JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

DOI:10.1021/jf102433u

Quantification of Chemically Reducing Species in the Phosphate Ion Catalyzed Degradation of Reducing Sugars

George P. Rizzi,*,[†] Eme E. Amba,[‡] and William R. Heineman[‡]

[†]Department of Chemistry, Miami University Middletown, Middletown, Ohio 45042, and [‡]Department of Chemistry, University of Cincinnati, 301 Clifton Court, Cincinnati, Ohio 45221-0172

Chemically reducing species formed during phosphate ion catalyzed degradation of reducing sugars were directly quantified by titration with 2,6-dichloroindophenol (Tillman's reagent) and by measurement of open circuit electrical redox potentials. Both techniques demonstrated a time-dependent increased production of chemically reducing species in 0.1 M phosphate buffer at 100 °C and the increasingly negative redox potentials observed were consistent with the formation of reductones. Cyclic voltammetry (CV) was investigated in an attempt to generate and observe the sugar-derived highly reactive reducing species in situ. CV analysis of a model Amadori compound, *N*-(1-deoxyfructos-1-yl)piperidine, indicated oxidative waves consistent with reductone formation, but chemical instability of the oxidation products formed precluded the electrochemical detection of highly electrophilic reducing species such as reductones.

KEYWORDS: Reductone; titration; redox potential; cyclic voltammetry; reducing sugars; Amadori compound

INTRODUCTION

The degradation of reducing sugars is a fundamental process for the formation of aroma, taste, and visual color compounds during food processing. The breakdown of reducing sugars generally involves dehydration or oxidation reactions, often leading to highly reactive α-dicarbonyl intermediates known collectively as deoxyosones. The key role of deoxyosones as reactive intermediates in carbohydrate chemistry suggested a further need to develop less invasive means for their detection and analysis and provided the incentive for this research. Deoxyosones have long been recognized as intermediates in food related and medically significant in vivo Maillard reactions (*1*). One generic class of deoxyosones, the α-hydroxymethylglyoxals (3-hydroxy-1,2-propanediones) or reductones, exhibit chemical reducing properties and have attracted particular attention both as flavor precursors and as potential redox coupling agents in related flavor-forming reactions (*2*). Deoxyosone formation by dehydration may be catalyzed by nitrogen bases or inorganic agents. In the Maillard reaction common to foods and biological systems, aldose and ketose sugars react with amines, amino acids, or proteins to form 3-deoxy-1,2-osones and reductones such as the 1-deoxy-2,3-osones. Similar products are formed catalytically from sugars via inorganic acid/base sugar degradation reactions in the absence of nitrogen bases. Because of their high reactivity, reductones are difficult to quantify per se in complex systems. For this reason, reductones are often detected and quantified as more stable derivatives such as quinoxalins or 1,2,4-triazines. Derivatization techniques are useful when applied with care, but artifacts frequently result from concomitant sugar/reagent reactions (*3*).

*To whom correspondence should be addressed. Phone: (513) 761-0816. E-mail: georgerizzi@yahoo.com. Address: Dr. G. P. Rizzi, 542 Blossomhill Lane, Cincinnati, OH 45224-1406.

Reducing species including Amadori compounds and presumably reductones have been estimated directly in Maillard reactions by titration with ferricyanide (*4*) or Tillman's reagent (2,6-dichloroindophenol) (*5*) and by color changes in formazan-forming redox indicators (*6*). The presence of highly reactive reductones has also been observed electrochemically during polarography of sugars (*7*) or by open circuit potential measurements in model Maillard reactions of *β*-alanine and reducing sugars (*8*). In theory, the cyclic voltammetry (CV) of Amadori compounds can also provide qualitative information of reductones formed in situ during the oxidation cycle by observing current responses in subsequent cycles. However, a recent CV analysis of Fru-Val [*N*-(1-deoxy-*β*-D-fructopyranose-1-yl)-L-valine] displayed a sole anodic (oxidation) wave at ca. 1.0 V but no evidence of reduction products (*9*). In a previous study, we observed and grossly quantified (by OPD derivatization) the formation of α-dicarbonyls in the phosphate-ion catalyzed degradation of reducing sugars (*10*). In this earlier work, OPD derivatives representing nine α-dicarbonyls including thee reductones, namely 3-hydroxy-2-oxopropanal, 4-hydroxy-2,3-butanedione, and 4,5-dihydroxy-2,3-pentanedione, were isolated and identified (by UV and proton NMR) from a phosphate-ion catalyzed degradation of D-ribose. In the present work, we describe the direct analysis of chemically reducing species in phosphate-ion-catalyzed sugar degradation reactions by the use of Tillman's reagent. In addition, we present open circuit electrochemical potential data to confirm the relative levels of reducing species present. Finally, a reexamination of the CV approach to reductone detection/analysis is described using a model Amadori compound, *N*-(1-deoxyfructos-1-yl)piperidine.

