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The detection of some dicarbonyl intermediates arising from the degradation of Amadori compounds (the Maillard reaction) ☆

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Abstract

Three Amadori compounds, *N*-(1-deoxy-D-fructos-1-yl)-glycine (**1**), N^{ϵ}-(1-deoxy-D-fructos-1-yl)-N^{α}-formyl-L-lysine (**2**) and *N,N*-di-(1-deoxy-D-fructos-1-yl)-glycine (**3**) were incubated (37°C) in buffered solutions having pH values corresponding to the pK_a of the substituted amino group in the presence of aminoguanidine. The dicarbonyl intermediates that were produced were trapped as the stable triazine derivatives (**6a, 6b** and **7**), and their yields were measured as a function of reaction time. The triazines derived from both 1-deoxy-2,3-D-*erythro*-hexodiulose (''1-deoxyglucosone'', **5**) and 3-deoxy-D-*erythro*-hexos-2-ulose (''3-deoxyglucosone'', **4**) were detected as the trimethylsilyl derivatives by GLC and were unequivocally identified by comparison with authentic standards and by GLC–MS. Varying the pH of the incubation mixture had little effect on the ratio or the nature of the dicarbonyl compounds produced. This represents an unusual situation, wherein two different dicarbonyl intermediates (one produced, presumably, as a result of initial 1,2-enolization and the other, presumably as a result of 2,3-enolization) were identified in the same Maillard reaction mixture.

Keywords: Amadori compounds; Maillard reaction; Dicarbonyl sugars

1. Introduction

The initial phase of the Maillard reaction involves the reaction of reducing sugars with amino groups to give 1-amino-1-deoxy-2-ketose derivatives (Amadori compounds).

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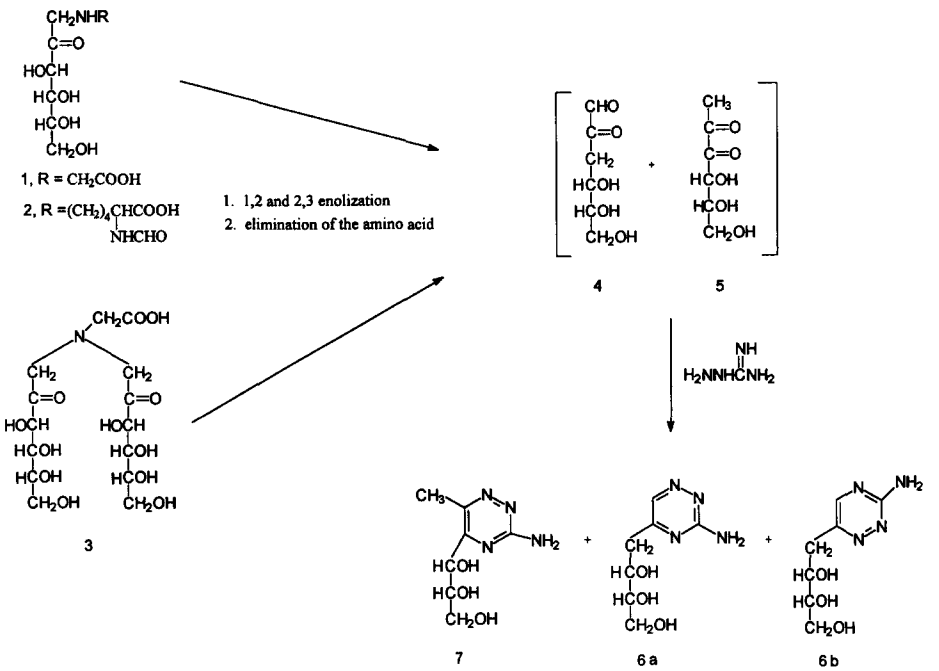

Scheme 1.

The latter are unstable and undergo a complex series of degradation reactions, with the resulting formation of colored pigments (non-enzymatic browning), UV-absorbing compounds (heterocyclic derivatives), and carbonyl-containing compounds.

There is abundant experimental evidence to indicate that highly reactive deoxy-dicarbonyl compounds are produced as initial intermediates in the reaction, and that these serve as precursors of, or play a significant role in the formation of, many of the final products in the overall reaction [1–6] (Scheme 1). One of the first such intermediates isolated was 3-deoxy-D-*erythro*-hexos-2-ulose ("3-deoxyglucosone", **4**), which has been shown to serve as a precursor of 2-furaldehydes, which are also associated with the Maillard reaction. Intermediate **4** was originally isolated as a result of the controlled degradation of an Amadori compound [3], and has since been isolated from a number Maillard reaction mixtures as well, and from food preparations, wherein the Maillard reaction has occurred.

