

Sugar Fragmentation in the Maillard Reaction Cascade: Formation of Short-Chain Carboxylic Acids by a New Oxidative α -Dicarbonyl Cleavage Pathway

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The formation of short-chain carboxylic acids was studied in Maillard model systems (90 °C, pH 6–10) with emphasis on the role of oxygen and water. The total amount of acetic acid formed did not depend on the reaction atmosphere. In the presence of labeled dioxygen or water (¹⁸O₂, H₂¹⁷O), labeled oxygen was partially incorporated into acetic acid. Thermal treatment of 1-deoxy-D-erythro-2,3-hexodiulose (**1**) and 3-deoxy-D-erythro-hexos-2-ulose in the presence of ¹⁷O-enriched water under alkaline conditions led to acetic and formic acid, respectively, as indicated by ¹⁷O NMR spectroscopy. The suggested mechanism involves an oxidative α -dicarbonyl cleavage leading to an intermediary mixed acid anhydride that releases the acids, e.g., acetic and erythronic acid, from **1**. Similarly, glyceric and lactic acids were formed from 1-deoxy-3,4-hexodiuloses, corroborated by complementary analytical techniques. This paper provides for the first time evidence for the direct formation of acids from C₆- α -dicarbonyls by an oxidative mechanism and incorporation of a ¹⁷OH group into the carboxylic moiety. The experimental data obtained support the coexistence of at least two newly described reaction mechanisms leading to carboxylic acids, i.e., (i) a hydrolytic β -dicarbonyl cleavage as a major pathway and (ii) an alternative minor pathway via oxidative α -dicarbonyl cleavage induced by oxidizing species.

KEYWORDS: Maillard reaction; acetic acid; glyceric acid; oxidative α -dicarbonyl cleavage; hydrolytic β -dicarbonyl cleavage; labeling studies

INTRODUCTION

The degradation of sugars to low molecular weight compounds has been widely studied in aqueous alkaline solutions (1–5) and in the presence of amines (6–8), basically resulting in carboxylic acids (e.g., lactic, glycolic, formic, acetic, pyruvic, erythronic, threonic, and saccharinic acids) and carbonyl compounds (e.g., methylglyoxal, acetol, reductones, and 2,3-butanedione). There are three main reaction mechanisms operating in most instances: (i) benzilic acid type rearrangement, (ii) retro-aldol cleavage, and (iii) splitting of dicarbonyl intermediates. While the first two mechanisms are well-understood and experimentally proven, sugar fragmentation via α -dicarbonyl splitting remains largely hypothetical.

Considering the large number of citations in the literature, the hydrolytic α -dicarbonyl cleavage mechanism seems to be well-established. It has repeatedly been employed to explain the formation of acids (e.g., acetic and formic acids) from sugars or sugar intermediates under alkaline conditions (5, 9), upon

roasting (10), pressure cooking (11), and through the Maillard reaction cascade in the presence of amino acids (12, 13). Ginz and co-workers (10) reported some not expected ¹³C NMR signals of labeled acetic acid obtained under rather drastic reaction conditions (240 °C, 15 min), which could not be explained by the hydrolytic α -dicarbonyl cleavage mechanism. Tressl and Rewicki (14) suggested a disproportionation process to explain the formation of acetic acid by an α -dicarbonyl cleavage of 1-deoxy-2,3-hexodiulose (**1**) “with, up to now, obscure mechanism”. Interestingly, none of the research groups mentioned above has made an attempt to prove this hypothesis, for example, by identifying the corresponding carbonyl compound, i.e., erythrose, as the C₄ counterpart of acetic acid in reaction systems based on glucose.

The splitting of β -dicarbonyl sugars was first proposed by Hayami (15) as an alternative, and later confirmed by Weenen (16), to explain the formation of acetol and glyceric acid from pentose and hexose sugars. In general, this mechanism has rarely been used in mechanistic studies, despite the fact that it occurs in a wide pH range (pH 3–11) and is favored in the presence of phosphate buffer. Recently, Davidék and co-workers (17) reported strong evidence for the hydrolytic β -dicarbonyl cleav-

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age as a major sugar degradation pathway. However, the experimental data have also indicated alternative mechanisms, pointing to oxidative sugar degradation yielding various carboxylic acids.

