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Mechanistic Pathway for the formation of Maltoxazine from Intact 1-[(2′-Carboxyl)pyrrolidinyl]-1-deoxy-D-fructose (Amadori–Proline)

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Neutral loss experiments and B/E linked-field scan mass spectrometric analysis of 1-[(2′-carboxyl)-pyrrolidinyl]-1-deoxy-D-fructose (Amadori–proline) have indicated that maltoxazine can be formed by a unimolecular decomposition of the proline Amadori compound without passing through a 3-deoxyosone intermediate as suggested in the literature. Further evidence was obtained from model studies of the proline Amadori compound in the presence of excess free proline and other amino acids, which indicated that the addition of free proline did not increase maltoxazine formation and that the addition of other amino acids did not decrease its formation, as would be expected if the reaction proceeded through a bimolecular interaction between the free proline and a 3-deoxyosone. A plausible mechanism for the formation of maltoxazine, consistent with the experimental observations, is presented.

Keywords: *Maltoxazine; mechanism of formation; proline Amadori compound; EIMS; GC/MS*

INTRODUCTION

The 1,2- and 2,3-enolizations of reducing sugars and Amadori products (**1**) under acid/base catalysis conditions initiate β-elimination reactions which eventually lead to the formation of reactive intermediates such as 1-deoxyosone and 3-deoxyosone (**2**). Formation of these intermediates from the Amadori products is accompanied by amino acid release. Both intermediates are considered to be important precursors in the subsequent formation of degradation products in Maillard systems. However, in principle, intact Amadori products can also undergo acid/base-catalyzed thermal degradations without deoxyosone formation, to produce a variety of other reactive intermediates that retain the amino acid moiety, such as 1-amino acid-1,4-dideoxy-2,5-dicarbonyl compounds (**5**). In fact, if the amount of free amino acid detected in the decomposition mixtures of pure Amadori products can be considered an indication of the extent of deoxyosone formation, then data available from the literature indicate that, at least in the case of the tryptophan Amadori compound, only about 50% of the Amadori compound undergoes decomposition (at 140 °C) to produce free tryptophan in the initial phase of the reaction, after which the concentration of tryptophan drops due to its further reactions (Yaylayan and Forage, 1992). This observation is compatible with the suggestion that decompositions from intact Amadori compounds are equally important. The proposition of the intermediacy of deoxyosones in rationalizing Maillard reaction products is not always necessary, if alternate mechanistic pathways can be envisaged directly from the Amadori compound, especially for products that retain the amino acid moiety at C-1 of the sugar residue, to avoid a recombination step to the same carbon atom from which it was originally cleaved. Such an approach will diminish the number of mechanistic steps proposed. Supporting evidence that maltol, for example, is formed directly from the intact Amadori product, without passing through the 1-deoxyosone pathway, has been presented (Yaylayan and Mandeville, 1994).

8-Oxo-1,2,3,3a,5,6,7,8-octahydrocyclopenta[*d*]pyrrolo-[2,1-b][1,3]oxazine (maltoxazine) has been identified in barley, malt, and numerous Maillard model systems containing proline. One postulated mechanism (Tressl et al., 1993) for the formation of maltoxazine involves the reaction of free proline with 3-deoxyosone (**2**) produced by the acid-catalyzed 1,2-enolization of Amadori products. However, proline-containing model systems have quite basic pH values, which does not encourage such 1,2-enolizations. In fact, all of the model studies with proline Amadori compounds produced 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one, the 2,3-enolization indicator, as the major product. In addition, it was demonstrated by Tressl et al. (1989) that maltoxazine formation was greatly favored under basic conditions (pH 8). In this paper we propose a new mechanism of maltoxazine formation initiated by base catalyzed 2,3-enolization of the proline Amadori product without formation of deoxyosones. Mills and Hodge (1976) have identified maltoxazine in the thermal decomposition mixtures of Amadori–proline alone.

MATERIALS AND METHODS

All reagents and chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). The synthesis of Amadori–proline was performed according to published procedures (Vernin et al., 1992).