More recently, Ledl's group have reported the detection of 1-deoxy-2,3-D-*erythro*-hexodiulose ("1-deoxyglucosone", **5**), isolated as the quinoxaline derivative) [6], a second putative intermediate, as a result of the controlled degradation of an Amadori compound. The same group also reported evidence for a 1,4-dideoxy-2,3-dicarbonyl derivative as well [7]. Because "deoxyosones" are highly reactive and unstable compounds, it has not been possible to directly examine their rates of formation or their yields during Maillard reactions since they undergo further degradation under conditions of their formation.

We have recently reported that dicarbonyl sugar derivatives react rapidly and irreversibly with aminoguanidine to give stable 3-aminotriazine derivatives (**6a, 6b** and **7**, Scheme 1). The reactivity of aminoguanidine was originally tested using several 5-and 6-carbon "osones" and 3-deoxy derivatives [8] thereof, and, more recently, we reported a synthesis of the triazine **7** derived from the reaction of aminoguanidine with **5** [9]. All of the dicarbonyl derivatives examined were found to react rapidly in a matter of minutes to give substituted triazines, which have been structurally characterized [8,10]. The fact that conversion to triazine derivatives is rapid, that the triazines produced are stable, and that aminoguanidine reacts only slowly and reversibly with D-glucose [11] suggests that aminoguanidine would be useful as a reagent to trap dicarbonyl intermediates produced in the Maillard reaction and allow their quantitation during such reactions.

In this paper, we wish to report a study of the degradation of several Amadori compounds derived from glycine and L-lysine, namely compounds **1, 2** and **3** (Scheme 1) in the presence of aminoguanidine in buffered solutions corresponding to the pK_a of their substituted amino groups. During this reaction, triazine derivatives consistent with the formation of both **4** and **5** (Scheme 1) were detected, identified and quantitated as a function of time.

2. Experimental

General methods.—Mass spectra were collected on a Kratos MS-25 spectrometer interfaced with a DS-55 data handling system. GLC was performed using a Varian 3400 instrument in the split mode. GLC parameters: initial temperature, 120°C, followed by a 2-min hold and then a ramp of 8°C per min to a final temperature of 250°C. TLC was performed on silica gel plates (Whatman K5F) using 7:3:0.3 (v/v) $CHCl_3$–MeOH–H_2O as irrigant. Detection was affected with UV light and by spraying with 5% H_2SO_4 in ethanol, followed by charring at 120°C. The Amadori compounds, *N*-(1-deoxy-D-fructos-1-yl)-glycine (**1**) and N^{ϵ}-(1-deoxy-D-fructos-1-yl)-N^{α}-formyl-L-lysine (**2**) were prepared as described by Mossine et al. [12]. *N,N*-Di-(1-deoxy-D-fructos-1-yl)-glycine (**3**) was synthesized as described by Anet [13]. The standard triazine derivatives, 3-amino-5- and -6-[(2*S*, 3*S*)-2,3,4-trihydroxybutyl]-1,2,4-triazines (**6a** and **6b** and 3-amino-5-[(D-*threo*)-1,2,3-trihydroxypropyl]-6-methyl-1,2,4-triazine (**7**) were prepared as described earlier [8,9]. The acid dissociation constants for **3** were determined exactly as those were measured earlier for **1** and **2** [12]. Conditions and other details for these determinations are reported in Table 1.

General reaction conditions.—The experimental protocol was the same for **1, 2** and **3**. In a typical experiment, 50 mg of **1, 2** or **3** were dissolved in 5 mL of phosphate buffer at pH 8.2 (for **1**), pH 9.0 (for **2**) and pH 5.2 (for **3**), and 1.5 molar equiv of aminoguanidine (bicarbonate salt) and 0.1 molar equiv of perseitol (internal standard) was added to the solution. The solution was sterile-filtered into sterilized, 10 mL screw-capped vials. These solutions were incubated in a water bath at 37°C. Aliquots (0.5 mL) were removed at intervals, placed in 3 mL Reactivials™ and evaporated to dryness in a stream of air at 37°C. The residue was then converted to the trimethylsilyl derivative (TMS) using 0.2 mL of pyridine and 0.2 mL of BSTFA (*N,O*-bis(trimethyl-

silyl)trifluoroacetimide) at 70°C for 45 min. Aliquots (1 μL) were injected on a 0.2 mm × 25 m Quadrex 007OV-17 GLC column. GLC retention times for the TMS derivatives of the standards were: **6a** = 16.6 min, **6b** = 16.75 min, **7** = 15.4 min, and, for perseitol = 14.01 min. Molar yields of triazine derivatives **6a** + **6b** and **7** were calculated by comparison with the perseitol (internal standard) peak. The structures of the triazine derivatives produced in the incubation solutions were confirmed by comparison of their TLC characteristics with known standards (R_f, **6a** = 0.5, **6b** = 0.45, **7** = 0.48). In addition, the GLC–MS of the triazines (TMS derivatives) produced in the incubation solutions were identical to those of authentic standards. For all three compounds, a molecular ion peak (m/z 488) was observed, as well as a peak corresponding to loss of a methyl group (m/z 473).