This paper deals with the formation of short-chain carboxylic acids through Maillard type reactions under cooking conditions. An in-depth mechanistic study was performed to elucidate a minor pathway of acid formation and to clarify the validity of the hydrolytic α -dicarbonyl cleavage hypothesis leading to carboxylic acids.

MATERIALS AND METHODS

Materials. The following chemicals of analytical grade were commercially available: glycine, D-(+)-glucose, L-threonic acid (hemicalcium salt), D-erythronic acid γ -lactone, *N,N*-dimethylformamide, 1-(trimethylsilyl)-1*H*-imidazole, *N,O*-bis(trimethylsilyl)trifluoroacetamide, lactic acid, L-glyceric acid (hemicalcium salt), methoxamine hydrochloride, and ammonium formate (Fluka/Aldrich, Buchs, Switzerland); monosodium dihydrogenphosphate, disodium monohydrogenphosphate, and phosphoric acid (85%) (Merck, Darmstadt, Germany); acetonitrile (Mallinckrodt Baker, Deventer, Holland); D-[3-¹³C]glucose (99%), D-[6-¹³C]glucose (99%), D-[1,2-¹³C₂]glucose (99%), ¹⁸O-dioxygen (¹⁸O₂, 93–94%), H₂¹⁷O (10%-enriched), and H₂¹⁸O (10%-enriched) (Cambridge Isotope Laboratories, Andover, MA); [2,2,2-³H₃]-acetic acid (Isotec, Miamisburg, OH); 3-deoxy-D-erythro-hexos-2-ulose (2) (Toronto Research Chemicals, Ontario, Canada); sodium hydroxide (NaOH) 46–48% solution (Fisher Scientific, Pittsburgh, PA); ultrapure deionized water (specific resistivity ≥ 18.2 M Ω ·cm, Milli-Q-system, Millipore, Bedford, MA); argon (99.998% purity) (Carbagas, Lausanne, Switzerland). 1-Deoxy-D-erythro-2,3-hexodiulose (1) was synthesized as recently described (18).

Degradation of Glucose. A solution of glycine (0.3 mmol) and glucose (0.3 mmol) in phosphate buffer (3 mL, 0.2 mol/L, pH 8) was placed in a flask (10 mL) under argon. The solution was degassed by three cycles of vacuum and argon. Finally, the solution was bubbled with argon (10 min) and heated in a silicone bath (90 °C, 15 h). Similar experiments were performed replacing argon by ¹⁸O-dioxygen or air and water by ¹⁸O-enriched water (10%). All experiments were performed at atmospheric pressure. Each sample was prepared at least in duplicate.

Quantification of C₄-Aldonic Acids. After the reaction was cooled to room temperature (RT), an aliquot of the reaction mixture (0.1 mL) was diluted 10 times with water and directly analyzed by high-performance anion exchange chromatography (HPAEC). Each sample was injected twice.

Quantification of Acetic Acid by Stable Isotope Dilution Assay (19). An aliquot of the reaction mixture (0.3 mL) was spiked with a defined amount of [²H₃]acetic acid in water (600 μ g), acidified to pH 3 with phosphoric acid (85%), and immediately extracted with diethyl ether (1 mL, 1 min). The extract was dried over anhydrous sodium sulfate and analyzed by gas chromatography–mass spectrometry (GC-MS). Each sample was injected twice. An independent experiment with unlabeled acetic acid and ¹⁸O-enriched water confirmed that under the conditions used in this study, there was no exchange between acetic acid and ¹⁸O-enriched water.

