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

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In short-time-heated mixtures of lactose and N^{α}-acetyllysine 1-[N^{ϵ}-(N^{α}-acetyllysinyl)]-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; antioxiative; 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 (•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 (ADIBA) were from Fluka (Buchs, Switzerland). Sodium linoleate and Tween 20 were purchased from Sigma (St. Louis, MO). Methanol LiChrosolv, chromatography grade, was obtained

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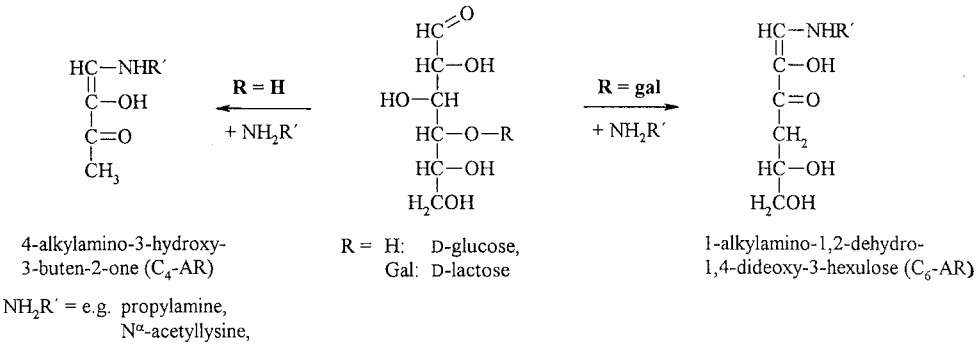
Scheme 1. Formation of C_4-AR and C_6-AR from D-Glucose or Lactose, Respectively



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 F_{254}.

Apparatus. UV spectra were recorded on a Perkin-Elmer UV−vis spectrometer Lambda 20 and ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra with a JEOL 400 GSX spectrometer using $(CH_3)_4Si$ 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 CH_4) 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 (C_6-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 C_6-AR was extracted with ethyl acetate. The analytically pure compound was immediately used for the assays.

3-Hydroxy-4-(propylamino)-3-buten-2-one (C_4-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 C_4-AR was purified by distillation at 150 °C and 0.4 Torr, which yields colorless crystals with a melting point of 47 °C: ^{1}H NMR ($CDCl_3$, COSY) δ 0.90 (t, 3 H, J = 7.3 Hz, CH_3-CH_2), 1.54 (sext, 2 H, J = 7.3 Hz, CH_3-CH_2), 2.07 (s, 3 H, $CH_3C=O$), 3.11 (dt, 2 H, J = 7.3 and 6.8 Hz, $N-CH_2$), 4.5 (br, 1 H, NH), 6.57 (d, 1 H, J = 12.4 Hz, CH=C); ^{13}C NMR ($CDCl_3$) δ 11.0 (CH_3-CH_2), 21.0 ($CH_3-C=O$), 24.5 (CH_3-CH_2), 49.9 (CH_2-N), 131.0 (=C−OH), 131.7 (=CH−N), 186.4 (C=O); UV (CH_3OH) λ_{max} 321 nm (log ϵ = 4.3); CI-MS *m/z* 144 ($M^+ + 1$). Anal. Calcd for $C_7H_{13}NO_2$: 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 (C_4-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 C_4-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 C_6-AR and C_4-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 C_6-AR and C_4-AR in methanol and of AA in water as indicated), and the reaction was started by addition of 8 μL of $CuSO_4$ (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 [Abs_{0min} − Abs_{20min}(sample)]/[Abs_{0min} − Abs_{20min}(blank)] × 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 $FeCl_3$/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 μg/5 μL) 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 μmol/12 μL) and stirring was continued. After 30 min, phenoxyacetic acid (156 μg/200 μL) 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: ^{1}H NMR (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, $CH-CH_2$), 8.27−8.29 (d, 1H, J = 8.0 Hz, $NH-CH-CH_2$), 9.04 (d, 1H, J = 9.5 Hz, $NH-CHO$), 10.51 (d, 1H, J = 9.5 Hz, $NH-CHO$), 11.02 (s, 1H, $CO-NH-CO$); ^{1}H NMR (DMSO-d_6 + D_2O) 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, $CH-CH_2$), 9.04 (s, 1H, $NH-CHO$); FABMS (Xe, 7 kV, glyerol), 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 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: ^{1}H 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, $C=CH-CHO$), 5.61 (m, 1H, $OCH-CH_3$), 5.87 (dt, 1H, J = 5.9 and 14.6 Hz, $CH_2-CH=C$), 8.21 (d, 2H, 8 Hz, $CH=C-COO$), 8.30 (d, 2H, 8 Hz, $CH=C-NO_2$). 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%).

