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Chemistry of *L*-ascorbic Acid Related to Foods

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ABSTRACT

A review of reactions of L-*ascorbic acid related to its antioxidant property in foods, preservation of vitamin C activity, removal of oxygen in headspace of containers, browning reaction, formation of combined forms of vitamin C, and anaerobic loss.*

INTRODUCTION

L-Ascorbic acid (AH_2) is the trivial name for L-*threo*-2-hexenono-1,4-lactone, the molecule responsible for preventing scurvy. L-Ascorbic acid or vitamin C is ubiquitous and has multiple functions in all metabolically active plant and animal cells. Szent-Györgi (1979) regarded the development of L-ascorbic acid as a mile-stone in evolution, which he compared to the appearance of light and oxygen.

One of the principal biochemical reactions of L-ascorbic acid is to destroy toxic free radicals (hydroxyl and perhydroxyl) resulting from the metabolic products of oxygen. In this role, the mixture of L-ascorbic acid and its oxidation product dehydroascorbic acid (A) is thought of as a 'redox buffer' (Sapper *et al.*, 1982*b*). When terminating free radicals, AH_2 is converted to A, which is then recycled to AH_2 by reductase enzymes and cofactors. Besides the redox functions in cells, other physiological actions of L-ascorbic acid (Loewus & Loewus, 1987) may be related to the compound's chelation with

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metals and complexing with protein (Gorman & Clydesdale, 1983; Fleming & Bensch, 1983).

The uses of L-ascorbic acid, including those in food, continue to increase because of the compound's vitamin C activity, useful properties, and low toxicity. Here we focus on selected chemical reactions of L-ascorbic acid, and we demonstrate, by example, their importance in foods.

PRODUCTION OF L-ASCORBIC ACID

Jaffe (1984) estimated world production of L-ascorbic acid is greater than 30 000 MT/year. L-Ascorbic acid is presently produced by a slight modification of the scheme first devised by Reichstein & Grüssner (Fig. 1, Crawford & Crawford, 1980). The yields of the individual steps in Fig. 1 are greater than 90%, and the overall yield of L-ascorbic acid from D-glucose is

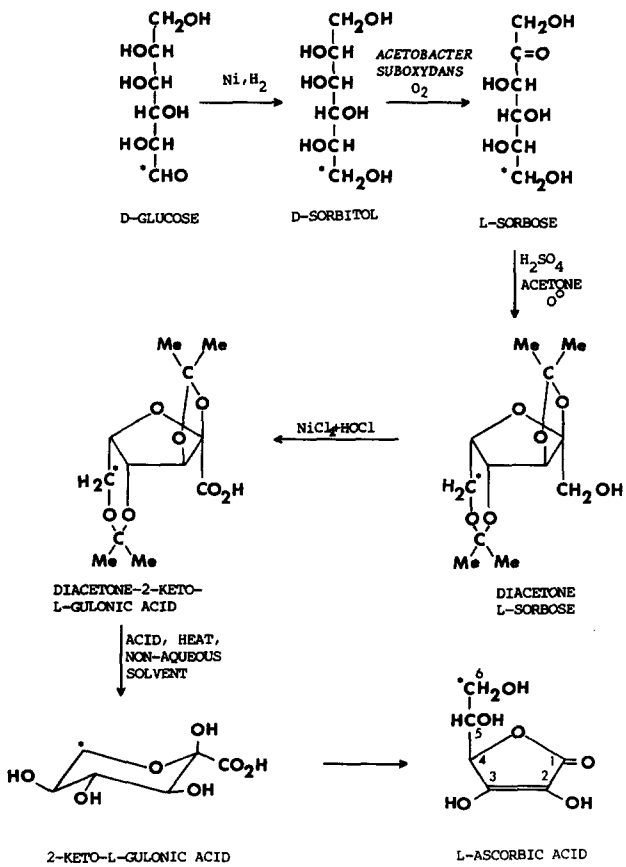

Fig. 1. Reichstein-Grüssner synthesis of L-ascorbic acid.

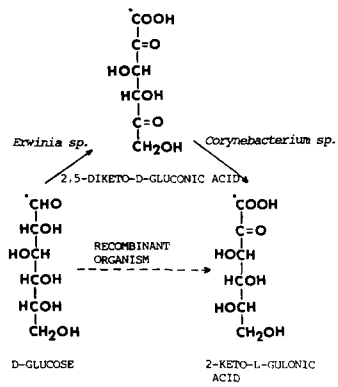

Fig. 2. Fermentation of D-glucose to 2-keto-L-gulonic acid, a key intermediate in the synthesis of L-ascorbic acid by tandem fermentation through 2,5-diketo-D-gluconic acid or by a single fermentation step using a recombinant microorganism.

approximately 50%. A second industrial method currently being examined is based on the production of the key intermediate L-*xylo*-2-hexenonic acid (2-keto-L-gulonic acid) by a single recombinant bacterium (Anderson *et al.*, 1985) or by a step-wise fermentation using two organisms (Fig. 2, Sonoyama *et al.*, 1982). In Figs 1 and 2, the C-1 carbon atom of D-glucose is marked with an asterisk to show that the carbon chain of L-ascorbic acid is inverted in the Reichstein–Grüssner synthesis but not in the alternate route.

Structure, physical properties and ionization

L-Ascorbic acid is one of a pair of enantiomers having the 2-hexenono-1,4-lactone structure (Fig. 3). The other pair is the D- and L-isoascorbic acids, which also are referred to as D- and L-erythorbic. In the old literature, D- and L-xyloascorbic and D- and L-araboascorbic acids were pseudonymes for D- and L-ascorbic acids and D- and L-isoascorbic acids, respectively.

When L-ascorbic acid is boiled in strong alcoholic potassium hydroxide, a

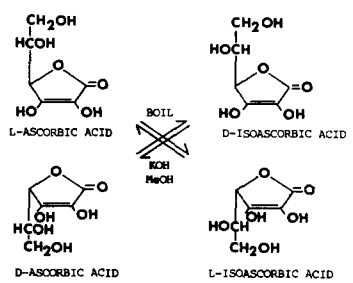

Fig. 3. Diasteriomers of 2-hexenono-1,4-lactone and their interconversion.

50/50 mixture of L-ascorbic and L-isoascorbic acid is obtained (Brenner *et al.*, 1964). Following acidification, L-isoascorbic acid is obtained by fractional crystallization. In a similar way, D-isoascorbic acid, which is commercially available, can be converted to give pure D-ascorbic acid (Fig. 3). Based on L-ascorbic acid with 100% vitamin C activity, the other three stereoisomers have 0–5% activity (Hay *et al.*, 1967).

D-Isoascorbic acid can be substituted for L-ascorbic acid in most functional uses in foods. However, this is not always true. The bread-improving action of L-ascorbic acid is stereospecific; the other three isomers have negligible action (Lillard *et al.*, 1982). From a nutritional standpoint, use of D-isoascorbic acid in foods is regulated due to its possible interference with the bioavailability of L-ascorbic acid (Hornig *et al.*, 1974).

L-Ascorbic acid may exist in at least five tautomeric forms, three of which are shown in Fig. 4. L-Ascorbic acid crystallizes (Hvoslef, 1982) as tautomer 1, and it exists in that form in aqueous solution (Paukstellis *et al.*, 1982).

The 2-OH and 3-OH of L-ascorbic acid are acidic and ionize with pK_1 4·17 and pK_2 11·79, respectively (Crawford & Crawford, 1980). The monobasic salts are readily isolated, but the dibasic salts are unstable. The crystal structures of the sodium and calcium salts show the metal is associated with O–3 in tautomer 1 (Fig. 4, Hvoslef, 1982). In an aqueous solution of the salt, however, ^{13}C–^{13}C spin coupling constants favor tautomer 3 (OH-1 ionized), whereas tautomers 2 and 3 are equally favored in the L-ascorbate dianion (Berger, 1984). While most derivatives of L-ascorbic acid have the structure of tautomer 1, tautomer 2 exists in both 2-*C*-benzyl-L-*xylo*-3-hexulosono-1,4-lactone (Jackson & Jones, 1965) and in ascorbigen (Fig. 5, Kiss & Neukom, 1966). Tautomer 3 is found in 1-*O*-methyl-L-ascorbic acid (Fig. 5, Lu *et al.*, 1984).