Identification of C₄-Aldonic Acids, Glyceric Acid, and Lactic Acid. An aliquot of the reaction mixtures (0.2 mL) was freeze-dried, and the residue was dissolved in a mixture of acetonitrile and *N,N*-dimethylformamide (0.2 mL, 1:1, v/v). *N*-(Trimethylsilyl)imidazole (0.15 mL) and *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.15 mL) were added, and the mixture was treated in an ultrasonic bath (10 s). Finally, the mixture was heated (70 °C, 10 min) and analyzed by GC-MS (20).

Samples for ¹⁷O NMR Experiments. A solution of glycine (0.35 mmol) and D-glucose, 3-deoxy-D-erythro-hexos-2-ulose (2), or 1-deoxy-D-erythro-2,3-hexodiulose (1), each 0.35 mmol, in ¹⁷O-enriched water (10%, 3.5 mL) was adjusted to pH 10 with NaOH in a flask (10 mL) and thermally treated in a silicone bath (90 °C, 3 h). After the reaction was cooled to RT, an aliquot of the reaction mixture (0.7 mL) was directly analyzed by ¹⁷O NMR spectroscopy (18).

GC-MS. Acetic Acid. The analyses were performed on a GC 6890A coupled to an MSD 5973 (both Agilent, Palo Alto, CA) using a DB-Wax capillary column (30 m \times 0.25 mm; film thickness, 0.25 μ m; J&W Scientific, Folsom, CA). The operating conditions were as previously described for method 1 (17). Quantification was performed by stable isotope dilution assay (19) in the scan mode by measuring the molecular ions of analyte and labeled internal standard. The naturally occurring percentage of ¹³C (1.10%) was considered in the calculations.

Trimethylsilyl (TMS) Derivatives of C₄-Aldonic Acids, Glyceric Acid, and Lactic Acid. The analyses were performed on a GC 6890A coupled to an MSD 5973 (both Agilent) using a HP-PONA capillary column (50 m \times 0.20 mm; film thickness, 0.50 μ m; Agilent). The operating conditions were as previously described for method 2 (17).

HPAEC. This was performed on a Dionex ion chromatography system (DX-500, Dionex, Sunnyvale, CA) composed of an autosampler (model AS-50 with a 25 μ L sample loop), a gradient pump (model GP-50) with on-line degas, and an electrochemical detector (model ED-40) as described in ref 21 with some modifications.

Analysis of Erythronic and Threonic Acid. The separation was accomplished on a CarboPac PA1 anion exchange column (250 mm \times 4 mm i.d.) and a CarboPac PA1 guard column (50 mm \times 4 mm i.d.) (Dionex). The eluents used were water (A) and NaOH (300 mmol/L, B) with the following gradient: 0–35 min, 0–100% B. Each analytical cycle was followed by cleaning and a regeneration phase of the column with NaOH (300 mmol/L, 15 min) and equilibration of the column using the initial gradient conditions (10 min). The flow rate was 1 mL/min. Erythronic acid (RT = 18.9 min) and threonic acid (RT = 19.5 min) were quantified with an electron capture detector (ECD) equipped with a gold working electrode. The electrode pulse potentials were as follows: E1 = 0.1 V, 0–400 ms; E2 = –2.0 V, 410–420 ms; E3 = 0.6 V, 430 ms; and E4 = –0.1 V, 440–500 ms. Quantification was based on calibration curves by comparing the peak area with that of standard solutions containing known amounts of pure compounds. Each sample was injected twice. The solutions and eluents were prepared using ultrapure deionized water (specific resistivity ≥ 18.2 M Ω cm). NaOH solutions used as eluents were prepared by diluting a carbonate free 50–52% (w/w) NaOH solution in water previously degassed with helium gas.

NMR Spectroscopy. ¹⁷O NMR spectra were recorded on a Bruker DPX-360 spectrometer, equipped with a 5 mm BBO grad probe head operating at 48.82 MHz (18). The instrumental setting was as follows: spectral width, 100 kHz; 8K data points; 90° pulse angle; 9.90 μ s; 200 ms acquisition delay; 41 ms acquisition time; and 70000–120000 scans were required. The spectra were recorded with sample spinning, without lock at RT. The pulse sequence was P1 (90°), P2 (90°), and P3 (90°). The signal-to-noise ratio was improved by applying a 20 Hz exponential broadening factor to the FID (free induction decay) prior to the Fourier transformation. Samples obtained from model reactions were diluted 20 times with nonlabeled water prior to ¹⁷O NMR spectroscopy. Water was used as a reference for calibrating the chemical shift (δ 0.00 ppm).

