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Degradation of Glucose: Reinvestigation of Reactive α-Dicarbonyl Compounds[†]

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Maillard reactions influence the formation of flavor and color in processed foods in an important way. Reducing sugars and amino acids ultimately react to stable end products. To elucidate the complex formation pathways a vast number of experiments have been published. α-Dicarbonyl compounds are accepted as important key intermediates. In the present work the Maillard degradation of glucose in the presence of lysine was reinvestigated. α-Dicarbonyl compounds were trapped with *o*-phenylenediamine to give stable quinoxalines of D-*arabino*-hexos-2-ulose (glucosone), N^6-(3,6-dideoxyhexos-2-ulos-6-yl)-L-lysine, 1-deoxy-D-*erythro*-2,3-hexodiulose (1-deoxyglucosone), 3-deoxy-D-*erythro*-hexos-2-ulose (3-deoxyglucosone), ethanedial (glyoxal), 2-oxopropanal (methylglyoxal), 3,4-dihydroxy-2-oxobutanal (threosone), 1-hydroxy-2,3-butanedione (1-deoxythreosone), 4-hydroxy-2-oxobutanal (3-deoxythreosone), 4,5-dihydroxy-2-oxopentanal (3-deoxypentosone) and 4,5-dihydroxy-2,3-pentanedione (1-deoxypentosone). Multilayer countercurrent chromatography (MLCCC) was used for the first time to separate quinoxalines from ethyl acetate and aqueous extracts of reaction mixtures. The purity and identity of isolated compounds was confirmed by NMR, HPLC−UV and HR-MS. Aerated and deaerated incubations of [^{13}C]-labeled glucose in presence of lysine and degradations of glucosone and 3-deoxyglucosone allowed insights into the formation pathways. Within this context the formation of 1-deoxypentosone and the importance of N^6-(3,6-dideoxyhexos-2-ulos-6-yl)-L-lysine (Lederer's glucosone) was established.

KEYWORDS: Maillard reaction; glucose; α-dicarbonyl compounds; quinoxalines; 1-deoxypentosone; N^6-(3,6-dideoxyhexos-2-ulos-6-yl)-L-lysine; multilayer countercurrent chromatography (MLCCC)

INTRODUCTION

The Maillard reaction is a very complex series of reactions. The knowledge of the underlying mechanisms is important to understand changes occurring during storage and processing of foods, but also pathological changes *in vivo* (*1*, *2*). The more stable reaction products are relatively easy to establish. However, key Maillard intermediates remain difficult to elucidate due to their high reactivity. One major class of these compounds is α-dicarbonyls. α-Dicarbonyl structures result from autoxidation of glucose (*3*), from degradation of Amadori products (*4*) or from fragmentation of deoxyosones. In foods and in biological systems α-dicarbonyl compounds are also generated by degradation of fatty acids (*5*) and triosephosphates (*6*). Retro-aldol reactions and α- and *β*-dicarbonyl cleavages are the major pathways leading to the formation of α-dicarbonyl compounds (*7*). The modern view of the Maillard reaction includes reactions of α-dicarbonyls regardless of their origin.

With a few exceptions α-dicarbonyl structures cannot be detected directly because they are too reactive. Thus, the use of several trapping reagents has been reported. However, the proper use of such chemicals is critical for qualitative and quantitative investigations, as they alter not only the amine content but also the redox status of the particular research system (*8*). In addition to the use of trapping reagents, countless setups of different model Maillard systems were used for mechanistic studies focusing mainly on selected α-dicarbonyl compounds. Thus, with the present work we deliver a comprehensive study of the major dicarbonyls arising from the degradation of glucose in the presence of lysine in an aqueous setup at 50 °C. *o*-Phenylenediamine (OPD) was selected for it is the most frequently used trapping reagent. Incubations were conducted in presence of OPD to isolate authentic reference material. For mechanistic inquiries incubations were done in absence of OPD, which was added at the time of sampling.

MATERIALS AND METHODS

Chemicals. D(+)-Glucose, methanol (HPLC grade), potassium dihydrogen phosphate, dipotassium hydrogen phosphate trihydrate and diethylenetriaminepentaacetic acid were obtained from Merck (Darmstadt, Germany). Quinoxaline, 2-methylquinoxaline, boric acid, Brij 35, 2-mercaptoethanol, *o*-phthaldialdehyde *N,O*-bis(trimethylsilyl)acetamide, [1-^{13}C]glucose and [6-^{13}C]glucose were from Sigma-Aldrich (Taufkirchen, Germany). L-Lysine monohydrochloride, *o*-phenylenediamine (OPD), pyridine and heptafluorobutyric acid were purchased from Fluka (Taufkirchen, Germany). Methanol-d_4 was obtained from Chemotrade (Leipzig, Germany), and ethyl acetate was supplied by Roth (Karlsruhe, Germany). Glucosone and 3-deoxyglucosone were synthesized according to ref *8*.

[†]Dedicated to the late Markus O. Lederer (10.01.2003)

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Analytical HPLC−UV. A Jasco (Gross-Umstadt, Germany) quaternary gradient unit pump PU 2080 with degasser LG-2080-02 and a 715 plus Waters autosampler (Eschborn, Germany) were used. Elution of materials was monitored by a Kontron UV-detector (Munich, Germany) operating at 320 nm. Chromatographic separations were performed on stainless steel columns (Eurospher 100-5 C18, 250 × 4.6 mm) by Knauer (Berlin, Germany) using a flow rate of 1 mL/min. The mobile phase used consisted of water (solvent A) and MeOH/water (7:3 (v/v), solvent B). To both solvents (A and B), 0.6 mL/L heptafluorobutyric acid (HFBA) was added. Samples were injected at 20% B; the gradients then were changed linearly to 40% B in 40 min and to 70% B in 15 min and were held at 70% B for 5 min, and then they were changed to 100% B in 30 min and were held for 10 min. Then the gradient was changed to 20% B in 5 min and was held at 20% B for 20 min. Values for quantified quinoxalines are expressed as the means of at least three independent determinations. Data showed coefficients of variation of less than 5%.

