JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 5685–5691 5685
DOI:10.1021/jf100140h

# Oxygen-Dependent Fragmentation Reactions during the Degradation of 1-Deoxy-D-*erythro*-hexo-2,3-diulose

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With this work, we report on further insights into the chemistry of 1-deoxy-D-*erythro*-hexo-2,3-diulose (1-deoxyglucosone, 1-DG). This α-dicarbonyl plays an important role as a highly reactive intermediate in the Maillard chemistry of hexoses. Degradation of 1-DG in the presence of the amino acid L-alanine led to the formation of several products. Lactic acid and glyceric acid were found to be major degradation products. Their formation was dependent on the presence of oxygen. Therefore, a mechanism is postulated based on oxidation leading to a tricarbonyl intermediate. Carbonyl cleavage of this structure should then give rise to carboxylic acids. This mechanism was supported by the isotope distribution observed during degradation of different $^{13}$C-labeled D-glucose isotopomers. Furthermore, we identified 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (γ-pyranone) to be capable of rehydration forming 1-DG to a minor extent and therefore leading to the same degradation products. The formation of carboxylic acids from γ-pyranone was also dependent on the presence of oxygen in agreement with the postulated oxidative fragmentation. Finally, we investigated the formation of aldehydes expected as retro-aldol products formed within the degradation of 1-DG. Results seemed to rule out this reaction as an important degradation pathway under the conditions investigated herein.

**KEYWORDS: Maillard reaction; 1-deoxyhexo-2,3-diulose; 1-deoxyglucosone; 1-DG; α-dicarbonyl compounds; dicarbonyl cleavage; oxidative degradation; tricarbonyl; carboxylic acids**

## INTRODUCTION

The term Maillard reaction is used today as a synonym for a complicated network of different reaction pathways. In the historic view, these reactions are initiated by reaction of reducing sugars and amino acids or other compounds bearing an amino group. Maillard reactions provide positive and negative aspects, for example, the formation of flavor and color of processed foods. Highly reactive intermediates, with an α-dicarbonyl moiety, are able to react with amino acids and thereby influence the nutritional value of processed foods (review in ref *1*). Furthermore, with the discovery of glycated hemoglobin (*2*, *3*), it became obvious that Maillard reactions also occur in vivo; thus, the formation of so-called advanced glycation end products (AGE) is discussed in the context of aging and complications like diabetes, Alzheimer's, or arteriosclerosis (*4*, *5*). Therefore, Maillard reactions and, in particular, the chemistry of α-dicarbonyl compounds are issues of vital interest in food chemistry as well as in medical science. The α-dicarbonyl compound 1-deoxy-D-*erythro*-hexo-2,3-diulose (1-deoxyglucosone, 1-DG) (**1**) is the key intermediate in the Maillard-induced degradation of hexoses. Because of its high reactivity, it was for a long time only accessible via indirect analytical means like trapping reactions (*6*). Successful synthesis of **1** (*7*) enabled us to investigate the chemical properties of 1-DG and its degradation products in detail. We recently reported on the degradation of 1-DG in aqueous model systems in the presence of the amino acid L-alanine. Carboxylic acids and dicarbonyl as well as hydroxycarbonyl compounds were among the degradation products verified. Their formation was explained nonoxidatively via hydrolytic β-dicarbonyl cleavage (*8*). In this work, we extend the range of products identified, as well as the knowledge on reaction pathways important to 1-DG degradation.

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## MATERIALS AND METHODS

**Materials.** The following chemicals of analytical grade were commercially available: *N*,*O*-*bis*-(trimethylsilyl)acetamide with 5% trimethylchlorosilane, L-alanine, L(+)-erythrulose, *o*-phenylenediamine (OPD), heptafluorobutyric acid, L-(+)-lactic acid, 2,3,5-triphenyltetrazolium chloride (Fluka/Sigma-Aldrich, Seelze, Germany), dipotassium hydrogen phosphate trihydrate, potassium dihydrogen phosphate (Merck, Darmstadt, Germany), calcium sulfate, diethyl ether, ethyl acetate (EtOAC) (Roth, Karlsruhe, Germany), diethylenetriaminepentaacetic acid (Sigma-Aldrich, Taufkirchen, Germany), D-glyceric acid calcium salt dihydrate (Aldrich/Sigma-Aldrich, Steinheim, Germany), and Dowex 50 W × 8 ($H^+$-form, 50−100 mesh) (Serva/Boehringer, Ingelheim-Heidelberg, Germany). 1-Deoxy-4,5-*O*-isopropylidene-D-*erythro*-hexo-2,3-diulose and 1-DG were synthesized as described in ref *7*.

**Degradation of 1-DG.** Degradation of 1-DG was carried out under aerated and deaerated conditions according to ref *8*. Incubation solutions (1-DG/L-alanine, 42 mM in 0.1 M phosphate buffer, pH 7.4) were heated (37 and 50 °C), and samples were taken over time and analyzed by GC

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Published on Web 04/08/2010

after derivatization. Values were expressed as means of at least three independent determinations.

