Free Radical Formation from Secondary Amines in the Maillard Reaction

Richard L. Roberts and Roger V. Lloyd*

Department of Chemistry, University of Memphis, Memphis, Tennessee 38152-6060

ESR spectroscopy has been used to study the formation of free radicals in the Maillard browning reaction, from the reaction between glycolaldehyde or glyceraldehyde and *N*,*N*′-dialkylethylenediamines (alkyl = methyl, ethyl, *iso*-propyl, *tert*-butyl) in either aqueous buffer or in methanol. Two series of cyclic cation radicals are formed. The first is a 1:1 sugar:amine product in which the nitrogen atoms in 1 ethylenediamine molecule are bridged by a sugar molecule. The second, and more stable, is a 2:2 product in which a substituted dihydropyrazine cation radical is formed by the bridging of two amines with two sugars. In addition, nitroxide radicals are also observed when methanol is used as the solvent. A mechanism for the formation of these radicals in the early stages of the Maillard reaction is proposed.

Keywords: *Maillard reaction; cation radicals; ESR; dihydropyrazines*

INTRODUCTION

The Maillard reaction, or amine glycation, between amines and carbonyl compounds has been known for many years to yield an extremely complex mixture of products, which ultimately forms brown polymeric materials known as melanoidins (Hodge, 1953). Pyrazines, which are responsible for many of the flavors and aromas of prepared foods, represent a major class of the numerous compounds involved in the reaction pathway (Parliment and Epstein, 1973; Seifert *et al*., 1972). Considerable research has been conducted into their formation via the Maillard reaction, and more than 70 different pyrazines have been identified in foods (Manley and Fargerson, 1970; Newell *et al*., 1967). Many model systems have been studied in attempts to determine the source of these pyrazine compounds, although as yet there is no report of a reaction pathway involving an alkylamine (Akimaya *et al*., 1978; Kohler and Odell, 1970; Namiki, 1988; Namiki and Hayashi, 1973; Rizzi, 1988; Shibamato and Bernhard, 1976, 1977a,b, 1978; Shibamato *et al*., 1979; Shibamato and Russell, 1977). The formation of methyl-substituted pyrazines with glycine as the nitrogen source has been described, but free radicals were not implicated as intermediates (Keyhani and Yaylayan, 1996).

Recently, free radicals have been detected in the early stages of the Maillard reaction (Namiki, 1988), and the pathway for their formation is also poorly understood. Namiki and Hayashi (1973, 1975) used ESR spectroscopy to observe free radicals produced by the reaction between primary amines and sugars, and identified the free radical products as *N*,*N*′-dialkylpyrazinium radical cations. Their results showed that the alkyl side chains on the pyrazinium nitrogen atoms were derived from the amine, while the carbon atoms of the pyrazinium ring came from the sugar. It is possible that these cation radicals are the immediate precursors of the neutral pyrazines observed at the later stages of the reaction.

One of the major difficulties in studying these free radicals is that the spectra often consist of only a broad singlet, with insufficient resolution to propose a structure. By following the reaction of simple pure compounds (glucose and lysine) at elevated temperature (100 °C), Namiki and Hayashi (1983) observed an ESR spectrum with a resolved hyperfine structure that was gradually transformed into the broad singlet ESR spectrum characteristic of melanoidins. Most primary amines reacted with different sugars to yield ESR spectra with similar hyperfine structure, suggesting that cleavage of the sugar molecule occurred during the reaction process. Further investigation revealed that pentoses and hexoses undergo fission at the C2 carbon prior to the formation of the *N*,*N*′-dialkylpyrazinium radical cation (Namiki and Hayashi, 1981; Hayashi and Namiki, 1981).

Given the importance of the Maillard reaction to the food supply and to food preparation, a better understanding of the mechanism of product formation from this reaction is important. The presence of free radicals is of interest since they are formed at an early stage in the reaction sequence and are known as well to be present in the final reaction mixture. In our experiments, we have obtained free radicals from simple model compounds to more clearly define the formation of the pyrazine ring system. The reactions studied are those of glycolaldehyde or glyceraldehyde with *N*,*N*′-dialkylethylenediamines. The two sugars have been used as model compounds by many researchers studying the Maillard reaction (Namiki and Hayashi, 1975), and the *N*,*N*′-dialkylethylenediamines represent an amine source that could serve directly as templates for a pyrazine ring, although diamines are not as commonly found in food systems.

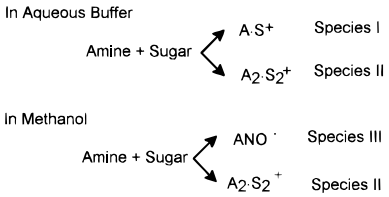
EXPERIMENTAL PROCEDURES

The amines and sugars were from Sigma Chemical (St. Louis, MO) and used as received. The amines were *N*,*N*′-dialkylethylenediamine (alkyl = methyl, ethyl, isopropyl, *tert*-butyl), and the sugars were glycolaldehyde or glyceraldehyde. Buffer solutions (phosphate or TRIS, 1.5 M, pH = 8.50) were prepared with water purified by reverse osmosis (Osmonics, Inc., Minnetonka, MN). For most experiments, the buffer solutions were stirred with Chelex 100 cation-exchange resin (Bio-Rad Laboratories, Richmond, CA) for at least 24 h prior to use, to remove adventitious metal ions. Experiments run in methanol were not buffered or pH adjusted.