RESULTS AND DISCUSSION

As recently reported (17), thermal degradation of 2,4-pentanedione (120 °C, pH 6–10, up to 4 h) resulted in almost equimolar amounts of acetic acid and acetone, which was explained by a hydrolytic β -dicarbonyl cleavage mechanism. Similar trials with 2,3-pentanedione led to significantly lower but still nearly equimolar amounts of acetic acid and propanoic acid. It was assumed that this minor degradation pathway might proceed via an oxidative α -dicarbonyl cleavage mechanism (Figure 1A) as no acetaldehyde or propionaldehyde was detected, which would have supported a hydrolytic mechanism. In analogy, an oxidative α -dicarbonyl cleavage of 1-deoxy-D-erythro-2,3-hexodiulose (1) should lead to acetic acid and erythronic acid (Figure 1B).

Role of Molecular Oxygen. To evaluate the validity of this hypothesis, specifically designed experiments were performed to study the formation of acetic acid from glucose heated in

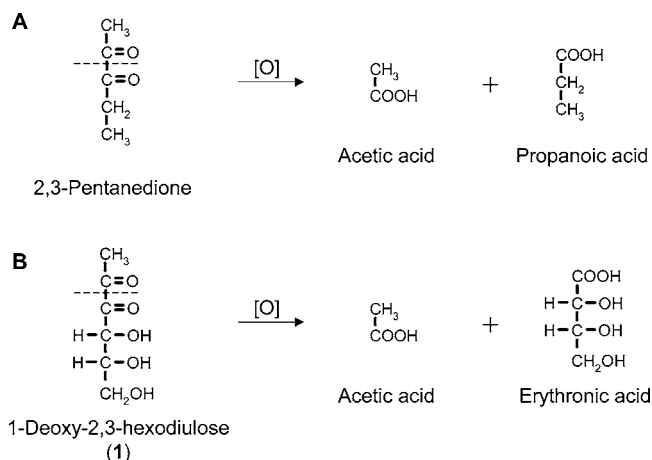


Figure 1. Schematic presentation of the formation of (A) acetic acid and propanoic acid from 2,3-pentanedione and (B) acetic acid and erythronic acid from 1-deoxy-D-erythro-2,3-hexodiulose (1) by an oxidative α -dicarbonyl cleavage pathway.

Table 1. Formation of Acetic Acid from Glucose Heated in the Presence of Glycine under Air, Argon, and ^{18}O -Dioxygen

reaction systems ^a	concentration of acetic acid (mmol/L) ^b		
	nonlabeled	labeled	total
D-glucose/glycine/argon	30.5 ± 0.4	0	30.5 ± 0.4
D-glucose/glycine/air	32.3 ± 3.3	0	32.3 ± 3.3
D-glucose/glycine/ $^{18}\text{O}_2$	22.9 ± 0.3 ^c	10.6 ± 0.2 ^c	33.5 ± 0.5

^a Glycine (0.3 mmol) and D-glucose (0.3 mmol) were reacted (15 h, 90 °C) in a phosphate buffer (3 mL, 0.2 mol/L, pH 8) at atmospheric pressure. ^b The data obtained by GC-MS showed variation coefficients of ≤10%. ^c MS data of acetic acid isotopomers as m/z (%): 43 (100), 45 (95), 47 (45), 60 ($^{16}\text{O-M}^+$), 50), 62 ($^{18}\text{O-M}^+$, 35).

the presence of glycine (15 h, 90 °C, pH 8) under air, argon, and ^{18}O -dioxygen. As shown in **Table 1**, independently of the atmosphere in which the reaction was carried out, the total amount of acetic acid formed was about 32 mol %. Interestingly, the MS spectrum of acetic acid formed in the presence of ^{18}O -dioxygen contained the ions at m/z 60 and m/z 62, corresponding to the molecular ions of unlabeled and mono- ^{18}O -labeled acetic acid, respectively. The MS data clearly indicated that only one ^{18}O atom was incorporated into acetic acid. The concentration of ^{18}O -labeled acetic acid was about half of that of nonlabeled acetic acid.