**Degradation of 3,5-Dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (*γ*-Pyranone).** *γ*-Pyranone was synthesized according to ref *9*. In brief, a solution of glucose and piperidine in ethanol was refluxed under introduction of argon. After the addition of acetic acid and further heating, the concentrated reaction solution was diluted with water, and *γ*-pyranone was extracted with EtOAc. The crude product was purified using column chromatography (silica gel) and high-vacuum distillation. Finally, the product was recrystallized from hexane.

**Degradation of $^{13}$C-Labeled Glucose.** Different $^{13}$C-labeled D-glucose isotopomers (labeled positions 1, 2, 3, 5, and 6) (12.6 *μ*mol, respectively) were dissolved together with L-alanine (12.6 *μ*mol) in phosphate buffer (0.1 M, pH 7.4, 0.3 mL). The incubation solution was reacted at 50 °C for 7 days under aerobic conditions. After the solution was cooled to room temperature, an aliquot (50 *μ*L) was measured by GC-LCI-MS after silylation for glyceric acid and by liquid chromatography−mass spectrometry (LC-MS$^2$) directly for lactic acid.

**Derivatization Reactions.** *Trimethylsilyl Derivatives*. Trimethylsilyl derivatives were obtained by adopting the method described in ref *8*. Data for silylated compounds analyzed by gas chromatography−mass spectrometry (GC-MS) showed standard deviations < 10 mmol/mol 1-DG, resulting in coefficients of variation < 5%.

*Benzimidazole Derivatives*. Samples were spiked with OPD (4.95 *μ*mol) dissolved in water (30 *μ*L) and kept for 5 h at room temperature prior to injection into the high-performance liquid chromatography−ultraviolet (HPLC-UV) system. Benzimidazoles were monitored at $\lambda_M$ = 272 nm. Quantification was carried out by comparison of peak areas obtained at 272 nm with those of standard solutions containing known amounts of pure authentic benzimidazoles (data obtained with HPLC-UV showed standard deviations < 1.5 mmol/mol 1-DG, resulting in coefficients of variation < 5%). Reference compounds were synthesized as follows.

*2-Methyl-1H-benzimidazole*. OPD (1.1 g, 10.2 mmol) was dissolved in phosphate buffer (20 mL, 0.1 M, pH 7.4). Acetaldehyde (381 *μ*L, 6.8 mmol) was added, and the mixture was maintained for 3 h at room temperature. After dilution with water (20 mL), the solution was extracted with three volumes of 50 mL of EtOAc. The combined organic extracts were dried over calcium sulfate and evaporated to dryness in vacuo. The crude product was chromatographed on a silica gel column (3 cm × 15 cm) with EtOAc/hexane (2/98, v/v) as an eluent. Fractions with target material [thin-layer chromatography (TLC), $R_f$ 0.15, same solvent system] were combined, and the organic solvent was removed. The residue was further purified using a LiChroprep RP-18 column with water/methanol (70/30, v/v) as the eluent. Fractions containing the target material were combined, yielding the product as a colorless solid (149 mg, 1.13 mmol, 17%). $^1$H NMR (200 MHz in $CD_3OD$): *δ* 2.57 (s, $-CH_3$), 7.20 (m, 2H), 7.50 (m, 2H) ppm. $^{13}$C NMR (200 MHz in $CD_3OD$): *δ* 14.5, 115.4, 123.7, 139.4 153.2 ppm. High-resolution mass spectrometry (HR-MS) verified a molecular mass of *m*/*z* 132.0684 (found) [*m*/*z* 132.0687 calculated for $C_8H_8N_2$ ($M^{+\bullet}$)]. NMR data were in line with refs *10* and *11*.

*1H-Benzimidazol-2-ylmethanol*. OPD (866 mg, 8 mmol) was dissolved in phosphate buffer (20 mL, 0.1 M, pH 7.4). Glycolaldehyde dimer (303 mg, 2.5 mmol) was added, and the mixture was maintained for 3 h at room temperature. After dilution with water (20 mL), the solution was extracted with three volumes of 50 mL of EtOAc. The combined organic extracts were dried over calcium sulfate and evaporated to dryness in vacuo. The crude product was chromatographed on a silica gel column (3 cm × 15 cm) with EtOAc/hexane (2/98, v/v) as the eluent. Fractions with target material (TLC, $R_f$ 0.12, same solvent system) were combined, and the organic solvent was removed. The residue was further purified using a LiChroprep RP-18 column with water/methanol (90/10, v/v) as the eluent. Fractions containing the target were combined, yielding the product as a colorless solid (142 mg, 0.96 mmol, 19%). $^1$H NMR (200 MHz in $CH_3OD$): *δ* 4.88 (s, 2H, $-CH_2OH$), 7.22 (m, 2H), 7.56 (m, 2H) ppm. HR-MS (after silylation) verified a molecular mass of *m*/*z* 220.1032 (found) [*m*/*z* 220.1032 calculated for $C_{11}H_{16}ON_2Si$ ($M^{+\bullet}$)].