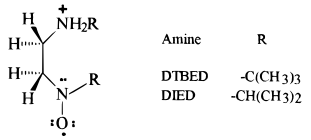
* Corresponding author (e-mail rlloyd@cc.memphis.edu).

S0021-8561(96)00902-8 CCC: $14.00

Scheme 1

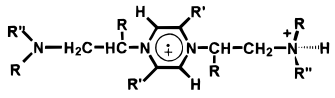


Scheme 2



Amine	R
DTBED	-C(CH3)3
DIED	-CH(CH3)2

Scheme 3



Amine	R	R"	Sugar	R'
DMED	CH_3	H	GLYCOL	H
DEED	CH_2CH_3	H	GLYCER	CH_2OH
DIED	$CH(CH_3)_2$	H		
DTBED	$C(CH_3)_3$	H		
TMED	CH_3	CH_3		

For sample preparation, a weighed portion of the amine was added to 10 mL of buffer in a glass vial to obtain the desired final concentration of amine (usually 0.5 M). The sugar was weighed into a separate glass vial, for a final sugar/amine mole ratio of (usually) 1:1. Both vials were then sealed with rubber serum caps, vented with polyethylene tubing, and purged with nitrogen for at least 30 min. These purged samples were then unsealed and mixed in a nitrogen-filled glove bag, and a quartz ESR flat cell was filled and stoppered in the glove bag. Therefore, all spectra run in aqueous buffer were obtained in the absence of oxygen. Spectra were obtained at room temperature (25 ± 3 °C) with either Varian E-4 or E-104 ESR spectrometers at various times after mixing and stored on a computer for later analysis. First-order ESR spectra were simulated with a readily available software package (Duling, 1994). The *g* values when measured were obtained by comparison with DPPH ($g = 2.0036$) as an external standard.

RESULTS

The reactions of *N,N′*-dialkylethylenediamines with glyceraldehyde or glycolaldehyde in solution produced at least three different series of free radicals. The particular radicals observed depended upon a variety of factors in addition to the starting materials, including the solvent and the buffer. The reactions are outlined in Scheme 1 (A = amine and S = sugar).

Within approximately 20 min after mixing, the reaction of DMED, DEED, or DIED with GLYCOL yielded a detectable ESR spectrum, although the solution remained colorless (Scheme 1, species I). A typical spectrum is shown in Figure 1 together with a simulation based upon the parameters in Table 1. This radical has only been observed with glycolaldehyde and not with glyceraldehyde as the sugar, despite numerous attempts to detect it. The species I radical is a transient species with a lifetime of approximately 2 h at room temperature.

In all cases, a second radical species was detected within 3−4 h after mixing (Scheme 1, species II), and the solution took on the brown color characteristic of the Maillard reaction. When the aqueous buffer was not pretreated with ion-exchange resin, the resulting ESR spectra had broad peaks (Figure 2A) and decayed

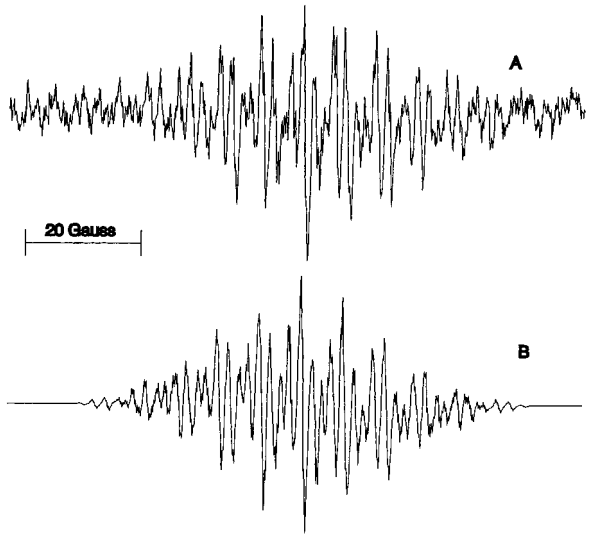


Figure 1. ESR spectrum of the species I radical from reaction of *N,N′*-dimethylethylenediamine with glycolaldehyde in aqueous buffer pretreated with Chelex resin: (A) experimental spectrum; (B) computer simulation of panel A, based on the parameters in Table 1.