Proline/Sugar Systems. Aqueous solutions (200 μL) containing 0.1 M fructose or glucose or 3-*O*-methyl-D-glucose and 0.1 M proline were heated in capped vials at 150 °C for 10 min on a heating block. The resulting brown mixtures were extracted five times with a total of 1 mL of ether and concentrated to a final volume of 200 μL under nitrogen using

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a Reacti-vap evaporator. Two microliters of the final concentrate was injected into GC/MS for analysis.

Amadori–Proline Systems. The synthesis of Amadori–proline was performed according to published procedures (Vernin et al., 1992). Amadori–proline (0.1 g) in 200 μL of water (1.8 M) was heated alone and in the presence of either L-alanine (0.1 g), D-proline (0.1 g), or *N*-methyl-L-proline (0.05 g). The resulting solutions were extracted with a total volume of 1 mL of diethyl ether and concentrated to a final volume of 200 μL. Two microliters of this solution was injected into GC/MS for analysis.

GC/MS Analysis. A Hewlett-Packard GC/mass selective detector (5890 GC/5971B MSD) was used for the analyses and acquisition of the electron impact mass spectra. Two microliters of sample solutions were injected in a splitless mode into a fused silica capillary column (DB-1, 30 m length X 0.32 mm i.d. X 0.25 μm film thickness; Supelco, Inc.). The initial column temperature (70 °C) was increased after 5 min at a 5 °C/min rate and maintained at 250 °C for 10 min. Carrier gas (helium) flow rate was 0.85 cm^3/min; injection port temperature was 250 °C; capillary direct MS interface temperature, 285 °C; ion source temperature, 280 °C; and the ionization voltage was 70 eV. The mass range was 33–350 amu, and electron multiplier voltage was 1500 V.

Linked-Field Scan and Neutral Loss Experiments. Linked-scan experiments on the collision-induced decompositions were performed on a VG-7070-EHF medium-resolution mass spectrometer that was equipped with a VG-11-250 data system. The analyses were performed at 70 eV at a resolving power of 1000 and a source temperature and pressure of 180 °C and 1 × 10^{-6} Torr (1 Torr = 133.3 Pa), respectively. The sample was introduced via a direct inlet probe heated to 180 °C.

RESULTS AND DISCUSSION

Linked-field scan mass spectrometric studies have demonstrated that some electron impact induced unimolecular fragmentation reactions have their counterparts in solution phase chemistry. It has been documented, for example, that β-carbolines can be formed from tryptophan Amadori products under electron impact conditions as well as in solution phase reactions at high temperatures (Yaylayan et al., 1990a,b, 1991). Similarly, pyrrolidine derivatives can be formed from lysine Amadori compounds under both conditions (Apriyantono and Ames, 1993). This type of correlation can help to distinguish between unimolecular and bimolecular mechanistic events associated with the reactions of Amadori products in model systems. It has been argued that the proline Amadori compound dissociates into 3-deoxyosone (**2** in Scheme 1) and then produces maltoxazine by a bimolecular interaction with free proline (Tressl et al., 1993). Since bimolecular interactions are not possible under the high vacuum conditions of the mass spectrometer, products identifiedin the mass spectrum of proline Amadori can originate only through unimolecular reactions. Table 1 shows products identified in the electron impact mass spectrum of proline Amadori (Yaylayan et al., 1993) and in its aqueous thermal decomposition mixtures (150 °C, 10 min). Maltoxazine, maltol, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one have been identified in both cases, whereas hydroxymaltol, being an oxidation product of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one, has been detected only in the thermal decomposition mixtures (Yaylayan and Mandeville, 1994). In addition, the 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one peak (*m/z* 144) was the second major peak in the mass spectrum of the proline Amadori compound (Yaylayan et al., 1993), and in the solution phase it was the major product formed. Plausible unimolecular mechanisms through ortho-elimination reactions have been proposed for the formation of maltol and 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one directly from the intact Amadori product (Yaylayan and Mandeville, 1994). On the other hand, the structure of the ion at *m/z* 179 in the mass spectrum of the proline Amadori product has been assigned to maltoxazine on the basis of B/E linked-field scan data shown in Table 2. To confirm the formation of maltoxazine from Amadori products in solution phase, aqueous solutions of the proline Amadori compound were decomposed alone and in the presence of free L-alanine, L-proline, or *N*-methyl-L-proline, in separate experiments, at 150 °C for 10 min. The mixtures were extracted with diethyl ether and analyzed by GC/MS. The results shown in Table 3 indicate that acid/base catalysis by amino acids is important for the formation of maltoxazine. In addition, these observations are not compatible with the proposition that maltoxazine is formed through the reaction of free proline with 3-deoxyosone (Scheme 1), in which case the addition of free proline was expected to increase maltoxazine formation relative to L-alanine and *N*-methyl-L-proline, by increasing the rate of the bimolecular reaction between 3-deoxyosone and L-proline. *N*-Methylproline, being the most basic amine (a tertiary amine) and less nucleophilic than other amino acids, most effectively catalyzed the unimolecular decomposition of the proline Amadori com-