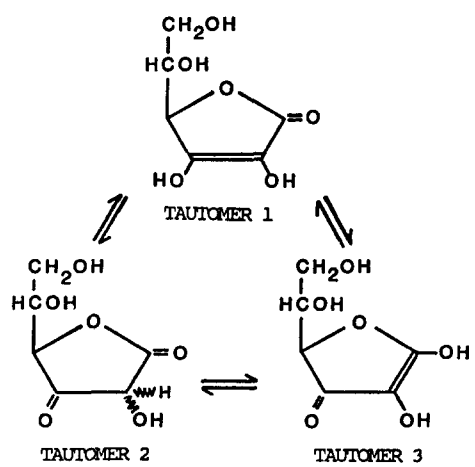

Fig. 4. Three tautomeric forms of L-ascorbic acid.

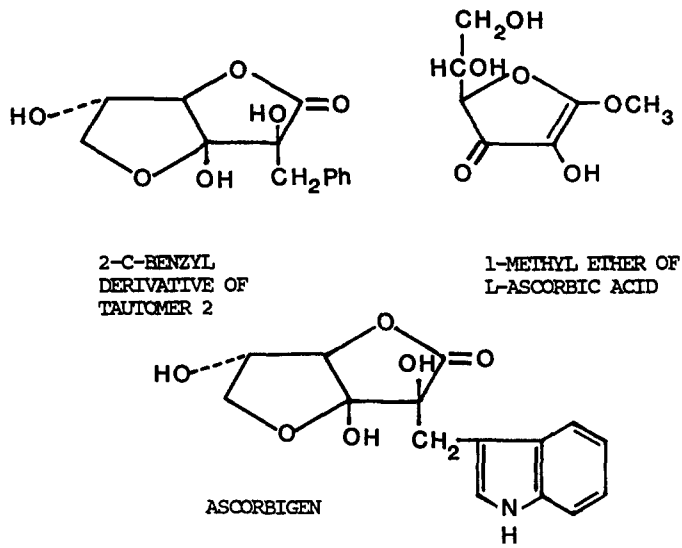

Fig. 5. Derivatives of tautomers 2 and 3 of L-ascorbic acid.

The unusual stability of L-ascorbic acid's lactone ring to alkali comes from the involvement of the lactone carbon in the delocalization of the negative charge of the monoanion to give a π-bonding system involving 5-atoms (Fig. 6). Disruption of the conjugated π system changes the behavior of the molecule. For example, the first ionization of 1-*O*-methyl-L-ascorbic acid gives a 2-oxyanion that cannot be delocalized. The presence of the 3-carbonyl group in the 1-methyl ether greatly increases the acidity (pK 9·1) of H-4 leading to facile racemization at C-4 (Fig. 7, Lu *et al.*, 1984).

Jaffe (1984) tabulated the physical properties of L-ascorbic acid, some of which are given in Table 1. The commercial forms of AH_2 available to the

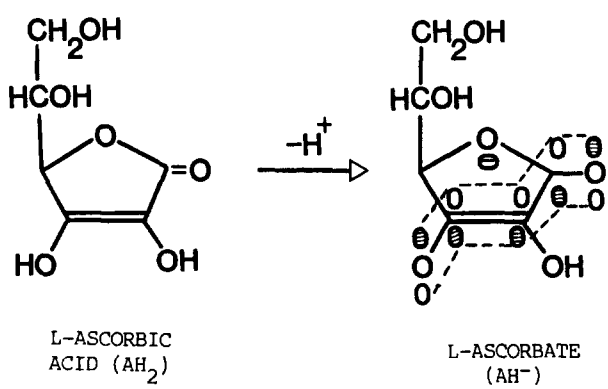

Fig. 6. Resonance stabilization of the lactone ring of L-ascorbate to alkali.

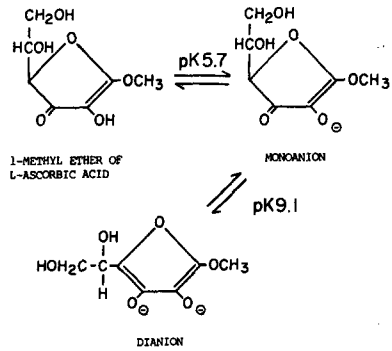

Fig. 7. Ionization of the 1-methyl ether of L-ascorbic acid with racemization at C-4

TABLE 1
Physical Properties of L-Ascorbic Acid

appearance	white colorless crystals
mp	190–2° (dec)

optical activity $[\alpha]_D^{25} + 21°$ (c 1·0, water)
$[\alpha]_D^{25} + 48°$ (c 1·0, methanol)

solubility, g/100 ml

water	33	ethanol	2
propylene glycol	5	glycerol	1
95% ethanol	3·3	lipid solvents	insoluble

UV (Bond *et al.*, 1972)

pH 2: λ_{max} 243 nm, ε mM 10·0
pH 7: λ_{max} 265 nm, ε mM 16·5

IR (Lohmann *et al.*, 1984)

3 540 cm^{-1}, 6-OH; 3 425 cm^{-1}, 3-OH;
3 330 cm^{-1}, 5-OH; 3 232 cm^{-1}, 2-OH.
1 759 cm^{-1}, C=O; 1 675 cm^{-1}, C=C.

NMR (Paukstellis *et al.*, 1982)

^1H in D_2O; δ (ppm), signal spliting, J (Hz), and assigned atom.
At pH 2 4·97, d, $J_{4,5}$ 1·8, H-4
4·09, ddd, $J_{5,6}$ 5·8, $J_{5,6'}$ 7·3, H-5
3·76, m, H-6, H-6'
At pH 7 4·50, d, $J_{4,5}$ 4·0, H-4

^{13}C in D_2O, δ (ppm), (assigned atom)
At pH 2 174·0 (C-1), 156·4 (C-3), 118·8 (C-2)
77·1 (C-4), 69·9 (C-5), 63·1 (C-6)
At pH 7 173·7 (C-1), 162·1 (C-3), 122·5 (C-2)
79·2 (C-4), 70·6 (C-5), 63·6 (C-6)

food industry are L-ascorbic acid and its sodium and calcium salts and 6-palmitate ester.

One-electron reduction by L-ascorbic acid reaction

Chemistry

The most important chemical property of L-ascorbic acid (AH_2) is its ease of oxidation either by a one- or two-electron transfer. When one electron is transferred in the rate-limiting step (Fig. 8), then the first product formed from L-ascorbic acid is the L-ascorbic acid radical ($AH^{\cdot}$), variously termed monodehydroascorbic acid or semidehydroascorbic acid (Bielski, 1982). The

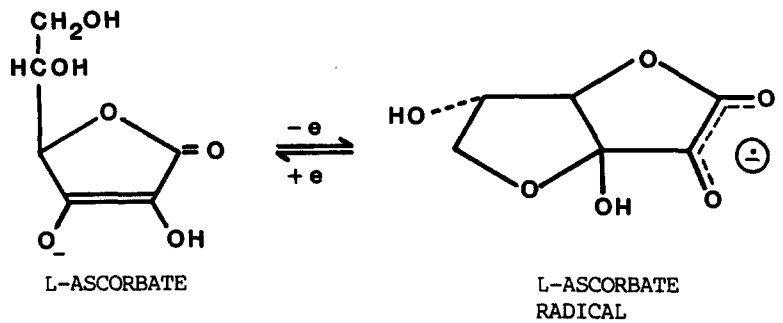

Fig. 8. One-electron oxidation of L-ascorbate to L-ascorbate radical.

