

Assessment of the Antioxidative and Prooxidative Activities of Two Aminoreductones Formed during the Maillard Reaction: Effects on the Oxidation of β -Carotene, N^α -Acetylhistidine, and *cis*-Alkenes

Monika Pischetsrieder,* Francesco Rinaldi, Ursula Gross, and Theodor Severin*

Institut für Pharmazie und Lebensmittelchemie der Universität München, Sophienstrasse 10, 80333 München, Germany

In short-time-heated mixtures of lactose and N^α -acetyllysine 1-[N^ϵ -(N^α -acetyllysyl)]-1,2-dehydro-1,4-dideoxy-3-hexulose (C_6 -AR) is formed as main product, whereas 3-hydroxy-4-(alkylamino)-3-buten-2-one (C_4 -AR) can be obtained in high yields from the Maillard reaction of glucose. Because both compounds have aminoreductone structure, their antioxidative (AOA) and prooxidative activities (POA) were determined and compared to those of ascorbic acid (AA). Concentration-dependent AOA was determined by measuring oxidative degradation of carotene induced by a radical starter. POA in the presence of metal ions was tested in three different systems: oxidation of carotene in emulsion, of N^α -acetylhistidine in aqueous solution, and of *cis*-alkenes in organic solvent. C_4 -AR possesses in all model systems AOA and POA, respectively, which are very similar to those of AA. C_6 -AR acts also as antioxidant and prooxidant, but its activity is weaker compared to those of C_4 -AR and AA. In the carotene assay the substances displayed POA in the presence of several metal ions, such as Cu^{2+} , Mn^{2+} , and Fe^{3+} /EDTA, but the activity with the latter is considerably lower.

Keywords: Aminoreductones; Maillard reaction; antioxidative; prooxidative

INTRODUCTION

During the Maillard reaction reducing sugars react with amino groups of amino acids or proteins and a variety of products are formed depending on the reaction conditions. In addition to other effects, Maillard products have strong reducing properties that can prevent oxidative spoilage of processed foodstuffs, such as beer (Yoshimura et al., 1997; Nicoli et al., 1997; Karastogiannidou and Ryley, 1994.). Several Maillard products with reductone structure that possess reducing properties have been identified (Ledl and Schleicher, 1990). However, it is not clear how much of the total antioxidative activity of Maillard products in food can be assigned to these reductones. Because several studies suggest that protein-bound Maillard products are responsible for scavenging reactive oxygen species (Alaiz et al., 1997; Kato, 1992), other compounds apart from reductones must be active.

On the other hand, it is known that the majority of antioxidants which are naturally present or added to foodstuffs can also enhance free radical damage of other components and therefore act as prooxidants in biological systems (Aruoma, 1996). This process has been thoroughly investigated for ascorbic acid (AA), and it was found that in addition to its well-known antioxidative properties, AA generates in the presence of metal ions and oxygen reactive oxygen species, such as H_2O_2 (Kalus et al., 1982), hydroxyl radicals ($\cdot OH$) (Wong et al., 1981), superoxide (O_2^-) (Nakanishi et al., 1985), or metal-peroxo complexes [e.g., $O_2-Cu(I)$] (Uchida and Kawakishi, 1989). As a result, peptides (Steinhart et

al., 1995), enzymes (Shinar et al., 1983), polysaccharides (Wong et al., 1981), DNA (Samuni et al., 1983), and lipids (Kanner et al., 1977) are oxidatively degraded. Therefore, it has to be considered that Maillard products in food and in vivo can also promote the oxidative damage of other components. It must be assumed that Maillard products can form H_2O_2 similarly to AA in the presence of metal ions and oxygen, which then generates in a Fenton reaction reactive oxygen species (ROS), such as hydroxyl radicals.

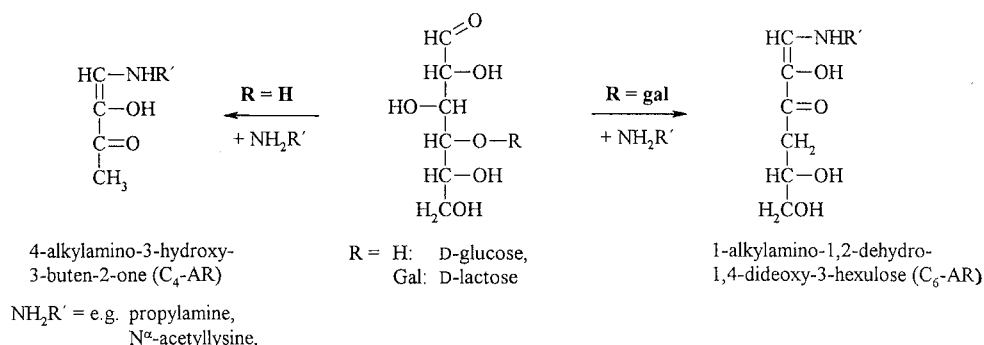
When we investigated Maillard reaction mixtures of disaccharides, 1-(alkylamino)-1,2-dehydro-1,4-dideoxy-3-hexulose (C_6 -AR) was obtained as the main product, whereas 3-hydroxy-4-(alkylamino)-3-buten-2-one (C_4 -AR) is formed in high yields when monosaccharides are reacted (Scheme 1). Both the C_6 -AR from lactose and the C_4 -AR from glucose possess a β -aminoreductone structure (Scheme 1). It is known that products with a reductone structure, such as AA, have reducing properties (Euler and Eistert, 1957). Furthermore, it was deduced that the N-analogous α - and β -aminoreductones have similar properties and that the electron-donating effect of the amino group even increases the reducing character (Pischetsrieder and Severin, 1997). However, this assumption has not clearly been proven so far.