Postcolumn Fluorescence-HPLC. A Jasco (Gross-Umstadt, Germany) ternary gradient unit 980-PU-ND with degasser DG-980−-50, autosampler 851-AS, column oven set at 20 °C and fluorescence detector FP-920 was used. Chromatographic separations were performed on stainless steel columns (YMC Hydrosphere C18, 250 × 4.6 mm, S-5 μm, 12 nm, Dinslaken, Germany) using a flow rate of 1.0 mL/min. The mobile phase used was water (solvent A) and MeOH/water (7:3 v/v; solvent B). To both solvents (A and B) 1.2 mL/L HFBA was added. Samples were injected at 2% B and run isocratically for 15 min, and the gradient was then changed to 100% B in 5 min and held at 100% for 10 min. The fluorescence detector was attuned to 340 nm for excitation and 455 nm for emission. Prior, a postcolumn derivatization reagent was added at 0.5 mL/min. This reagent consisted of 0.8 g of *o*-phthaldialdehyde, 24.73 g of boric acid, 2 mL of 2-mercaptoethanol and 1 g of Brij 35 in 1 L of H_2O adjusted to pH 9.75 with KOH.

HPLC−Electrospray Ionization Mass Spectrometry (HPLC−ESI-MS). For mass determination an Applied Biosystem 4000 Q Trap linear ion trap quadrupole LC/MS/MS equipped with Turbo Spray source was employed: positive ion mode, capillary 4.5 kV, nebulizer gas flow 60 mL/min, declustering potential 50 V. For the MS experiments the enhanced resolution scan mode with the linear ion trap was used, entry barrier of the trap was set at 8 V, and a scan rate of 250 amu/s and a stop site of 0.03 amu were used.

Nuclear Magnetic Resonance Spectroscopy (NMR). NMR spectra were recorded on Varian Unity Inova 500 instrument (Darmstadt, Germany). Chemical shifts are given relative to external Me_4Si. Final structural evidence of quinoxalines were achieved by ^{1}H and ^{13}C NMR, as well as heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) NMR experiments and by HR-MS.

Accurate Mass Determination (HR-MS). The high-resolution positive and negative ion ESI mass spectra (HR-MS) were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker, Daltonics, Billerica, MA) equipped with an Infinity cell, a 7.0 T superconducting magnet (Bruker, Karlsruhe, Germany), a radio frequency (RF)-only hexapole ion guide and an external electrospray ion source (APOLLO, Agilent, off-axis spray). Nitrogen was used as drying gas at 150 °C. The samples were dissolved in methanol and the solutions were introduced continuously via a syringe pump at a flow of 120 μL h^{-1}. The data were acquired with 256 *k* data points and zero filled to 1024 *k* by averaging 32 scans.

Incubation of Glucose and Lysine in the Presence of OPD. To isolate α-dicarbonyl quinoxalines, glucose (42 mM) and L-lysine monohydrochloride (42 mM) and OPD (5 mM) were dissolved in phosphate buffered solution (0.1 M, pH 7.4) and incubated at 50 °C for 7 days under aerated and deaerated conditions. Deaerated conditions were achieved in presence of diethylenetriaminepentaacetic acid (1 mM) and by gassing with argon. This setup was also used for incubations with [1-^{13}C] and [6-^{13}C] labeled glucose.

Incubation of Glucose and Lysine with OPD Added after the Sampling. To investigate the formation of α-dicarbonyl compounds, glucose (42 mM) and L-lysine monohydrochloride (42 mM) were dissolved in phosphate buffered solution (0.1 M, pH 7.4) and incubated at 50 °C for 7 days under aerated and deaerated conditions. OPD (5 mM) was added at the time of sampling. Samples were then reincubated for 5 h at 20 °C and subsequently analyzed.

Degradation of 3-Deoxyglucosone and Glucosone. Glucosone (42 mM) and 3-deoxyglucosone (42 mM) were incubated with lysine (42 mM) at 50 °C for 0 h, 1 h, 3 h, 5 h, 8 h, 24 and 48 h under aerated and deaerated conditions to investigate the formation of related α-dicarbonyls. OPD was added at the various time points to form quinoxalines and the resulting solution was reincubated 5 h at 20 °C before analysis.

Multilayer Countercurrent Chromatography (MLCCC). The MLCCC system (Ito, Multilayer Separator-Extractor Model, P.C. Inc., Photomac) was equipped with a chromatography pump (model 6000A) by Waters (Eschborn, Germany), a Kontron UV-detector (Munich, Germany) operating at 320 nm and a sample injection valve with a 10 mL sample loop. Eluted liquids were collected in fractions of 9 mL with a fraction collector (LKB Ultrorac 2070). Chromatograms were recorded on a plotter (Servogor 210). The multilayer coil was prepared from 1.6 mm i.d. poly(tetrafluoroethylene) (PTFE) tubing. The total capacity was 270 mL. The MLCCC was run at a revolution speed of 800 rpm and a flow rate of 2 mL/min in head to tail modus. Ethyl acetate was used as the stationary phase, water (saturated with ethyl acetate) as the mobile phase. The coil was first filled with the stationary phase. The mobile phase was applied during rotation. Samples were dissolved in 10 mL of water and ethyl acetate 1:1 (v/v) and injected after breakthrough of the mobile phase.