MATERIALS AND METHODS

Materials. Sugars D-xylose, D-glucose, D-ribose, D-galactose, D-mannose, D-arabinose, D-fructose, and sugar alcohols D-xylitol and D-sorbitol

© 2010 American Chemical Society
Published on Web 08/16/2010

as well as L-ascorbic acid, [2,6-dichloro-4-[(4-hydroxyphenyl)imino]-2,5-cyclohexadiene-1-one, Na salt (DCIP), aka 2,6-dichloroindophenol sodium, *o*-phenylenediamine (OPD), potassium monohydrogen phosphate, and potassium dihydrogen phosphate were all analytical grade commercial products and used directly without further purification. *N*-(1-Deoxyfructos-1-yl)piperidine was synthesized as described previously (*8*). Phosphate buffer (0.10 M), pH 7.1 was prepared by adding 0.10 M KH_2PO_4 to 0.10 M K_2HPO_4. Preparative TLC was done as previously described on Sigma-Aldrich silica gel GF plates (0.25 mm layer thickness) using 95:5 v/v acetonitrile/water as solvent (*10*).

Reaction Procedures. For electrochemical measurements, 30.0 mL of pH 7.1 0.10 M phosphate buffer was added to solid sugars or sugar alcohols to obtain initial concentrations of 0.10 M. Solutions were heated at reflux for various times (t) and cooled rapidly (< 1 min) to 23 °C before measuring final potentials, E_f. Data are reported as changes in EMF, $E_f - E_b$, where E_b was the potential observed in unheated buffer at the time of each experiment. Xylose and xylitol data plotted in **Figure 3** are mean values from three independent experiments shown ±1 SD. Data for 4 h reactions shown in **Figure 4** were obtained from single experiments with exception of the already mentioned triplicate xylose/xylitol measurements (**Figure 3**).

Titrimetric analyses of reducing species content employed similar, but separate reactions. During each reaction, 1.00 mL aliquots were withdrawn at various times and rapidly cooled to ca. 23 °C using a prechilled hypodermic syringe. The cooled, pale-yellow aliquots were rapidly titrated under inert atmosphere (propane was used in lieu of more common inert gases which were unavailable at the time) to a sharp blue or blue–green end point with standardized 1 mM DCIP solution in the common phosphate buffer. Small volumes of titrant were accurately measured by dispensing them from tared hypodermic syringes. DCIP solution was standardized periodically by titrating weighed quantities of L-ascorbic acid in phosphate buffer. DCIP solution was stored at 23 °C in actinic glassware to minimize photo decomposition and freshly prepared every 2–3 days. Data points in **Figure 2** expressed in μmol of ascorbic acid equivalents/mmol of initial carbohydrate are averages of values from two independent experiments. Measurement precision in replicate titration experiments was ca. ±15%.