Table 1. Hyperfine Splitting Constants for Species I Radicals from *N,N′*-Dialkylethylenediamines and Glycolaldehyde[a]

	amine		
	DMED	DEED	DIED
nitrogen	7.43 (2)	7.40 (2)	7.18 (2)
ring hydrogens (double bond)	5.37 (2)	5.02 (2)	5.02 (2)
ring hydrogens (CH_2)	2.43 (4)	2.75 (4)	2.94 (4)
nitrogen substituents			
CH_3	7.06 (6)	0.32 (6)	0.27 (6)
CH_2		6.96 (4)	
CH			6.81 (2)

[a] All values are in Gauss. The numbers in parentheses represent the number of magnetically equivalent nuclei. Refer to Scheme 4.

within approximately 4 h to a broad singlet (Figure 2B). In contrast, spectra observed in buffer systems treated with ion-exchange resin showed considerable resolved fine structure (Figure 2C) and had significantly longer lifetimes. It was found in a separate experiment that deliberate addition of 5 mM ferrous sulfate to a solution exhibiting spectrum Figure 2C caused its immediately conversion to a spectrum that was identical to Figure 2A. Because of these results, buffers were routinely treated with Chelex ion-exchange resin before use.

Examples of the species II spectra are shown in Figures 3 (DMED with GLYCER), 4 (DMED with GLYCOL), and 5 (DIED with GLYCER), together with computer simulations based on the data in Tables 2 and 3. These spectra can be very long-lived once formed. For example, the radical obtained from the reaction of 0.5 M DMED and 0.5 M glyceraldehyde in 1.5 M Chelex-treated phosphate buffer at pH = 8.25 has been detected 9 months after it was originally prepared (prepared under nitrogen and sealed in an aqueous flat cell). Once exposed to the atmosphere, the spectrum eventually decays to the broad singlet, which is itself detectable for several months more.

Because of the low solubility of DTBED in water, some reactions were carried out in methanol. The reaction of DIED or DTBED with either GLYCER or GLYCOL in the presence of oxygen gave a nitroxide radical (species III, Scheme 1) with a lifetime of several

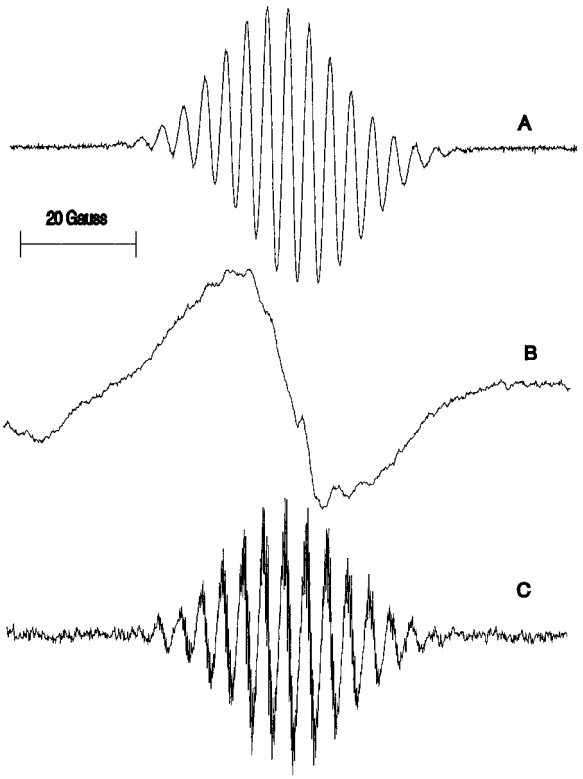


Figure 2. (A) ESR spectrum of the species II radical formed from *N*,*N*′-dimethylethylenediamine with glyceraldehyde in aqueous buffer. (B) Sample of spectrum A after an additional 4 h at room temperature. (C) Same as panel A except buffer pretreated with ion-exchange resin.

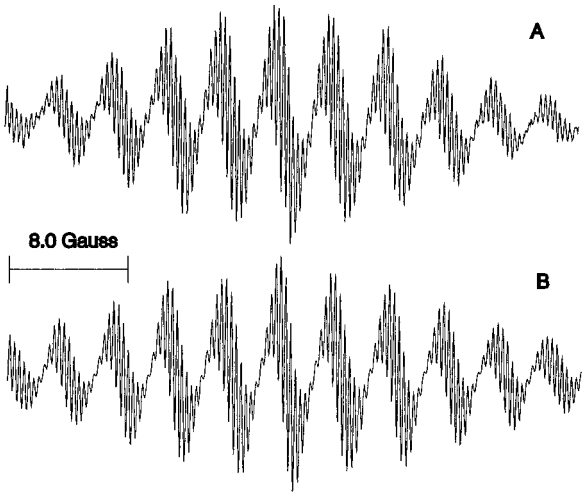


Figure 3. ESR spectrum of the species II radical formed from *N*,*N*′-dimethylethylenediamine with glyceraldehyde in aqueous buffer pretreated with ion-exchange resin (spectrum 2C), expanded scale: (A) experimental spectrum; (B) computer simulation of panel A, based on the parameters in Table 3.

hours. Formation of the nitroxide required the presence of both sugar and oxygen in the methanol solution. A typical spectrum is shown in Figure 6 (DTBED and GLYCOL), with a simulation based upon the data in Table 4. Following the disappearance of the nitroxide radical, the reaction of either amine with GLYCOL gave spectra similar to those of species II in aqueous solution. For DIED and GLYCOL, the spectrum was identical to that observed in aqueous buffer for the same reactants (Table 2).