Therefore, the purpose of this study was to investigate the prooxidant and antioxidant activities of C_4 -AR and C_6 -AR in detail.

MATERIALS AND METHODS

Reagents. AA was purchased from Roth (Karlsruhe, Germany), and β -carotene, diethylenetriaminepentaacetic acid (DTPA), and α, α' -azodiisobutyramidine dihydrochloride (ADI-BA) were from Fluka (Buchs, Switzerland). Sodium linoleate and Tween 20 were purchased from Sigma (St. Louis, MO). Methanol LiChrosolv, chromatography grade, was obtained

* Author to whom correspondence should be addressed (telephone ++49-89-5902-387; fax ++49-89-5902-447; e-mail pischets@pharmchem.uni-muenchen.de).

Scheme 1. Formation of C₄-AR and C₆-AR from D-Glucose or Lactose, Respectively

from Merck (Darmstadt, Germany). Deionized water was distilled before use for HPLC. Preparative thin-layer chromatography (TLC) was performed using 20×20 cm glass plates coated with a 0.5 mm thickness of silica gel 60 F₂₅₄.

Apparatus. UV spectra were recorded on a Perkin-Elmer UV-vis spectrometer Lambda 20 and ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra with a JEOL 400 GSX spectrometer using (CH₃)₄Si as internal standard. Chemical shifts are reported in parts per million. Mass spectral analyses were obtained with an HP 5989 A MS engine (CI with CH₄) and positive FABMS data with a Kratos MS 80 RFA spectrometer.

HPLC. Analytical HPLC was performed with a Merck L-7100 gradient pump, a Merck L-7450 photodiode array detector including Merck-Hitachi model D-7000 Chromatography Data Station software (Merck, Darmstadt). For quantification DAD-System-Manager software D-7000 Chromatography Data Station (Merck-Hitachi) with manual baseline correction was used. For preparative HPLC a Merck L-6250 pump, a Merck L-4000 UV detector, and a Merck D-2500 chromatointegrator were used. The mixtures were separated on a column packed with Nucleosil 100-5 (RP 18, 125 × 3 mm i.d., guard cartridge, 8 × 3 mm) from Macherey & Nagel (Düren, Germany). For elution a gradient was used of 0–100% B from 0 to 25 min and 100% B from 25.1 to 40 min at a flow rate of 0.5 mL/min (solvent A, 50 mM triethylammonium acetate, pH 5.8; solvent B, methanol).

GC/MS. The GC/MS system consists of a Hewlett-Packard (Waldbronn, Germany) gas chromatograph HP 5890 series II coupled with a Hewlett-Packard HP 5971A msd mass spectrometer. Separations were performed on an Optima fused silica capillary column 1701-0.25 μm (0.25 mm × 25 m; Macherey & Nagel) with a helium flow of 0.5 mL/min (split: 1/10). Oven temperature was programmed as follows for detection of 2-cyclohexen-1-one: 4 min at 60 °C, from 60 to 80 °C at a rate of 2 °C/min, from 80 to 260 °C at a rate of 20 °C/min, and at 260 °C for 15 min. For the detection of 2-hydroxy-3-hexene the program was as follows: 4 min at 40 °C, from 40 to 70 °C at a rate of 1 °C/min, from 70 to 260 °C at a rate of 20 °C/min, and at 260 °C for 15 min. Electron impact mass spectra were recorded under the following conditions: capillary direct interface, 280 °C; ionization voltage, 70 eV; mass range, *m/z* 50–400; electron multiplier voltage, 2450 V; scan rate, 1.5 scans/s. Spectra were obtained using HP G1034C MS ChemStation software.

Preparation of the Aminoreductones. 1-(Butylamino)-1,2-dehydro-1,4-dideoxy-3-hexulose (C₆-AR) was prepared as described before (Pischetsrieder et al., 1997). Briefly, lactose was heated with butylamine in phosphate buffer at pH 7.0 for 30 min at 100 °C, and C₆-AR was extracted with ethyl acetate. The analytically pure compound was immediately used for the assays.

3-Hydroxy-4-(propylamino)-3-buten-2-one (C₄-AR) was isolated from a Maillard reaction mixture. Thirty milliliters of an aqueous solution of propylamine (0.25 M) and glucose (0.25 M) was adjusted with phosphoric acid to pH 7.1 and was heated for 1 h at 100 °C. The mixture was extracted three times with 30 mL of ethyl acetate, and the solvent of the organic layer was evaporated. The residue was dissolved in

methanol and separated by preparative HPLC on a LiChrosorb RP 18, 250/2–7 μm column (Merck) with an eluent of 20% methanol in 50 mM ammonium formate and a flow rate of 10 mL/min. The fractions between 30 and 40 min were collected, unified, and extracted four times with 40 mL of ethyl acetate. The unified organic layers were evaporated, and C₄-AR was purified by distillation at 150 °C and 0.4 Torr, which yields colorless crystals with a melting point of 47 °C: ¹H NMR (CDCl₃, COSY) δ 0.90 (t, 3 H, *J* = 7.3 Hz, CH₃–CH₂), 1.54 (sext, 2 H, *J* = 7.3 Hz, CH₃–CH₂), 2.07 (s, 3 H, CH₃C=O), 3.11 (dt, 2 H, *J* = 7.3 and 6.8 Hz, N–CH₂), 4.5 (br, 1 H, NH), 6.57 (d, 1 H, *J* = 12.4 Hz, CH=C); ¹³C NMR (CDCl₃) δ 11.0 (CH₃–CH₂), 21.0 (CH₃–C=O), 24.5 (CH₃–CH₂), 49.9 (CH₂–N), 131.0 (=C–OH), 131.7 (=CH–N), 186.4 (C=O); UV (CH₃OH) λ_{max} 321 nm (log ε = 4.3); CI-MS *m/z* 144 (M⁺ + 1). Anal. Calcd for C₇H₁₃NO₂: C, 58.70; H, 9.16; N, 9.79. Found: C, 59.04; H, 8.93; N, 9.61.