Isolation of Deoxyosones as OPD Derivatives. In two separate experiments, 30.0 mL of pH 7.12 0.10 M phosphate buffer was added to 461 and 467 mg of D-xylose, and the mixtures were heated at reflux for 2 h. After being cooled rapidly to 23 °C, the reaction mixtures were combined, treated with ca. 50 mg OPD (30 min at 50 °C), extracted with 3 × 5 mL of CH_2Cl_2, and solvent evaporated to afford 10.5 mg of solid residue. Preparative TLC afforded ca. 2 mg of polar quinoxalin derivatives from the R_f region (ca. 0.40–0.60) previously associated with pentose-derived deoxyosone OPD derivatives (*10*). UV–vis analysis confirmed the presence of quinoxalins with characteristic absorptions at 237 and 318 nm (*10*). The major TLC product, R_f 0.50, was isolated by repeated preparative TLC and tentatively identified as a quinoxalin, possibly 2-(1,2-dihydroxyethyl)-3-methylquinoxalin, the OPD derivative of 4,5-dihydroxy-2,3-pentanedione, by its positive ion electrospray mass spectrum: MH^+ 205 and MH_2^+ 206 amu; $C_{11}H_{12}N_2O_2$, MW = 204 amu.

Instrumental Analyses. Open circuit potential measurements were made similar to those previously described (*8*). Briefly, a combination Pt redox electrode (Orion model 977800) containing a Pt working electrode and a Ag/AgCl reference electrode was coupled with a Corning model 240 pH meter (millivolt mode). Observed potential changes are expressed as the difference $E_f - E_b$, where E_f and E_b are voltages (in mV) observed at 23 °C in cooled reaction solutions and in unheated buffer respectively. Data from xylose and xylitol (**Figure 3**) are averaged values from three independent experiments ±1 SD. Results with other carbohydrates (**Figure 4**) were obtained from single measurements with an approximate precision of ±15% of stated values. UV–vis data were obtained in MeOH solution using a computer interfaced Perkin-Elmer Lambda 35 spectrophotometer using a slit width of 1 nm. Electrospray ionization MS (EI/MS) employed a Micromass Platform LCZ instrument using direct injection diffusion with source and desolvation temperatures of 130 and 300 °C, respectively. Both positive (+) and negative (−) modes were used to optimize the detection of analyte ions examined in MeOH solution. For cyclic voltammetry, a rotating disk electrode RDE-2 workstation and BAS-100B/W software were used to obtain electrochemical measurements. A three-electrode system was used for all experiments. The working electrode was a glassy carbon rotating disk electrode tip (3 mm diameter) from Bioanalytical Systems Inc. The reference electrode was a Ag/AgCl wire (0.5 mm diameter) and the auxiliary electrode was a Pt wire (0.5 mm). A 2 mM solution of *N*-(1-deoxy-D-fructose-1-yl)piperidine was made in pH 7.14 0.10M phosphate buffer, and the working electrode was lowered onto a 30 μL drop of analyte solution on a hydrophobic (siliconized) slide to provide sufficient electrode contact. The applied-potential was initially scanned anodically from −1.5 V to +1.5 V, followed by a similar but reversed polarity (cathodic) scan, both at a rate of 50 mV/s. Because experiments were done in the presence of air, small cathodic peaks were always observed (−0.6 V) due to the reduction of dioxygen.

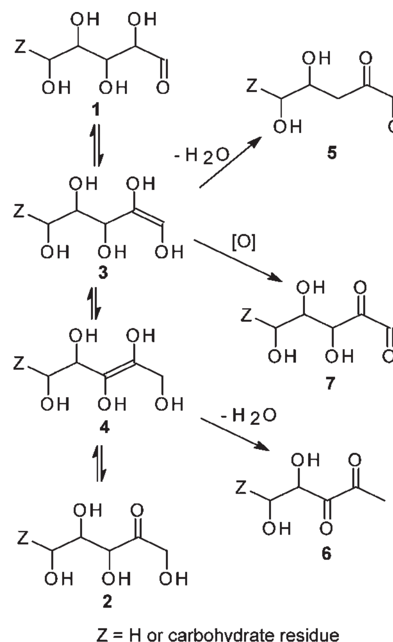

Figure 1. Formation of 1,2-dicarbonyl compounds from reducing sugars.