3. Results and discussion

The two dicarbonyl derivatives **4** and **5** studied herein were chosen because they are thought to constitute major intermediates in the Maillard reaction. Compound **4** has been known for a number of years and has been suggested to serve as a precursor of 2-furaldehydes [3], which frequently are produced during Maillard reactions and which appear to be a major of source of the UV-absorbing compounds produced in the reaction. Intermediate **5** has never been isolated as such, but Ledl and his group have trapped it as the quinoxaline derivative (reaction with phenylenediamine) during the degradation of an Amadori compound, thus verifying that it is produced in such reactions [6]. This intermediate is thought to serve as the precursor of methylfuranones, which are also produced in the reaction and which serve as major food flavor and aroma precursors [14].

Our initial studies showed that the reaction of **4** with aminoguanidine [8] gives two isomeric triazines (5-and 6-substituted isomers) that are readily separated by GLC. The presence of both isomers was also observed in this study. For the case of intermediate **5**, which has never been isolated as such, the authentic triazine derivative was prepared by reaction of the 4-*O*-acetyl-deoxy-5,6-*O*-isopropylidene-2,3-D-*erythro*-hexodiulose derivative with aminoguanidine, followed by removal of the blocking groups. This data is reported in a separate paper [9]. Thus, authentic samples of the triazines derived from both **4** and **5** are now available.

Table 1
Acid dissociation constants of carboxyl groups (K_{a1}) and amino groups (K_{a2}) of Amadori compounds in aqueous solutions [a]

Compound	**1** [b]	**2** [b]	**3** [c]
pK_{a1}	2.20	3.08	1.76 ± 0.02
pK_{a2}	8.18	9.02	5.18 ± 0.01

[a] $T = 298 \pm 0.1$ K, I = 0.2 M (KNO_3).
[b] Ref. [12].
[c] This work.

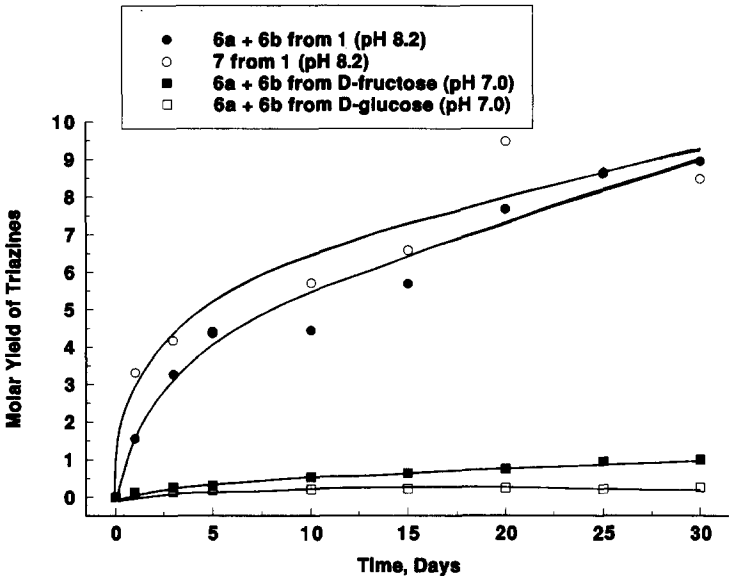

Fig. 1. Molar yields of triazines (**6a** + **6b** and **7**) produced from "fructose glycine" (**1**) at the pH corresponding to its pK_a (pH = 8.2), and from D-fructose and D-glucose at pH 7.0.

The formation of **4** vis-a-vis **5** from an Amadori compound has been the subject of considerable debate over the years. Anet suggested [15] that this may be a function of the pK_a of the amino group and predicted that the optimum conditions for the formation of **4** were at a solution pH corresponding to the pK_a of the amino group of the Amadori compound. Under these conditions, the Amadori compound would be expected to undergo an initial 1,2-enolization, with elimination of the amine portion with the resultant release of **4** (as the enol tautomer) into the solution. Conversely, the formation of **5** would be expected to take place at a more basic pH (relative to the pK_a of the Amadori compound) and would involve an initial 2,3-enolization, followed by elimination of the amine substituent. According to this hypothesis, a degradation at a pH corresponding to the pK_a of the Amadori compound would be expected to give significantly higher yields of **4** relative to **5**.

In order to test this hypothesis, the Amadori compounds were incubated at a solution pH corresponding to their pK_a values. The pK_a values for the three compounds were determined experimentally and are shown in Table 1. The data on yields of **6a** + **6b** and **7**, as produced from **1**, **2** and **3** are shown in Figs 1–3.