L-ascorbic acid radical ($AH^{\cdot}$) is a strong acid with pK0·45, and at physiological pH it exists as a radical anion ($A^{\cdot-}$). The structure of $A^{\cdot-}$ appears to be bicyclic as indicated by electron spin resonance (Sapper *et al.*, 1982*a*), although others (Lohmann *et al.*, 1984; Lohmann & Holtz, 1984; Abe *et al.*, 1984) believe a C_2—C_3 double bond is present in $A^{\cdot-}$.

Free radicals that damage lipids and other readily oxidized compounds in foods and tissue are terminated by reduction with L-ascorbate. In the reaction one electron is transferred to the free radical from AH^- giving ascorbate radical ($A^{\cdot-}$). Aqueous mixtures with AH^- invariably contain $A^{\cdot-}$. But the concentration of $A^{\cdot-}$ is maintained at a low level (approximately $0{\cdot}7 \times 10^{-6}$M, Sapper *et al.*, 1982*a*; Iyanagi *et al.*, 1985) by a disproportionating reaction. $A^{\cdot-}$ is both a good oxidizing agent and a good reducing agent; it reacts with itself (Bielski *et al.*, 1981; Sawyer *et al.*, 1982) to yield a 1:1 mixture of L-ascorbic acid and dehydroascorbic acid (Fig. 9).

Example in food

It is generally accepted that oxidative rancidity in lipids is initiated when a free radical attacks a lipid molecule to give an alkyl radical. This initiation

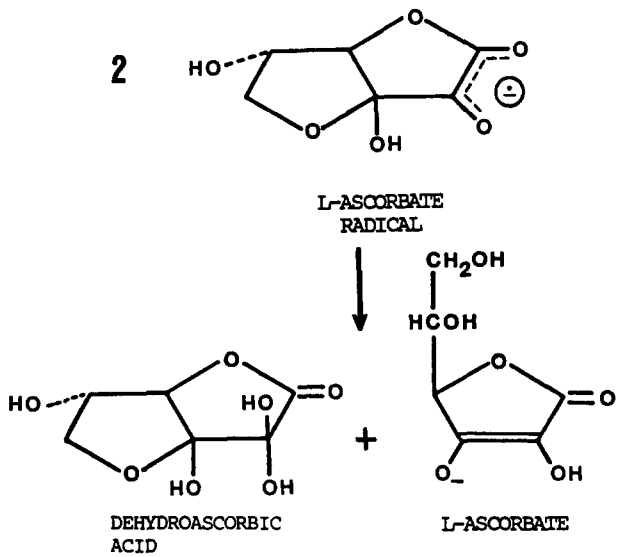

Fig. 9. Dismutation reaction of L-ascorbate radical.

step is terminated (Lambelet *et al.*, 1985) in the presence of L-ascorbyl 6-palmitate (AP, Fig. 10). The AP radical in Fig. 10 cannot form a bicyclic structure; instead, the unpaired electron is delocalized over six atoms. Furthermore, the AP radical is believed to disproportionate to AP and dehydroascorbyl 6-palmitate in the same fashion as $A^{\dot{-}}$.

Due to their reactivity with free radicals, ascorbyl palmitate (AP) and AH_2 behave as typical antioxidants: they prevent peroxide formation in lipids and delay the onset of the chain reaction that causes deterioration of

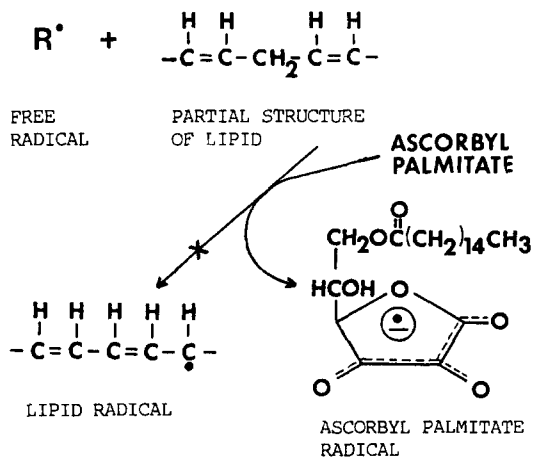

Fig. 10. Inhibition of reaction between free radical and lipid by L-ascorbyl 6-palmitate.

vegetable oils, animal fat, fish, margarine, milk, vitamin A, and carotenoids (Cort, 1982). It is known that the 2- and 3-hydroxyl groups of L-ascorbic acid must be unsubstituted for antioxidant activity and that fatty acid esterification of OH-6 and/or OH-5 of AH_2 increases lipid solubility (Cort, 1982; Takahashi *et al.*, 1986).

AP is generally a more effective antioxidant when used in combination with tocopherol (Cort, 1982). Tocopherol appears to react first with the free radicals (Lambelet *et al.*, 1985), and AP or AH_2 then reduces the tocopherol radical to regenerate tocopherol and give ascorbate radical (Wayner, 1986). The regeneration of tocopherol persists until all ascorbate is consumed (Lambelet *et al.*, 1985).

Ascorbic acid (AH_2) reacts with superoxide radical ($O_2^{\cdot-}$), perhydroxyl radical ($HO_2^{\cdot}$), hydroxyl radical ($HO^{\cdot}$) and singlet oxygen (Fessenden & Verma, 1978; Nanni *et al.*, 1980; Cabelli & Bielski, 1983). Those reactions by AH_2 retard lipid autoxidation.

Ascorbate radical is the initial oxidation product of two enzyme reactions that occur in plants (Loewus & Loewus, 1987). Ascorbate oxidase is the enzyme that oxidizes L-ascorbic acid in the presence of oxygen as shown by the overall reaction below:

$$2 \text{ Ascorbate} + O_2 \longrightarrow 2 \text{ Dehydroascorbic acid} + 2H_2O$$

Ascorbate peroxidase is another plant enzyme that oxidizes AH_2, but it uses hydrogen peroxide instead of oxygen as the electron acceptor.

$$\text{Ascorbate} + H_2O_2 \longrightarrow \text{Dehydroascorbic acid} + 2H_2O$$

Two-electron reduction of dioxygen by L-ascorbic acid

Chemistry

The reaction of L-ascorbate with dioxygen in the presence of transition metal ions is of prime importance in ascorbate chemistry. The transition metals, especially cupric and ferric ions, catalyze the autoxidation by joining together L-ascorbate and oxygen in a proposed ternary complex (Martell, 1982). Then, two π-electrons from L-ascorbate in the complex are thought to shift to oxygen through the transition metal ion. The complex dissociates, and the products are dehydroascorbic acid (A), hydrogen peroxide, and the metal ion (Fig. 11). In most foods, A is rapidly hydrolyzed to 2,3-diketogulonic acid, a reaction that is non-reversible and results in loss of vitamin C activity.

The metal-catalyzed reaction between O_2 and AH_2 increases when acidity is decreased from pH 1·5 to 3·5. This confirms that L-ascorbate monoanion is involved in the intermediate complex. Autoxidative loss of AH_2 accelerates above approximately pH 7 when side reactions become important.

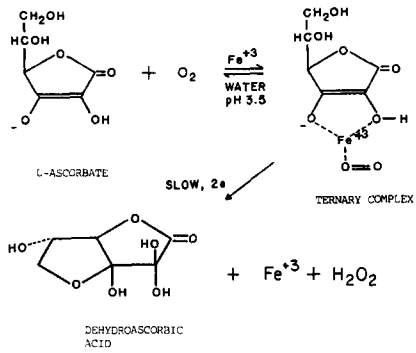

Fig. 11. Reaction of dioxygen with L-ascorbate in the presence of Fe(III).