Following the hypothesis of an oxidative α -dicarbonyl cleavage pathway, C_4 -aldonic acids were expected as counterparts of acetic acid formed from glucose. Indeed, erythronic acid has been reported as a sugar degradation product, however, always under oxidative alkaline conditions, e.g., from cellobiose (22) and pentose sugars (1). The latter authors also found threonic, glyceric, glycolic, and formic acids. Moreover, Ishizu et al. (23) found glucosaccharinic acids and erythronic acid as degradation products of **1** in an aqueous alkaline solution at RT in the presence of air.

HPAEC-ECD analysis (**Figure 2**) indicated several peaks of which two could be attributed to C_4 -aldonic acids, the expected counterpart of acetic acid formed from glucose. The presence of erythronic and threonic acids was verified by the reference compounds, thus supporting the oxidative α -dicarbonyl cleavage hypothesis. An unequivocal identification of erythronic and threonic acid was achieved by GC-MS after TMS derivatization. Mass spectral data of the TMS derivatives of erythronic and

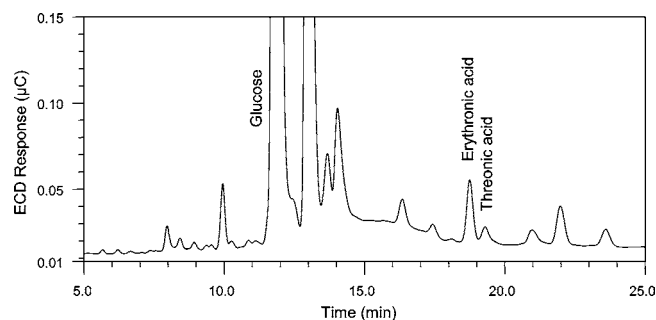


Figure 2. HPAEC-PAD chromatogram of a model reaction system containing glucose and glycine heated in the presence of ^{18}O -dioxygen (90 °C, 15 h, pH 8) showing erythronic and threonic acid as reaction products.

threonic acids formed from D-glucose under ^{18}O -dioxygen or from selected ^{13}C -labeled glucose isotopomers under air are listed in **Table 2**. Only unlabeled C_4 -aldonic acids were formed from the [1,2- $^{13}\text{C}_2$]glucose isotopomer, as indicated by m/z 409. On the other hand, the [3- ^{13}C]glucose and [6- ^{13}C]glucose isotopomers formed only singly labeled C_4 -aldonic acids (m/z 410). The [3- ^{13}C]glucose isotopomer formed erythronic and threonic acids labeled at the carboxyl group (C-1 atom). This is in agreement with the ions at m/z 221 and m/z 293, both of them containing the C-1 carbon atom, which were formed by a McLafferty type rearrangement involving a γ -hydrogen atom and the β -TMS group, respectively (24). The [6- ^{13}C]glucose isotopomer formed erythronic and threonic acids labeled at the terminal hydroxymethyl group, as indicated by the ion at m/z 206 representing vicinal diol end groups. In the presence of ^{18}O -dioxygen, glucose formed both unlabeled and ^{18}O -labeled erythronic and threonic acids (m/z 409, 411). The ions at m/z 222 and 294 clearly indicate that ^{18}O was incorporated into the carboxyl group of both acids. The ratio between labeled and unlabeled aldonic acids was 1/3 to 2/3, respectively. Only traces of lactones corresponding to the aldonic acids were found by the silylation method (data not shown). Contrary to acetic acid, the total concentration of the two C_4 -aldonic acids was dependent on the atmosphere in which the reaction was performed and reached about 0.7, 2.2, and 5.1 mmol/L under argon, air, and ^{18}O -dioxygen, respectively. This may be due to preferred sugar degradation by β -dicarbonyl cleavage, which does not release aldonic acids.

