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3-Deoxypentosulose: An α-Dicarbonyl Compound Predominating in Nonenzymatic Browning of Oligosaccharides in Aqueous Solution

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The thermal degradation of D-glucose, maltose, and maltotriose in aqueous solution was investigated under caramelization (no glycine) and Maillard (with glycine) conditions. Degradation of the sugar and α-dicarbonyls product was monitored. Under both caramelization and Maillard reaction conditions, 3-deoxypentosulose was the predominating α-dicarbonyl compound formed from maltose and maltotriose. In the absence of an amino compound, however, 3-deoxypentosulose is formed in much lower concentration. It was concluded that 3-deoxypentosulose is formed by a pathway specific for oligo- and polysaccharides since this α-dicarbonyl is formed from the α-1→4 glucans such as maltose and maltotriose but not from glucose. For its formation, a retro Claisen reaction of an enolization product of 1-amino-1,4-dideoxyhexosulose is proposed as the route to its formation. 1-Amino-1,4-dideoxyhexosulose could be formed by vinylogous α-elimination from the 2,3-enediol structure after Amadori rearrangement, favored by planar alignment of the bonds between C1 and C4. Subsequent rearrangement by keto−enoltautomerization leads to a 1-imino-3-keto structure. In this structure, attack of a hydroxyl anion, provided by water at neutral pH, could cause a splitting off of the C1. This reaction gives rise to formic acid or formamide and a pentose derivative, which reacts further to give 3-deoxypentosulose.

KEYWORDS: Nonenzymatic browning; Maillard reaction; α-dicarbonyl compounds; thermal degradation; oligosaccharides; 3-deoxypentosulose

INTRODUCTION

The Maillard reaction between monosaccharides and amino acids has been investigated thoroughly in aqueous solution. Ledl (*1*) reviewed the investigation that had been done and stressed the role of compounds with an α-dicarbonyl structure as key intermediates in the initial and advanced stage. The formation of reaction products with an α-dicarbonyl moiety was reported mainly for the Maillard reaction of monosaccharides. Besides the formation of osuloses by the elimination of water, Weenen (*2*) suggested pathways in which monosaccharides are degraded into sugar fragments with an α-dicarbonyl moiety by retro aldolization or α-cleavage. Hofmann (*3*) focused on the formation of glyoxal in the reaction of D-glucose and D-xylose with L-alanine. Because it was formed to a considerable amount in a very early stage of the reaction, they proposed its formation via cleavage of glucosone and xylosone, respectively, which they postulated to be oxidation products of the Schiff's base. Also, 3-deoxyhexosulose and 1-deoxyhexosulose were quantified, reaching a maximum concentration at a later stage of the reaction.

The reaction of pentoses to form α-dicarbonyls was also investigated. 3-Deoxy-pentosulose was synthesized first (*4*) and was found in Maillard reaction mixtures, caramelization, and pyrolysis of pentoses (*5*, *6*). When Beck et al. (*7*) published the formation of 1-amino-1,4-dideoxyhexosuloses, they speculated that it might be formed preferentially from disaccharides and secondary amines. However, 1,4-dideoxyhexosulose, as a reaction product of this α-dicarbonyl, was also detected in aqueous mixtures of glucose/*β*-alanine (*8*).

Recently, the formation of aminoreductones with a 4-deoxy-moiety from lactose was found by Pischetsrieder et al. (*9*). The reductone was detected in an early stage of the Maillard reaction and was proposed to be the first stable intermediate of the 1-amino-1,4-dideoxyhexosulose.

The degradation of disaccharides in aqueous solutions during the Maillard reaction has so far been described via two major pathways. Kroh (*10*) reported the breakdown of oligo- and polysaccharides in aqueous solution to 1,4-linked malto-oligosaccharides and glucose by hydrothermolysis. Moreover, fission of the acetal bond can be achieved by elimination reactions after the glucose unit at the reducing end has reacted to give heterocyclic rings (*11*).

In earlier investigations, it was demonstrated that *o*-phenylenediamine is an appropriate means of monitoring α-dicar-

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bonyl production in the Maillard reaction (*12*). For the reaction of oligosaccharides during Maillard reaction in quasi water free systems, a specific degradation pathway was previously identified (*13*). The formation of specific α-dicarbonyl compounds in the degradation of oligosaccharides in aqueous solution has now been investigated. *o*-Phenylenediamine was used as a trapping reagent in our investigations on the Maillard reaction in a quasi water free system.