Of the compounds studied, **2** was the most stable Amadori compound under these conditions. After 30 days of incubation (pH 9.0), the yield of triazine derivatives was approximately 10%, and the yields of both **6a** + **6b** and **7** were significant, showing that both **4** and **5** are produced in the reaction. The yield of **7** was slightly higher than **6a** + **6b** throughout the reaction (Fig. 2).

Compound **1** was less stable than **2**, and, after 30 days of incubation (pH 8.2), the yield of triazine derivatives was approximately 17.5% (Fig. 1). Again, the yield of **7** was

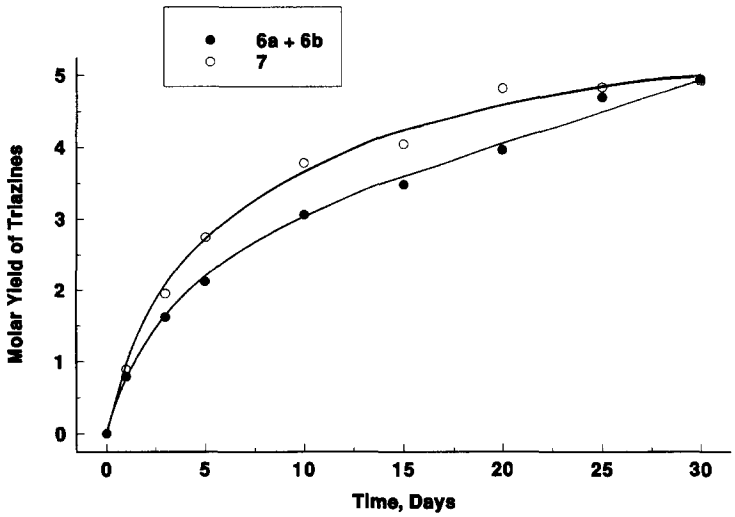

Fig. 2. Molar yields of triazines (**6a** + **6b** and **7**), produced from "fructose lysine" (**2**) at the pH corresponding to its pK_a (pH = 9.0).

higher than for **6a** + **6b** throughout the reaction. Fig. 1 also shows data on the reactivity of both D-fructose and D-glucose at pH 7.0. As expected, both sugars are considerably more stable than any of the Amadori compounds, and, D-fructose gives considerably higher yields of triazines **6a** and **6b**, but the overall yields after 30 days of reaction did

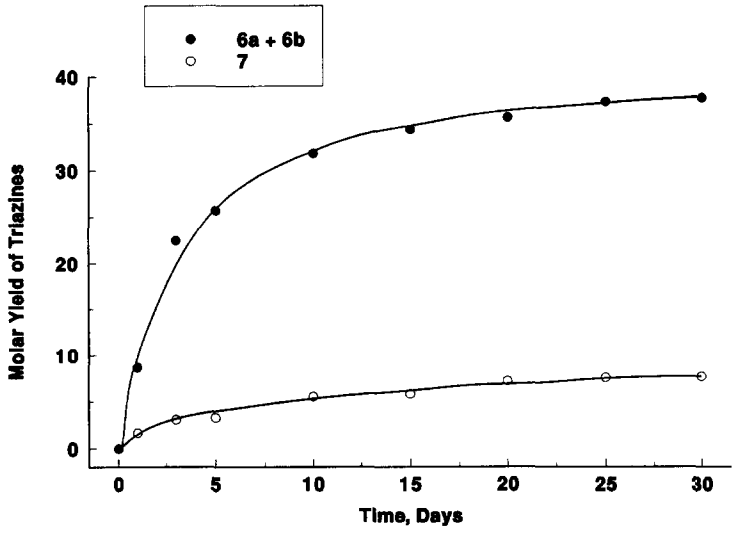

Fig. 3. Molar yields of triazines (**6a** + **6b** and **7**) produced from "difructose glycine" (**3**) at the pH corresponding to its pK_a (pH = 5.2).

not exceed 1%, attesting to the fact that it is inherently less stable than D-glucose. It is also noteworthy that the yields of **7** from D-fructose and D-glucose were negligible.

The most unstable compound was **3**. In this case, after 30 days of incubation at pH 5.2, the yield of triazine derivatives reached 45% (Fig. 3). In this case, however, the yield of **6a** + **6b** was approximately five-fold higher than that for **7**.

We cannot presently explain the high reactivity of **3** relative to **1** and **2**, nor why it yields larger amounts of **4** during the reaction. Repetition of the above experiments at different pH values, (pH 7.0, pH above and below the pK_a, respectively, data not shown) show that the yields and the ratios of the triazines are not significantly different from that shown in Figs 1–3, i.e., an explanation for the instability of the Amadori compounds does not appear to be a function on pH nor is it related to the pK_a of the amino groups contained by them.

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