RESULTS AND DISCUSSION

Previously we observed how the presence of phosphate ion accelerated the formation of visible color in Maillard reactions of β-alanine with various reducing sugars (*10*). It was then established that phosphate had catalyzed sugar degradation and assisted color formation by contributing to the formation of 1,2-dicarbonyl Maillard reaction intermediates. Reducing sugars, e.g. aldoses **1** and ketoses **2** shown in **Figure 1** (portrayed in open-chain reactive form), are susceptible to myriad isomerization, dehydration, or oxidation reactions, leading to relatively more reactive 1,2-dicarbonyl products. Many of these reactions are catalyzed by acid/base reactions or by bidentate polyatomic anions like phosphate. A key step in the formation of 1,2-dicarbonyls is the enolization of **1** and **2** to form ene-diols **3** and **4**, respectively. Dehydration of ene-diols leads directly to the well-established Maillard reaction intermediates 3-deoxy-1,2-osones **5** and 1-deoxy-2,3-osones **6**. Oxidation of ene-diols can also form carbohydrate osones **7**, and this particular transformation, e.g. glucose → glucosone, has been extensively studied for its possible role in in vivo processes (*11*). Chemically, ene-diols can be powerful reducing agents, and their involvement in coupled redox processes within the Maillard reaction is highly likely. Reductones, i.e. α-hydroxymethylglyoxals (3-hydroxy-1,2-propanediones) like compounds **6** and **7**, are especially prone to ene-diol redox activity as evidenced by their previously observed chemical and electrochemical behavior(*7*).

Titration Experiments Using 2,6-Dichloroindophenol (DCIP). The formation of reducing species was quantified in model systems

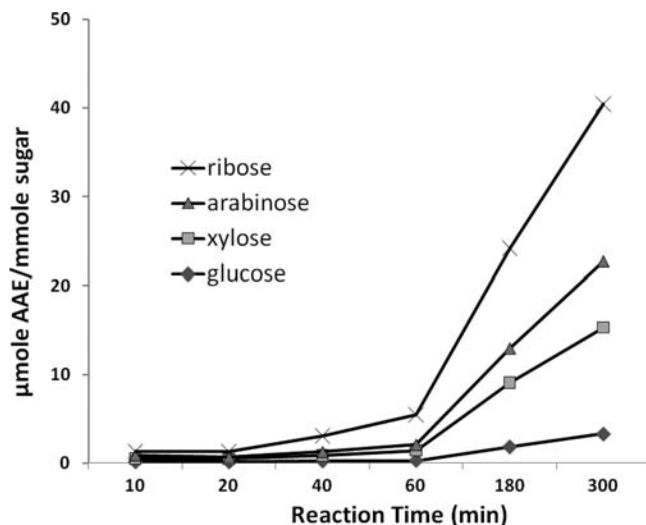

Figure 2. DCIP titration of reducing species generated from aldose sugars in 0.10 M pH 7.1 phosphate buffer at 100 °C.

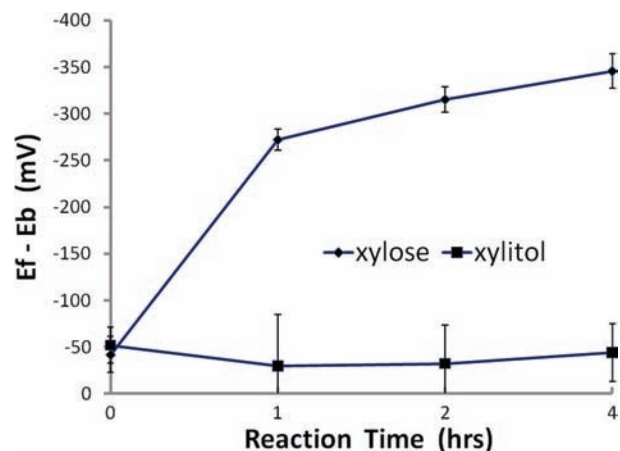

Figure 3. Comparison of open-circuit electrode potentials developing with time by xylose and xylitol (control) in aqueous 0.10 M pH 7.1 phosphate buffer solution at 100 °C.