Alternatively, 3-hydroxy-4-(propylamino)-3-buten-2-one (C₄-AR) was synthesized from 1-bromo-2,3-butanedione. Thus, 1-bromo-2,3-butanedione (165 mg, 1 mmol), which was prepared according to the literature (Dow Chemical Co., 1958), was dissolved in 1 mL of tetrahydrofuran and kept on ice. Two millimoles of ice-cold propylamine was added dropwise to the mixture and stirred for 30 min at room temperature. After filtration, the solvent was evaporated and the residue was separated by column chromatography on silica gel (solvent isopropyl ether/ethyl acetate 2:3, detection of C₄-AR with 2,6-dichlorindophenone). Spectral data were identical with those of the isolated product.

Antioxidant Assay. Antioxidant activity was assayed according to a modified method of Chuda et al. (1996). Solutions of β-carotene (0.5 mg in 0.5 mL), sodium linoleate (20 mg in 0.2 mL), and Tween 20 (200 mg in 1 mL) in chloroform were vigorously mixed, and the solvent was removed under a stream of nitrogen. The mixture was dissolved in 100 mL of water, and to 45 mL of this solution was added 4 mL of buffer (0.2 M phosphate buffer, pH 6.8, containing 5 mg/mL DTPA). The reagent solution (3.1 mL) was mixed with 100 μL of the sample (various concentrations of C₆-AR and C₄-AR in methanol and of AA in water as indicated), and the reaction was started by addition of 5 μL of 0.5 M ADIBA in phosphate buffer/DTPA. The blanks were prepared exactly in the same way, but water or methanol was added instead of sample. After 50 min of reaction in the dark, the absorbance was measured at 470 nm. The antioxidative activity (AOA) was calculated after subtraction of the blank as follows: (Abs_{0min} – Abs_{50min})/Abs_{0min}.

The results are the mean of two to four independent experiments.

Prooxidant Assay Using β-Carotene/Sodium Linoleate. Reagent solution was prepared as described above with the exception that 0.2 M triethylammonium acetate, pH 7.0, was used as buffer, unless otherwise noted. This reagent solution (3.1 mL) was mixed with 100 μL of the sample (various concentrations of C₆-AR and C₄-AR in methanol and of AA in water as indicated), and the reaction was started by addition of 8 μL of CuSO₄ (3.2 mg/mL in water). As blanks, 100 μL of methanol or water was used. Absorbance at 470 nm was

measured before and after 20 min of reaction in the dark. Prooxidative activity (POA) was calculated as $[\text{Abs}_{0\text{min}} - \text{Abs}_{20\text{min}}(\text{sample})]/[\text{Abs}_{0\text{min}} - \text{Abs}_{20\text{min}}(\text{blank})] \times 100$. The results are the mean of two independent experiments.

To determine the influence of different metal ions, instead of CuSO_4 , 8 μL of MnCl_2 (4.0 mg/mL) or $\text{FeCl}_3/\text{EDTA}$ (3.3 mg of FeCl_3 and 7.6 mg of EDTA/mL) was used.

Prooxidant Assay Using *N*^α-Acetylhistidine. CuSO_4 (16 $\mu\text{g}/5 \mu\text{L}$) was added to an aqueous solution of *N*^α-acetylhistidine (0.22 mg/mL), and the mixture was stirred vigorously in an open vial. The reaction was started by the addition of the sample (2 $\mu\text{mol}/12 \mu\text{L}$) and stirring was continued. After 30 min, phenoxyacetic acid (156 $\mu\text{g}/200 \mu\text{L}$) was added as an internal standard and the solution was immediately injected into the HPLC. Separation was performed on a column packed with Lichrosorb 5C (RP 18, 250 × 4.6 mm i.d.) from Macherey & Nagel with an elution gradient of 0–25% B from 0 to 7 min and 55–100% B from 7.1 to 30 min at a flow rate of 0.8 mL/min (solvent A, 10 mM ammonium formate, pH 7.5; solvent B, methanol). The substances were detected at 215 nm.

Identification of *N*-Formyl-*N'*-(*N*^α-acetyl-β-aspartyl)-urea. *N*^α-Acetylhistidine (550 mg) and 725 mg of AA were dissolved in 200 mL of water. After the addition of 9.6 mg of CuSO_4 in 3 mL of water, the mixture was stirred for 24 h at room temperature and the excess of copper ions was removed by an Amberlite IR 120 column. The eluate was lyophilized and redissolved in 30 mL of water, filtered through a membrane (0.2 μm pore size), and purified by preparative HPLC. Separation was performed on a Supelcosil column LC-18-DB, 250 × 21.2 mm, 5 μm particle size (Supelco, Bellefonte, PA) using water as eluent with a flow rate of 12 mL/min. Each time 2 mL of the solution was injected and the compounds were detected at a wavelength of 207 nm. The fractions that eluted between 11.3 and 12.9 min were collected, lyophilized, and subjected to spectral analyses: ¹H NMR ($\text{DMSO}-d_6$) δ 1.85 (s, 3 H, CH_3CO), 2.76–2.82 (dd, 1H, $J = 6.9$ and 16.6 Hz, COCH_aH_b), 2.94–3.00 (dd, $J = 5.9$ and 16.6 Hz, 1H, COCH_aH_b), 4.58–4.64 (m, 1H, $\text{CH}-\text{CH}_2$), 8.27–8.29 (d, 1H, $J = 8.0$ Hz, $\text{NH}-\text{CH}-\text{CH}_2$), 9.04 (d, 1H, $J = 9.5$ Hz, $\text{NH}-\text{CHO}$), 10.51 (d, 1H, $J = 9.5$ Hz, $\text{NH}-\text{CHO}$), 11.02 (s, 1H, $\text{CO}-\text{NH}-\text{CO}$); ¹H NMR ($\text{DMSO}-d_6 + \text{D}_2\text{O}$) δ 1.85 (s, 3 H, CH_3CO), 2.76–2.82 (dd, 1H, $J = 6.9$ and 16.6 Hz, COCH_aH_b), 2.94–3.00 (dd, $J = 5.9$ and 16.6 Hz, 1H, COCH_aH_b), 4.58–4.61 (dd, 1H, $J = 6.1$ and 6.8 Hz, $\text{CH}-\text{CH}_2$), 9.04 (s, 1H, $\text{NH}-\text{CHO}$); FABMS (X_e , 7 kV, glycerol), m/z 246 [M + H]; UV (H_2O) λ_{max} 206 nm.