MATERIALS AND METHODS

Thermal Treatment. *Caramelization: Mono-, Di-, or Trisaccharide with o-Phenylenediamine.* Samples of 0.5 mL of 0.25 M solution of each sugar D-glucose (Merck, water free), maltose monohydrate (Merck), or maltotriose (Fluka, >93% high-performance liquid chromatography (HPLC)) and 0.25 M trapping reagent (*o*-phenylenediamine, recrystallized from water) were heated for timed intervals up to 240 min at 100 ± 1 °C in sealed tubes by means of a thermoblock (behrotest ET 1, behr Labor Technik). Experiments were carried out in duplicate. After the samples were heated, the samples were cooled, and after appropriate dilution (methanol; Merck, suprasolv), they were subjected to HPLC and high-perfomance thin-layer chromatography (HPTLC).

Maillard Reaction: Mono-, Di-, or Trisaccharide with Glycine and o-Phenylenediamine. Samples (0.5 mL) of 0.25 M solutions of D-glucose, maltose monohydrate, or maltotriose, respectively, and 0.5 mL each of 0.25 M glycine (Serva) and 0.25 M *o*-phenylenediamine were heated for timed intervals up to 240 min at 100 ± 1 °C in sealed tubes. Samples were treated and analyzed as above.

Synthesis of Reference Material. The synthesis of 2-methyl-3-(1′,2′,3′-trihydroxypropyl)quinoxaline, 2-(2′,3′,4′-trihydroxybutyl)quinoxaline, 2-(*arabino*-1′,2′,3′,4′-tetrahydroxybutyl)quinoxaline, and 2-methyl-3-(2′,3′-dihydroxypropyl)quinoxaline was performed as described by Hollnagel and Kroh (*13*). The synthesis of 2-(2′,3′-dihydroxypropyl)-quinoxaline was performed with slight variations according to Glomb (*14*). ^{1}H nuclear magnetic resonance (NMR) (Brucker AM 300, 300 MHz) in CD_3OD: δ 3.09 (dd, 1H), 3.24 (dd, 1H), 3.61 (dd, 2H), 4.15 (m, 1H), 7.76 (m, 2H), 8.02 (m, 2H), 8.83 (s, 1H). ^{13}C NMR (300 MHz) in CD_3OD: δ 41.05, 67.07, 72.9, 129.5, 129.62, 130.65, 131.4, 142.0, 143.18, 147.9, 156.97. Mass spectrum of the acetylated compound (Hewlett-Packard 5989B): *m*/*z* 288 (0.03%, M^+), 229 (5), 228 (4), 215 (0.2), 187 (15), 186 (10), 169 (42), 157 (24), 144 (37), 129 (3), 117 (4), 43 (100).

HPLC/DAD. Instrumentation: degasser (Degasys DG-13000, Knauer); pump (Shimadzu LC 10 AT); thermostat (Haake F3, Fisons); guard column (Nucleosil 120-5 C_{18}, Macherey-Nagel); column (Nucleosil 5 C_{18}, Macherey-Nagel, 250 mm × 4.6 mm i.d., 5 μm); detector (Kontron 440); flow, 1.0 mL/min; temperature, 30 °C; injection volume, 20 μL; eluent: solvent A, methanol; solvent B, water (both HPLC grade); detection wavelength, 320 nm, full scan 190−440 nm; gradient, 0−5 min 5% A, 5−25 min 5−50% A, 25−30 min 50−100% A, and 30−40 min 100% A.

Gas Chromatography/Mass Spectroscopy (GC/MS) and Gas Chromatography/Flame Ionization Detector (GC/FID). Extraction and derivatization of the reference material was carried out according to ref *12*. GC/MS: Analytical GC was performed on a Hewlett-Packard 5890 Series II gas−liquid chromatograph equipped with a Hewlett-Packard 5989B mass spectrometer (in EI mode) and a fused-silica capillary column DB-5HT (J&W; 30 m × 0.32 mm i.d., 0.1 μm film). Carrier gas, He; detector/injector temperature, 280 °C; temperature program, initial temperature 120 °C, held for 5 min, 120−200 °C at 10 °C/min, held at 200 °C for 5 min, 200−280 °C at 10 °C/min, held at 280 °C for 9 min. Column effluents were analyzed by electron ionization mass spectrometry (range: *m*/*z* 40−800 amu). The identities of the separated compounds were confirmed by comparison with those of independently synthesized standards.

HPTLC. *Monosaccharides.* HPTLC plates (Merck, Kieselgel 60, 20 cm × 10 cm) were used; the eluent consisted of chloroform (Merck, HPLC grade), acetic acid (Merck, 96%), methanol (Merck suprasolv), and water (60/18/12.5/5; v/v/v/v); and the plates were developed twice according to ref *10*.