*1-(1H-Benzimidazol-2-yl)ethane-1,2-diol*. OPD (452 mg, 4.2 mmol) was dissolved in phosphate buffer (20 mL, 0.1 M, pH 7.4). Glyceric aldehyde (189 mg, 2.1 mmol) was added, and the mixture was maintained for 16 h at room temperature. After dilution with water (20 mL), the solution was extracted with three volumes of 50 mL of EtOAc. The combined organic extracts were dried over calcium sulfate and evaporated to dryness in vacuo. The residue was purified using a LiChroprep RP-18 column with water/methanol (90/10, v/v) as the eluent. Fractions with target material (TLC, $R_f$ 0.12, same solvent system) were combined, yielding the product as a colorless solid (199 mg, 1.12 mmol, 53%). $^1$H NMR (200 MHz in $CH_3OD$): *δ* 3.88 [dd, 1H, $^3J$ = 6.7 Hz, $^2J$ = 11.4 Hz, −CHaHb(OH)], 4.05 [dd, 1H, $^3J$ = 4.0 Hz, $^2J$ = 11.2 Hz, −CHaHb-(OH)], 5.02 [dd, 1H, $^3J$ = 4.0 Hz, $^3J$ = 6.5 Hz, −CH(OH)], 7.23 (m, 2H), 7.58 (m, 2H) ppm. HR-MS (after silylation) verified a molecular mass of *m*/*z* 322.1537 (found) [*m*/*z* 322.1533 calculated for $C_{15}H_{26}O_2N_2Si_2$ ($M^{+\bullet}$)].

**GC-EI-MS.** Samples were analyzed on a Thermo Finnigan Trace GC Ultra coupled to a Thermo Finnigan Trace DSQ (both Thermo Fisher Scientific GmbH, Bremen, Germany) using the parameters and temperature program given in ref *8*.

**GC-LCI-MS.** GC-MS with liquid chemical ionization was performed on the instrument described above for glyceric acid isotope label experiments. Methanol was used as a reactant gas. Mass spectra were obtained at 70 eV (source, 190 °C; emission current, 80 *μ*A) in full scan mode (mass range *m*/*z* 50−650).

**LC-MS$^2$.** LC-MS$^2$ was applied for lactic acid isotope label experiments in negative-ion mode. Mass transitions for multiple reaction monitoring (MRM) mode were set according to ref *12*. The optimized parameters for mass spectrometry were as follows: *m*/*z* 89 → 43 (DP, −50.00; CE, −19.20; CXP, 0.00), *m*/*z* 89 → 45 (DP, −48.90; CE, −15.20; CXP, 0.00), *m*/*z* 90 → 43 and *m*/*z* 90 → 44 (DP, −50.00; CE, −19.20; CXP, 0.00), *m*/*z* 90 → 45 and *m*/*z* 90 → 46 (DP, −48.90; CE, −15.20; CXP, 0.00); the dwell time was always 75.00 ms. $H_2C{=}CH{-}O^-$ with *m*/*z* 43 (unlabeled species) or *m*/*z* 44 (labeled species) represents $-HCOH-CH_3$ of the lactic acid molecule. $^-$COOH with *m*/*z* 45 (unlabeled species) or *m*/*z* 46 (labeled species) represents −COOH of the lactic acid molecule. A Jasco PU-2080 Plus quaternary gradient pump with degasser and a Jasco AS-2057 Plus autosampler (Jasco, Gross-Umstadt, Germany) were used. Chromatographic separations were performed on a stainless steel column (Eurospher 100−5 C18, 250 mm × 4.0 mm, 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), 0.8 mL/L formic acid was added. Samples were injected at 100% A (held 5 min), and the gradient then changed to 100% B in 5 min (held 15 min) and then changed to 100% A in 5 min (held 15 min). Lactic acid eluted at $t_R$ = 4.0 min. The mass analyses were performed using an Applied Biosystems API 4000 quadrupole instrument (Applied Biosystems, Foster City, CA) equipped with an API source using an electrospray ionization (ESI) interphase. The LC system was connected directly to the probe of the mass spectrometer. Nitrogen was used as the sheath and auxiliary gas.

**HPLC-UV.** A Jasco PU-2089 Plus quaternary gradient pump with degasser was used combined with a Jasco AS-2055 Plus autosampler. Elution of benzimidazoles (glyceric aldehyde **11** at 27.8 min, glycolaldehyde **12** at 30.7 min, and acetaldehyde **13** at 38.9 min) and 1-DG-quinoxaline **10** (23.5 min) was monitored by a Jasco UV-2075 Plus UV detector (all Jasco, Gross-Umstadt, Germany). Chromatographic separation was carried out on a stainless steel column (Eurospher 100-5 C18, 250 mm × 4.6 mm) by Knauer (Berlin, Germany) using the system described in our previous paper (*8*).

**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, United States) equipped with an Infinity cell, a 7.0 T superconducting magnet (Bruker, Karlsruhe, Germany), a radio frequency-only hexapole ion guide, and an external electrospray ion source (APOLLO, Agilent, off-axis spray). Nitrogen was used as the 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 *μ*L/h. The data were acquired with 256k data points and zero filled to 1024k by averaging 32 scans.

**Nuclear Magnetic Resonance Spectroscopy (NMR).** $^1$H and $^{13}$C experiments were performed on a Bruker AC-200 (Bruker, Rheinstetten, Germany).