consisting of aldose sugars (C_0 = 0.10M) in 0.10 M potassium phosphate buffer initially at pH 7.1. Mixtures were heated at reflux (ca. 100 °C), and aliquots taken at time intervals were rapidly cooled to ca. 23 °C and immediately titrated with standardized 1 mM DCIP in common phosphate buffer. DCIP, also known as Tillman's reagent, is often used to quantify ascorbic acid (a compound with reductone properties) in foods. Ascorbic acid served to standardize DCIP solution in this work. DCIP reagent is deep-blue (λ_{max} 605 nm) in its commercially available oxidized form. During titration, each drop of reagent was rendered instantly colorless by reaction with reducing species present. Return of blue color signaled the stoichiometric end point. Sharp color change end points were observed over a 10−300 min reaction span ranging initially from colorless = >deep blue in 10−60 min but becoming pale-yellow = > greenish−blue in latter stages due to the coformation of yellow-colored reaction products. Evidence from control experiments suggested that the observed DCIP reaction was due to the presence of reductones in the phosphate-catalyzed reaction mixtures. Thus control experiments using two independent buffers had shown that reductones were formed from an aldose (ribose) in pH 7.1 aqueous solution at 100 °C and that phosphate ion was required for their formation under these conditions (*10*). In the present study, the DCIP titrant showed no evidence of reaction with unheated sugar solutions in phosphate buffer or with previously heated and cooled aqueous sugar solutions. Moreover, the DCIP reagent showed zero titer with our model Amadori compound, *N*-(1-deoxyfructos-1-yl)piperidine, in pH 7.1 phosphate buffer at 22 °C. Reaction pH remained nearly constant during 300 min, occasionally falling to as low as 6.8 in final aliquots taken. Formation of reducing species shown in **Figure 2** is expressed as µmol of ascorbic acid equivalents (AAE) produced per initial mmol of carbohydrate. The stoichiometry of the DCIP/reducing sugar reaction product(s) was assumed to be the same as for DCIP/ascorbic acid. A smooth accelerated increase of reducing species with time was seen for four aldose sugars (**Figure 2**). Pentose sugars favored glucose in the production of reducing species with a yield trend in the order ribose > arabinose > xylose > glucose. Glucose was selected as a representative aldohexose for comparison because of its homologous stereochemical relationship to xylose. The high degree of reactivity observed for ribose and arabinose may have resulted from the relatively large percentages of free carbonyl (0.05% and 0.03% open-chain form, respectively) exhibited by these sugars in aqueous solution (*12*). The relatively low reactivity of glucose may have been a consequence of its extremely low (0.002%) percentage of free carbonyl in solution (*12*). Reducing species formation seemed to level off at 300 min, and no maxima in production were observed. Titration beyond 300 min was not feasible due to excessive reaction color, and the maximum chemical yield of reducing species observed was 1.7%, with ribose after 300 min. Previously, Hofmann et al. used DCIP to analyze an equimolar (1.5 molar) glucose/alanine Maillard reaction (*5*) and reported a maximum yield of ca. 60 µmol of AAE/mmol glucose (6.0% yield) after 50 min at reflux temperature in 0.5 M phosphate buffer at pH 7. The presence of a reductone in the xylose reaction was tentatively confirmed by a trapping experiment with *o*-phenylenediamine (OPD). OPD treatment of a 2 h xylose/buffer reaction, followed by methylene chloride extraction and preparative TLC, provided a mixture of quinoxalin derivatives recognized by TLC R_f values and characteristic UV absorptions at 237 and 318 nm. Further TLC analysis afforded a single homogeneous compound, R_f 0.50, tentatively identified as a quinoxalin, possibly 2-(1,2-dihydroxyethyl)-3-methylquinoxalin [M = 204 amu], the OPD derivative of 1-deoxy-2,3-xylosone (**6**, Z = H) [4,5-dihydroxy-2,3-pentanedione] via its positive ion electrospray MS signature at MH^+ 205 and MH_2^+ 206 amu.