Species I Radical. On the basis of the observed hyperfine splittings and the number of equivalent nuclei

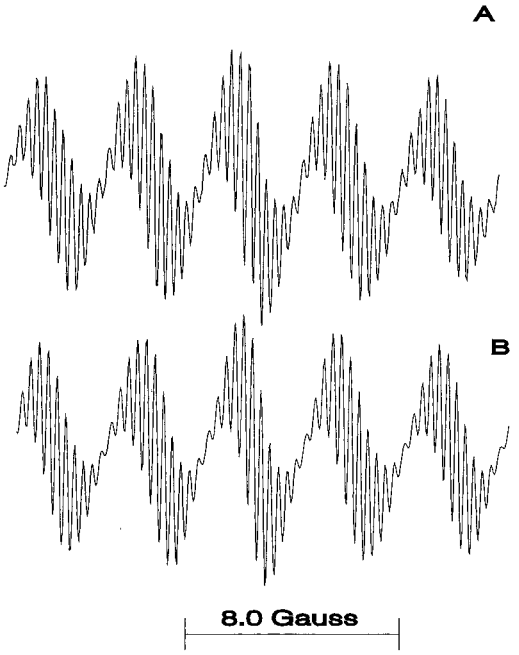


Figure 4. ESR spectrum of the species II radical from reaction of *N*,*N*′-dimethylethylenediamine with glycolaldehyde in aqueous buffer pretreated with Chelex resin: (A) experimental spectrum (expanded scale); (B) computer simulation of panel A, based on the parameters in Table 2.

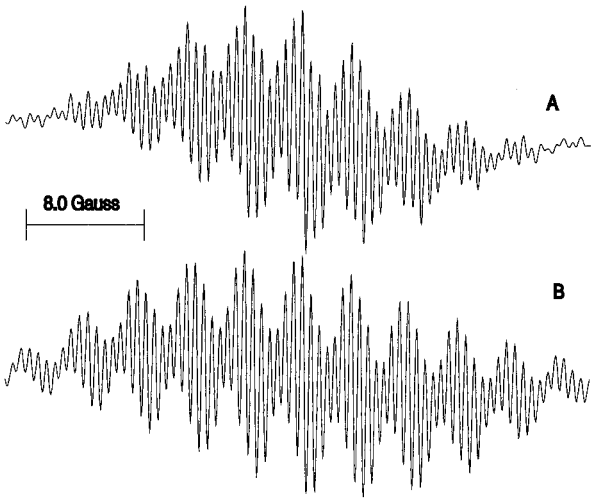


Figure 5. ESR spectrum of the species II radical from reaction of *N*,*N*′-diisopropylethylenediamine with glycolaldehyde in aqueous buffer pretreated with Chelex resin: (A) experimental spectrum (expanded scale); (B) computer simulation of panel A, based on the parameters in Table 3.

in the computer simulation, in particular two equivalent nitrogen atoms, we propose the structure shown in Scheme 4 for the species I radicals. The species I radical spectra completely disappeared before the species II radical could be detected, suggesting that species I is not a direct precursor of species II. TMED did not form a species I radical but formed the species II radical directly, which is also consistent with the proposed structure, since the tertiary nitrogen could not participate in ring formation.

Species II Radical. The species II radicals are the major and most long-lived radical products of our reactions. We propose the general structure shown in Scheme 3, an *N*,*N*′-disubstituted pyrazinium radical cation. The major hyperfine splittings observed are consistent within the series (Tables 2 and 3) and are

Table 2. Hyperfine Splitting Constants for Species II Radicals from *N,N′*-Dialkylethylenediamines and Glycolaldehyde[a]

	amine (solvent)			
	DMED (aq)	DEED (aq)	DIED (aq or methanol)	DTBED (methanol)
N (ring)	7.33 (2)	7.21 (2)	7.46 (2)	7.47 (2)
H (ring, R′=H)	3.35 (4)	4.69 (4)	4.68 (4)	4.58 (4)
side chain				
H (CH)	8.00 (2)	7.37 (2)	6.85 (2)	7.49 (2)
			2.19 (2)	
H (CH_2)	0.32 (4)	2.00 (4)	2.81 (4)	1.89 (4)
		1.27 (4)		
N	0.31 (2)	0.34 (2)	0.08 (2)	
R (=CH_3)	0.02 (6)			

[a] See Scheme 3 and footnotes for Table 1.