Prooxidant Assay Using *cis*-Hexenes. To 3 mL of acetonitrile (HPLC grade, Merck) were added 0.1 mmol of C_4 -AR, AA, or 5,6-*O*-isopropylideneascorbic acid (Micheel and Hasse, 1936), 150 μL of a solution of 10% FeCl_3 in water, and 0.6 mmol of cyclohexen or *cis*-3-hexene, respectively. Air was bubbled through the mixture for 100 min with stirring. After the reaction, 50 μL of cycloheptanol in methanol (12.1 mg/mL) was added as internal standard, and the organic solvent was removed under reduced pressure at room temperature. The residue was diluted with 3 mL of ethyl acetate and dried over Na_2SO_4 anhydrous, and the solution was filtered through a membrane of 0.45 μm pore size (Chromafil, Macherey & Nagel). The products were identified by comparing of retention time and mass spectrum of those of the authentic reference compound. 2-Cyclohexen-1-one was obtained from Fluka, and 2-hydroxy-3-hexene was synthesized according to a modified method of Kothe et al. (1994). Selenium dioxide (3.5 mmol) was suspended in 4 mL of methylene chloride and 1.6 mL of 80% *tert*-butyl hydroperoxide in bis(*tert*-butylperoxide), 0.08 mL of water, and 0.08 mL of *tert*-butyl alcohol were added. *cis*-3-Hexene (3.5 mmol) was added to the stirred reaction mixture. After 24 h of further stirring, the solvent was evaporated, and the residue was suspended in 5 mL of water and extracted three times with 5 mL of ethyl acetate. After the pooled organic layers were evaporated, an oil was obtained that contained 2-hydroxy-3-hexene as identified by GC/MS. For further characterization of the product, the oil was derivatized for 15 min at room temperature in an excess of *p*-nitrobenzoyl chloride in pyridine. The product was isolated by preparative TLC with an eluent of diisopropyl

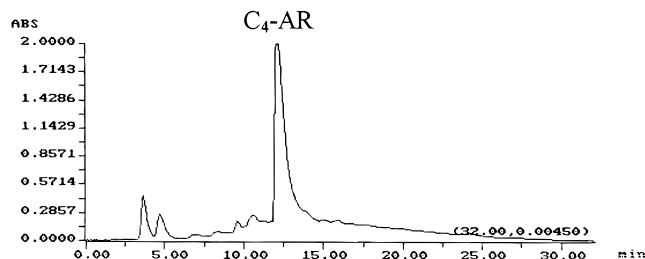


Figure 1. HPL-chromatogram of a reaction mixture of glucose (0.25 M) and propylamine (0.25 M), which was heated in phosphate buffer, pH 7.0, for 1 h at 100 °C. The substances were detected in a wavelength range from 230 to 450 nm.

ether–heptane (3:2) (R_f value = 0.80), and after elution from the silica gel with ethyl acetate, *p*-nitrobenzoic acid (3-hexen-2-yl) ester was obtained as colorless crystals: ¹H NMR (CDCl_3) δ 1.01 (t, 3H, $J = 7.2$ Hz, CH_3-CH_2), 1.46 (d, 3H, $J = 6.1$ Hz, CH_3-CHO), 2.09 (quin, 2H, $J = 7.2$ Hz, CH_3-CH_2), 5.57 (m, 1H, $\text{C}=\text{CH}-\text{CHO}$), 5.61 (m, 1H, $\text{OCH}-\text{CH}_3$), 5.87 (dt, 1H, $J = 5.9$ and 14.6 Hz, $\text{CH}_2-\text{CH}=\text{C}$), 8.21 (d, 2H, 8 Hz, $\text{CH}=\text{C}-\text{COO}$), 8.30 (d, 2H, 8 Hz, $\text{CH}=\text{C}-\text{NO}_2$). The NMR data were in accordance with those which are reported in the literature for acetic acid (3-hexen-2-yl) ester (Hansson et al., 1990). 2-Hydroxy-3-hexene: retention time, 6.9 min; MS, m/z (relative intensity) 85 (15%), 71 (100%), 69 (20%), 57 (20%), 55 (20%). 2-Cyclohexen-1-one: retention time, 6.9 min; MS, m/z (relative intensity) 96 (35%), 68 (100%), 55 (10%), 51 (10%).

RESULTS AND DISCUSSION

Previous studies have revealed that 1-(alkylamino)-1,2-dehydro-1,4-dideoxy-3-hexulose (C_6 -AR) is the main product, which can be detected by HPLC/UV, when lactose is heated with alkylamines, such as *N*^α-acetyllysine (Pischetsrieder et al., 1997). 3-Hydroxy-4-(alkylamino)-3-buten-2-one (C_4 -AR) has previously been isolated from reaction mixtures of glucose and alkylamines, but the yields have been rather low (Ledl and Severin, 1979). In course of these experiments HPLC analysis was used to investigate the Maillard reaction of glucose in detail, and it was found that under certain reaction conditions, such as heating for 1 h at 100 °C and neutral pH, C_4 -AR is the major UV-absorbing product which could be detected (Figure 1).