Glucosone-Quinoxaline (Q, MLCCC, t_R 45−60 min). HR-MS: *m/z* 273.0849 (found); *m/z* 273.0846 (calculated for $C_{12}H_{14}N_2O_4Na$) $[M + Na]^+$. The NMR data were in line with Hollnagel and Kroh (*9*).

Lederer's Glucosone-Q (MLCCC, t_R 60−70 min). HR-MS: *m/z* 363.2029 (found); *m/z* 363.2027 (calculated for $C_{18}H_{27}N_4O_4$) $[M + H]^+$. NMR results were according to Biemel et al. (*10*).

3-Deoxyglucosone-Q (MLCCC, t_R 75−95 min). HR-MS: *m/z* 257.0896 (found); *m/z* 257.0896 (calculated for $C_{12}H_{14}N_2O_3Na$) $[M + Na]^+$. NMR results were corresponding with Nedvidek et al. (*11*).

1-Deoxyglucosone-Q (MLCCC, t_R 100−150 min). HR-MS: *m/z* 257.0896 (found); *m/z* 257.0896 (calculated for $C_{12}H_{14}N_2O_3Na$) $[M + Na]^+$. The NMR data of 1-deoxyglucosone-Q were in line with Glomb et al. (*12*).

3-Deoxythreosone-Q (MLCCC, t_R 580−700 min). HR-MS: *m/z* 197.0687 (found); *m/z* 197.0685 (calculated for $C_{10}H_{10}N_2ONa$) $[M + Na]^+$. NMR results were in line with Usui et al. (*13*).

1-Deoxythreosone-Q (MLCCC, t_R 1250−1530 min). ^{1}H NMR (500 MHz, CD_3OD): δ [ppm] = 8.05 (m, 1H), 8.01 (m, 1H), 7.74 (m, 2H), 4.92 (s, 2H), 2.75 (s, 3H). ^{13}C NMR (CD_3OD): δ [ppm] = 155.9, 154.8, 142.5, 141.7, 131.1, 130.6, 129.7, 129.0, 64.6, 21.8. HR-MS: *m/z* 197.0688 (found); *m/z* 197.0685 (calculated for $C_{10}H_{10}N_2ONa$) $[M + Na]^+$.

Threosone-Q (MLCCC, t_R 210−275 min). HR-MS: verified *m/z* 213.0633 (found); *m/z* 213.0634 (calculated for $C_{10}H_{10}N_2O_2Na$) $[M + Na]^+$. NMR results were corresponding with Usui et al. (*13*).

3-Deoxypentosone-Q (MLCCC, t_R 100−150 min). HR-MS: *m/z* 227.0789 (found); *m/z* 227.0790 (calculated for $C_{11}H_{12}N_2O_2Na$) $[M + Na]^+$. NMR results were in line with Bravo et al. (*14*).

Glyoxal-Q and Methylglyoxal-Q. These quinoxalines were isolated from MLCCC (t_R 1250−1530 min) and from the stationary phase, respectively. Both quinoxalines were verified in comparison to commercial available materials.

RESULTS

The formation of α-dicarbonyl compounds arising from glucose in the presence of lysine was studied in aerated and deaerated model systems. In the literature the molar yields of α-dicarbonyls formed in Maillard reaction systems are reported to be extremely low (0.01−2.0%). Therefore for isolation of authentic reference material we chose incubations conducted in presence of OPD. Reactive α-dicarbonyls were trapped at formation and resulting quinoxalines accumulated during time.

Identification of α-Dicarbonyl Quinoxalines with HPLC−UV. A large number of quinoxalines were monitored by HPLC−UV

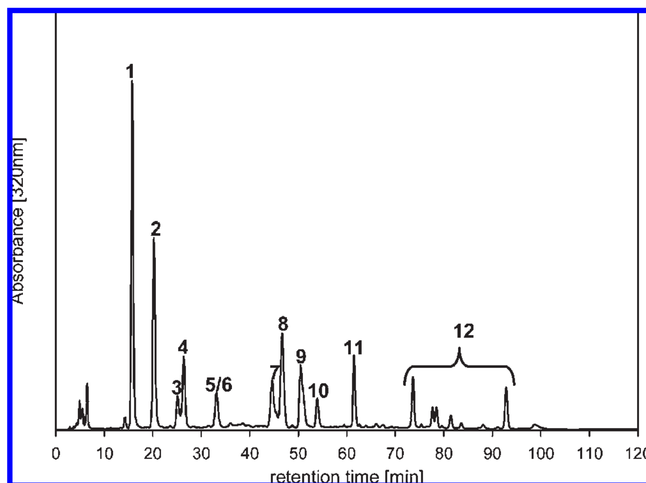

Figure 1. HPLC−UV chromatogram from aerated incubation monitored at 320 nm. Retention times are given in parentheses. Quinoxalines of **1**, glucosone (15.8 min); **2**, 1-deoxyglucosone (20.3 min); **3**, threosone (25.2 min); **4**, 3-deoxyglucosone (26.5 min); **5**, 3-deoxypentosone (33.3 min); **6**, 1-deoxypentosone (33.3 min); **7**, 3-deoxythreosone (44.9 min); **8**, 1-deoxythreosone (47.1 min); **9**, Lederer's glucosone (51.2 min); **10**, glyoxal (54.3 min); **11**, methylglyoxal (61.7 min); **12**, artifacts (73−92 min).