Electrochemical Potential Measurements. Electrical redox measurements were used to estimate the degree of reductone formation during phosphate-catalyzed degradation of reducing sugars. Reductones will exhibit open circuit potentials, as indicated by the half-cell reaction described in (*8*). For EMF (electromotive force) measurement, solutions of carbohydrates (0.10M) in pH 7.1 0.10 M phosphate buffer were heated at reflux for various times followed by rapid cooling to room temperature. EMF values were obtained at ca. 23 °C with a combination electrode containing a platinum working electrode [cathode (+)] and an Ag/AgCl (saturated KCl) reference electrode [anode (−)]. The open circuit voltage E_{oc} was taken as the difference $E_f - E_b$, where E_f was the reaction EMF and E_b the voltage observed in unheated buffer. And, as in the case of Maillard reactions (*8*), increased negative values of E_{oc} were correlated to increasing concentration of reductones. Results obtained for xylose and related sugar alcohol xylitol shown in **Figure 3** show for xylose a rapid buildup of reductones with time. With xylose, E_{oc} ranged from −42 ± 19 mV initially to −346 ± 15 mV after 4 h at 100 °C. A parallel experiment with (nonreducing) xylitol showed nearly complete absence of reductone activity with E_{oc} of only −52 ± 19 mV at 4 h. Similar results were obtained after 4 h at 100 °C with ribose

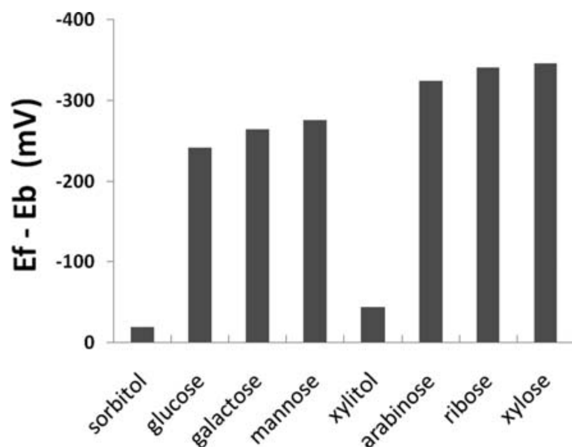

Figure 4. Changes in open-circuit electrode potential for various aldohexose sugars after 4 h heating in 0.10 M pH 7.2 phosphate buffer at 100 °C. Unreactive sugar alcohols sorbitol and xylitol served as electroinactive controls.

and arabinose and with hexoses glucose, galactose, and mannose and a related (nonreducing) sugar alcohol, sorbitol (**Figure 4**). In general hexoses, e.g. glucose (E_{oc}: 242 mV) produced less reductones than pentoses under comparable conditions, facts that are consistent with our DCIP titration results, which indicated a steady increase of chemically reducing species as a function of reaction time.

Cyclic Voltammetry of an Amadori Compound. Cyclic voltammetry (CV) was investigated as a possible method for observing and measuring highly reactive reducing materials like reductones in situ. In this automated, cyclic redox process, a compound is first oxidized by exposure to a gradually increasing positive voltage and oxidation products are immediately reduced with applied voltage of reversed polarity. The short duration between cycles makes CV an ideal method for observing certain highly reactive reaction intermediates. Amadori compounds **8** (**Figure 5**) are key intermediates in Maillard reactions of amino acids and reducing sugars. Under oxidative conditions, Amadori compounds have been shown to generate reductones by various electron transfer processes (*13*), which can be described generally as shown in **Figure 5**. Because it is also possible to oxidize Amadori compounds electrochemically, we sought to test the possibility of using cyclic voltammetry (CV) to study the formation and behavior of reductones generated in situ. The oxidative formation of glucosone **7** (Z = CH_2OH) from an Amadori compound has been known since the 1950s and was most recently reviewed and studied by Baker et al. (*14*). A series of reactions describing the observed oxidation is shown in **Figure 5**. Apparently, the reaction proceeds via 1,2-enolization of **8** to form an eneaminol **9** followed by electron transfer (oxidation) and hydrolysis to form the osasone (reductone) **7**. In cases reported, the highly reactive reductone products were identified and/or quantified only after chemical derivatization.