To investigate the AOA of C_4 -AR and C_6 -AR, a linoleic acid/carotene mixture was oxidized in the absence of metal ions by the addition of ADIBA, which starts radical reactions, and the activity of the samples to prevent carotene degradation was measured. The results are summarized in Figure 2. It was found that the AOA of C_4 -AR is over a wide concentration range almost the same as that of AA, and an antioxidative effect can even be observed in concentrations as low as 0.015 mM. C_6 -AR is a weaker antioxidant, which is still effective in a concentration of 0.02 mM.

It is known that in the presence of metal ions antioxidants can be effective as prooxidants against hydrophilic compounds, such as proteins, and lipophilic compounds, such as unsaturated lipids. Therefore, we developed two assay systems, one using *N*^α-acetylhistidine as a model for proteins and one using linoleate/carotene as an example for lipids. *N*^α-Acetylhistidine was used because it is known that particularly histidine residues are prone to degradation during the metal ion-mediated oxidation of proteins and peptides (Cheng et al., 1992). It was suggested that histidine forms a complex with copper, which is directly reduced by the

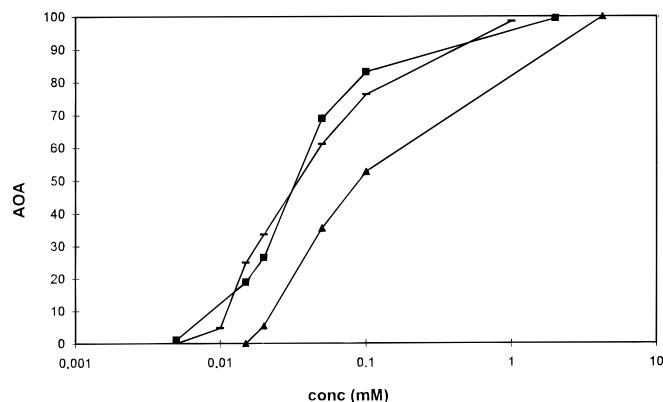


Figure 2. AOA dependent on the concentration of AA (—), C₄-AR (■), and C₆-AR (▲) tested for the ADIBA-induced oxidation of carotene. Conc, concentration of the sample which was added to the test solution.

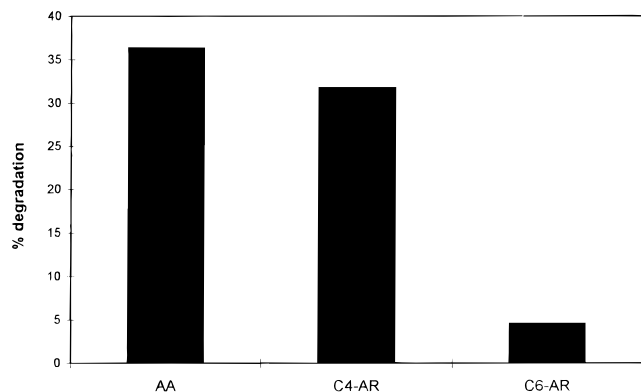


Figure 3. Oxidative degradation of *N*-acetylhistidine in the presence of Cu²⁺ and AA, C₄-AR, or C₆-AR.

antioxidant and reacts with oxygen to give a strongly oxidizing oxo-copper species (Uchida and Kawakishi, 1990). However, in another study it was suggested that H₂O₂ plays a role in the oxidation process (Cheng et al., 1992). We developed an HPLC system to monitor the degradation of *N*-acetylhistidine during the reaction with C₄-AR or C₆-AR, Cu²⁺, and O₂. To confirm that degradation of *N*-acetylhistidine is due to oxidation and not due to other processes, the main reaction product, which could be detected by HPLC, was isolated and identified as *N*-formyl-*N*-(*N*-acetyl-β-aspartyl)urea. This compound has been previously postulated as an intermediate of the metal ion/AA-induced oxidative degradation of *N*-acetylhistidine, but it has not been isolated so far (Uchida and Kawakishi, 1990). However, since several products, including aspartate and asparagine, are known to be formed during this reaction, degradation of the educt, and not product formation, has been quantified in this assay.

The results were compared to AA, which is known to be a very potent prooxidant in the presence of metal ions. Copper alone did not show significant reaction, and this sample was therefore used as blank.

As shown in Figure 3, it was found that C₆-AR causes the degradation of 4.6% of *N*-acetylhistidine. On the other hand, C₄-AR was much more effective, causing a loss of 31.8% of the amino acid, which is comparable to the result of AA (36.4%).

In the linoleate/carotene system results similar to those for the *N*-acetylhistidine test were obtained (Figure 4). The dose-dependent curve for C₄-AR is very similar to the curve for AA, with increasing POA