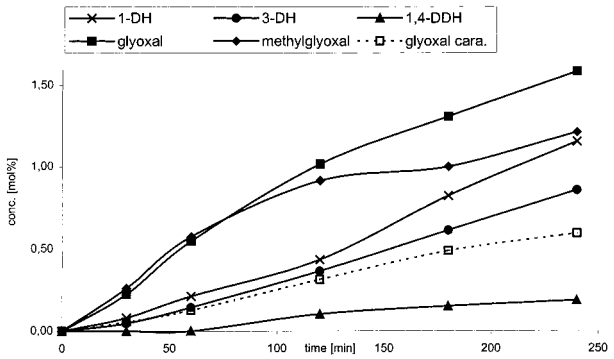

Figure 1. Formation of α-dicarbonyls detected as quinoxalines in the Maillard reaction (D-glucose/glycine/*o*-phenylenediamine, solid lines) and in caramelization (D-glucose/*o*-phenylenediamine, dotted line). 1-DH, 1-deoxyhexosulose; 3-DH, 3-deoxyhexosulose; 1,4-DH, 1,4-dideoxyhexosulose; all compounds as quinoxalines.

Di- and Trisaccharides. HPTLC plates were developed twice in an AMD chamber (CAMAG) using an eluent mixture of chloroform, methanol, and water (50/40/8; v/v/v) in which 2.5−2.8 mg of boric acid was dissolved. Detection was performed in both systems with diphenylamine/aniline/phosphoric acid as a spray reagent and heating (120 °C for 5 min).

HPAEC/PAD. Instrumentation: pump (Dionex GP 40); column (2 × PA-100, 250 mm × 4 mm i.d., Dionex); detector (PAD, Dionex); oven (HPLC column oven 2155, Pharmacia); temperature, 25 °C; injection volume, 20 μL; flow, 0.5 mL/min; eluent: solvent A, 0.15 M NaOH; solvent B, 1 M sodium acetate in 0.15 M NaOH; gradient, 0−10 min 100% A and 10−60 min 0−60% B.

RESULTS AND DISCUSSION

To investigate the identity and the concentration of α-dicarbonyls, a trapping agent (*o*-phenylenediamine) was directly added to the reaction mixture. With this approach, all of the α-dicarbonyls formed could be analyzed in their accumulated concentration. The trapping agent was added prior to the reaction in order to trap all of the α-dicarbonyl compounds formed. In preliminary experiments with post derivatization, a lower concentration of α-dicarbonyls was detected. This method further allows the difference due to the varying reactivity of α-dicarbonyls, as was reported by Glomb and Pfahler (*4*), to be minimized. Using reference material for quinoxalines of hexosuloses as well as of sugar fragments with an α-dicarbonyl moiety, a wide range of α-dicarbonyl compounds could be determined quantitatively. To investigate the effect of the amino acid on the formation of α-dicarbonyls only, experiments were carried out with and without glycine whereas OPD was present in both types of reaction mixtures.

Characterization of the complex Maillard reaction mixture was performed by observation of (i) the formation of α-dicarbonyls, (ii) degradation of the starting carbohydrate, and (iii) subsequent formation of carbohydrates with a lower degree of polymerization (dp) than the starting compound by hydrothermolysis.

Formation of α-Dicarbonyls from Mono- and Oligomeric Carbohydrates. For the quantification of α-dicarbonyl compounds formed, the reaction of D-glucose under caramelization and Maillard reaction conditions was investigated as a starting point for further interpretation of the results from oligosaccharides. In the caramelization model, only glyoxal was detected (as its quinoxaline) with a yield of 0.6 mol % after 240 min (**Figure 1**).

In the presence of glycine, the formation of α-dicarbonyls was enhanced as expected. Glyoxal formation from D-glucose

Table 1. Formation of α-Dicarbonyls Detected as Quinoxalines (mol %) in Caramelization of Maltose (Maltose/*o*-Phenylenediamine)

time (min)	glyoxal	methylglyoxal	3-deoxypentosulose
30	0.03	0.02	0.04
60	0.07	0.07	0.06
120	0.20	0.03	0.23
180	0.29	0.02	0.45
240	0.31	0.02	0.63

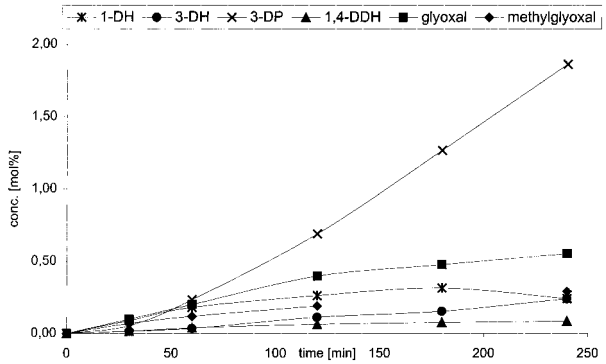

Figure 2. Formation of α-dicarbonyl compounds detected as quinoxalines in the Maillard reaction of maltose/glycine/*o*-phenylenediamine. 1-DH, 1-deoxyhexosulose; 3-DH, 3-deoxyhexosulose; 3-DP, 3-deoxypentosulose; 1,4-DH, 1,4-dideoxyhexosulose; all compounds as quinoxalines.

increased to 1.6 mol %, and also, methylglyoxal was formed in considerable quantities (1.2 mol % after 240 min; **Figure 1**). The formation of glyoxal was fastest during the first 60 min; subsequently, the rate of its formation decreased slowly. However, the concentration of glyoxal and methylglyoxal still increased throughout the last interval of the investigated time period.