Table 1. Formation of Quinoxalines from Incubations of Glucose and Lysin in Presence of OPD after 7 days

	mmol/mol glucose	
α-dicarbonyl quinoxalines of	aerated	deaerated
glucosone	44.5	5.5
1-deoxyglucosone	6.9	23.5
3-deoxyglucosone	4.6	5.0
Lederer's glucosone	4.1	8.2
1-deoxypentosone	1.1	0.6
3-deoxypentosone	0.5	0.2
1-deoxythreosone	4.4	14.8
3-deoxythreosone	1.5	4.6
threosone	8.4	2.0
methylglyoxal	3.9	4.0
glyoxal	3.3	1.6

originating from glucosone, 1-deoxyglucosone, threosone, 3-deoxyglucosone, 3-deoxypentosone, 1-deoxypentosone, 3-deoxythreosone, 1-deoxythreosone, N^6-(3,6-dideoxyhexos-2-ulos-6-yl)-L-lysine (Lederer's glucosone), glyoxal and methylglyoxal (**Figure 1**). Quinoxalines of other α-dicarbonyls were identified only in negligible amounts (e.g., 1-amino-1,4-dideoxyglucosone) and are not further considered herein. Peaks eluting from 70 to 100 min were assigned to artifact formation stemming from oxidation of OPD, but not to the generation of quinoxaline structures. Quantitative data on the formation of target structures for this setup is shown in **Table 1**.

Isolation of Quinoxalines with MLCCC. Quinoxalines were isolated after extraction with ethyl acetate and from the aqueous phase. In the aqueous phase the polar quinoxalines e.g. of glucosone, 1-deoxyglucosone and Lederer's glucosone were found whereas the ethyl acetate extract contained the more nonpolar quinoxalines e.g. of 1-deoxythreosone, 3-deoxythreosone, glyoxal and methylglyoxal. MLCCC proved to be superior to preparative RP-HPLC and silica gel flash chromatography with regard to selectivity and capacity. Especially the chromatography of the aqueous phase led to significant losses of material with the solid stationary phase based techniques. The purity and identity of isolated compounds were confirmed by NMR and HR-MS. The isolated quinoxaline compounds are depicted in **Figure 2**.

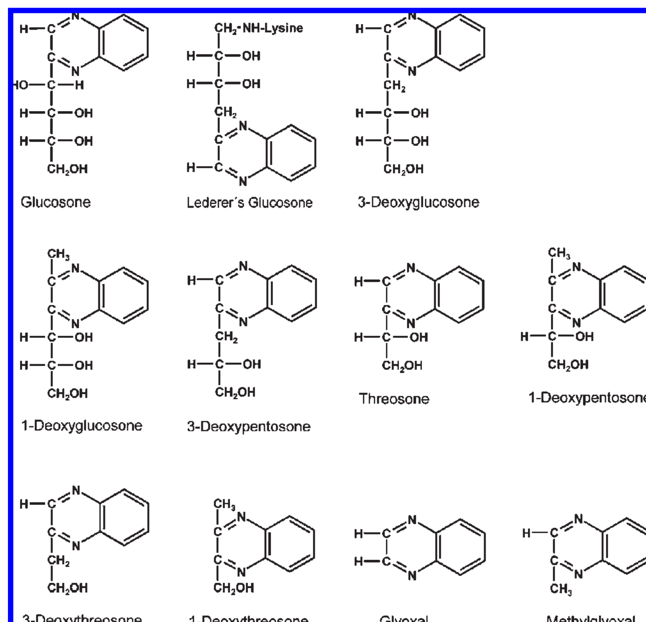

Figure 2. Identified α-dicarbonyl quinoxalines.

Unexpectedly, 1-deoxypentosone-quinoxaline (Q) was established as a novel hexose degradation intermediate and was isolated and identified from MLCCC (t_R 300−370 min). ^{1}H NMR (500 MHz, CD_3OD): δ [ppm] = 8.08 (m, 1H), 7.98 (m, 1H), 7.75 (m, 2H), 5.15 (dd, ^{1}H, ^3J = 6.2 Hz, ^3J = 5.6 Hz, −C*H*(OH)), 4.03 (dd, 1H, ^2J = 11.4 Hz, ^3J = 5.4 Hz, −CHa*H*b-(OH)), 3.96 (dd, 1H, ^2J = 11.4 Hz, ^3J = 6.3 Hz, −C*H*aHb(OH)), 2.84 (s, 3H, $-CH_3$). ^{13}C NMR (CD_3OD): δ [ppm] = 156.5, 154.7, 143.8, 141.7, 131.2, 130.5, 129.8, 128.8, 72.8, 66.3, 22.3. HR-MS verified a molecular mass of m/z 227.0790 (found); m/z 227.0791 (calculated for $C_{11}H_{12}N_2O_2Na$) $[M + Na]^+$). This structure was so far only reported for pentose based Maillard systems (*15*).

Incubation of Glucose and Lysine (without OPD). For mechanistic studies glucose and lysine were incubated in the absence of OPD to avoid artifact formation. Samples were then reincubated with OPD for 5 h under carefully controlled conditions to give the same above quinoxalines, however at much lower concentrations following a different kinetic. In support of the literature (*8*) 5 h reaction time was verified to be sufficient for complete reaction. The formation of α-dicarbonyl quinoxalines retaining the full glucose carbon backbone and of fragmentation products is shown in **Figure 3** and **Figure 4**, respectively.

3-Deoxyglucosone-Q (2.2 mmol/mol glucose, at 7 days) was the major product in this experimental setup and increased independent from oxygen during time. Glucosone-Q was formed preferentially under aeration. 1-Deoxyglucosone-Q increased 4-fold under deaerated conditions (0.05 vs 0.2 mmol/mol glucose). In parallel, Lederer's glucosone-Q levels found were small under aeration; they increased in absence of oxygen (0.04 vs 0.2 mmol/mol glucose). 3-Deoxythreosone-Q levels increased in the absence of oxygen (0.15 vs 0.23 mmol/mol glucose, at 7 days). 1-Deoxythreosone-Q levels increased 2-fold under deaerated conditions (0.05 vs 0.11 mmol/mol glucose). The formation of methylglyoxal-Q did not depend on the presence of oxygen (0.08 vs 0.08 mmol/mol glucose). Glyoxal-Q was formed preferentially under aeration (0.12 vs 0.06 mmol/mol glucose). Threosone-Q levels reached three times the yield under aerated conditions (0.25 vs 0.08 mmol/mol glucose).