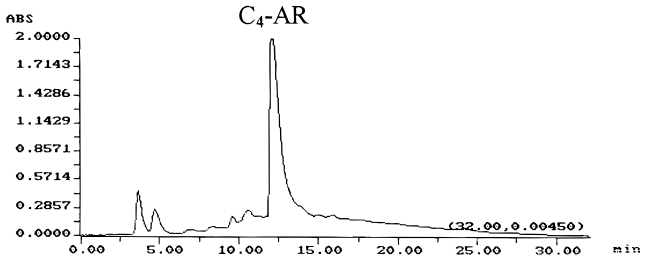


Figure 1. HPL-chromatogram of a reaction mixture of glucose (0.25 M) and propylamine (0.25 M), which was heated in phosphate buffer, pH 7.0, for 1 h at 100 °C. The substances were detected in a wavelength range from 230 to 450 nm.

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

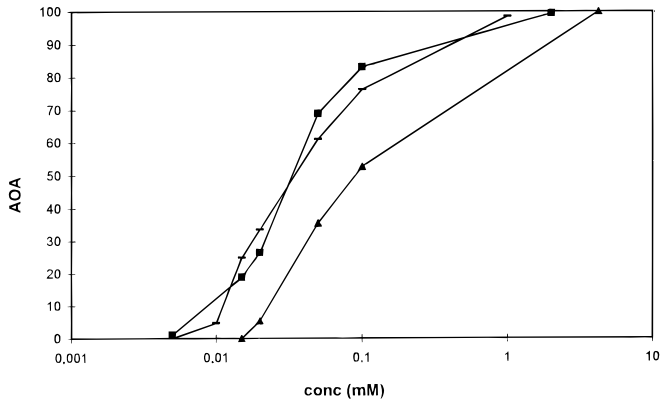


Figure 2. AOA dependent on the concentration of AA (—), C_4-AR (■), and C_6-AR (▲) tested for the ADIBA-induced oxidation of carotene. Concn, concentration of the sample which was added to the test solution.

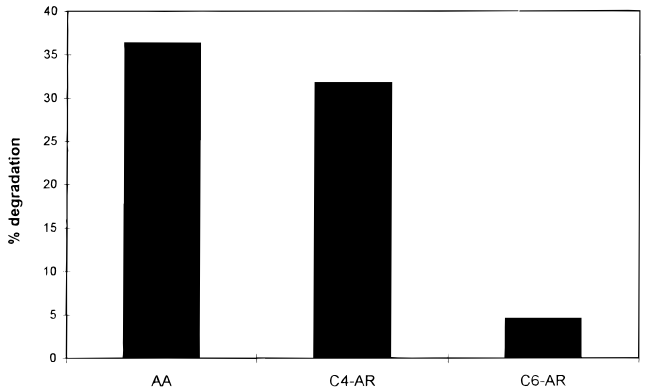


Figure 3. Oxidative degradation of N^{α}-acetylhistidine in the presence of Cu^{2+} and AA, C_4-AR, or C_6-AR.

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 H_2O_2 plays a role in the oxidation process (Cheng et al., 1992). We developed an HPLC system to monitor the degradation of N^{α}-acetylhistidine during the reaction with C_4-AR or C_6-AR, Cu^{2+}, and O_2. To confirm that degradation of N^{α}-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^{α}-acetyl-β-aspartyl)urea. This compound has been previously postulated as an intermediate of the metal ion/AA-induced oxidative degradation of N^{α}-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 C_6-AR causes the degradation of 4.6% of N^{α}-acetylhistidine. On the other hand, C_4-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^{α}-acetylhistidine test were obtained (Figure 4). The dose-dependent curve for C_4-AR is very similar to the curve for AA, with increasing POA

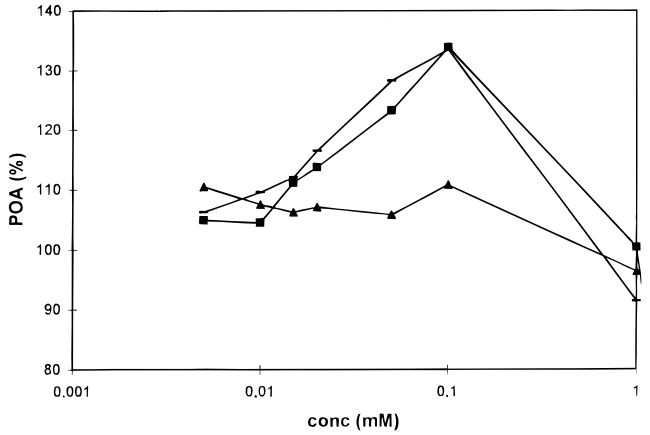


Figure 4. POA dependent on the concentration of AA (—), C_4-AR (■), and C_6-AR (▲) tested for the Cu^{2+}-induced oxidation of carotene. Concn, concentration of the sample which was added to the test solution.