In contrast to caramelization, in the Maillard reaction, hexose derivatives with an α-dicarbonyl moiety could also be determined. 1-Deoxyhexosulose was formed to 1.1 mol % after 240 min, whereas 3-deoxyhexosulose was formed in a slightly lower amount at the same reaction time (0.9 mol %, **Figure 1**). The formation of 1,4-dideoxyhexosulose was almost negligible with 0.2 mol % after the observed time range. In general, the formation of hexosuloses was sluggish in the early stage of the reaction and increased after 120 min.

With disaccharides, solutions containing maltose and *o*-phenylenediamine showed the formation of glyoxal (0.3 mol % after 240 min; **Table 1**) and traces of methylglyoxal. However, the HPLC chromatogram indicated that a so far unidentified quinoxaline was predominating. The retention time suggested that this quinoxaline should be less polar than that of 3-deoxyhexosulose and more polar than that of 1,4-dideoxyhexosulose. GC/MS revealed that it was the quinoxaline of a pentose derivative. Comparison with reference material obtained by independent synthesis confirmed by several chromatographic and spectroscopic measures that the observed peak has to be assigned to the quinoxaline of 3-deoxypentosulose. It was quantified with 0.63 mol % formed after 240 min in caramelization of maltose (maltose/*o*-phenylenediamine; **Table 1**). After an induction phase of 60 min, the formation of 3-deoxypentosulose proceeded at a more or less constant rate.

In the presence of glycine, the concentration of 3-deoxypentosulose was significantly higher; after 240 min, 1.86 mol % (**Figure 2**) was formed. The shape of the graph suggests that its formation did not stop at that stage of the reaction, and the reaction was still proceeding. Other detected α-dicarbonyl compounds were formed at considerably lower concentrations than 3-deoxypentosulose. The starting pH measured prior to reaction (D-glucose/glycine/*o*-phenylenediamine) was neutral and dropped slowly over the reaction time to 5. When glycine was added, glyoxal reached a concentration of 0.55 mol % after 240 min, and also, methylglyoxal formation was increased. 1-Deoxyhexosulose and 3-deoxyhexosulose were formed under these conditions as well but in relatively low concentrations of 0.24 mol % each after 240 min (**Figure 2**). The production of all detected α-dicarbonyl compounds except 3-deoxypentosulose decreased after 120 min. In contrast to the reaction of maltose/glycine/*o*-phenylenediamine in a quasi water free system, where 1,4-dideoxyhexosulose was formed to 18 mol % (*13*), this α-dicarbonyl does not play an important role in aqueous solution. Under the chosen reaction conditions, 3-deoxypentosulose is the predominating α-dicarbonyl, and it can be assumed that 3-deoxypentosulose is formed from the reducing end of disaccharides.

Table 2. Formation of α-Dicarbonyls Detected as Quinoxalines (mol %) in Caramelization (Maltotriose/*o*-Phenylenediamine; cara) and in the Maillard Reaction (Maltotriose/glycine/*o*-Phenylenediamine; MR)[a]

time	glyoxal		3-deoxypentosulose		1,4-dideoxyhexosulose	
(min)	cara	MR	cara	MR	cara	MR
30	0.02	0.09	0.01	0.07	nd	0.02
60	0.06	0.18	0.05	0.28	nd	0.05
120	0.16	0.45	0.19	0.92	nd	0.09
180	0.27	0.52	0.35	1.73	nd	0.11
240	0.36	0.59	0.52	2.42	nd	0.11

[a] nd is not detected.

In caramelization models with maltotriose, similar observations were made as for maltose. In addition to glyoxal (0.36 mol % after 240 min), only 3-deoxypentosulose (0.52 mol % after 240 min) could be identified and determined (**Table 2**). The addition of glycine to the reaction mixture strongly accelerated the formation 3-deoxypentosulose to give 2.4 mol % after 240 min. Together with 3-deoxyhexosulose, only glyoxal (0.6 mol % after 240 min) and a small amount of 1,4-dideoxyhexosulose (0.1 mol % after 240 min) were formed (**Table 2**). This observation underlines the importance of the formation of 3-deoxypentosulose in the Maillard reaction of oligosaccharides in aqueous solution.