Table 3. Hyperfine Splitting Constants for the Species II Radical from *N,N′*-Dialkylethylenediamines and Glyceraldehyde[a]

	amine		
	DMED	DEED	DIED
N (ring)	7.35 (2)	7.90 (2)	7.27 (2)
H (ring)	3.99 (2)	4.64 (2)	4.55 (2)
CH_2 (ring)	3.98 (4)	3.32 (4)	3.41 (4)
side chain			
H (CH)	7.06 (2)	6.68 (2)	6.61 (2)
			0.56 (2)
H (CH_2)	0.37 (4)	1.90 (2)	1.16 (2)
		0.66 (4)	
N	0.05 (2)	0.18 (2)	0.13 (2)
CH_3	0.32 (6)		

[a] See Scheme 3 and footnotes for Table 1.

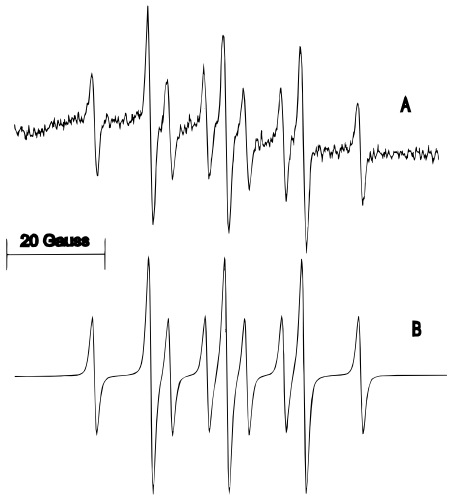


Figure 6. ESR spectrum of the species III nitroxide radical from the reaction of *N,N′*-di-*tert*-butylethylenediamine with glycolaldehyde in methanol: (A) experimental spectrum; (B) computer simulation of panel A, based on the parameters in Table 4.

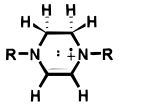
also in agreement with literature values for 1,4-dialkyl substituted pyrazinium cation radicals (Ahn and Johnson, 1969; Cope and Trumbull, 1960; Kaim, 1983; Ollis *et al.*, 1983; Pine, 1970). Namiki and Hayashi (1983) proposed the same general structure to explain their observed ESR spectra from the reaction of primary amines and sugars, which included glycolaldehyde and glyceraldehyde. However, their spectra were not as well resolved, making it difficult to assign unique structures. This may have been a result of the fact that their reactions were run at elevated temperatures without buffering or removal of metal ions.

Table 4. Hyperfine Splitting Constants for the Species III Nitroxide Radical from *N,N′*-Dialkylethylenediamines and Sugars in Methanol[a]

	amine, sugar			
	DIED, GLYCOL	DIED, GLYCER	DTBED, GLYCOL	DTBED, GLYCER
N	15.23 (1)	15.48 (1)	15.41 (1)	15.65 (1)
H	11.33 (2)	11.47 (2)	11.48 (2)	11.62 (2)
	4.90 (1)	4.95 (1)		
g value	2.0059			2.0063

[a] See Scheme 2 and footnotes for Table 1.

Scheme 4



Amine	R
DMED	CH_3
DEED	CH_2CH_3
DIED	$CH(CH_3)_2$

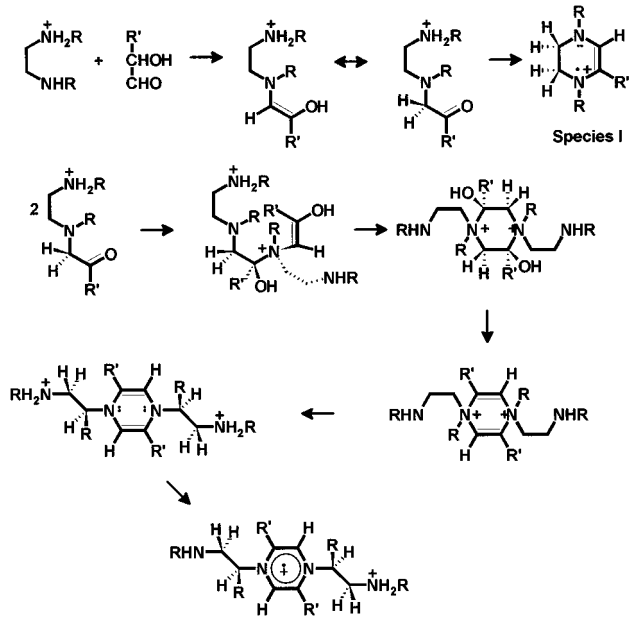
TMED, which was used in one experiment, has only one secondary nitrogen atom available for formation of the pyrazine ring in the species II structure. An ESR spectrum was observed from TMED and glycolaldehyde over the same time frame (approximately 3 h) as DMED and glycolaldehyde. As would be expected from the proposed structure (Scheme 3), the two spectra were identical.