The ternary complex explains the more rapid oxidation of AH_2 by a mixture of oxygen and the metal ion (highest valence state) than by the metal ion alone (Martell, 1982). Complexing of ferric ion with ascorbate (AH^-) is a much faster reaction than AH_2 reduction of the ferric ion, and the ferric $^{\cdot}AH^-$ complex is stable between pH 2 and 8 (Gorman & Clydesdale, 1983). Furthermore, the Fe(III) $^{\cdot}AH^-$ complex is very much more stable than the Fe(II) $^{\cdot}AH^-$ complex. Kalus & Filby (1982) presented evidence that the ascorbate radical ($A^{\dot{-}}$) is not an intermediate in the reaction between L-ascorbic acid and dioxygen. The detection of $A^{\dot{-}}$ was thought to be due to secondary reactions involving oxidized products from L-ascorbate.

A number of metal chelating agents is known to retard the autoxidation of L-ascorbate, including ethylenediamine tetraacetate (EDTA), oxalate, citrate, phosphates, histidine, uric acid, sugars, acidic polysaccharides, and flavonoids. Agents that complex with L-ascorbic acid, such as proteins, EDTA, and histidine (Fleming & Bensch, 1983), also may retard the autoxidation. Evidence of possible complexing between AH_2 and protein is known from the activation of myrosinase with AH_2 (Ohtsuru & Hata, 1979).

Figure 11 shows that one mole of hydrogen peroxide is released per mole of ascorbate lost. Recently, a 74% yield of H_2O_2 was observed in one reaction where 0·1 mM aqueous AH_2 was heated 5 min at 75° and pH 6

$$AH_2 + O_2 + METAL^+ \longrightarrow A + H_2O_2$$
$$AH_2 + H_2O_2 \longrightarrow A + 2H_2O$$
$$2AH_2 + O_2 \longrightarrow 2A + 2H_2O$$

$$\text{THEORY: } \frac{1\ cm^3\ AIR}{3.3\ mg\ AH_2} \qquad \text{FOUND: } \frac{1\ cm^3\ AIR}{3.4\text{-}3.6\ mg\ AH_2}$$

Fig. 12. Overall loss of L-ascorbic acid (AH_2) reacted with dioxygen in water, and stoichiometry between air and AH_2.

The ternary complex in Fig. 11 suggests that limiting the concentration of any one of the three reactants (Table 2) should diminish the rate of loss of L-ascorbate. Because of the many factors in Table 2 that influence the autoxidation reaction, it is not always possible to predict the stability of AH_2 in foods. The prediction is complicated by other reducing substances in foods, oxidative enzymes, light-degradation and acid-catalyzed dehydration of L-ascorbic acid. Furthermore, in relatively dry foods, the acidic products of oxidized L-ascorbate may chelate metals in the vicinity of the remaining vitamin and thereby change the reaction rate.

L-Ascorbic acid is added to canned or bottled fruits, vegetables, beer, wine, and other beverages to prevent development of oxidative off-flavors and off-color, especially during pasteurization (Bauernfeind, 1982). In this use, the oxygen in the headspace is reduced to water by ascorbate (Fig. 12).

An attractive approach to preserve vitamin C in foods is to add chemically modified forms that resist oxidation. L-Ascorbate 2-phosphate, for example, is at least 10 to 20 times more resistant to O_2 than AH_2 (Seib, 1985). The 2-phosphate ester is a fully active form of vitamin C in primates (Machlin *et al.*, 1979), but it has not been approved for food use.

Other reductions of food components by L-ascorbic acid

The mechanism by which ascorbate reduces various food components are not well understood. Here we mention three such reactions.

Reduction of nitrous acid

Acidification of nitrate salts gives nitrous acid, which is an oxidant that reacts with L-ascorbic acid. If crystals of AH_2 are added to an aqueous sodium nitrite solution, bubbles of nitric oxide immediately appear (Cort, 1982). It is postulated that the reaction proceeds through L-ascorbate 2-nitrite, which decomposes to give nitric oxide and the L-ascorbate radical (Fig. 14).

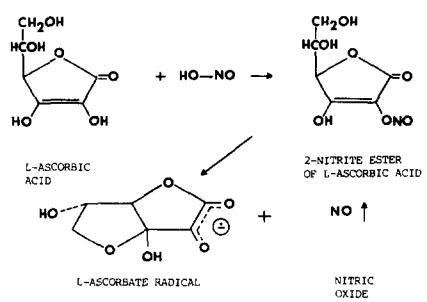

Fig. 14. Reduction of nitrous acid by L-ascorbate.

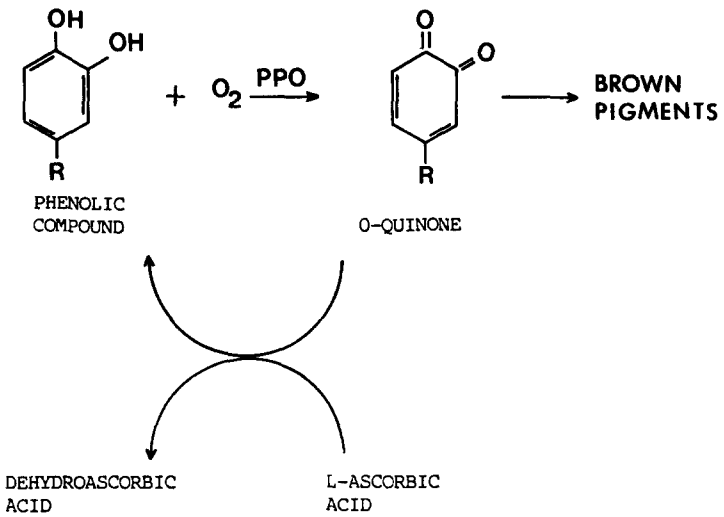

Fig. 15. L-Ascorbic acid reduction of *ortho*-quinone formed during enzymic browning.

The reduction of nitrous acid to nitric oxide by AH_2 is important in the curing of meats (Ranken, 1981; Bauernfeind, 1982) and in preventing *N*-nitrosamine formation (Kim *et al.*, 1982). High concentrations of ascorbic acid (>1 000 ppm) are needed to substantially limit *N*-nitrosamine levels. Excess ascorbic acid destroys most of the nitrous acid, which is the precursor to the *N*-nitrosylating agents.

Reduction of oxidized phenolics

Enzymic browning occurs in fruits and vegetables that have low levels of endogenous AH_2 and active polyphenol oxidase (PPO). Upon cutting or damaging, oxygen and phenolic substrates react by enzyme catalysis to yield quinones, which polymerize rapidly to give brown pigments. Ascorbic acid reduces quinones back to the phenolic form, thereby reversing the reaction until L-ascorbate becomes exhausted (Bauernfeind, 1982; Fig. 15).

Reduction of metal ions

L-Ascorbic acid may preserve or promote the reduced oxidation state of a metal ion in food (Hay *et al.*, 1967). The oxidation state is an important variable in mineral nutrition (Solomons & Viteri, 1982; Keypour *et al.*, 1986). Moreover, the reduction of transition metals by AH_2 is implicated in its 'pro-oxidant' effect, which is observed on occasion (Cort, 1982). This seemingly contradictory nature of a strong reducing agent acting as an accelerator of oxidative deterioration of flavor or color (e.g. carotenoids) in a food is due to a Fenton-type reaction (Walling, 1975). In the scheme shown in Fig. 16, ferrous ions, which are formed by reduction of ferric ions by AH_2,

$$Fe^{+3} + AH^- \longrightarrow Fe^{+2} + A^{\cdot} + H^+$$
$$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + HO^- + HO^{\cdot}$$

Net: $AH^- + H_2O_2 \longrightarrow A^{\cdot} + HO^{\cdot} + H_2O$

AH^- = L-ascorbate

$A^{\cdot}$ = L-ascorbate free radical

Fig. 16. Fenton-type reaction responsible for 'pro-oxidant' effect of L-ascorbate.

react with hydrogen peroxide to release hydroxyl radicals that bleach pigments and propagate chain reactions of radicals. The hydrogen peroxide shown in Fig. 16 may be replaced by an alkyl peroxide, in which case an alkoxy radical is formed instead of a hydroxyl radical. Peroxides arise in foods due to reactions of dioxygen. The pro-oxidant effect does not occur if the concentration of AH_2 in a food is large enough to quench the radicals released by the Fenton reaction.