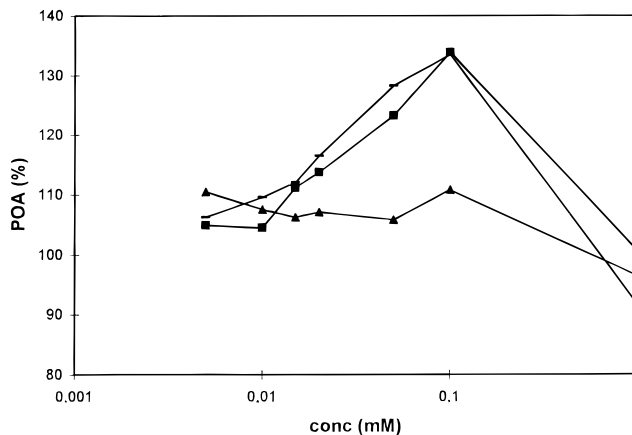


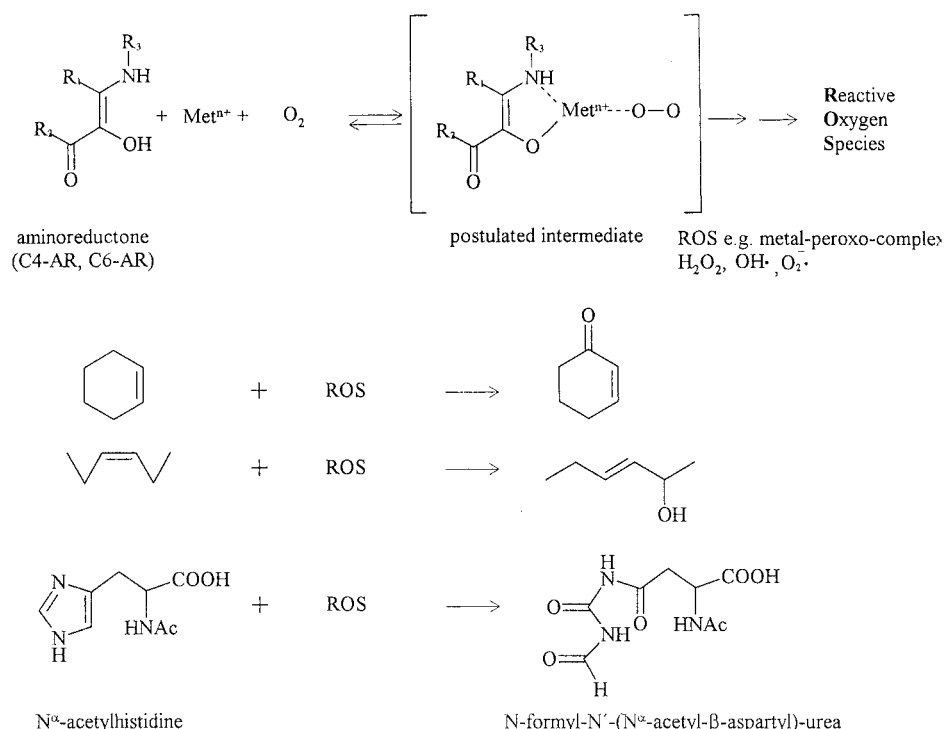
Figure 4. POA dependent on the concentration of AA (—), C₄-AR (■), and C₆-AR (▲) tested for the Cu²⁺-induced oxidation of carotene. Conc, concentration of the sample which was added to the test solution.

between 0.005 and 0.1 mM sample. In concentrations >0.1 mM the AOA of both compounds becomes predominant, resulting in a decrease of POA. The effect that AA enhances at lower concentrations oxidation but acts as an antioxidant at high concentrations has been described in the literature (Steinhart et al., 1993; Mahoney and Graf, 1986). The values for the C₆-AR were again significantly lower.

Because Fenton-type reactions were described for various metal ions, we tested if C₄-AR and C₆-AR can display their POA also in the presence of other metals. Therefore, in the linoleate/carotene assay copper was replaced by MnCl₂ or FeCl₃/EDTA. The samples displayed POA in the presence of all tested metal ions. For Mn²⁺ results similar to those for Cu²⁺ were obtained, whereas Fe³⁺ caused less than half of the POA.

It is known that AOA or POA against lipids can be highly dependent on the model system chosen (Hopia et al., 1996), particularly if the lipids were used in emulsions, bulk systems, or organic solvents. Therefore, a second assay was used to determine the POA of C₄-AR against lipids in the presence of Fe³⁺. *cis*-Alkenes, such as cyclohexene and *cis*-3-hexene, were used as model compounds for oxidation of oleic acid in organic solvent. The reaction products were analyzed by GC/MS and identified by comparing retention times and mass spectra with those of authentic reference compounds. It was found that oxidation in the α-position of cyclohexene leads to the formation of 2-cyclohexen-1-one, whereas the open chain alkene is oxidized to give 2-hydroxy-3-hexene (Scheme 2). The reactivity was again compared to this one of AA which was used directly in suspension or as 5,6-isopropylidene derivative, which is soluble in organic solvent. It was found that AA, 5,6-isopropylidene AA, and C₄-AR produce the same reaction products in similar yields. Very recently, Barton and Delanghe (1998) showed that cyclohexanone is in a similar way the main product when cyclohexane is oxidized by an Udenfriend's system.

The mechanism of the POA of aminoreductones has not been elucidated so far and seems to be dependent on the reaction conditions. It can be suggested, however, that in the first step the aminoreductone forms a reactive complex with the metal and oxygen (Scheme 2). The analogous complex with AA was described by Hamilton as the oxidant of Udenfriend systems (Hamilton, 1974; Elstner, 1990; Uchida and Kawakishi, 1990). Because of the similar structure and oxidative behavior

Scheme 2. Oxidation of *cis*-Alkenes and *N*^α-Acetylhistidine in the Presence of Aminoreductones and Metal Ions

of reductones, such as AA, and aminoreductones, it is likely that the complex indicated in Scheme 2 can be formed and can be an intermediate of the oxidation reactions. This complex can then either oxidize directly the substrate (Elstner, 1990), or superoxide radicals can be released. After dismutation of the latter and a Fenton reaction, H₂O₂ and OH· can be formed as reactive oxygen species (ROS). Against our expectations, oxidation of *cis*-alkenes could be observed only when Fe³⁺ was used, whereas Fe²⁺ did not show an effect. This observation can possibly be explained by the well-known fact that the tendency of Fe³⁺ to form complexes is much higher compared to that of Fe²⁺. It is possible that synergistic effects of AA and C₄-AR occur in this oxidation system; however, this was not investigated in the scope of this project.

