants, antioxidants, and the degenerative disease of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915–7922.
- Aoyama, S.; Iwakami, M. On the role of fatty acid peroxides in atherogenesis. *Jpn. Heart J.* **1965**, *6*, 128–143.
- Belch, J. J. F. Free radicals and their scavenging in stroke. *Scott. Med. J.* **1992**, *37*, 67–68.
- Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
- Donohue, T. M., Jr.; Tuma, D. J.; Sorrell, M. F. Acetaldehyde adducts with proteins: binding of [¹⁴C]acetaldehyde to serum albumin. *Arch. Biochem. Biophys.* **1983**, *220*, 239–246.
- Finholt, P.; Paulsen, R. B.; Higuchi, T. Rate of anaerobic degradation of ascorbic acid in aqueous solution. *J. Pharm. Sci.* **1963**, *52*, 948–954.
- Glavind, J.; Hartmann, S.; Clemmesen, J.; Jessen, K. E.; Dam, H. Studies on the role of lipoperoxides in human pathology. II. The presence of peroxidized lipids in the atherosclerotic aorta. *Acta Pathol. Microbiol. Scand.* **1952**, *30*, 1–6.
- Gutteridge, J. M. C. Iron promoters of the fenton reaction and lipid peroxidation can be released from hemoglobin by peroxides. *FEBS Lett.* **1986**, *201*, 291–295.
- Iwakami, M. Peroxides as a factor of atherosclerosis. *Nagoya J. Med. Sci.* **1965**, *28*, 50–66.
- Jialal, I.; Grundy, S. M. Preservation of the endogenous antioxidants in low density lipoprotein by ascorbate but not probucol during oxidative modification. *J. Clin. Invest.* **1991**, *87*, 597–601.
- Kurata, T.; Sakurai, Y. Degradation of L-ascorbic acid and mechanism of nonenzymic browning reaction. Part III. Oxidative degradation of L-ascorbic acid (Degradation of dehydro-L-ascorbic acid). *Agric. Biol. Chem.* **1967**, *31*, 177–184.
- Lam, C. W.; Casanova, M.; Heck, H. D. Decreased extractability of DNA from proteins in the rat nasal mucosa after acetaldehyde exposure. *Fundam. Appl. Toxicol.* **1986**, *6*, 541–550.
- Lissi, E. A.; Encina, M. V. Polymerization photosensitized by carbonyl compounds. *J. Polym. Sci., Polym. Chem. Ed.* **1979**, *17*, 2791–2803.
- Lopez, M. G.; Feather, M. S. The production of threose as a degradation product from L-ascorbic acid. *J. Carbohydr. Chem.* **1992**, *11*, 799–806.
- Miyake, T.; Shibamoto, T. Quantitative analysis of acetaldehyde in foods and beverages. *J. Agric. Food Chem.* **1993**, *41*, 1968–1970.
- Niemela, K. Oxidative and non-oxidative alkali-catalysed degradation of L-ascorbic acid. *J. Chromatogr.* **1987**, *399*, 235–243.
- Niyati-Shirkhodae, F.; Shibamoto, T. Formation of toxic aldehydes in cod liver oil after ultraviolet irradiation. *J. Am. Oil Chem. Soc.* **1992**, *69*, 1254–1256.
- Pryor, W. A. Editorial commentary. In *Free radicals and peroxides in the etiology of cancer. Oncology overview*; Pryor, W. A., Ed.; National Cancer Institute: Bethesda, MD, 1987; pp vii–xiii.
- Retsky, K. L.; Freeman, M. W.; Frei, B. Ascorbic acid oxidation product(s) protect human low density lipoprotein against atherogenic modification. *J. Biol. Chem.* **1993**, *268*, 1304–1309.
- Sadrzadeh, S. M. H.; Graf, E.; Panter, S. S.; Hallaways, P. E.; Eaton, J. W. Hemoglobin: a biologic Fenton reagent. *J. Biol. Chem.* **1984**, *259*, 14354–14356.
- Shin, D.-B.; Feather, M. S. The degradation of L-ascorbic acid in neutral solutions containing oxygen. *J. Carbohydr. Chem.* **1990**, *9*, 461–469.
- Steinbrecher, U. P. Role of superoxide in endothelial-cell modification of low-density lipoproteins. *Biochim. Biophys. Acta* **1988**, *959*, 20–30.
- Stocker, R.; Bowry, V. W.; Frei, B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does α -tocopherol. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1646–1650.
- Taylor, A. Role of nutrients in delaying cataracts. *Ann. N. Y. Acad. Sci.* **1992**, *669*, 111–123.
- Terada, K.; Ohmura, T. Stability of dehydroascorbate in vitro. I. *Nippon Nogei Kagaku Kaishi* **1966**, *40*, 196–200.
- Tuma, D. J.; Sorrell, M. F. Covalent binding of acetaldehyde to hepatic proteins: Role in alcoholic liver injury. *Prog. Clin. Biol. Res.* **1985**, *183*, 3–17.
- Umano, K.; Shibamoto, T. Analysis of headspace volatiles from overheated beef fat. *J. Agric. Food Chem.* **1987**, *35*, 14–18.
- Yasuhara, A.; Shibamoto, T. Determination of physical and spectral data on thiazolidines for trace aldehyde analysis. *Agric. Biol. Chem.* **1989**, *53*, 2273–2274.
- Yasuhara, A.; Shibamoto, T. Determination of volatile aliphatic aldehydes in the headspace of heated food oils by derivatization with 2-aminoethanethiol. *J. Chromatogr.* **1991**, *547*, 291–298.
- Yang, N. C.; Yang, D.-D. H. Photochemical reaction of ketones in solution. *J. Am. Chem. Soc.* **1958**, *80*, 2913–2914.

Received for review January 10, 1995. Revised manuscript received April 12, 1995. Accepted April 17, 1995.* This work was supported in part by a grant-in-aid from SUNTORY Limited, Osaka, Japan.

JF950021Q

* Abstract published in *Advance ACS Abstracts*, June 1, 1995.