Dehydroascorbic acid

Structure and chemistry

The structure and reactions of dehydroascorbic acid (A) have been reviewed by Tolbert & Ward (1982) and Seib (1985). The best method of producing A is to bubble oxygen through an ethanolic solution of ascorbic acid in the presence of charcoal. Removal of charcoal and ethanol gives syrupy A, and dehydration of the syrupy product by refluxing in methyl ethyl ketone gives 64% crystalline dimer (Fig. 17). Dissolving the dimer in water immediately releases two molecules of the monomeric form of A.

In aqueous solution dehydroascorbic acid exists predominantly in the bicyclic structure having a 6,3-hemiketal ring, a 1,4-lactone ring, and a C-2

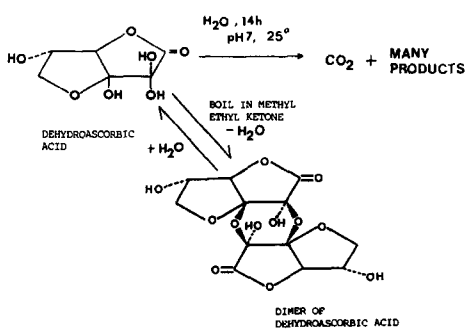

Fig. 17. Stability of dehydroascorbic acid (A) in water and formation of its dimer.

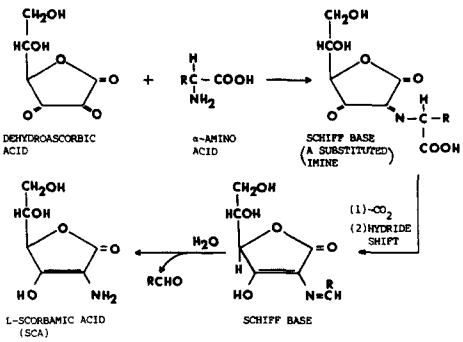

Fig. 18. Strecker degradation between dehydroascorbic acid and an amino acid to give an aldehyde and scorbamic acid.

gem-diol group (Fig. 17) as opposed to its diketo form (Fig. 18). Dehydroascorbic acid decarboxylates at pH 7, whereas it has fair stability in acid at pH 2–5 (Fig. 17). Dehydroascorbic acid can be quantitatively reduced back to AH_2 by hydrogen sulfide, dithiothreitol, reduced glutathione and stannous ions plus thiourea, among others (Tolbert & Ward, 1982; Doner, 1984). The reversible oxidation-reduction of L-ascorbic acid-dehydroascorbic acid in aqueous solution distinguishes that stereoisomer from isoascorbic acid, the stereoisomer whose dehydro-form rapidly decomposes in aqueous solution. L-Ascorbic acid and its dehydro-form have the same vitamin C activity.

Dehydroascorbic acid and browning in foods

Dehydroascorbic acid (A) is though to be involved in the browning of citrus juices and some dehydrated foods (Kurata *et al.*, 1973). It has been postulated that a Strecker degradation between A and an amino acid initiates this browning reaction (Fig. 18), although A forms brown products in the absence of amino compounds (Otsuka *et al.*, 1986). In the Strecker degradation, the initial product is a Schiff base that decarboxylates and then undergoes a hydride shift to give a second Schiff base or substituted imine intermediate. The substituted imine hydrolyzes to release an aldehyde and scorbamic acid (SCA). It is well known that aldehydes released by the Strecker degradation of some amino acids have offensive odors and flavors.

The amino-reductone, L-scorbamic acid, is presumed to be an important intermediate in the browning reaction of A and in the formation of an assortment of colored compounds (Hayashi, 1986; Hayashi *et al.*, 1983). SCA reacts with a molecule of A to give a red pigment (Fig. 19). The red pigment is a weak base, because it is a nitrilo derivative with extensive delocalization of the electrons on its nitrogen, a fact that gives it the red color. The red pigment can be isolated (yield approximately 1%) from

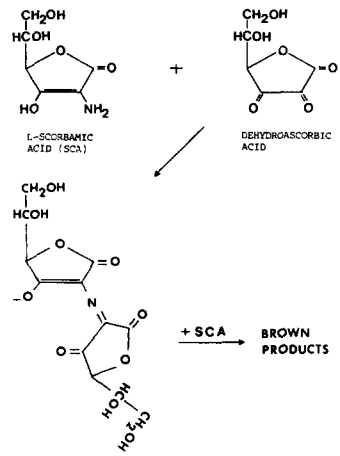

Fig. 19. Reaction of scorbamic acid with dehydroascorbic acid to give a red pigment and brown products.

reactions done in alcohol, but it has not been obtained from an aqueous medium to date. Scorbamic acid has been synthesized by independent routes and shown to form the red pigment when heated with A or the dehydro-form of SCA. SCA has not been isolated from reactions between A and amino acids. Under ordinary conditions, the red pigment undergoes further reactions with SCA to give brown reaction products (Fig. 19).

A series of interesting colored compounds containing bound ascorbyl moieties have been isolated in low yield (Hayashi & Namiki, 1979; Hayashi *et al.*, 1983) when the red pigment is heated with AH_2 in 95% ethanol. Under those reducing conditions, the conversion of the red pigment to brown products is relatively suppressed. The initial reduction product formed from the red pigment is a colorless secondary amine that condenses with a third molecule of A to give an unstable intermediate (Fig. 20). The unstable intermediate is next reduced by AH_2 to give an isolatable radical (by preparative tlc) that is blue in color. Finally, the blue radical is reduced by AH_2 to give tris(2-deoxy-2-ascorbyl) amine, a pale yellow solid whose structure is well characterized. The sequence of reactions giving the red → blue → pale yellow colors can be reversed by air oxidation. When the pale-yellow solid is dissolved in air-saturated aqueous phosphate buffer at pH 7 and 25°C, a blue color (radical) forms within 2 min followed by loss of blue color over a period of approximately 4 h with formation of the red pigment (Hayashi & Namiki, 1979).

The red pigment also is formed when dehydroascorbic acid reacts with proteins containing ε-amino groups. At 5% moisture and 60°C, casein and ovalbumin react with A to give a rapid red coloration, but zein, being low in lysine, reacts weakly (Hayashi *et al.*, 1985). At 20% moisture, casein and

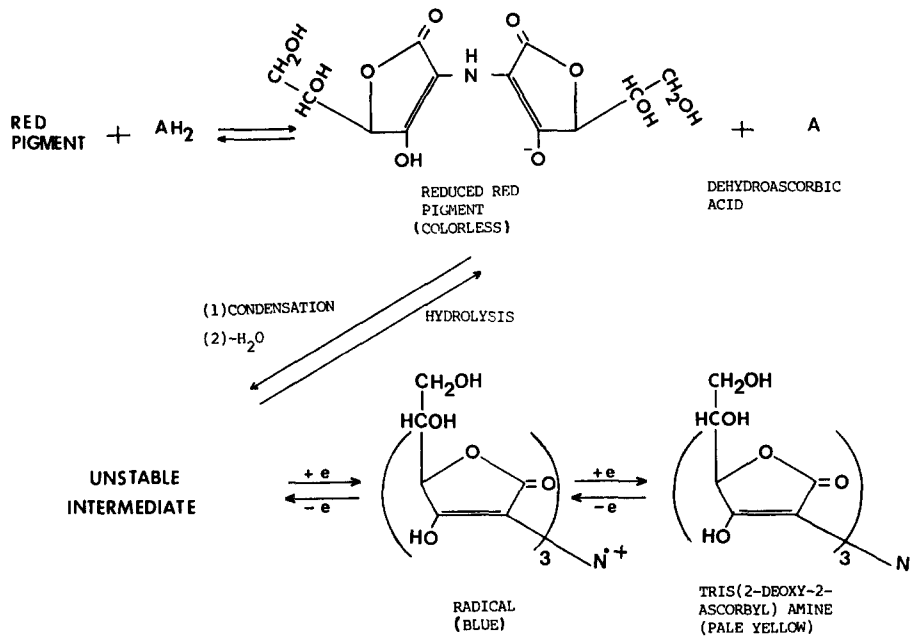

Fig. 20. Reaction of red pigment with excess L-ascorbic acid.

ovalbumin reaction mixtures brown rapidly and without a visible red color. The red pigment in the reacted protein is soluble in water, and its spectral characteristics match those of the red pigment made from free amino acids and A (Fig. 19).