Scheme 1. Proposed Mechanistic Pathways for the Formation of Maltoxazine from Proline Amadori Product

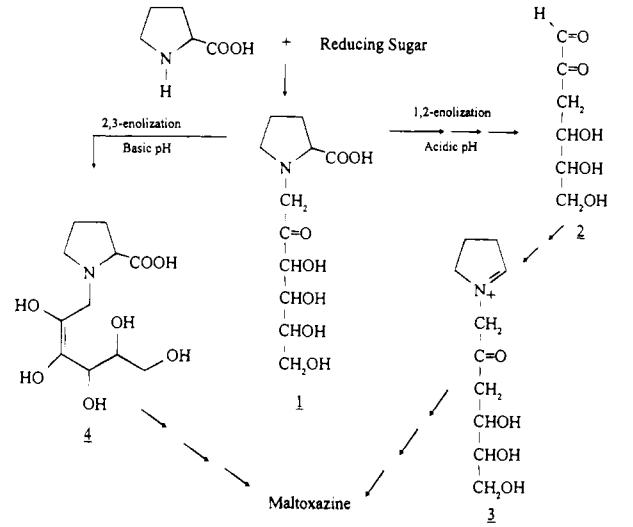


Table 1. Compounds Identified in the Electron Impact Mass Spectrum (EIMS) of Proline Amadori and in Its Aqueous Thermal Decomposition Mixtures

EIMS	thermal decomposition[a]	EIMS	thermal decomposition[a]
maltoxazine	maltoxazine	pyranone	pyranone[b]
maltol	maltol	–	hydroxymaltol

[a] Heated at 150 °C for 10 min. [b] 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one.

Table 2. B/E Linked-Field Scan Data of Ion *m/z* 179 Generated from EI of Proline Amadori Products

precursor ion	corresponding product ions[a]
m/z 179	179, 178, 160, 150, 136, 123, 110, 108
maltoxazine[b]	179, 178, 161, 150, 136, 123, 122, 118, 110, 108

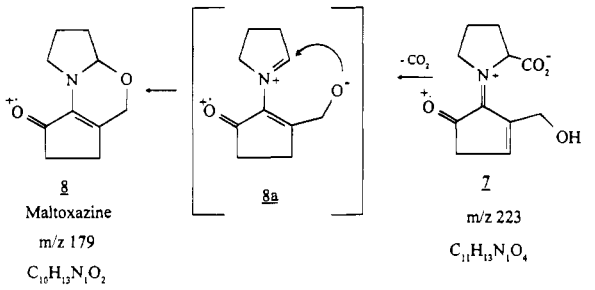
[a] Ions higher than 100 amu. [b] Normal spectrum.

Table 3. Relative Chromatographic Peak Areas of Maltoxazine from Proline Amadori Alone or in the Presence of Amino Acids

system[a]	maltoxazine[b]
proline Amadori	tr[c]
proline Amadori + L-proline	1.0
proline ARP[d] + L-alanine	1.2
proline ARP[d] + *N*-methyl-L-proline	3.8

[a] Heated at 150 °C for 10 min. [b] 8-Oxo-1,2,3,3a,5,6,7,8-octahydrocyclopenta[*b*]pyrrolo[2,1-*b*][1,3]oxazine. [c] Trace. [d] Amadori rearrangement product.