Species III Radical. The reaction of either sugar with DIED or DTBED in methanol in the presence of oxygen yields the nitroxide radical III (Scheme 2). The *g* values (2.0061 ± 0.0002) are only consistent with nitroxides and are considerably different from those expected for alkylamino radicals (~2.0036) (Brand *et al.*, 1984; Cohen and Hoffman, 1974; Coppinger and Swalen, 1961; Kawamura *et al.*, 1967; Roberts and Winter, 1978). Since both sugar and oxygen are required for formation of III, it is likely that reactive oxygen species (such as hydroxyl, superoxide, or hydrogen peroxide) formed during autoxidation of the sugar (Hunt et al., 1988; Thornally *et al.*, 1984; Thornally and Stern, 1984; Wolff and Dean, 1987) are responsible for oxidation of the amines to nitroxides (Thomas, 1960; Wolff *et al.*, 1984). Similar radicals were not observed with DMED and DEED, suggesting that steric hindrance by the bulky *tert*-butyl and isopropyl groups reduces the rate of radical−radical recombination reactions.

DISCUSSION

A simplified mechanism for the formation of species I and II is shown in Scheme 5. The amines are shown as protonated, consistent with the high values of pK_{a2} (10.1−10.4) for substituted ethylenediamines (Rappoport, 1967). In agreement with our experimental results, species I is not a direct intermediate leading to species II. Species I is formed by intramolecular ring closure of the initial ketamine condensation product, while species II is formed by dimerization of the ketamine. It is important to note that with glyceraldehyde as the sugar, only a single radical species was obtained, specifically, the symmetric 2,5-disubstituted species II, with no detectable amount of another species, such as the 2,6-disubstituted radical. The latter radical, if present, would be readily identified owing to the magnetically nonequivalent ring nitrogen atoms (Kaim, 1983; Ahn and Johnson, 1969). Thus, the second step

Scheme 5



in the reaction is postulated as a dimerization, rather than as consecutive reactions of the original ketamine with a second sugar molecule and subsequently with a second amine. The product of the latter reaction sequence would be the 2,6- instead of the 2,5-disubstituted pyrazinium cation radical, or an equimolar mixture of 2,5- and 2,6- radicals, depending on the order of reaction with amine and the sugar. In either case, the 2,6- radical would be present in substantial abundance.

The structure also requires an unusual alkyl group migration, specifically, two 1,2-alkyl shift reactions. The products of the Hoffmann elimination reaction that might otherwise be expected owing to the presence of two beta hydrogens (Cope and Trumbull, 1960) are not consistent with the observed radical products. In order to form the species II radical, the ring compound formed from the reaction of the *N*,*N*′-dialkylethylenediamine and the sugar must instead undergo a Stevens rearrangement. The mechanism of this reaction involves the loss of a proton on the carbon directly adjacent to the nitrogen in the ring (*i.e.*, the alpha carbon) to form the appropriate ylide. The removal of this hydrogen normally requires very basic conditions, but in the precursor to the species II radicals, the alpha proton is highly acidic and easily removed, especially when aided by the steric strain of the alkyl substituents on the ring nitrogens. The next step in the Stevens rearrangement is debatable. Evidence indicates that the reaction proceeds by a solvent caged radical pair mechanism resulting in the 1,2-shift of the alkyl group, although an ion-pair mechanism has also been proposed (Hennion and Shoemaker, 1970; Ollis *et al*., 1983; Pine, 1970; Pine et al., 1970).

The antiaromatic, eight electron, six membered ring formed by the Stevens rearrangement has been proposed previously by Namiki and co-workers to account for the radical obtained from the reaction of primary amines and sugars (Hayashi *et al*., 1977). They suggested a single-electron transfer (SET) reaction to an unspecified receptor. These experiments were performed with no attempt to exclude oxygen, and the counterion may have been the superoxide anion. Recently, Yim prepared a cross-linked radical by the reaction of methylglyoxal with alanine and made a similar suggestion of SET (Yim *et al*., 1995). Their observed hyperfine splittings are consistent with those found for our species II radicals, and they observed increased radical production under anaerobic conditions, also in agreement with our results.

Since species I and II did not require the presence of oxygen or transition metal ions for their formation, we believe that they were formed by single-electron transfer reactions rather than metal-catalyzed oxidations. We were unable to detect an anion radical in solution, but it is possible that small levels of oxygen were present.

CONCLUSIONS

The ring system of a major class of Maillard reaction products, the pyrazines, can easily be formed from open-chain reactants. From the reaction of *N*,*N*′-dialkylethylenediamines and small sugars we have shown that secondary amines yield free radical products from the Maillard reaction similar to those found for primary amines. The species II radical was found to be a persistent radical product of all of the *N*,*N*′-dialkyl substituted ethylenediamines and sugar systems studied and appears to play an important role in the Maillard reaction pathway. The spectra of radicals found in melanoidins are similar to those observed in this study from exposure of solutions containing the species II radical to atmospheric oxygen or to the ferrous ion. Both are found to be broad singlets without fine structure, suggesting that the radical could on the direct pathway leading to melanoidin formation in the Maillard reaction. The other two radicals observed, species I and III, have not previously been reported as part of the Maillard reaction. The species I radicals were only detected with glycolaldehyde as the reactant sugar, indicating that they probably represent minor alternative pathways not directly involved in the formation of species II.