The reaction between protein and dehydroascorbic acid has been proposed (Schmandke, 1981; Pfeilsticker & Marx, 1986) to explain the improvement of bread when AH_2 is added to a bread formula. It is suggested that dehydroascorbic acid acts to cross-link the gluten and thereby increases the elasticity of dough.

Reactions of the 2-carbanion of L-ascorbate

Chemistry

Jackson & Jones (1965) demonstrated the reactivity of the 2-carbanion of L-ascorbate when they attempted to prepare its 3-*O*-benzyl ether. Reaction of potassium L-ascorbate with benzyl chloride in acetone/water gave equal yields of 3-*O*-benzyl-L-ascorbic acid and 2-*C*-benzyl-L-*xylo*-3-hexulosono-1,4-lactone (Fig. 21). The 2-carbanion of L-ascorbate also is involved in the alkali-catalyzed rearrangement of 2,3-di-*O*-methyl-L-ascorbic acid to give the bicyclic 2,3-di-*O*-methyl-L-*xylo*-3-hexulosono-1,4-lactone (isodimethyl-ascorbic acid) (Seib, 1985), and in reactions with α,β-unsaturated carbonyl compounds (Fodor *et al.*, 1983; 1986).

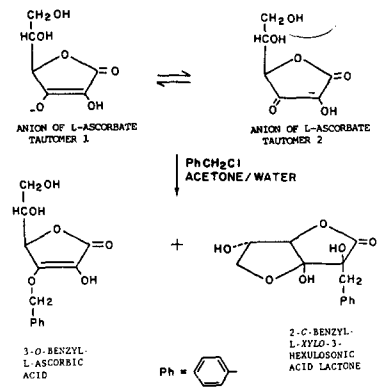

Fig. 21. Benzylation of L-ascorbate.

Example in food

The formation of ascorbigen (Kutacek *et al.*, 1960) in species of *Brassica oleracea* (kale, cabbage, cauliflower, kohlrabi, etc.) appears to be an example of the ability of the C-2 carbanion on ascorbic acid to react with electrophiles (carbonium ions). Plants in the cabbage family contain a glycosinolate called glucobrassicin (Fig. 22). Upon disruption of the plant cells, the enzyme myrosinase (thioglucoside glucohydrolase) catalyzes the hydrolysis of the thioglucosidic bond to give glucose, sulfate, and an intermediate isothiocyanate. It is hypothesized that the C-2 anion of tautomer 2 of L-ascorbate (Fig. 4) then replaces the thiocyanate ion in the intermediate to form ascorbigen.

Kiss & Neukom (1966) synthesized ascorbigen in high yield starting from 3-hydroxymethylindole. They also showed that in water at pH 2 and 37° ascorbigen releases L-ascorbic acid slowly. Ascorbigen is a combined form of vitamin C that should be relatively stable to oxygen. However, its biological activity relative to L-ascorbic acid is 15–20% (Matano & Kato, 1967).

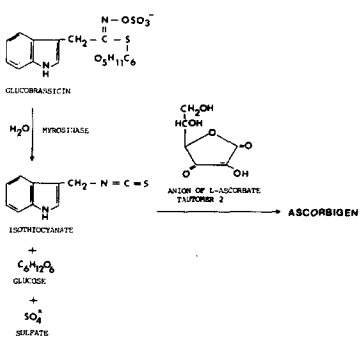

Fig. 22. Mechanism of formation of ascorbigen in ground cabbage.

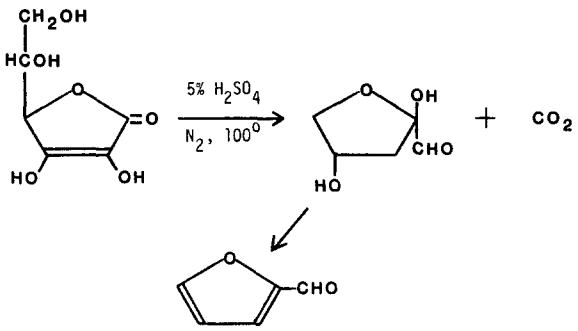

Fig. 23. Acid-catalyzed anaerobic degradation of L-ascorbic acid.

Besides ascorbigen, a second combined form of L-ascorbic acid was discovered recently in plants. Elaeocarpusin is a water-soluble tannin formed by condensation of the C-2 carbanion of L-ascorbate with the tannic acid geraniin (Okuda *et al.*, 1986; Tanaka *et al.*, 1986). Since geraniin is widely distributed in plants, elaeocarpusin may occur in plant foods.

Acid-catalyzed or anaerobic degradation of L-ascorbic acid

Chemistry

In hot acid, L-ascorbic acid is known to decarboxylate and dehydrate to give almost quantitative yields of furfural and carbon dioxide (Fig. 23, Feather & Harris, 1973). A detailed mechanism for the acid-catalyzed degradation of AH_2 has been proposed based on several products isolated from the reaction (Huelin *et al.*, 1971; Goshima *et al.*, 1973; Seib, 1985).

Example in foods

The acid-catalyzed degradation of L-ascorbic acid is thought to be responsible for anaerobic loss of vitamin C in foods, such as canned grapefruit and orange juices, which have a pH of approximately 3·5 (Kefford *et al.*, 1959; Smoot & Nagy, 1980). At 50°C, the juices lose 70–95% of L-ascorbic acid in 12 weeks; the degradation rection is zero-order with respect to AH_2 and has a Q_{10} of 2·5–3·0. The anaerobic loss of AH_2 is often one-tenth the rate of loss under aerobic conditions.

NOTE ADDED ON PROOF

L-Ascorbic acid (AH_2) retards enzymic browning by at least two mechanisms (Golan-Goldhirsh *et al.*, 1984). AH_2 chemically reduces

benzoquinone intermediates to colorless *o*-dihydroxyphenols, and it also irreversibly denatures polyphenoloxidase (PPO). Evidence (Golan-Goldhirsh *et al.*, 1987) suggests that PPO is denatured mainly by Cu^{+2}-catalyzed oxidative cleavage of imidazole groups of the histidine residues on the enzyme to aspartic acid and urea. The oxidation is mediated through a quaternary complex thought to contain the imidazole group, cupric ion, L-ascorbic acid radical ($AH^{\cdot}$), and O_2.

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REFERENCES

Abe, Y., Horii, H. & Taniguchi, S. (1984). A theoretical study on the oxidation of triose reductone in reference to L-ascorbic acid. *Can. J. Chem.*, **64**, 360–5.

Anderson, S., Marks, C. B., Lazarus, R., Miller, J., Stafford, K., Seymour, J., Light, D., Rastetter, W. & Estell, D. (1985). Production of 2-keto-L-gulonate, an intermediate in L-ascorbate synthesis, by a genetically modified *Erwinia herbicola*. *Science*, **230**, 144–9.

Bauernfeind, J. C. (1982). Ascorbic acid technology in agricultural, pharmaceutical, food, and industrial applications. In *Ascorbic Acid: Chemistry, Metabolism and Uses*, ed. P. A. Seib & B. M. Tolbert, Adv. Chem. Ser. No. 200. American Chemical Society, Washington, DC, 395–497.

Berger, S. (1984). ^{13}C–^{13}C-Spin-spin coupling constants as a probe for mesomeric structures: Vitamin C. *J. Chem. Soc., Chem. Commun.*, **1984**, 1252–3.

Bielski, B. H. J. (1982). Chemistry of ascorbic acid radicals. In *Ascorbic Acid: Chemistry, Metabolism and Uses*. ed P. A. Seib & B. M. Tolbert, Adv. Chem. Ser. No. 200. American Chemical Society, Washington, DC, 81–99.