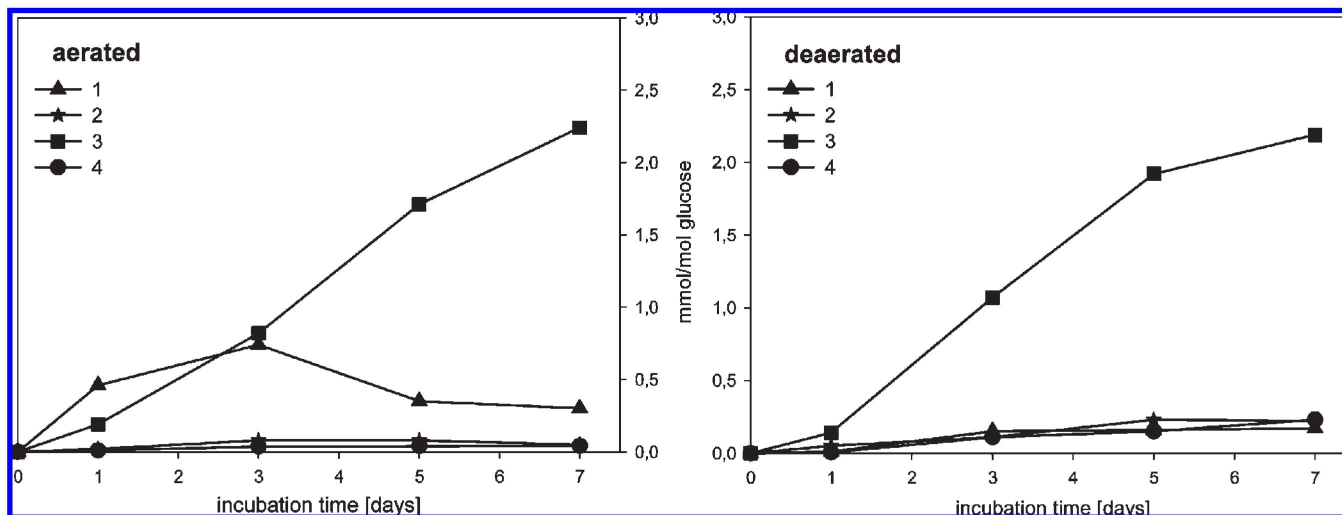

Figure 3. Formation of osone quinoxalines under aerated and deaerated conditions, glucosone (**1**), 1-deoxyglucosone (**2**), 3-deoxyglucosone (**3**) and Lederer's glucosone (**4**).

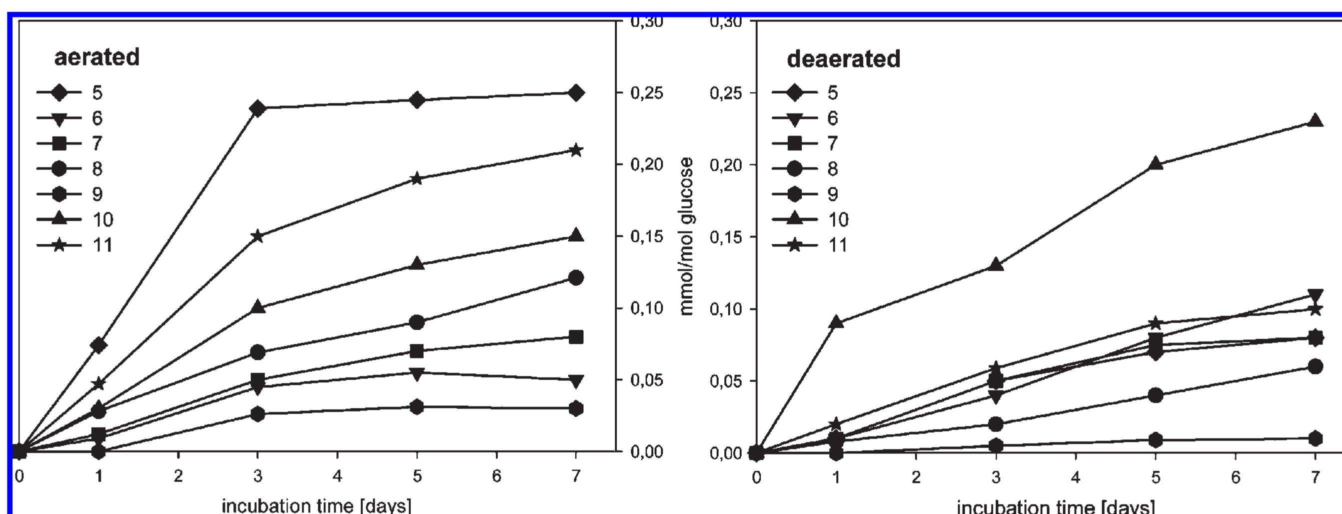

Figure 4. Formation of C_2−C_5 α-dicarbonyl fragment quinoxalines under aerated and deaerated conditions, threosone (**5**), 1-deoxythreosone (**6**), methylglyoxal (**7**), glyoxal (**8**), 1-deoxypentosone (**9**), 3-deoxythreosone (**10**) and 3-deoxypentosone (**11**).

3-Deoxypentosone-Q levels found were small under deaeration; unexpectedly, they increased in presence of oxygen (0.21 vs 0.10 mmol/mol glucose). Almost the same paradox relationship was obtained for 1-deoxypentosone-Q (0.03 vs 0.01 mmol/mol glucose).