## RESULTS

**Degradation of 1-DG.** Recently, we described silylation of a 1-DG/L-alanine incubation (42 mM in phosphate buffer 0.1 M, pH 7.4) leading to several signals in GC-MS (*8*). Major signals

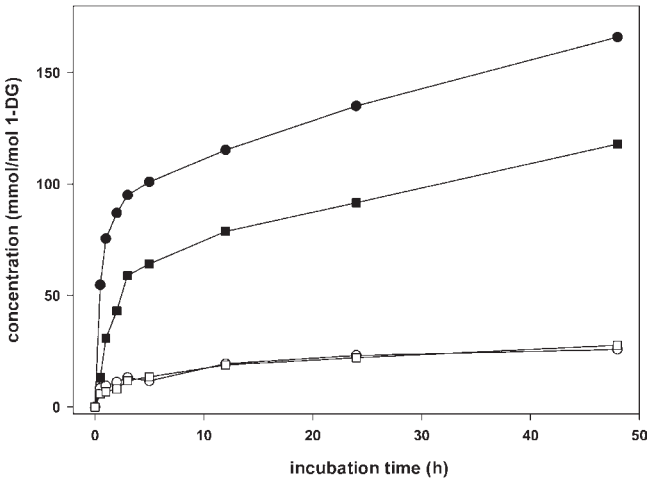


**Figure 1.** Time−course formation of lactic acid (●, 37 °C; ■, 50 °C) in incubations of 1-DG and L-alanine (42 mM, pH 7.4, respectively) under aeration (full symbols) and under deaeration (open symbols).

were assigned to glyceric acid **3**, erythrulose **4**, γ-pyranone **5**, and 4-hydroxy-2-(hydroxymethyl)-5-methylfuran-3(2*H*)-one **6** (furan-3-one). Another product formed from **1** was lactic acid **2**. **Figure 1** shows the formation of lactic acid over a period of 48 h at two different temperatures (37 and 50 °C) under aerated and deaerated conditions. The formation of lactic acid was significantly preferred under aerated conditions. Up to 166 mmol lactic acid per mol 1-DG was formed at 37 °C after 48 h (aerated conditions), while incubation under deaerated conditions yielded only 26 mmol/mol 1-DG. The formation of lactic acid proceeded at a higher rate within the first 2 h, that is, about half of the total amount (87 mmol/mol 1-DG) was already formed. Later on, the concentration of lactic acid increased at a lower rate but still nearly linear over the whole time monitored. As reported before, the half-life of 1-DG was about 0.5 h under the given conditions (*7*). Incubations at 50 °C resulted in significantly lower quantities of lactic acid as compared to incubations at 37 °C. For example, only 118 mmol/mol 1-DG was obtained after 48 h under aeration. Still, the difference to deaeration was obvious (28 mmol/mol 1-DG). Interestingly, lactic acid followed almost the same pattern in the rate of formation and in concentration as glyceric acid. The largest differences between these two compounds were observed at 37 °C under aerated conditions. Here, the formation of glyceric acid was favored (266 vs 166 mmol/mol 1-DG) under aerated conditions. As with lactic acid, there was a significant increase for glyceric acid under aeration and a significant decrease for comparable incubations conducted at 50 °C.

Because of the lack of stable isotope-labeled **1**, we investigated the degradation of $^{13}$C-labeled glucose in the presence of L-alanine to gain more information on the occurring reaction pathways leading to the formation of glyceric acid and lactic acid. In GC-LCI-MS after silylation, *m*/*z* 323 related to unlabeled glyceric acid (M + 1), while *m*/*z* 324 indicated label incorporation (M + 2). Glyceric acid always incorporated the label from C-5 and C-6 but not from C-1 to C-3 of glucose. This means that glyceric acid is formed exclusively from the lower three carbon atoms of glucose, confirming data from Davidek et al. (*13*). In contrast, results obtained for lactic acid from LC-MS$^2$ analyses were not as clear-cut and are depicted in **Table 1**. Selected mass transitions (*12*) not only allowed us to monitor label incorporation but also allowed us to locate the exact position of the label in the −COOH versus the −CHOH−$CH_3$ part of the lactic acid molecule. The data unequivocally showed that 48% of lactic acid formed during glucose degradation originated from C-1 to C-3

**Table 1.** Degradation of Different $^{13}$C-D-Glucose Isotopomers Revealed the Formation of Discrete Lactic Acid Species from Different Parts of the Original Carbon Backbone

| glucose backbone | origin of lactic acid | | | | | |
|---|---|---|---|---|---|---|
| | distribution (%) | | | | | |
| | 48 | 0 | 36 | 0 | 8 | 8 |
| C-1 | CHO | $CH_3$ | COOH | | | |
| C-2 | HCOH | HCOH | HCOH | | | |
| C-3 | HOCH | COOH | $CH_3$ | $CH_3$ | COOH | $CH_3$ | COOH | $CH_3$ |
| C-4 | HCOH | | | HCOH | HCOH | | | |
| C-5 | HCOH | | | COOH | $CH_3$ | | | |
| C-6 | $CH_2OH$ | | | | | COOH | $CH_3$ |