For our CV experiments, we chose as a model compound a previously synthesized Amadori compound, *N*-(1-deoxyfructos-1-yl) piperidine (*8*). The compound was chosen because of its availability in a highly purified crystalline state and because it had been thoroughly characterized previously. Furthermore, it was anticipated that the relatively simple structure of a model Amadori compound would facilitate the interpretation of electrochemical results. Results of CV analysis of *N*-(1-deoxyfructos-1-yl)piperidine are shown in **Figure 6**. A 2 mM solution of the Amadori compound in 0.1 M pH 7.14 phosphate buffer was subjected to analysis with a glassy carbon electrode. As the potential

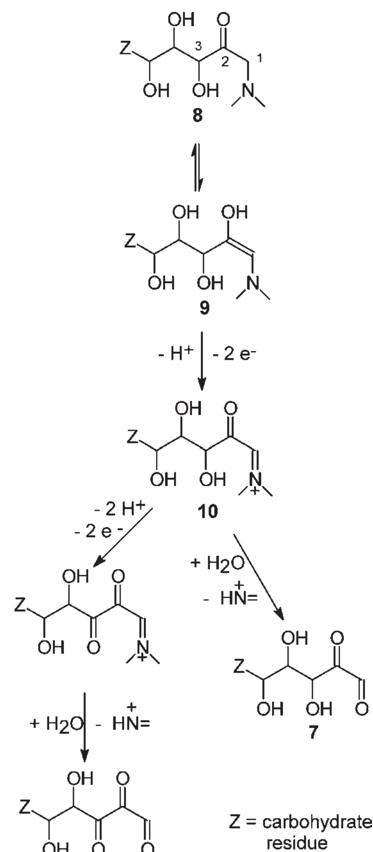

Figure 5. Reductone formation via oxidation of Amadori compounds.

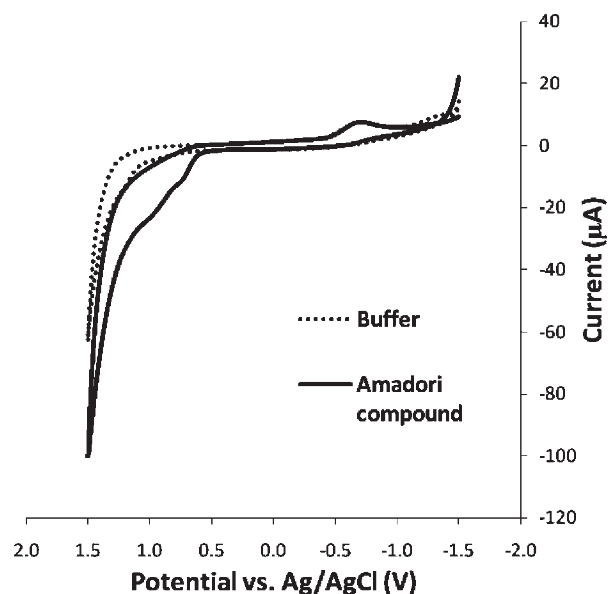

Figure 6. Cyclic voltammogram of *N*-(1-deoxyfructos-1-yl)piperidine in 0.10 M pH 7.14 phosphate buffer. Lower curves represent oxidative scans.

was scanned positively from −1.5 V to +1.5 V at 50 mV/s, two anodic waves were observed at ca. + 0.7 V and +0.9 V (vs Ag/AgCl reference) [lower curve]. Conceivably, the two oxidation waves represent sequential oxidation of the eneaminol **9** and its immediate oxidation product, the iminoosone **10**. However, when the applied potential was immediately swept negatively from +1.5 V to −1.5 V [upper curve], no waves (other than the reduction of dissolved oxygen) were observed to indicate that the oxidation process was reversible. That is, products with expected reductone