Determination of Lysine in Incubation of Glucose and Lysine (without OPD). The low yields of α-dicarbonyls observed in the literature and herein prompted us to quantify lysine as one of the incubation educts. The present conditions starting from 42 mM at 50 °C led to a decrease of 30% within 7 days.

Incubation of [^{13}C]-Labeled Glucose. Further insights to the precise 1-deoxypentosone formation pathway from glucose were achieved by application of D-[1-^{13}C]glucose and D-[6-^{13}C]glucose to the above model system. Mass spectra were obtained in the enhanced resolution mode by coupled HPLC−mass spectrometry (HPLC−ESI-MS). Comparison of the product ion spectra allowed distinct differentiation of the labeled position in the respective quinoxaline derivative and, thus, also in the originating α-dicarbonyl molecule. The analysis of percent distribution of the observed isotopomers (M + 1 and M + 2) is shown in **Table 2**. M + 1 relates to the protonated molecular ions of an unlabeled structure, whereas M + 2 indicates incorporation of the label from glucose.

Degradation of 3-Deoxyglucosone and Glucosone. In this experiment 3-deoxyglucosone had a half-life of 40 h. However no additional quinoxalines were formed. In contrast from glucosone, with a half-life of 8 h, the formation of 1-deoxypentosone-Q, 3-deoxypentosone-Q, pentosone-Q, threosone-Q, methylglyoxal-Q and glyoxal-Q was established (**Table 3**). 3-Deoxypentosone-Q was formed preferentially under deaeration (2.9 vs 6.1 mmol/mol glucosone, after 8 h). 1-Deoxypentosone-Q levels also did not depend on the presence of oxygen (0.3 vs 0.9 mmol/mol glucosone). Threosone-Q levels increased 2-fold under aerated conditions (7.5 vs 3.5 mmol/mol glucosone). Methylglyoxal-Q and glyoxal-Q levels were low (0.3 and 0.7 mmol/mol glucosone, respectively) and independent from oxygen. In addition, small traces of pentosone were detected under aeration.

DISCUSSION

All structures monitored in the present study have been established before in Maillard literature. However, this is the first time

Table 2. Percent Label Distribution Identified in Glucose/Lysine/OPD Incubations by ESI-LC/MS/MS

		1-^{13}C-glucose		6-^{13}C-glucose	
α-dicarbonyl quinoxalines of	*m/z*	3[a]	7[a]	3[a]	7[a]
intermediates with C6-carbon backbone	M + 2	100	100	100	100
1-deoxypentosone	M + 1	100	100	0	0
	M + 2	0	0	100	100
3-deoxypentosone	M + 1	100	100	0	0
	M + 2	0	0	100	100
1-deoxythreosone	M + 1	100	100	0	0
	M + 2	0	0	100	100
3-deoxythreosone	M + 1	100	100	0	0
	M + 2	0	0	100	100
threosone	M + 1	100	100	0	0
	M + 2	0	0	100	100
glyoxal	M + 1	47.9	51.2	72.2	69
	M + 2	52.1	48.8	27.8	31
methylglyoxal	M + 1	66.8	68.2	52.9	52.9
	M + 2	33.2	31.8	47.1	47.1

[a] Days of incubation.

Table 3. Degradation of Glucosone (8 h)[a]

	mmol/mol glucosone	
α-dicarbonyl quinoxalines of	aerated	deaerated
threosone	7.5	3.5
3-deoxypentosone	2.9	6.1
1-deoxypentosone	0.3	0.9
methylglyoxal	0.3	0.3
glyoxal	0.7	0.7

[a] Pentosone was identified in traces.

that all of the α-dicarbonyls relevant to the Maillard reaction induced degradation of glucose have been verified. Incubations in presence of OPD yielded sufficient amounts of quinoxalines for isolation and unequivocal structure elucidation. In order to clarify the formation of α-dicarbonyls different experiments were conducted from glucose and from α-dicarbonyl precursors. In these cases, OPD was added after incubation to not alter the actual concentration and the mechanistic relationship of target structures.

Glyoxal is the simplest α-dicarbonyl compound detected preferentially under aeration in incubation of glucose-lysine. Thornalley et al. (*16*) assumed its direct formation by retro-aldol scission of glucose. Hofmann et al. (*17*) suggested glucosone as the glyoxal precursor considering its formation by cleavage of C_2-C_3 bond. Yaylayan and Keyhani (*18*) proposed a mechanism based on dehydration of aldohexoses (a loss of two water molecules from C_3-C_4 and C_5-C_6) and retro-aldol cleavage between C_2-C_3. Hayashi and Namiki (*19*) proposed a hypothesis of glyoxal formation based on oxidation of the glycolaldehyde-imine arising from retro-aldol fragmentation of the glycosylamine. In the present work the formation pathway described by Hofmann et al. (*17*) was verified. However, yields were extremely low and alternative pathways of formation were not further explored. The analysis of percent distribution of the observed isotopomers indicated that glyoxal involved C_1-C_2 (49%) and C_5-C_6 (31%) of glucose skeleton. Thus, 20% must be attributed to the C_2-C_5 region of glucose based on other mechanisms, most likely to the degradation of C4 and C5 fragments discussed below. Methylglyoxal was formed in equal yields under aeration and deaeration. Formation of methylglyoxal from hexoses by cleavage of the C_3-C_4 bond of 1-deoxyglucosone was proposed by Hollnagel and Kroh (*20*). Similarly, methylglyoxal can be generated by scission of glyceraldehyde from 2-ene-2,3-diols of hexoses (*16*). Weenen (*21*) suggested 3-deoxyglucosone as the precursor for formation of methylglyoxal, which was confirmed by Yaylayan and Keyhani (*18*). The formation of methylglyoxal was not dependent on oxygen in incubation of glucose-lysine clearly indicating the absence of oxidative mechanisms. This diminishes the relevance of the present finding that methylglyoxal was also formed in minor quantities from glucosone. Incubation with [^{13}C]-labeled glucose proved that methylglyoxal stems only to 32% from the C_1-C_3, but to 47% from the C_4-C_6 region. Thus, 21% must be attributed to the C_2-C_5 region of glucose. Major alternative pathways to the ones described above must exist. For glyoxal and methylglyoxal formation it is important to consider that the found isotope distribution might be a function of reaction temperature and also of the respective amino acid used. The reaction conditions most likely will alter the contribution of the different mechanisms and thus the resulting percentages of label incorporation.