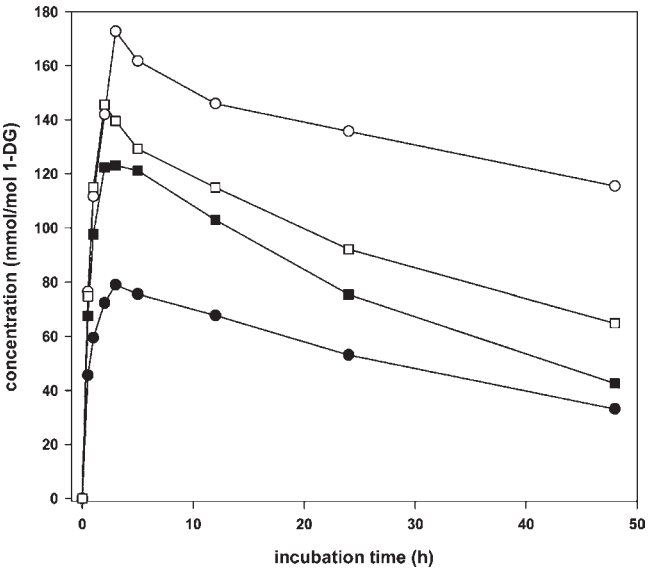


**Figure 2.** Formation of 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (γ-pyranone) **5** (1-DG/L-alanine, 42 mM in 0.1 M phosphate buffer, pH 7.4; ●, 37 °C; ■, 50 °C) under aeration (full symbols) and under deaeration (open symbols).

with the −COOH group strictly located at C-3. Thirty-six percent resulted from C-3 to C-5 with the −COOH group strictly at C-5. The remaining 16% was verified from C-4 to C-6 with the −COOH group assigned to C-4 and C-6 in equal parts.

Another product identified in the degradation of **1** was γ-pyranone **5**. **Figure 2** shows the time−course formation of **5** under aerated and deaerated conditions (37 and 50 °C). Maximum yield resulted after 2−3 h (173 mmol/mol 1-DG, 37 °C under deaeration) that decreased in later samples. Formation was preferred under deaeration; for example, 146 mmol/mol 1-DG was detected after 12 h (37 °C) under deaerated conditions vs 67 mmol/mol 1-DG under aerated conditions. Yields obtained at 50 °C showed fewer differences (114 vs 103 mmol/mol 1-DG after 12 h).

We also expected the formation of aldehydes during the degradation of **1**. Therefore, samples taken at various time points were reincubated with OPD and analyzed by HPLC-UV. In general, we observed a rather fast formation of aldehydes within the first 5 h. As indicated in **Figure 3** for acetaldehyde, concentrations did almost not change at later incubation times. The highest yields were obtained for acetaldehyde and glycolaldehyde at 50 °C under aerated conditions (30 and 29 mmol/mol 1-DG at 12 h, respectively), while glyceric aldehyde was only formed up to

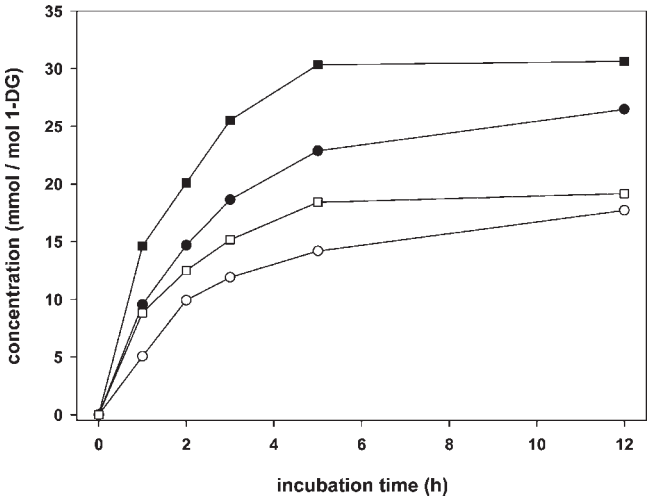


**Figure 3.** Formation of acetaldehyde (1-DG/L-alanine, 42 mM in 0.1 M phosphate buffer, pH 7.4).

13 mmol/mol 1-DG. For all three aldehydes, yields responded significantly to aeration. We also used this trapping reaction to investigate a possible Strecker degradation of the applied α-alanine. Incubations in the presence of β-alanine yielded 50% of the amount of acetaldehyde obtained in upper incubations, while concentrations of glycolaldehyde and glyceric aldehyde did not change. The postincubation trapping conditions were optimized for coinciding α-dicarbonyl structures. Experiments for the conversion of acetaldehyde, glycolaldehyde, and glyceric aldehyde monitored by authentic benzimidazols yielded only recoveries of 27% for acetaldehyde, 38% for glycolaldehyde, and 76% for glyceric aldehyde. These relatively poor yields could not be significantly improved by changing the reaction conditions. However, stringent reaction conditions led to an acceptable coefficient of variation of smaller than 5% and also avoided artifact formation (*8*, *14*, *24*). Both parameters, α-dicarbonyls and aldehydes, can therefore be quantified by the same trapping reaction.