Degradation of the Starting Compound by Caramelization and Maillard Reaction. Degradation of D-glucose in models resembling caramelization proceeded rather slowly as compared to quasi water free reaction mixtures. This finding is consistent with the relatively low concentrations of quinoxalines detected. When glycine was present, D-glucose was degraded faster than in absence of glycine. After 60 min, almost half of the D-glucose was converted, whereas in caramelization at that reaction time more than two-thirds of the carbohydrate remained unreacted. After 240 min in Maillard reaction mixtures, (D-glucose/glycine/*o*-phenylenediamine) there was still 35.7 mol % glucose present in contrast to caramelization reaction mixtures, which contained 44.9 mol % D-glucose (**Table 3**). The slower degradation of the carbohydrate in aqueous solution can be assigned to the high dilution (0.25 M) of the starting compound, resulting in a low reaction rate. This finding is consistent with the relatively low concentrations of quinoxalines that were formed in aqueous solution.

The degradation of maltose was not significantly effected by glycine. Although conversion of the disaccharide proceeded somewhat faster in the Maillard reaction, the values determined

Table 3. Degradation (mol %) of D-Glucose, Maltose, and Maltotriose in the Maillard Reaction (MR) with Glycine (D-Glucose/Glycine/*o*-Phenylenediamine) and in Caramelization (cara) without Glycine (D-Glucose/*o*-Phenylenediamine)[a]

	D-glucose		maltose		maltotriose	
time (min)	MR	cara	MR	cara	MR	cara
0	100	100	100	100	100	100
30	46	71	52	67	41	71
60	54	68	53	57	38	63
120	45	59	51	53	36	55
180	nr	55	43	43	29	44
240	34	45	33	34	28	49

[a] nr is not resolved.

Table 4. Formation of D-Glucose in Caramelization of Maltose (Maltose/*o*-Phenylenediamine) and in the Maillard Reaction of Maltose (Maltose/Glycine/*o*-Phenylenediamine) in mol %

time (min)	D-glucose from maltose/*o*-phenylenediamine	D-glucose from maltose/glycine/*o*-phenylenediamine
30	7.58	5.41
60	7.83	7.39
120	9.34	9.60
180	11.22	10.01
240	11.41	9.68

for maltose after 240 min of reaction time did not differ significantly (**Table 3**). However, with maltotriose, the enhancing effect of glycine on carbohydrate degradation was observed as for D-glucose. Especially after 30 min, the difference was very distinct, whereas in caramelization at that time 70.7 mol % was still present, in the Maillard reaction only 45.8 mol % remained (**Table 3**). After the first hour in both cases, the rate of degradation decreased markedly, which might be due to the diminished concentration of starting compound. In the absence of glycine, somewhat more than half of the maltotriose was converted after 240 min (48.7 mol %). In Maillard reaction mixtures, 28.5 mol % maltotriose was found in the reaction mixture after 240 min. The degradation of the trisaccharide proceeded at a similar rate as D-glucose, although monosaccharides are expected to be more reactive. It can be assumed that degradation of oligosaccharides in aqueous solution occurs not only by reaction of the OH group at the reducing end with the amino compound but also by hydrolysis of the glycosidic bonds (*15*).

Hydrothermolysis of Oligomeric Carbohydrates. To elucidate the relevance of hydrolytic cleavage, carbohydrates with a lower dp than the starting oligosaccharides were determined. In caramelization mixtures containing maltose, D-glucose was formed in considerable quantities, up to 11.4 mol % after 240 min (**Table 4**). That means that at least 5.7% of the maltose was converted by hydrolytic cleavage at the end of the observed time range.

In the Maillard reaction, about 10 mol % D-glucose was detected as well (**Table 4**), but it has to be considered that now other degradation mechanisms besides hydrolysis take place, which include the reaction of the glucose unit at the reducing end to 3-deoxypentosulose and formic acid as well as to aminoreductones (*9*). Therefore, it can assumed that under Maillard reaction conditions more disaccharide is degraded by fission of the glycosidic bond than in caramelization. Moreover, in the presence of glycine, D-glucose is able to react in terms of the Maillard reaction given the higher reactivity as compared