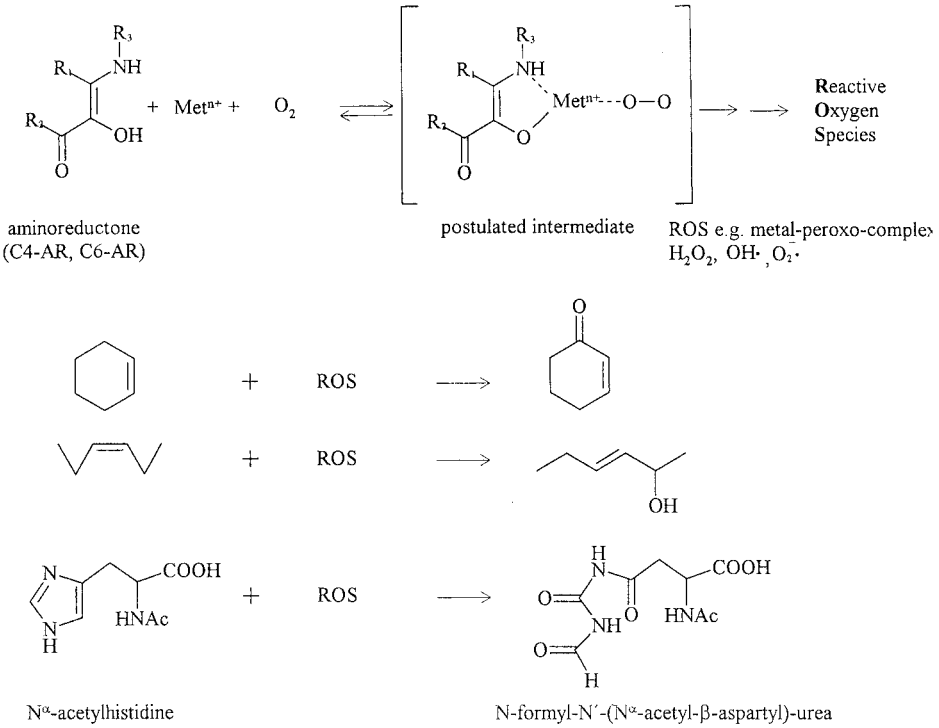
between 0.005 and 0.1 mM sample. In concentrations >0.1 mM the AOA of both compounds becomes predominant, resulting in a decrease of POA. The effect that AA enhances at lower concentrations oxidation but acts as an antioxidant at high concentrations has been described in the literature (Steinhart et al., 1993; Mahoney and Graf, 1986). The values for the C_6-AR were again significantly lower.

Because Fenton-type reactions were described for various metal ions, we tested if C_4-AR and C_6-AR can display their POA also in the presence of other metals. Therefore, in the linoleate/carotene assay copper was replaced by $MnCl_2$ or $FeCl_3$/EDTA. The samples displayed POA in the presence of all tested metal ions. For Mn^{2+} results similar to those for Cu^{2+} were obtained, whereas Fe^{3+} 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 C_4-AR against lipids in the presence of Fe^{3+}. *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 C_4-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, H_2O_2 and OH• can be formed as reactive oxygen species (ROS). Against our expectations, oxidation of *cis*-alkenes could be observed only when Fe^{3+} was used, whereas Fe^{2+} did not show an effect. This observation can possibly be explained by the well-known fact that the tendency of Fe^{3+} to form complexes is much higher compared to that of Fe^{2+}. It is possible that synergistic effects of AA and C_4-AR occur in this oxidation system; however, this was not investigated in the scope of this project.

In summary, it can be stated that C_4-AR and C_6-AR are strongly reducing agents which have, dependent on the conditions, both antioxidative and prooxidative properties. In all assay systems the activity of C_4-AR was in the range of the activity of AA and significantly higher than that of C_6-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 C_4-AR and C_6-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 C_4-AR and C_6-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; C_4-AR, 3-hydroxy-4-(alkylamino)-3-buten-2-one; C_6-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.

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