**Degradation of 3,5-Dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (γ-Pyranone) (5).** As described above, γ-pyranone was found to be an important product formed from 1-DG. Given that γ-pyranone itself represents a reactive Maillard intermediate, one could expect that related degradation products should contribute to the overall spectrum of products formed during degradation of 1-DG. To test this hypothesis, γ-pyranone was synthesized independently and thermally treated (37 and 50 °C, respectively) in the presence of L-alanine under the same conditions as described above for 1-DG. **Figure 4** shows a total ion chromatogram obtained after silylation of an incubation sample (after 12 h at 50 °C). Interestingly, we monitored the same major products that were already found in the degradation of 1-DG. Signals were assigned to lactic acid **2**, glyceric acid **3**, erythrulose **4**, and furan-3-one **6**. Again, the formation of lactic acid and glyceric acid was preferred under aerated conditions, and the formation of glyceric acid exceeded the formation of lactic acid to some extent; for example, 30 mmol/mol γ-pyranone of glyceric acid vs 23 mmol/mol γ-pyranone lactic acid was formed after 12 h at 37 °C (aerated). At 50 °C, degradation resulted in 66 mmol/mol γ-pyranone of glyceric acid vs 61 mmol/mol γ-pyranone of lactic acid (12 h/aerated conditions). Furthermore, we verified furan-3-one **6** as a degradation product of **5**.

## DISCUSSION

Carboxylic acids represent stable degradation products formed during degradation of 1-DG and therefore in the Maillard

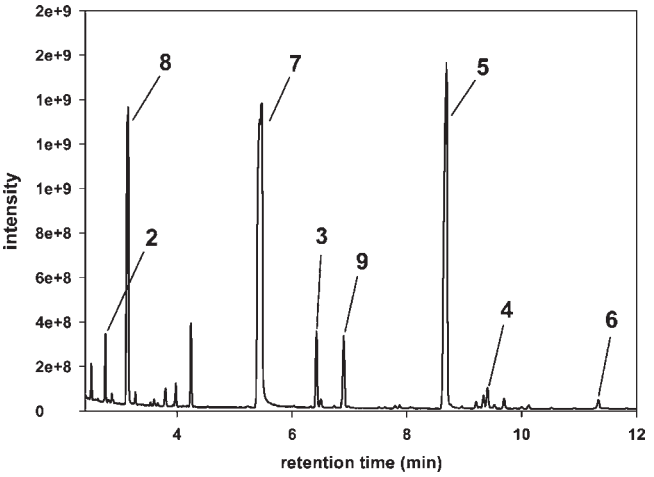


**Figure 4.** Total ion chromatogram of a γ-pyranone/L-alanine incubation mixture (42 mM in 0.1 M phosphate buffer, pH 7.4; after 12 h at 50 °C under deaerated conditions). Signals are trimethylsilyl derivatives of lactic acid **2**, L-alanine **8**, phosphoric acid **7**, glyceric acid **3**, an unknown compound **9**, γ-pyranone **5**, erythrulose **4**, and 4-hydroxy-2-(hydroxymethyl)-5-methyl-furan-3(2*H*)-one **6**.

chemistry of hexoses. The formation of glyceric acid from a glucose/glycine model system was recently reported by Davidek and co-workers (*13*). In an associated work (*15*), these authors described the formation of large amounts of acetic acid in the degradation of **1** under cooking conditions. This result was explained by a hydrolytic β-dicarbonyl cleavage of the isomeric 1-deoxyhexo-2,4-diulose induced by nucleophilic attack of a hydroxyl anion at the C-2 carbonyl function. This mechanism is also capable of explaining the formation of glyceric acid (nucleophilic attack at the C-4 carbonyl function). The corresponding counterpart in this mechanism is 1-hydroxypropan-2-one (acetol), which we established in our previous work (*8*). However, the formation of glyceric acid as well as of lactic was significantly favored under aerated conditions. This result cannot be explained on the basis of a simple hydrolytic cleavage reaction. Indeed, Davidek et al. also reported an additional oxidative α-dicarbonyl cleavage under cooking conditions (*13*). This mechanism is able to explain the formation of glyceric acid and lactic acid based on the oxidation of the isomeric 1-deoxy-hexo-3,4-diulose but was reported to occur only to negligible extent, which is in contrast to the significant yield that we found herein. In addition in our previous work, we were not able to verify erythronic acid as the corresponding counterpart of acetic acid, which is the major carboxylic acid during 1-DG degradation. A possible scheme was suggested earlier by Beck and Ledl to explain the oxygen-dependent formation of both acids. They reported on the formation of glyceric and lactic acid in a glucose/glycine model system and postulated formation as shown in **Figure 5** (*16*). In this mechanism, 1-DG, or more likely its corresponding α-oxo-enediol reductone structure, is oxidized to give the indicated tricarbonyl compound. Metal-catalyzed oxidations of Maillard intermediates have been published before (*17*). The cyclic form of this structure was suggested to undergo a β-dicarbonyl cleavage forming the glyceric acid ester of lactic acid. Hydrolysis then produces lactic acid and glyceric acid. They also suggested that the same pathway could be entered from γ-pyranone. This is in good agreement with our results on the degradation of γ-pyranone; that is, both acids were formed preferably under aerated conditions. The postulated ester was identified by Beck and Ledl during their investigation of the rather drastic oxidative treatment of γ-pyranone with iodine. These reports are in line with our