ABBREVIATIONS USED

DEED, *N*,*N*′-diethylethylenediamine; DIED, *N*,*N*′-diisopropylenediamine; DMED, *N*,*N*′-dimethylethylenediamine; DTBED, *N*,*N*′-di-*tert*-butylethylethylenediamine; TMED, *N*,*N*,*N*′-trimethylethylenediamine; GLYCER, glyceraldehyde; GLYCOL, glycolaldehyde.

ACKNOWLEDGMENT

We thank Dr. Peter Bridson and Dr. Theodore Burkey for helpful suggestions.

LITERATURE CITED

Ahn, M.-K.; Johnson, C. S., Jr., Electron Spin Relaxation in the N,N′-Dimethylpyrazine Cation Radical. *J. Chem. Phys.* **1969**, *50*, 632−644.

Akiyama, T.; Enomoto, Y.; Shibamoto, T. A New Method of Pyrazine Synthesis for Flavor Use. *J. Agric. Food Chem.* **1978**, *26*, 1176−1179.

Brand, J. C.; Cook, M. D.; Roberts, B. P. Silylaminyl Radicals. Part 3. Electron Spin Resonance Studies of Alkyl(trialkylsilyl)aminyl Radicals. *J. Chem. Soc., Perkin Trans. 2* **1984**, 1187−1196.

Cohen, A. H.; Hoffman, B. M. Acceptor Strengths of Some Group IV Lewis Acids by Electron Paramagnetic Resonance Studies of Their Complexes with Aliphatic Nitroxides. *Inorg. Chem.* **1974**, *13*, 1484−1491.

Cope, A. C.; Trumbull, E. R. Olefins from Amines: The Hoffmann Elimination Reaction and Amine Oxide Pyrolysis. *Org. React.* **1960**, *11*, 317−493.

Coppinger, G. M.; Swalen, J. D. Electron Paramagnetic Resonance Studies of Unstable Free Radicals in the Reaction of t-Butyl Hydroperoxides and Alkylamines. *J. Am. Chem. Soc.* **1961**, *83*, 4900−4902.

Duling, D. R. Simulation of Multiple Isotropic Spin-Trap EPR Spectra. *J. Magn. Reson., Ser. B* **1994**, *104*, 105−110.

Hayashi, T.; Namiki, M. On the Mechanism of Free Radical Formation During Browning Reaction of Sugars with Amino Compounds. *Agric. Biol. Chem.* **1981**, *45*, 933−939.

Hayashi, T.; Ohta, Y.; Namiki, M. Electron Spin Resonance Spectral Studies on the Structure of the Novel Free Radical Products Formed by the Reactions of Sugars with Amino Acids or Amines. *J. Agric. Food Chem.* **1977**, *25*, 1282−1287.

Hennion, G. F.; Shoemaker, M. J. Stevens Rearrangement of 3-Dimethylamino-3-methyl-1-butyne Methiodide. *J. Am. Chem. Soc.* **1970**, *92*, 1769−1770.

Hodge, J. E. Dehydrated Foods: Chemistry of Browning Reactions in Model Systems. *J. Agric. Food Chem.* **1980**, *1*, 928−943.

Hunt, J.; Dean, R.; Wolff, S. Hydroxyl Radical Production and Autoxidative Glycosylation. *Biochem. J.* **1988**, *256*, 205−212.

Kaim, W. Effects of Cyclic 8-π-Electron Conjugation in Reductively Silylated N-Heterocycles. *J. Am. Chem. Soc.* **1983**, *105*, 707−713.

Kawamura, T.; Matsunami, S.; Yonezawa, T. Solvent Effects on the g-Value of Di-t-butyl Nitric Oxide. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 1111−1115.

Keyhani, A.; Yaylayan, V. A. Elucidation of the Mechanism of Pyrazineone Formation in Glycine Model Systems Using Labeled Sugars and Amino Acids. *J. Agric. Food Chem.* **1996**, *44*, 2511−2516.

Koehler, P. E.; Odell, G. V. Factors Affecting the Formation of Pyrazine Compounds in Sugar-Amine Reactions. *J. Agric. Food Chem.* **1970**, *18*, 895−898.

Manley, C. H.; Fagerson, I. S. Major Volatile Components of the Basic Fraction of Hydrolyzed Soy Protein. *J. Agric. Food Chem.* **1970**, *18*, 340−342.

Namiki, M. Chemistry of Maillard Reactions: Recent Studies on the Browning Reaction Mechanism and the Development of Antioxidants and Mutagens. *Adv. Food Res.* **1988**, *32*, 115−184.

Namiki, M.; Hayashi, T. Development of Novel Free Radicals During the Amino-Carbonyl Reaction of Sugars and Amino Acids. *J. Agric. Food Chem.* **1975**, *23*, 487−491.

Namiki, M.; Hayashi, T. Formation of Novel Free Radical Products in an Early Stage of the Maillard Reaction. *Prog. Food Nutr. Sci.* **1981**, *5*, 81−91.

Namiki, M; Hayashi, T. A New Mechanism of the Maillard Reaction Involving Sugar Fragmentation and Free Radical Formation. *ACS Symp. Ser.* **1983**, *No. 215*, 21−46.