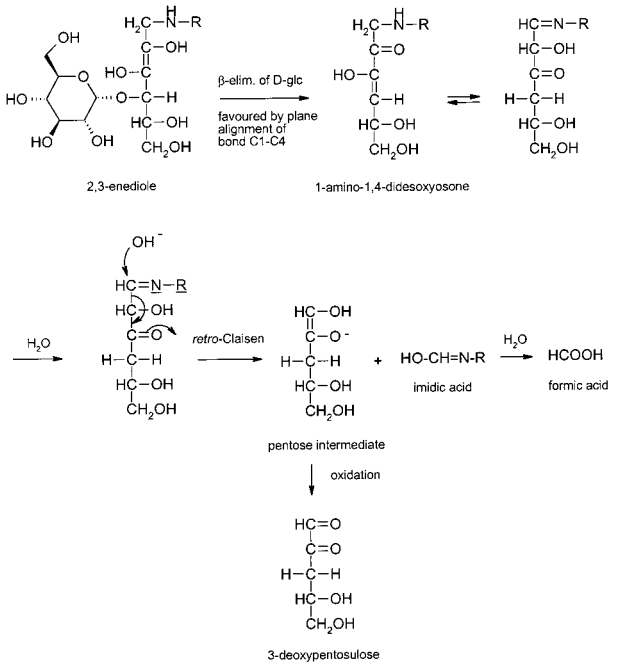

Figure 3. Proposed reaction pathway for the formation of 3-deoxypentosulose and formic acid.

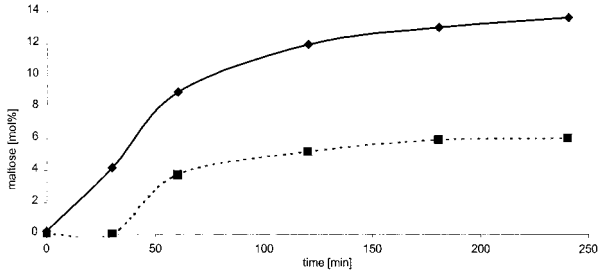

Figure 4. Formation of maltose in the Maillard reaction from maltotriose/glycine/*o*-phenylenediamine (solid line) and in caramelization maltotriose/*o*-phenylenediamine (dotted line).

with disaccharides, reducing the detected value of glucose stronger than in the absence of an amino acid.

The cleavage of the glycosidic bond in maltotriose gives rise to maltose. In caramelization, maltose was formed by hydrothermolysis to maximal 6 mol % maximum after 240 min (**Figure 4**). With participation of glycine, maltose concentration rose to 13.7 mol % at 240 min reaction time (**Figure 4**). This increase of maltose formation in the Maillard reaction cannot be assigned only to the formation of formic acid and 3-deoxypentosulose at the reducing end of the sugar molecule. Additionally, it has to be considered that oligosaccharides can also be degraded by the formation of aminoreductones (*9*); the α-dicarbonyl structure is not necessarily involved.

For better understanding of nonenzymatic browning, it is important to study the occurring reaction pathways in detail. Information about the mechanisms that lead to the formation of the various α-dicarbonyls could be of particular value.

The formation of sugar fragments with an α-dicarbonyl moiety was higher under Maillard reaction conditions than during caramelization from glucose. Whereas they are formed by retro aldolization of the sugar molecule itself under caramelization conditions, in the Maillard reaction, the Schiff's base as well as the ene-aminol can be starting compounds for cleavage reactions. Moreover, the amino acid can act as a base and catalyze retro aldolization reactions (*2*).

In both systems, an increase of the glyoxal concentration was observed throughout the reaction time when the trapping agent was present. In contrast, Hofmann et al. (*3*) observed a maximum concentration of glyoxal at a very early stage of the reaction with the observed yields of quinoxalines much lower as compared to the data presented here. Hofmann and coworkers suggested that glyoxal is formed via glucosone; however, under the chosen reaction conditions, no glucosone, as its corresponding quinoxaline, could be detected by GC/MS. The glyoxal we detected should therefore be formed by pathways suggested by Weenen (*2*).

In contrast to caramelization, in the Maillard reaction, hexose derivatives with an α-dicarbonyl moiety could also be determined. 1-Deoxyhexosulose was formed at 1.1 mol % after 240 min, whereas 3-deoxyhexosulose was formed in a slightly lower amount (0.9 mol %; **Figure 1**). The formation of 1,4-dideoxyhexosulose was almost negligible, with 0.2 mol % after the observed time period.

Hofmann (*3*) obtained different results for the reaction of D-glucose with alanine. They found that four times more 3-deoxyhexosulose was formed than 1-deoxyhexosulose, when the trapping reagent was added after thermal treatment. However, in this case, differences in the reactivity of the various α-dicarbonyls might not be fully accounted for. Nevertheless, when the trapping reagent was present throughout the entire reaction time, Feather et al. and Nedvidek (*16*, *17*) obtained results that confirm the present data. More 1-deoxyhexosulose than 3-deoxyhexosulose was found in mixtures of fructosylglycine and D-glucose/propylamine and fructosylpropylamine reaction mixtures as well (*17*). The fact that 1,4-dideoxyhexosulose was formed in such small amounts suggests that Strecker degradation or other reduction steps, which are necessary for the formation of 1,4-dideoxyhexosulose (*13*), are not favored in aqueous solution or that these reactions proceed more slowly than the reaction with *o*-phenylenediamine. In general, the formation of hexosuloses was sluggish in the early stage of the reaction and increased after 120 min.