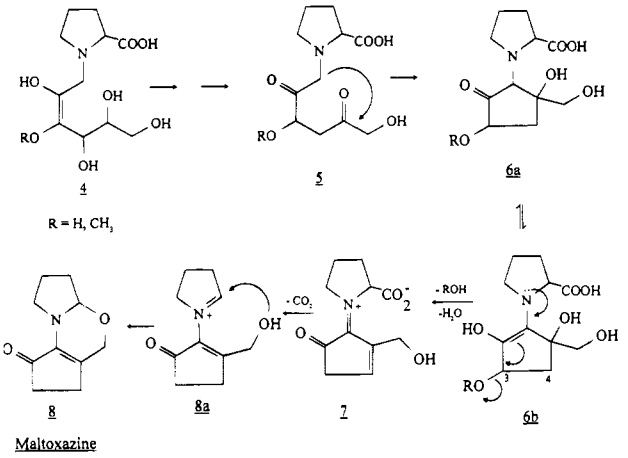
Scheme 2. Decarboxylation of the Ion at *m/z* 223 in the EI Mass Spectrum of Proline Amadori Compound and Formation of Maltoxazine



pound into maltoxazine, which is known to be favored under basic conditions (Tressl et al., 1989). Furthermore, experiments performed with [1-^{13}C]-D-glucose indicate that C-1 of D-glucose is directly attached to the nitrogen atom of L-proline (Tressl et al., 1993); this observation precludes 1-deoxyglucosone, the expected intermediate under basic conditions, to be the precursor of maltoxazine. On the basis of these observations we propose a unimolecular mechanism of decomposition of the proline Amadori compound to produce maltoxazine.

Mechanism of Maltoxazine Formation. Neutral loss experiments performed with the proline Amadori compund indicated that the ion at *m/z* 223 (**7** in Scheme 2) loses a carbon dioxide molecule (44 amu) to form the ion at *m/z* 179 (**8** in Scheme 2), which has been assigned to maltoxazine structure on the basis of B/E linked-field scan experiments (Table 2). Compound **7** can be regarded as the immediate precursor of maltoxazine. Scheme 2 illustrates the proposed conversion of *m/z* 223 to maltoxazine through the intermediate **8a**. The structure equivalent to **7** in solution phase chemistry has been incorporated in a mechanistic scheme illustrating the formation of maltoxazine from the proline Amadori compound without passing through the intermediate 3-deoxyosone (see Scheme 3). The pH of proline-containing model systems is quite basic, which promotes 2,3-enolization reactions, and according to Scheme 3, the 2,3-enediol form (**4**) of the proline Amadori compound undergoes a β-elimination reaction-,followed by enolization to produce a 1-proline-1,4-dideoxy-2,5-dicarbonyl compound (**5**). The zwitterionic form of this intermediate can initiate through the C-1 enolate anion a condensation at C-5 of the sugar moiety to produce the intermediate **6a**, which after enolization into **6b** can undergo a β-elimination at C-3 to produce compound **7** which has been identified as the immediate precursor of maltoxazine under electron impact conditions. The conversion of **6b** into **7** can be facilitated by replacing the C-3 hydroxyl group by a better leaving group, such as methoxy, and observing the effect on the maltoxazine formation. Indeed, when D-fructose, D-glucose, and 3-*O*-methyl-D-glucose were heated separately in the presence of proline in a sealed tube for 10

Scheme 3. Proposed Mechanism of Formation of Maltoxazine from Intact Proline Amadori Compound



min at 150 °C and the resulting brown solutions were extracted with diethyl ether, the GC/MS analysis of the extracts indicated that 3-O-methyl-D-glucose produced 3.6 times more maltoxazine relative to D-glucose. Table 4 lists the relative peak areas of maltoxazine identified in these model solutions. The increased formation of maltoxazine can be attributed to the ease of conversion of **6b** to **7**, when R is a methyl group, and not to the reactivity of 3-*O*-methyl-D-glucose relative to D-glucose, since both sugars had comparable concentrations of *aldehydeo* forms when analyzed by FTIR as a function of temperature. The resulting imminium ion **8a**, which can be deactivated by reaction with the solvent (water), can preferentially cyclize to produce maltoxazine, due to the entropy factor. One of the drawbacks of the mechanism proposed by Tressl et al. (1993) is that such imminium ion intermediates (**3**) are evoked early in the mechanistic scheme, much before the ring closure step, and this increases the likelihood of their deactivation by solvent (water) molecules.

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