Namiki, M.; Hayashi, T.; Kawakishi, S. Free Radicals Developed in the Amino-Carbonyl Reaction of Sugars With Amino Acids. *Agric. Biol. Chem.* **1973**, *37*, 2935−2936.

Newell, J. A.; Mason, M. E.; Matlock, R. S. Precursors of Typical and Atypical Roasted Peanut Flavor. *J. Agric. Food Chem.* **1967**, *15*, 767−772.

Ollis, D. W.; Rey, M.; Sutherland, I. O. Base Catalyzed Rearrangements Involving Ylide Intermediates. Part 15. The Mechanism of the Stevens [1,2] Rearrangement. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1009−1027.

Parliment, T. H.; Epstein, M. Organoleptic Properties of Some Alkyl-Substituted Alkoxy and Alkylthiopyrazines. *J. Agric. Food Chem.* **1973**, *21*, 714−716.

Pine, S. H. The Base-Promoted Rearrangement of Quaternary Ammonium Salts. *Org. React.* **1970**, *18*, 403−464.

Pine, S. H.; Catto B. A.; Yamagishi, F. G. Stevens Rearrangement of *N*,*N*,*N*-Trimethylneopentylammonium Iodide. *J. Org. Chem.* **1970**, *35*, 3663−3666.

Rappoport, Z., Ed.; *Handbook of Tables for Organic Compound Identification*, 3rd ed.; CRC Press: Boca Raton, FL, 1967; 437.

Rizzi, G. P. Formation of Pyrazines from Acyloin under Mild Conditions. *J. Agric. Food Chem.* **1988**, *36*, 349−352.

Roberts, B. P.; Winter, J. N. Homolytic Addition to N-Methylene-t-butylamine: A New Route to Aminyl Radicals. *J. Chem. Soc., Chem. Commun.* **1978**, 960−961.

Seifert, R. M.; Buttery, R. G.; Guadagni, D. G.; Black, D. R.; Harris, J. G. Synthesis and Odor Properties of Some Additional Compounds Related to 2-Isobutyl-3-methoxypyrazine. *J. Agric. Food Chem.* **1972**, *20*, 135−137.

Shibamoto, T.; Bernhard, R. A. Effects of Time, Temperature and Reactant Ratio on Pyrazine Formation in Model Systems. *J. Agric. Food Chem.* **1976**, *24*, 847−852.

Shibamoto, T.; Bernhard, R. A. Investigation of Pyrazine Formation Pathways in Sugar-Ammonia Model Systems. *J. Agric. Food Chem.* **1977**, *25*, 609−614.

Shibamoto, T.; Bernhard, R. A. Investigation of Pyrazine Formation Pathways in Glucose-Ammonia Model Systems. *Agric. Biol. Chem.* **1977**, *41*, 143−153.

Shibamoto, T.; Bernhard, R. A. Formation of Heterocyclic Compounds from the Reaction of L-Rhamnose with Ammonia. *J. Agric. Food Chem.* **1978**, *26*, 183−187.

Shibamoto, T.; Russell, G. F. A Study of the Volatiles Isolated from D-Glucose-Hydrogen Sulfide-Ammonia Model System. *J. Agric. Food Chem.* **1977**, *25*, 109−112.

Shibamoto, T.; Akiyama, T.; Sakaguchi, M.; Enomoto, U.; Masuda, H. A study of Pyrazine Formation. *J. Agric. Food Chem.* **1979**, *27*, 1027−1031.

Thomas, J. R. The Identification of Radical Products From the Oxidation of Diphenylamine. *J. Am. Chem. Soc.* **1960**, *82*, 5955−5956.

Thornalley, P.; Stern, S. The Production of Free Radical During the Autoxidation of Monosaccharides by Buffer Ions. *Carbohydr. Res.* **1984**, *134*, 191−204.

Thornalley, P.; Wolff, S.; Crabbe, J.; Stern, A. The Autoxidation of Glyceraldehyde and Other Simple Monosaccharides Under Physiological Conditions Catalyzed by Buffer Ions. *Biochim. Biophys. Acta* **1984**, *797*, 276−287.

Wolff, S. P.; Dean, R. T. Glucose Autoxidation and Protein Modification, The Potential Role of Autoxidative Glycosylation in Diabetes. *Biochem J.* **1987**, *245*, 243−250.

Wolff, S. P.; Crabbe, M. J. C.; Thornalley, P. J. The Autoxidation of Glyceraldehyde and Other Simple Monosaccharides. *Experientia* **1984**, *40*, 244−246.

Yim, H.-S.; Kang, S.-O.; Hah, Y.-C.; Chock, P. B.; Yim, M. B. Free Radicals Generated during the glycation reaction of Amino Acids by Methylglyoxal. *J. Biol. Chem.* **1995**, *270*, 28228−28233.

Received for review December 2, 1996. Accepted March 24, 1997.[⊗]

JF960902C

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1997.