In comparison with the experiments under quasi water free conditions (*13*), yields of the quinoxalines of the D-glucose model system were relatively low. This effect is most probably due to the higher dilution of starting material reducing the reaction rate. Furthermore, the product spectrum was shifted; while in quasi water free mixtures, 1,4-dideoxyhexosulose, methylglyoxal, and 3-deoxyhexosulose were formed, and in aqueous solution, glyoxal was the predominating α-dicarbonyl, followed by methylglyoxal and 1-deoxyhexosulose.

Under caramelization and Maillard reaction conditions, 3-deoxypentosulose is the predominating α-dicarbonyl from maltose. The presence of glycine strongly enhanced its formation. The fact that it is not formed from D-glucose indicates that the glycosidic bond causes a change in the type of degradation reactions taking place. Taking into account that fission of the hexose between C1 and C2 must have occurred for the formation of the pentose derivative, the deoxy moiety at C3 in the reaction product was originally the site of the glycosidic linkage.

A feasible reaction pathway towards 3-deoxypentosulose could be the formation of 1-amino-1,4-dideoxyhexosulose by vinylogous β-elimination from the 2,3-ene-diol structure after Amadori rearrangement, favored by planar alignment of the bonds between C1 and C4. Subsequent rearrangement by keto−enoltautomerization leads to a 1-imino-3-keto structure. In this structure, attack of a hydroxyl anion, provided by water at neutral pH (and in weak acid pH), or the participation of the carboxylate functionality of glycine could cause a splitting off of the C1. This reaction can be characterized as a retro Claisen ester condensation, giving rise to formic acid or formamide and a pentose derivative. The first reaction product that is suggested to be produced by this reaction pathway, formic acid, is frequently found in the Maillard reaction and causes, among other formed acids, the drop in pH that is typical for the Maillard reaction. The essential role of hydroxyl ion or carboxylate ion in retro Claisen ester condensation could explain why this reaction only predominates in aqueous systems (**Figure 3**).

The subsequent reaction of this pentose derivative can lead to 3-deoxypentosulose as it was detected in reaction mixtures of maltose. Other possible reaction products of this derivative such as 3-deoxypentulose, as ketose derivatives, or furfuryl alcohol have been found in model systems and food, e.g., in heat-treated milk. For example, Troyano et al. (*18*) published the formation of 3-deoxypentulose in milk and in alkaline model mixtures containing maltose or cellobiose. Formic acid was also proposed as a secondary product. Furfuryl alcohol, another possible product of the further conversion of the pentose derivative, was detected by Patton (*19*), who found furfuryl alcohol in milk as well as in neutral and alkaline model systems containing lactose or lactose and lysine. The formation was postulated to occur via the formation of 3-deoxyhexosulose at the reducing end of lactose and α-dicarbonyl cleavage yielding a galactosylated pentose derivative that eliminates the galactosyl residue and gives rise, after ring closure and dehydration, to furfuryl alcohol. The pH necessary for these reactions ranged between 6 and 8 and 4−6, respectively, which might be accomplished by the increase of hydrogen ion concentration throughout the Maillard reaction. In caramelization, one could imagine that a similar reaction pathway occurs also in the absence of an amino acid. Enolization, which is essential to form the 2,3-ene-diol as a precursor to the β-elimination, is also known to occur in the absence of the amino acid under the given reaction conditions. However, this reaction occurs much more slowly than in the presence of the amino compound, acting as a catalyst in enolization and β-elimination.

Reactions of oligosaccharides in aqueous solution proceed via various pathways. In the absence of an amino compound, the formation of α-dicarbonyls plays a minor role; therefore, subsequent reaction products of such reactive intermediates are formed to a minor extent throughout caramelization. Accordingly, the formation of brown pigments and flavor is rather slow when oligosaccharides are heated in an aqueous solution.

The addition of glycine enhanced the formation of α-dicarbonyl compounds markedly. For the reaction of oligosaccharides, the formation of 3-deoxypentosulose was described as the most abundant α-dicarbonyl, and a reaction pathway was proposed yielding the α-dicarbonyl, a carbohydrate with a lower dp, and formic acid as degradation products. Besides the formation of formic acid, the generation of some pentose derivatives such as 3-deoxypentulose or furfuryl alcohol can be explained by the proposed pathway. Under the chosen reaction conditions, glyoxal is most probably not formed from glucosone but is rather a reaction product of retro aldolization.

Because of the reaction with the amino compound, the formation of carbohydrates with a lower dp increased. Apparently, in the presence of an amino compound, conversion of the oligosaccharide and formation of color are not only accelerated as compared with caramelization but also there are some qualitative differences in the spectrum of intermediates formed in caramelization and the Maillard reaction. By the

reaction with the amino acid, certain degradation pathways are favoured in the degradation of oligosaccharides, which are significant neither in the Maillard reaction of monosaccharides nor in the caramelization of mono- or oligosaccharides. These findings are relevant for the understanding of changes of food components occurring throughout food processing, such as in milk and other dairy products.