Threosone formation was preferred under aerated conditions in incubation with glucose. Threosone is known as a degradation compound of dehydro-L-ascorbic acid (*22*). Usui (*13*) recently reported threosone during degradation of glucose. However, this author explained its formation via glucosone. In the present work threosone was formed from glucosone under aeration (0.8%), which confirmed the findings of Usui. However, a major alternative oxidative mechanism originating from 1-deoxyglucosone (1.4%) was established by Voigt and Glomb (*7*). 1-Deoxyglucosone was elucidated as the central intermediate in a common chemistry for all C4-dicarbonyls. In support, the analysis of percent distribution of the observed isotopomers indicated that threosone involved only the C_3-C_6 region (100%) of glucose skeleton, in parallel to that of 1-deoxythreosone and of 3-deoxythreosone, which were formed preferentially under deaeration in the present study. Alternative pathways were proposed by Usui et al. (*13*) for 3-deoxythreosone from 3-deoxyglucosone, glucosone and 3-deoxypentosone, which could not be attested from the present work.

Unexpectedly, two α-dicarbonyls with five carbon atoms were identified in lysine incubations of glucose and of glucosone in significant amounts, 3-deoxypentosone and 1-deoxypentosone. Nedvidek et al. (*11*) detected pentosone, 3-deoxypentosone and 1-deoxypentosone in heated alkaline solutions of pentoses. Hollnagel and Kroh (*23*) described 3-deoxypentosone as the predominating α-dicarbonyl compound formed from disaccharides. Usui (*13*) explained its formation from glucose via glucosone. Grandhee and Monnier (*24*) proposed a mechanism of 3-deoxypentosone formation from D-ribose. In contrast, 1-deoxypentosone was so far only related to the degradation of pentoses or pentose derivatives. Rizzi (*25*) reported on the formation in ribose degradation catalyzed by phosphate. Hauck et al. (*15*) isolated 1-deoxypentosone-Q after incubation of ribosephosphate isomerase with D-ribose-5-phosphate and OPD. Bravo et al. (*14*) established the formation of 3-deoxypentosone and 1-deoxypentosone in beer and related the structures to the degradation of pentose precursors. In the present work, 1-deoxypentosone was identified and quantified for the first time within the context of hexose Maillard degradation. The analysis of percent distribution of the observed isotopomers indicated that 3-deoxypentosone and 1-deoxypentosone involved C_2-C_6 (100%) of glucose skeleton. Both C5-dicarbonyls were monitored preferentially under aeration. This is in contrast to the C4-intermediates discussed above, and to 3-deoxyglucosone and 1-deoxyglucosone. 3-Deoxyglucosone accumulated independent from the presence of oxygen; 1-deoxyglucosone levels were significantly lower. Both C6-dicarbonyls are generated nonoxidatively via enolization and dehydration. However, the redox