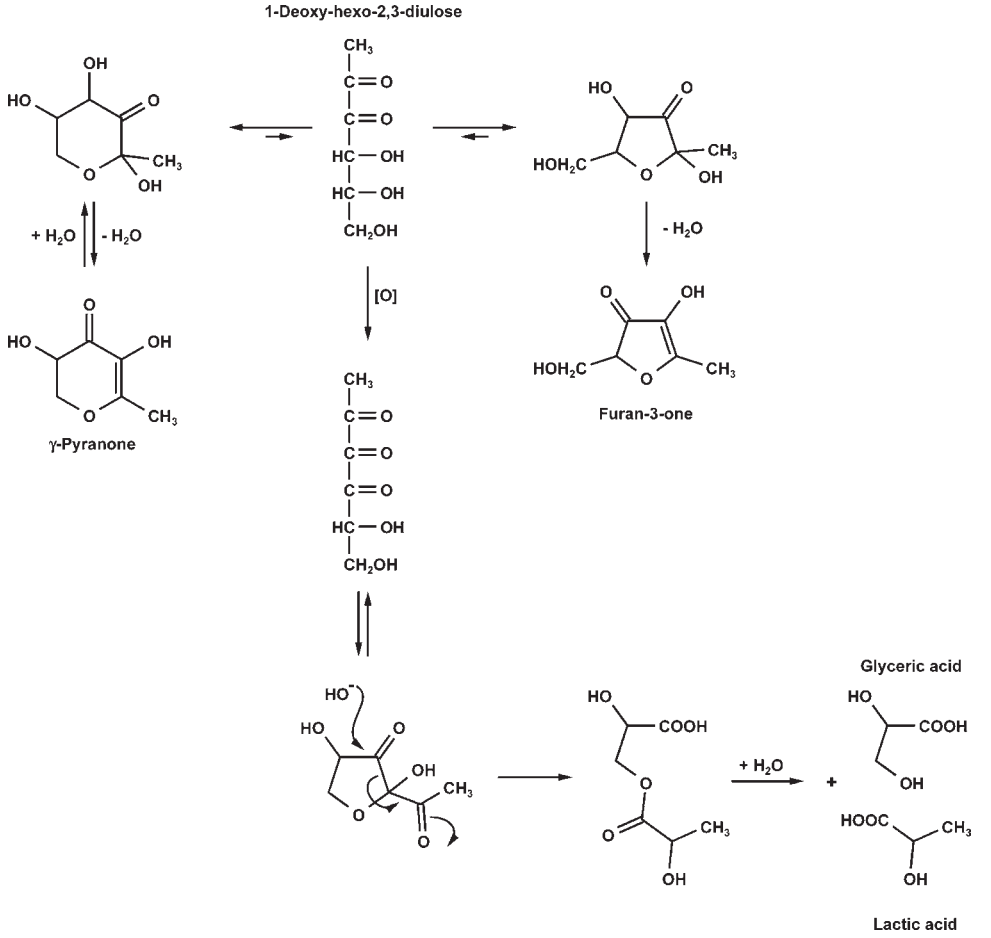


**Figure 5.** Formation of degradation products formed from **1**.

finding that $\gamma$-pyranone was capable to undergo hydration resulting in 1-DG and to give all degradation products already found during 1-DG degradation. The identification of furan-3-one (**6**) during degradation of $\gamma$-pyranone clearly verified this reaction. The change of the pyranoic to the furanoic cyclic structure can in fact only occur via participation of the open chain form of **1**. This kind of reversible hydration/dehydration was already suggested by Kim and Baltes (*9*). They investigated reactions of $^{13}$C-labeled $\gamma$-pyranone under rather drastic conditions (1 h at 150 °C in phosphate buffer pH 5.8 and roasting with sea sand up to 220 °C). Hydrolytic ring cleavage of **5** was used to explain the formation of several degradation products. An alternative reaction mechanism would be the direct $\beta$-dicarbonyl cleavage of the open chain form of the tricarbonyl structure (**Figure 6**). This mechanism requires a ketene intermediate. Hydration of these structures was indeed reported to result in carboxylic acids (*18*). However, it is not clear if such a highly reactive structure can be formed under the conditions of our experiments. Ketenes are usually generated by photolysis or pyrolysis and are useful building blocks in preparative organic chemistry (*19*−*21*). They are highly prone to nucleophilic additions; for example, reaction with alcohols readily gives esters. On the other hand, degradation of dehydroascorbic acid leads to the formation of glyoxylic acid (*22*, *23*). This reaction is in parallel to the proposed above mechanism. First, oxidation of a reductone forms the 1,2,3-tricarbonyl, which then undergoes $\beta$-dicarbonyl cleavage to give glyoxylic acid and threonic acid. Again, the most likely intermediate is a ketene. All mechanisms shown have in common that lactic acid is derived exclusively from the upper carbon skeleton of **1** (C-1 to C-3), while carbons 4−6 strictly form the backbone of glyceric acid. On the basis of our results obtained during the degradation of different $^{13}$C-labeled D-glucose isotopomers, only glyceric acid stems strictly from the lower glucose part. This is in line with above oxidation mechanisms and with nonoxidative mechanisms leading to acetol as the counterpart (*8*). To our surprise, results for lactic acid were not as definite. We observed 52% labeled lactic acid for the degradation of glucose originating from the lower carbon skeleton C-3 to C-6—a result that cannot be explained by above mechanisms. We therefore suggest that only 48% of lactic acid is formed by above direct oxidation of 1-DG, and 36% originates from oxidation of primary degradation products of 1-DG. A most likely candidate is 1-deoxythreosone (1-hydroxybutane-2,3-dione), which we already established earlier as an important structure. Oxidation of this reductone and hydrolytic dicarbonyl cleavage should readily give lactic acid and formic acid with the lactic carboxylic acid function strictly located at C-5 (**Figure 6**). The remainder 16% of lactic acid must be assigned to unknown mechanisms, obviously also due to the not complete conformity of glucose versus 1-DG reaction mixtures.