Bielski, B. H. J., Allen, A. O. & Schwarz, H. A. (1981). Mechanism of disproportionation of ascorbate radicals. *J. Am. Chem. Soc.*, **103**, 3516–8.

Bond, A. D., McClelland, B. W., Einstein, J. R. & Finamore, F. J. (1972). Ascorbic acid-2-sulfate of the brine shrimp, *Artemia salina*. *Arch. Biochem. Biophys.*, **153**, 207–14.

Brenner, G. S., Hinkley, D. F., Perkins, L. M. & Weber, S. (1964). Isomerization of the ascorbic acids. *J. Org. Chem.*, **29**, 2389–92.

Cabelli, D. E. & Bielski, B. H. J. (1983). Kinetics and mechanism for the oxidation of ascorbic acid/ascorbate by HO_2/O_2^- radicals. A pulse radiolysis and stopped-flow photolysis study. *J. Phys. Chem.*, **87**, 1809–12.

Cort, W. M. (1982). Antioxidant properties of ascorbic acid in foods. In *Ascorbic Acid: Chemistry, Metabolism and Uses*, ed. P. A. Seib & B. M. Tolbert, Adv. Chem. Ser. No. 200. American Chemical Society, Washington, DC, 533–50.

Crawford, T. C. & Crawford, S. A. (1980). Synthesis of L-ascorbic acid. *Adv. Carbohydr. Chem.*, **37**, 79–155.

Doner, L. W. (1984). Analysis of ascorbic acid and related compounds in fluids and

tissues. In *Trace Analysis.* Vol. 3. ed J. F. Lawrence, Academic Press, Inc., NY, 119.

Feather, M. S. & Harris, J. F. (1973). Dehydration reactions of carbohydrates. *Adv. Carbohydr. Chem.*, **28**, 161–224.

Fessenden, R. W. & Verma, N. C. (1978). A time-resolved electron spin resonance study of the oxidation of ascorbic acid by hydroxyl radical. *Biophys. J.*, **24**, 93–101.

Fleming, J. E. & Bensch, K. G. (1983). Effect of amino acids, peptides, and related compounds on the autoxidation of ascorbic acid. *Int. J. Peptide Protein Res.*, **22**, 355–61.

Fodor, G., Arnold, R., Mohacsi, T., Karle, I. & Flippen-Anderson, J. (1983). A new role for L-ascorbic acid: Michael donor to α,β-unsaturated carbonyl compounds. *Tetrahedron.*, **39**, 2137–45.

Fodor, G., Sussangkarn, K., Mathelier, H., Fang, K., Arnold, R., Flippen-Anderson, J. & Karle, I. (1986). Stereospecificity of a new reaction of L-ascorbic acid with *cis* and *trans* olefinic 1,4-dicarbonyl compounds. *J. Org. Chem.*, **51**, 3148–50.

Golan-Goldhirsh, A., Kahn, V. & Whitaker, J. R. (1984). Relation between structure of polyphenol oxidase and prevention of browning. In *Advances in Experimental Medicine and Biology*, ed. M. Friedmann. Plenum Press, New York, 437–56.

Golan-Goldhirsh, A., Osuga, D. T., Chan, A. O. & Whitaker, J. T. (1987). Effects of ascorbic acid and copper on proteins. Abstract HIST No. 26, 193rd National Meeting of the American Chemical Society, Denver, CO, USA, 5–10 April 1987.

Gorman, J. E. & Clydesdale, F. M. (1983). The behaviour and stability of iron–ascorbate complexes in solution. *J. Fd. Sci.*, **48**, 1217–20, 1225.

Goshima, K., Maezono, N. & Tokuyama, K. (1973). A novel degradation pathway of L-ascorbic acid under non-oxidative conditions. *Bull. Chem. Soc. Jpn.*, **46**, 902–4.

Hay, G. W., Lewis, B. A. & Smith, F. (1967). In *The Vitamins.* Vol. 1 (2nd edn), ed. W. H. Sebrell, Jr & R. S. Harris, Academic Press Inc., NY, 307–46.

Hayashi, T. (1986). Chemistry of ascorbic acid and its utilization for foods. *Nippon Shokuhin Kogyo Gakkaishi*, **33**, 456–62.

Hayashi, T. & Namiki, M. (1979). Tri(2-deoxy-2-L-ascorbyl) amine: A novel compound related to a fairly stable free radical. *Tetrahedron Lett.*, **46**, 4467–70.

Hayashi, T., Namiki, M. & Tsuji, K. (1983). Formation mechanism of the free radical products and its precursor by the reaction of dehydro-L-ascorbic acid with amino acid. *Agric. Biol. Chem.*, **47**, 1955–60.

Hayashi, T., Terao, A., Ueda, S. & Namiki, M. (1985). Red pigment formation by the reaction of oxidized ascorbic acid and protein in a food model system of low moisture content. *Agric. Biol. Chem.*, **49**, 3139–44.

Hornig, D., Weber, F. & Wiss, O. (1974). Influence of erythorbic acid on the vitamin C status in guinea-pigs. *Experientia*, **30**, 173–4.

Huelin, F. E., Coggiola, I. M., Sidhu, G. S. & Kennett, B. H. (1971). The anaerobic decomposition of ascorbic acid in the pH range of foods and in more acid solutions. *J. Sci. Food Agric.*, **22**, 540–2.

Hvoslef, J. (1982). Crystallography of the ascorbates. In *Ascorbic Acid: Chemistry, Metabolism and Uses*, ed. P. A. Seib & B. M. Tolbert, Adv. Chem. Ser. No. 200. American Chemical Society, Washington, DC, 37–57.

Isbell, H. S. & Frush, H. L. (1979). Oxidation of L-ascorbic acid by hydrogen peroxide: Preparation of L-threonic acid. *Carbohydr. Res.*, **72**, 301–4.

Iyanagi, T., Yamazaki, I. & Anan, K. F. (1985). One-electron oxidation-reduction properties of ascorbic acid. *Biochim. Biophys. Acta*, **806**, 255–61.

Jackson, K. G. A. & Jones, J. K. N. (1965). The *O*- and *C*-benzylation of L-ascorbic acid. *Can. J. Chem.*, **43**, 450–7.

Jaffe, G. M. (1984). Ascorbic acid. In *Encyclopedia of Chemical Technology*, Vol. 24 (3rd edn) ed K. Othmer, John Wiley and Sons, NY, 8–40.

Kalus, W. H. & Filby, W. G. (1982). The effect of inhibitors on the radical formation in aqueous solution of ascorbic acid. *Int. J. Vit. Nutr. Res.*, **52**, 44–9.

Kalus, W. H. & Filby, W. G. (1986). Loss of ascorbic acid during simulated industrial blanching experiment. *Lebensm. Wiss. d-Technol.*, **19**, 7–12.

Kefford, J. F., McKenzie, H. A. & Thompson, P. C. O. (1959). Effect of oxygen on quality and ascorbic acid retention in canned and frozen orange juices. *J. Sci. Food Agric.*, **10**, 51–63.

Keypour, H., Silver, J., Wilson, M. T. & Hamed, M. Y. (1986). Studies on the reactions of ferric ion with ascorbic acid. A study of solution chemistry with Mossbauer spectroscopy and stopped-flow techniques. *Inorg. Chim. Acta.*, **125**, 97–106.

Kim, Y. K., Tannenbaum, S. R. & Wishnok, J. S. (1982). In *Ascorbic Acid: Chemistry, Metabolism and Uses*, ed. P. A. Seib & B. M. Tolbert, Adv. Chem. Ser. No. 200. American Chemistry Society, Washington, DC, 571–85.

Kiss, G. & Neukom, H. (1966). About the structure of ascorbigens. *Helv. Chim. Acta*, **49**, 989–92.