electrochemical behavior were not observed. The same CV results were obtained in replicate experiments, and both oxidation waves were also observed at a faster scan rate of 100 mV/s. Similar CV results were reported by Chuang and Chou (*9*) in the analysis of the Amadori compound, Fru-Val, i.e. *N*-(1-deoxy-β-D-fructopyranos-1-yl)-L-valine. For Fru-Val, a single anodic wave was observed at ca. + 1.0 V, and likewise no evidence was found for reductone products in the cathodic scan. The irreversible nature of the CV results indicated that the oxidation products once formed were not available for subsequent electrochemical reduction. The expected reductone product may have reacted rapidly with piperidine liberated in the reaction (**Figure 5**) to form more stable products incapable of electrochemical detection. Apart from the failure of CV to detect reductone-like products per se, the method does offer a useful noninvasive way to quantify Amadori compounds themselves. The positive anodic waves associated with oxidation do offer a method for the detection and measurement of Amadori compounds. A recent practical example of this aspect is the development of a selective electrode for the measurement of glycated hemoglobin (HbA1C) in the blood of diabetic patients (*15*). In this example, CV was used to detect the oxidation wave associated with an Amadori compound derived from blood glucose and the pendant amino groups of blood protein. On the basis of our observations and those of others, we believe that CV methodology could be a useful noninvasive technique for quantifying the levels of Amadori compounds in foodstuffs or in model Maillard reactions.

ACKNOWLEDGMENT

We thank Mrs. Deborah Ewald for performing the electrospray mass spectral analysis.

LITERATURE CITED

(1) Nursten, H. *The Maillard Reaction*; The Royal Society of Chemistry: Cambridge, UK, 2005.

(2) Yaylayan, V. A.; Haffenden, L.; Chu, F. L.; Wnorowski, A. Oxidative pyrolysis and postpyrolytic derivatization techniques for the total analysis of Maillard model systems. *Ann. N.Y. Acad. Sci.* **2005**, *1043*, 41–54.

(3) Glomb, M. A.; Tschirnich, R. Detection of α-dicarbonyl compounds in Maillard reaction systems and in vivo. *J. Agric. Food Chem.* **2001**, *49*, 5543–5550.

(4) Masaki, N.; Matsuzaki, T.; Shigematsu, H. Formation of reducing substances in the Maillard reaction between D-glucose and γ-aminobutyric acid. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 806–807.

(5) Hofmann, T.; Bors, W.; Stettmaier, K. Studies on radical intermediates in the early stage of the non-enzymic browning reaction of carbohydrates and amino acids. *J. Agric. Food Chem.* **1999**, *47*, 379–390.

(6) Trang, V. T.; Takeuchi, H.; Kudo, H.; Aoki, A.; Katsuno, S.; Shimamura, T.; Sugiura, T.; Ukeda, H. Antimicrobial activity of aminoreductone against helicobacter pylori. *J. Agric. Food Chem.* **2009**, *57*, 11343–11348.

(7) Fedoronko, M. The electrochemistry of carbohydrates and their derivatives. *Adv. Carbohydr. Chem. Biochem.* **1974**, *29*, 168–171.

(8) Rizzi, G. P. Electrochemical study of the Maillard reaction. *J. Agric. Food Chem.* **2003**, *51*, 1728–1731.

(9) Chuang, S.-W.; Chou, T. Ch. Electrochemical behavior of an Amadori compound in pH controlled aqueous media. *Meet. Abstr. Electrochem. Soc.* **2008**, *801*, 510.

(10) Rizzi, G. P. Role of phosphate and carboxylate ions in Maillard browning. *J. Agric. Food Chem.* **2004**, *52*, 953–957.

(11) Wolff, S. P., Free radicals and glycation theory. In *The Maillard Reaction*; Ikan, R., Ed; John Wiley & Sons: New York, 1996; pp 73−88.

(12) Angyal, S. J. The composition of reducing sugars in solution: current aspects. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 15–68.

(13) Kawakishi, S.; Tsunehiro, J.; Uchida, K. Autoxidative degradation of Amadori compounds in the presence of copper ion. *Carbohydr. Res.* **1991**, *211*, 167–171.

(14) Baker, J. R.; Zyzak, D. V.; Thorp, S. R.; Baynes, J. W. Chemistry of the fructosamine assay: D-glucosone is the product of oxidation of Amadori compounds. *Clin. Chem.* **1994**, *40*, 1950–1955.

(15) Chuang, S.-W.; Rick, J.; Chou, T. Ch. Electrochemical characterization of a conductive polymer molecularly imprinted with an Amadori compound. *Biosens. Bioelectron.* **2009**, *24*, 3170–3173.

Received for review March 25, 2010. Revised manuscript received August 2, 2010. Accepted August 4, 2010.