Aldehydes were thought to be formed from **1** as a result of retro-aldol fragmentation. In view of our results, this has to be ruled out because of several reasons. In the case of glyceric aldehyde, the counterpart within retroaldolization is methylglyoxal. This compound was indeed identified in the OPD-derivatized samples as its corresponding quinoxaline (data not shown). However, amounts were only negligible and could not match the formation of glyceric aldehyde. The formation of glycolaldehyde via retro-aldol fragmentation should give rise to 1-hydroxybutane-2,3-dione (1-deoxythreosone), carrying the remaining C-1 to

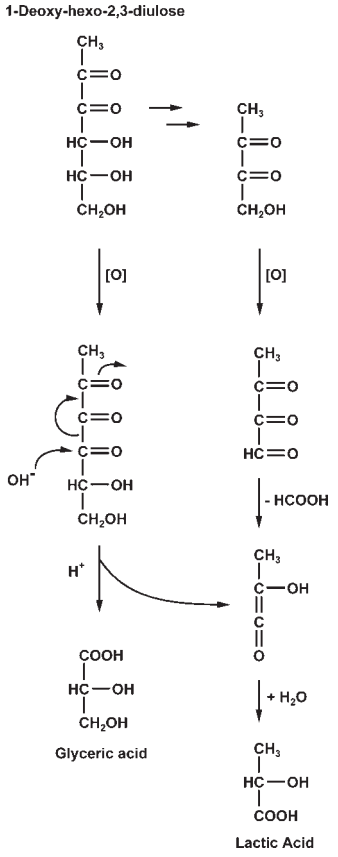


**Figure 6.** Alternative pathways of the formation of glyceric acid and lactic acid from **1**.

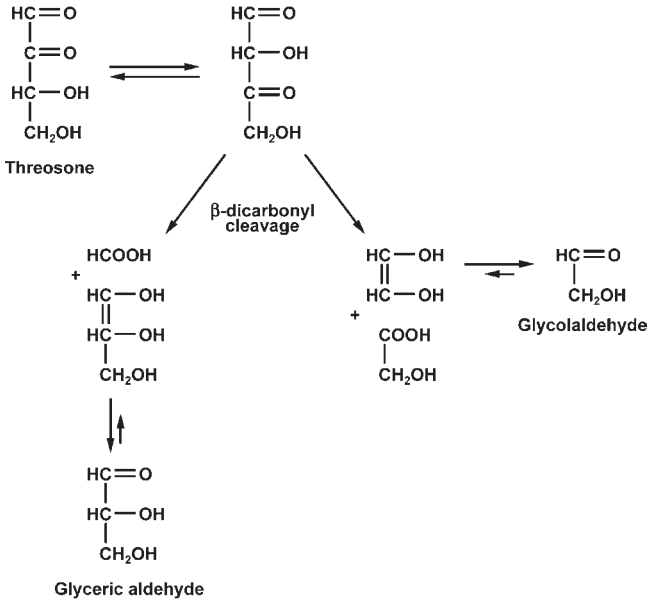


**Figure 7.** Formation of aldehydes from primary degradation products of **1**.

C-4 carbon skeleton. This is in contrast to recent experiments with [1-$^{13}$C]glucose performed in our working group (*24*), which only resulted in unlabeled 1-deoxythreosone. This clearly indicated that 1-deoxythreosone is exclusively derived from the C-3 to C-6 region of the carbon skeleton of **1**. A conformable reaction pathway was reported in our previous paper (*8*). Alternatively, glycolaldehyde and glyceric aldehyde can be explained by fragmentation of primary degradation products of **1**. **Figure 7** depicts $\beta$-dicarbonyl cleavage of 3,4-dihydroxy-2-oxo-butanal (threosone) to give both aldehydes and corresponding carboxylic acids. As threosone is formed by oxidation via an C-4-enediol from 1-DG (*8*), this mechanism thereby also explains the increase of aldehydes under aeration. Strecker degradation of the applied amino acid α-alanine would explain the formation of acetaldehyde from **1**. Experiments conducted in the presence of $\beta$-alanine indeed showed that half of the amount of acetaldehyde found originates from this reaction. However, given the rather low yields of acetaldehyde, this reaction is at most of minor importance for the degradation of **1** under the conditions covered by our experiments. Retro-aldol fragmentation of a putative 3,4-isomer of 1-DG would be a possible explanation for the formation of acetaldehyde in the absence of α-alanine, but this reaction should yield acetaldehyde and threosone in a 1:1 ratio. As above, the mismatched concentrations of both products and the independence from oxygen do not warrant this mechanism.

Oxygen-dependent reactions obviously play an important role in the direct degradation of 1-DG and therefore in the Maillard chemistry of hexoses. Furthermore, such reactions also occur from the primary degradation products of 1-DG and result in carboxylic acids as stable end products.

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**Received for review January 14, 2010. Revised manuscript received March 23, 2010. Accepted March 27, 2010.**