Kurata, T., Fujimaki, M. & Sakurai, Y. (1973). Red pigment produced by the reaction of dehydro-L-ascorbic acid with α-amino acid. *Agric. Biol. Chem.*, **37**, 1471–7.

Kutacek, M., Prochazka, Z. & Grunberger, D. (1960). Biogenesis of ascorbigen, 3-indolylacetonitrile, and indole-3-carboxylic acid from D,L-tryptophan-3-^{14}C in *Brassica oleracea* L., *Nature*, **187**, 61–2.

Lambelet, P., Saucy, F. & Loliger, J. (1985). Chemical evidence for inteactions between vitamins E and C. *Experientia*, **41**, 1384–8.

Lillard, D. W., Jr, Seib, P. A. & Hoseney, R. C. (1982). Isomeric ascorbic acids and derivatives of L-ascorbic acid: Their effect on the flow of dough. *Cereal Chem.*, **59**, 291–6.

Loewus, F. A. & Loewus, M. W. (1987). Biosynthesis and metabolism of ascorbic acid in plants. *CRC Crit. Rev. Plant Sci.*, **5**(1), 101–19.

Lohmann, W. & Holz, D. (1984). Structure of ascorbic acid and its biological function. I. ESR determination of ascorbyl radical in biological samples and model systems. *Biophys. Struct. Mech.*, **10**(4), 197–204.

Lohmann, W., Pagel, D. & Penka, V. (1984). Structure of L-ascorbic acid and its biological function. *Eur. J. Biochem.*, **138** 479–80.

Lu, P. W., Lillard, D. W., Jr, Seib, P. A., Kramer, K. J. & Liang, Y. T. (1984). Synthesis of the 2-methyl ether of L-ascorbic acid: Stability, vitamin activity, and carbon-13 nuclear magnetic resonance spectrum compared to those of the 1- and 3-methyl ethers. *J. Agric. Food Chem.*, **32**, 21–8.

Machlin, L. J., Garcia, F., Kuenzig, W. & Brin, M. (1979). Antiscorbutic activity of ascorbic acid phosphate in the rhesus monkey and the guinea pig. *Am. J. Clin. Nutr.*, **32**, 325–31.

Martell, A. E. (1982). Chelates of ascorbic acid: Formation and catalytic properties. In *Ascorbic Acid: Chemistry, Metabolism and Uses.* Ed P. A. Seib & B. M. Tolbert, Adv. Chem. Ser. No. 200. American Chemical Society, Washington, DC, 153–78.

Matano, K. & Kato, N. (1967). Studies on synthetic ascorbigen as a source of vitamin C for guinea pigs. *Acta Chem. Scand.*, **21**, 2886–7.

Nanni, E. J., Jr, Stallings, M. D. & Sawyer, D. T. (1980). Does superoxide ion oxidize catechol, α-tocopherol, and ascorbic acid by direct electron transfer? *J. Am. Chem. Soc.*, **102**, 4481–5.

Ohtsuru, M. & Hata, T. (1979). The interaction of L-ascorbic acid with the active center of myrosinase. *Biochim. Biophys. Acta*, **567**, 384–91.

Okuda, T., Yoshida, T., Hatano, T. & Ikeda, Y. (1986). Biomimetic synthesis of elaeocarpusin. *Heterocycles*, **24**, 1841–3.

Otsuka, M., Kurata, T. & Arakawa, N. (1986). Isolation and characterization of a degradation product derived from 2,3-diketo-L-gulonic acid. *Dev. Food Sci.*, **13**, 77–84.

Paukstellis, J. V., Mueller, D. D., Seib, P. A. & Lillard, D. W., Jr (1982). Nmr spectroscopy of ascorbic acid and its derivatives. In *Ascorbic Acid: Chemistry, Metabolism and Uses.* Ed P. A. Seib & B. M. Tolbert, Adv. Chem. Ser. No. 200. American Chemical Society, Washington, DC, 125–51.

Pfeilsticker, K. & Marx, F. (1986). An investigation into the reaction kinetics of L-ascorbic acid and L-dehydroascorbic acid in wheat flour doughs using a gaschromatographic/mass spectrometric method. *Z. Lebensm. Unters. Forsch.*, **182**, 191–5.

Ranken, M. D. (1981). The use of ascorbic acid in meat processing. In *Vitamin C, Ascorbic Acid.* Ed J. N. Counsell & D. H. Hornig, Applied Science Publishers, NJ, 105–22.

Sapper, H., Pleyer-Weber, A. & Lohmann, W. (1982*a*). ^{1}H-Nmr and ESR investigations on the structures of dehydroascorbic acid and the semidehydroascorbate radical. *Z. Naturforsch. C: Biosci.*, **37**, 129–31.

Sapper, H., Kang, S. O., Paul, H. H. & Lohmann, W. (1982*b*). The reversibility of the vitamin C redox system: Electrochemical reasons and biological aspects. *Z. Naturforsch. C: Biosci.*, **37C**, 942–6.

Sawyer, D. T., Chiericato, G., Jr & Tsuchiya, T. (1982). Oxidation of ascorbic acid and dehydroascorbic acid by superoxide ion in aprotic media. *J. Am. Chem. Soc.*, **104**, 6273–8.

Schmandke, H. (1981). Actual problems of research in food science. *Nahrung*, **25**, 39–47.

Seib, P. A. (1985). Oxidation, monosubstitution and industrial synthesis of ascorbic acid. *Int. J. Vit. Nutr. Res., Supplement*, **27**, 259–306.

Smoot, J. M. & Nagy, S. (1980). Effects of storage temperature and duration on total vitamin C content of canned single strength grapefruit juice. *J. Agric. Food Chem.*, **28**, 417–21.

Solomons, N. W. & Viteri, F. E. (1982). Biological interaction of ascorbic acid and mineral nutrients. In *Ascorbic Acid: Chemistry, Metabolism and Uses.* Ed P. A. Seib & B. M. Tolbert, Adv. Chem. Ser. No. 200. American Chemical Society, Washington, DC, 551–69.

Sonoyama, T., Tani, H., Matsuda, K., Kageyama, B., Tanimoto, M., Kobayashi, K., Yagi, S., Kyotani, H. & Mitsushima, K. (1982). Production of 2-keto-L-gulonic

acid from D-glucose by two-stage fermentation. *Appl. Environ. Microbiol.*, **43**, 1064–9.

Szent-Györgyi, A. (1979). Submolecular Biology and Cancer. Ciba Foundation Symposium 67, Excerpta Medica, Amsterdam. Cited in Eckert-Maksic, M., Bischof, P. & Maksic, Z. B. (1985). Vitamin C and its radicals: Tautomerism, electron structure and properties. *Croatica Chem. Acta*, **58**, 407–26.

Takahashi, M., Niki, E., Kawakami, A., Kumasaka, A., Yamamoto, Y., Kamiya, Y. & Tanaka, K. (1986). Oxidation of lipids. XIV. Inhibition of oxidation of methyl linoleate by fatty acid esters of L-ascorbic acid. *Bull. Chem. Soc. Jpn.*, **59**, 3179–83.

Tanaka, T., Nonaka, G. & Nishioka, I. (1986). Tannins and related compounds. Part 37. Isolation and structure elucidation of elaeocarpusin, a novel ellagitannin from *Elaeocarpus sylvestris* var. *Ellipticus. J. Chem. Soc., Perkin Trans. I.*, **1986**, 369–76.

Tolbert, B. M. & Ward, J. B. (1982). Dehydroascorbic acid. In *Ascorbic acid: Chemistry Metabolism and Uses.* Ed P. A. Seib & B. M. Tolbert, Adv. Chem. Ser. No. 200. American Chemical Society, Washington, DC, 101–23.

Walling, C. (1975). Fenton's reagent revisited. *Acc. Chem. Res.*, **8**, 125–31.

Wayner, D. D. M., Burton, G. W. & Ingold, K. U. (1986). The antioxidant efficiency of vitamin C is concentration-dependent. *Biochim. Biophys. Acta*, **884**, 119–23.