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LITERATURE CITED

(1) Ledl, F.; Schleicher, E. Die Maillard-Reaktion in Lebensmitteln und im menschlichen Körper- neue Ergebnisse zu Chemie, Biochemie und Medizin. *Angew. Chem*. **1990**, *102*, 597−734.

(2) Weenen, H. Reactive intermediates and carbohydrate fragmentation in Maillard chemistry. *Food Chem*. **1998**, *62*, 393−401.

(3) Hofmann, T.; Bors, W.; Stettmaier, K. Studies on radical intermediates in the early stage of the nonenzymatic browning of carbohydrates and amino acids. *J. Agric. Food Chem*. **1999**, *47*, 379−390.

(4) Glomb, M. A.; Pfahler, C. Synthese eines bedeutenden Maillard-Intermediats beim Abbau von Hexosen. *Lebensmittelchemie* **1999**, *53*, 16−17.

(5) Lederer, M.; Glomb, M.; Fischer, P.; Ledl, F. Reactive Maillard intermediates leading to colored products- 5-hydroxymethyl- and 5-(1,2-dihydroxyethyl)-3(2H)-furanone. *Z. Lebensm.-Unters. Forsch.* **1993**, *197*, 413−418.

(6) Kato, K.; Komorito, H. Pyrolysis of cellulose. V. Isolation and identification of 3-deoxyglycosones produced from D-glucose, D-xylose, and α-cellulose by heating. *Agric. Biol. Chem*. **1968**, *32*, 715−720.

(7) Beck, J.; Ledl, F.; Severin, T. Formation of 1-deoxy-D-erythro-2,3-hexodiulose from Amadori compounds. *Carbohydr. Res.* **1988**, *177*, 240−243.

(8) Nedvidek, W.; Ledl, F.; Fischer, P. Detection of 5-hydroxymethyl-2-methyl-3(2*H*)-furanone and of α-dicarbonyl compounds in reaction mixtures of hexoses and pentoses with different amines. *Z. Lebensm.-Unters. Forsch*. **1992b**, *194*, 222−228.

(9) Pischetsrieder, M.; Schoetter, C.; Severin, T. Formation of an aminoreductone during the Maillard reaction of lactose with N^{α}-acetyllysine or proteins. *J. Agric. Food Chem*. **1998**, *46*, 928−931.

(10) Kroh, L. W.; Jalyschko, W.; Häseler, J. Nonvolatile reaction products by heat-induced degradation of α-glucans. Part I: analysis of oligomeric maltodextrins and anhydrosugars. *Starch/Stärke* **1996**, *48*, 426−433.

(11) Kramhöller, B.; Pischetsrieder, M.; Severin, T. Maillard reactions of dextrins and starch. *Z. Lebensm.-Unters. Forsch*. **1993**, *197*, 227−229.

(12) Hollnagel, A.; Kroh, L. W. Formation of α-dicarbonyl fragments from mono- and disaccharides under caramelization and Maillard reaction conditions. *Z. Lebensm.-Unters. Forsch. A* **1998**, *207*, 50−54.

(13) Hollnagel, A.; Kroh, L. W. Degradation of oligosaccharides in nonenzymatic browning by formation of α-dicarbonyl compounds via a "peeling off" mechanism. *J. Agric. Food Chem.* **2000**, *48*, 6219−6226.

(14) Glomb, M. A. Synthese, Strukturaufklärung und Nachweis reaktiver Pentoseabbauprodukte. Thesis, Stuttgart, 1992.

(15) Häseler, J. Nichtflüchtige polare Verbindungen des thermisch induzierten Abbaus von α-Glucanen. Thesis, Berlin, 2000.

(16) Feather, M. S.; Mossine, V.; Hirsch, J. The Use of Aminoguanidine To Trap and Measure Dicarbonyl Intermediates Produced During the Maillard Reaction. In *Chemical Markers for Processed and Stored Foods*; Lee, T.-C., Kim, H.-J., Eds.; ACS Symposium Series 631; American Chemical Society: Washington, DC, 1996.

(17) Nedvidek, W. Zuckerabbauprodukte mit α-Dicarbonylstruktur. Thesis, Stuttgart, 1992a.

(18) Troyano, E.; Olano, A.; Jimeno, M. L.; Sanz, J.; Martinez-Castro, I. Isolation and characterization of 3-deoxypentulose and its determination in heated milk. *Dairy Res.* **1992**, *59*, 507−515.

(19) Patton, S. Studies of heated milk III. Mode of formation of certain furan compounds. *Dairy Sci*. **1950**, *33*, 904−910.

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