In summary, it can be stated that C₄-AR and C₆-AR are strongly reducing agents which have, dependent on the conditions, both antioxidative and prooxidative properties. In all assay systems the activity of C₄-AR was in the range of the activity of AA and significantly higher than that of C₆-AR. It must be assumed that both aminoreductones contribute considerably to the anti- and prooxidative effect of Maillard products. On the one hand, these aminoreductones can help to prevent oxidative spoilage of foodstuffs. On the other hand, at low ratios of aminoreductone to metal ions, the aminoreductones can enhance oxidative damage of proteins, lipids, or other components. In vivo under normal conditions metal ions are sequestered. However, there is strong evidence that under pathological conditions, such as cataract (Cook and McGahan, 1986), atherosclerotic lesions (Evans et al., 1995; Smith et al., 1992), and diabetes (Hunt, 1996), metal ions are released and can be detected in their free and harmful form. Very recently, it was shown that AA exhibits POA also in vivo, resulting in an increase of potentially mutagenic lesions (Podmore et al., 1998). In the presence of reducing agents metals can cause, for example, inactivation of enzymes (Stadtman and Oliver, 1991) or

damage of DNA (Kalus et al., 1982) or LDL (Jürgens et al., 1987). In these cases aminoreductones, which are formed during the Maillard reaction, can be of particular interest, because they are mostly bound to proteins and the close proximity of the reducing agent can greatly influence the oxidative damage of proteins and lipoproteins. As indicated above, AA influences oxidation of a wide range of molecules, including DNA and phospholipids. Since AA and the aminoreductones showed very similar behaviors in the here-applied assay systems, it can be deduced that C₄-AR and C₆-AR can have anti- or prooxidative activity on other components such as phospholipids and DNA. However, further experiments are needed to confirm this assumption.

Further investigations to find out to what extent C₄-AR and C₆-AR are formed in foodstuffs or in vivo are currently under progress.

ABBREVIATIONS USED

AA, ascorbic acid; ADIBA, α,α'-azodiisobutyramidine dihydrochloride; AOA, antioxidative activity; C₄-AR, 3-hydroxy-4-(alkylamino)-3-buten-2-one; C₆-AR, 1-(alkylamino)-1,2-dehydro-1,4-dideoxy-3-hexulose; DAD, diode array detection; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; POA, prooxidative activity.

LITERATURE CITED

- Alaiz, M.; Hidalgo, J.; Zamora, R. Comparative Antioxidant Activity of Maillard- and Oxidized Lipid-Damaged Bovine Serum Albumin. *J. Agric. Food Chem.* **1997**, *45*, 3250–3254.
- Aruoma, O. Assessment of Potential Prooxidant and Antioxidant Actions. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1617–1625.
- Barton, D. H.; Delanghe, N. C. The Selective Functionalization of Saturated Hydrocarbons. Part 46. An Investigation of Udenfriend's System under Gif Conditions. *Tetrahedron* **1998**, *54*, 4471–4476.