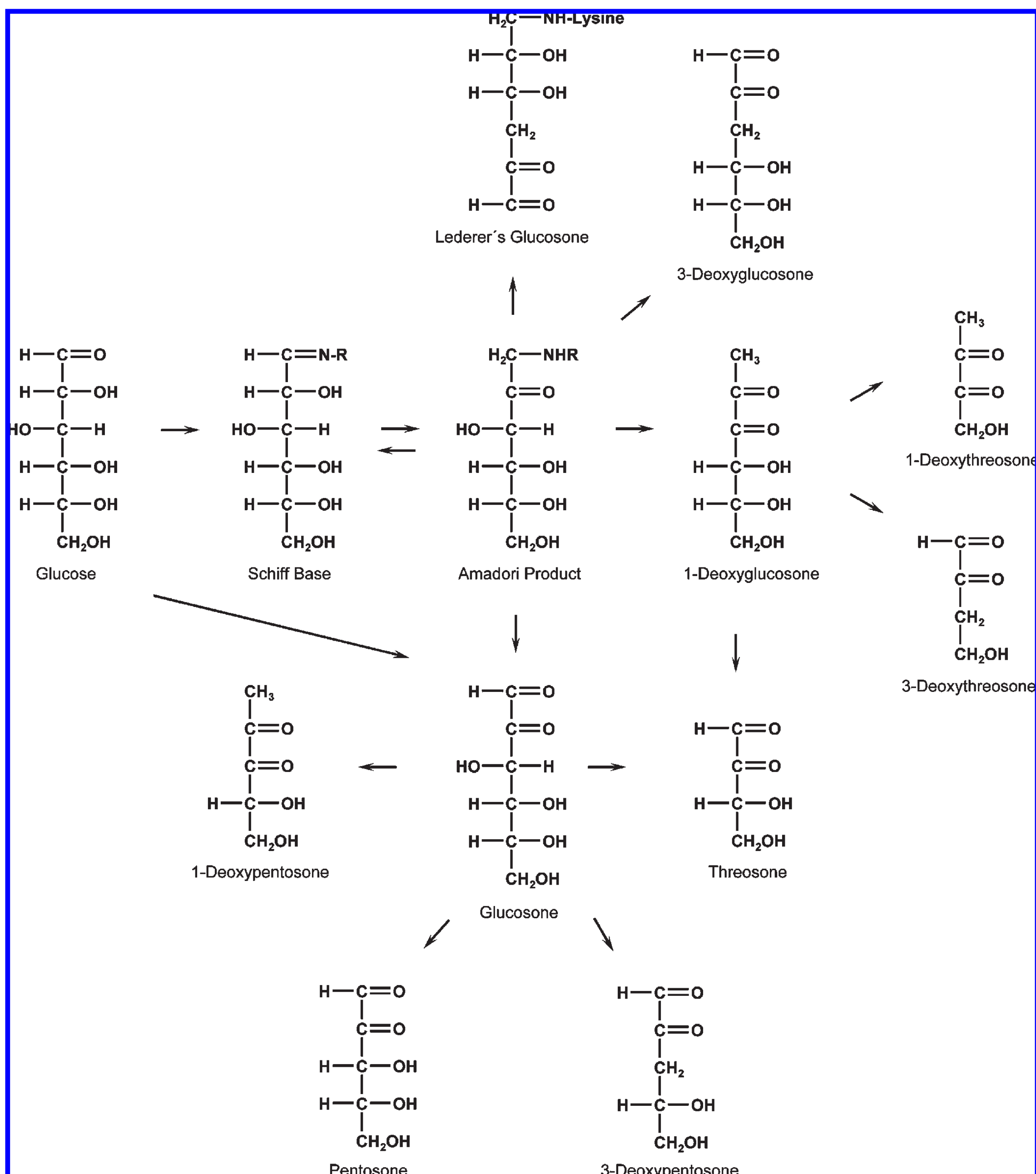

Figure 5. Formation of α-dicarbonyl compounds in glucose Maillard reaction systems.

reactivity of 1-deoxyglucosone is much higher due to its reductone structure resulting in a decrease under aeration (*8*). The difference in the pattern of the C5-dicarbonyl formation suggested a common precursor, which can only be formed via oxidative pathways. The major α-dicarbonyl formed during oxidative glucose degradation was glucosone. Indeed, when authentic glucosone was incubated, 3-deoxypentosone and 1-deoxypentosone were identified predominantly under deaeration. We therefore propose the formation of both C5-intermediates nonoxidatively from glucosone. The 1,3-tautomer is split into formic acid and an enediol via a hydrolytic β-dicarbonyl cleavage. The resulting 1,2-enediol then gives 3-deoxypentosone by dehydration, but should also readily isomerize to the 2,3-enediol to give 1-deoxypentosone. This mechanism of β-dicarbonyl cleavage with a pivotal enediol structure was confirmed for the degradation of 1-deoxyglucosone to give C4-dicarbonyls (*7*, *26*). In addition in the present work, formation of small amounts of pentosone was verified in aerated glucosone incubations, which is the direct oxidation product of that enediol intermediate. In further support, with about 7:1 the relation of 3-deoxypentosone to 1-deoxypentosone was almost identical in glucose and in glucosone incubations.

In this context the relevance of N^6-(3,6-dideoxyhexos-2-ulos-6-yl)-L-lysine (Lederer's glucosone) was established. Lederer's glucosone is similar to 3-deoxyglucosone with the terminal OH functional group substituted by the lysine N^{ε} amino moiety. The formation of this structure was established by the research group of Markus O. Lederer (*27*), who described the enolization along the entire carbohydrate backbone, ultimately leading to a 5,6-enediol which is prerequisite to eliminate water at C4 to form the deoxyosone. In incubations of glucose-lysine Lederer's glucosone was formed under deaeration. This is expected as enolization via enediole intermediates along the complete carbon backbone is highly susceptible to oxidative degradation.

The present study clearly depicts the advantages and disadvantages of the use of OPD as a trapping reagent. Incubations in

presence of OPD lead to accumulation of all α-dicarbonyls, which was helpful for isolation of quinoxalines. On the other hand, this forbids statements on actual concentrations and on reactivity of the participating structures. E.g. 3-deoxyglucosone was the single major dicarbonyl in the absence of OPD, whereas in the presence of OPD glucosone, 1-deoxyglucosone, Lederer's glucosone and 1-deoxythresosone were of much higher quantitative importance. This means that highly reactive reductone structures are degraded immediately after formation and especially once their direct precursor exhausts. At the same time more stable structures as the 3-deoxy-isomers accumulate even in absence of the trapping reagent due to lack of reactivity. This might at least in part explain the difference of the low yields of free α-dicarbonyls compared to the significant loss of lysine in the reaction mixtures. OPD was also reported to impose major oxidative stress on the particular research system (*8*). This lead to glucosone artifact formation in incubations of the Amadori product from glucose and lysine reacted at 37 °C due to the presence of OPD. If this is also relevant to the reaction mixtures studied herein, cannot be clarified by the conducted experiments.

In summary, we were able to present a thorough study for all α-dicarbonyl structures relevant to the degradation of glucose in presence of lysine using MLCCC as a successful technique to isolate quinoxalines of dicarbonyl compounds. This novel use is therefore highly suggestive of further applications for the polar reaction products found within Maillard chemistry. Lederer's glucosone was confirmed to be an important intermediate comparable to 1-deoxyglucosone. In addition, 1-deoxypentosone was unequivocally established as a novel α-dicarbonyl compound arising from glucose via glucosone under oxidative conditions. The complete reaction mechanisms are summarized by **Figure 5**. To enhance clarity the formation of glyoxal and methylglyoxal was excluded from the scheme and is discussed in depth above.

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