- Cheng, R.-Z.; Uchida, K.; Kawakishi, S. Selective Oxidation of Histidine Residues in Proteins or Peptides through the Copper(II)-Catalysed Autoxidation of Glucosone. *Biochem. J.* **1992**, *285*, 667–671.
- Chuda, Y.; Ono, H.; Mayumi, O.-K.; Nagata, T.; Tsushida, T. Structural Identification of Two Antioxidant Quinic Acid Derivatives from Garland (*Chrysanthemum coronarium* L.). *J. Agric. Food Chem.* **1996**, *44*, 2037–2039.
- Cook, C. S.; McGahan, M. C. Copper concentration in cornea, iris, normal, and cataractous lenses and intraocular fluids of vertebrates. *Curr. Eye Res.* **1986**, *5*, 69–76.
- Dow Chemical Co. U.S. Patent 282/555; *Chem. Abstr.* **1958**, *52*, 9198i.
- Elstner, E. *Der Sauerstoff. BI—Wissenschaftsverlag: Mannheim*, 1990.
- Euler, H.; Eistert, B. *Chemie und Biochemie der Reduktone und Reduktonate*; Ferdinand Enke Verlag: Stuttgart, 1957.
- Evans, P. J.; Smith, C.; Mitchinson, M. J.; Halliwell, B. Metal ion release from mechanically-disrupted human arterial wall. Implications for the development of atherosclerosis. *Free Radical Res.* **1995**, *23*, 465–469.
- Hamilton, G. A. In *Molecular Mechanisms of Oxygen Activation*; Hayaishi, O., Ed.; Academic Press: New York, 1974; p 405.
- Hansson, S.; Heumann, A.; Rein, T.; Akermark, B. Preparation of Allylic Acetates from Simple Alkenes by Palladium (II)-Catalyzed Acetoxylation. *J. Org. Chem.* **1990**, *55*, 975–984.
- Hopia, A. I.; Huang, S.-W.; Schwarz, K.; German, J. B.; Frankel, E. N. Effect of Different Lipid Systems on Antioxidant Activity of Rosemary Constituents Carnosol and Carnosic Acid with and without α -Tocopherol. *J. Agric. Food Chem.* **1996**, *44*, 2030–2036.
- Hunt, J. V. Ascorbic Acid and Diabetes Mellitus. In *Subcellular Biochemistry, Vol. 25: Ascorbic Acid: Biochemistry and Biomedical Cell Biology*; Harris, J. R., Ed.; Plenum Press: New York, 1996.
- Jürgens, G.; Hoff, H. F.; Chisolm, G. M.; Esterbauer, H. Modification of Human Serum Low-Density Lipoprotein by Oxidation—Characterization and Pathophysiological Implications. *Chem. Phys. Lipids* **1987**, *45*, 315–336.
- Kalus, W.; Filby, W.; Münzner, R. Chemical Aspects of the Mutagenic Activity of the Ascorbic Acid Autoxidation System. *Z. Naturforsch.* **1982**, *37C*, 40–45.
- Kanner, J.; Mendel, H.; Budowski, P. Prooxidant and Antioxidant Effects of Ascorbic Acid and Metal Salts in a β -Carotene-Linoleate Model System. *J. Food Sci.* **1977**, *42*, 60–64.
- Karastogiannidou, C.; Ryley, J. The Formation of Water-soluble Antioxidants in Chicken Held at 80 Degree C. *Food Chem.* **1994**, *51*, 215–220.
- Kato, H. Scavenging of Active Oxygen Species by Glycate Protein. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 928–931.
- Kothe, G.; Bagby, M. O.; Weisleder, D.; Peterson, R. E. Allylic Mono- and Di-hydroxylation of Isolated Double Bonds with Selenium Dioxide-tert-Butyl Hydroperoxide. NMR Characterization of Long-chain Enols, Allylic and Saturated 1,4-Diols, and Enones. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1661–1669.
- Ledl, F.; Schleicher, E. New aspects of the Maillard reaction in foods and in the human body. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 565–594.
- Ledl, F.; Severin, T. Formation of Aminoreductones from Glucose and Primary Amines. *Z. Lebensm. Unters. Forsch.* **1979**, *169*, 173–175.
- Mahoney, J. R.; Graf, E. Role of α -tocopherol, ascorbic acid, citric acid and EDTA as oxidants in model systems. *J. Food Sci.* **1986**, *51*, 1293–1296.
- Micheel, F.; Hasse, K. Über die 2-Deoxy-L-ascorbinsäure. *Chem. Ber.* **1936**, *69*, 879.
- Nakanishi, Y.; Isohashi, F.; Matsunaga, T.; Sakamoto, Y. Oxidative Inactivation of an Extramitochondrial Acetyl-CoA Hydrolase by Autoxidation of L-Ascorbic Acid. *Eur. J. Biochem.* **1985**, *152*, 337–342.
- Nicoli, M. C.; Anese, M.; Parpinel, M. T.; Franceschi, S.; Leric, C. R. Loss and/or formation of antioxidants during food processing and storage. *Cancer Lett.* **1997**, *114*, 71–74.
- Pischetsrieder, M.; Severin, T. New Aspects on the Maillard Reaction—Formation of Aminoreductones from Sugars and L-Ascorbic Acid. *Recent Res. Dev. Agric. Food Chem.* **1997**, *1*, 29–37.
- Pischetsrieder, M.; Schoetter, C.; Severin, T. Formation of an Aminoreductone during the Maillard Reaction of Lactose with N^α-Acetyllysine or Proteins. *J. Agric. Food Chem.* **1998**, *46*, 928–931.
- Podmore I. D.; Griffiths, H. R.; Herbert, K. E.; Mistry, N.; Mistry, P.; Lunec, J. Vitamin C exhibits pro-oxidant properties. *Nature* **1998**, *392*, 559.
- Samuni, A.; Aronovitch, J.; Godinger, D.; Chevion, M.; Czapski, G. On the Cytotoxicity of Vitamin C and Metal Ions. A Site-Specific Fenton Mechanism. *Eur. J. Biochem.* **1983**, *137*, 119–124.
- Shinar, E.; Navok, T.; Chevion, M. The Analogous Mechanism of Enzymatic Inactivation Induced by Ascorbate and Superoxide in the Presence of Copper. *J. Biol. Chem.* **1983**, *258*, 14778–14783.
- Smith, C.; Mitchinson, M. J.; Aruoma, O. I.; Halliwell, B. Stimulation of lipid peroxidation and hydroxyl-radical generation by the contents of human atherosclerotic lesions. *Biochem. J.* **1992**, *286*, 901–905.
- Stadtman, E. R.; Oliver, C. N. Metal-catalyzed Oxidation of Proteins. *J. Biol. Chem.* **1991**, *266*, 2005–2008.
- Steinhart, H.; Vollmar, M.; Sailer, C. Pro- and Antioxidative Effect of Ascorbic Acid on L-Tryptophan in the System Fe³⁺/Ascorbic Acid/O₂. *J. Agric. Food Chem.* **1993**, *41*, 2275–2277.
- Steinhart, H.; Meyer, K.; Vollmar, M. Stability of Tryptophan-Containing Peptides in the Presence of an L-Ascorbic Acid-Ferric Ion System. *J. Agric. Food Chem.* **1995**, *43*, 2321–2324.
- Uchida, K.; Kawakishi, S. Ascorbate-Mediated Specific Modification of Histidine-Containing Peptides. *J. Agric. Food Chem.* **1989**, *37*, 897–901.
- Uchida, K.; Kawakishi, S. Formation of the 2-Imidazolone Structure within a Peptide Mediated by a Copper(II)/Ascorbate System. *J. Agric. Food Chem.* **1990**, *38*, 1896–1899.
- Wong, S. F.; Halliwell, B.; Richmond, R.; Skowronek, W. R. The Role of Superoxide and Hydroxyl Radicals in the Degradation of Hyaluronic Acid Induced by Metal Ions and by Ascorbic Acid. *J. Inorg. Biochem.* **1981**, *14*, 127–134.
- Yoshimura, Y.; Iijima, T.; Watanabe, T.; Nakazawa, H. Antioxidative Effects of Maillard Reaction Products Using Glucose-Glycine Model System. *J. Agric. Food Chem.* **1997**, *45*, 4106–4109.

Received for review February 10, 1998. Revised manuscript received May 19, 1998. Accepted May 19, 1998.

JF980118N