Mechanism of the Oxidative α -Dicarbonyl Cleavage. The formation of short-chain carboxylic and aldonic acids was further studied by ^{17}O NMR in model systems containing glucose or C_6 -deoxy- α -dicarbonyl intermediates. Glucose/glycine, 1-deoxy-D-erythro-2,3-hexodiulose (**1**), or 3-deoxy-D-erythro-hexos-2-ulose (**2**) was reacted in an aqueous solution, using 10% ^{17}O -enriched water, under alkaline conditions (pH 10) at 90 °C for 180 min. Alkaline pH conditions were chosen to minimize oxygen exchange between water and acids. To confirm this assumption, unlabeled acetic acid was heated under the same experimental conditions (90 °C, 180 min, pH 10) in 10% ^{17}O -enriched water. No oxygen exchange between acetic acid and ^{17}O -enriched water could be observed by GC-MS (data not shown) under the experimental conditions used, thus confirming the validity of the results presented below.

The corresponding ^{17}O NMR spectra are shown in **Figure 3**, indicating various compounds with ^{17}O incorporated. Thanks to ^{17}O enrichment, the spectra were relatively clear despite the complex composition of the well-colored sample. The observed signals corresponded only to oxygen originating from ^{17}O -enriched water, which provided a direct insight into the

Table 2. Characteristic Mass Spectra (MS) Fragments of TMS Derivatives^a of Erythronic (E-OH) and Threonic (T-OH) Acid Formed from Glucose and Selected ¹³C-Labeled Glucose Isotopomers under Air or ¹⁸O-Dioxygen

$$\begin{array}{c}
 \text{COOTMS} \\
 | \\
 \text{a} \text{---} \text{C} \text{---} \text{HOTMS} \text{---} \text{b} \\
 | \\
 \text{C} \text{---} \text{HOTMS} \\
 | \\
 \text{CH}_2\text{OTMS}
 \end{array}$$

reaction systems ^b	mass spectra data, <i>m/z</i> (%) ^c									
		[M - 15] ⁺	[M - 15 - TMSOH] ⁺	[a + TMS] ⁺ ^d	[a + H] ⁺ ^d	[b] ⁺				
[1,2- ¹³ C ₂]glucose/glycine/air	E-OH:	409 (2)	319 (2)	292 (62)	220 (29)	205 (27)				
	T-OH:	409 (2)	319 (2)	292 (51)	220 (25)	205 (21)				
[3- ¹³ C]glucose/glycine/air	E-OH:	410 (2)	320 (4)	293 (74)	221 (40)	205 (31)				
	T-OH:	410 (1)	320 (6)	293 (48)	221 (22)	205 (23)				
[6- ¹³ C]glucose/glycine/air	E-OH:	410 (2)	320 (2)	292 (65)	220 (31)	206 (22)				
	T-OH:	410 (2)	320 (5)	292 (56)	220 (28)	206 (15)				
D-glucose/glycine/ ¹⁸ O ₂	E-OH:	411 (1)	409 (1)	321 (1)	319 (2)	294 (29)	292 (48)	222 (12)	220 (24)	205 (29)
	T-OH:	411 (1)	409 (1)	321 (2)	319 (4)	294 (17)	292 (46)	222 (8)	220 (24)	205 (24)

^a The carboxylic group (C-1) of the C₄-aldonic acid TMS derivatives corresponds to the C-3 of glucose. ^b Glycine (0.3 mmol) and D-glucose or various ¹³C-labeled glucose isotopomers (0.3 mmol) were reacted in a phosphate buffer (3 mL, 0.2 mol/L, pH 8; 15 h, 90 °C). ^c Only major and characteristic ions are shown. Characteristic signals marked in bold are discussed in the text. ^d McLafferty rearrangement products.

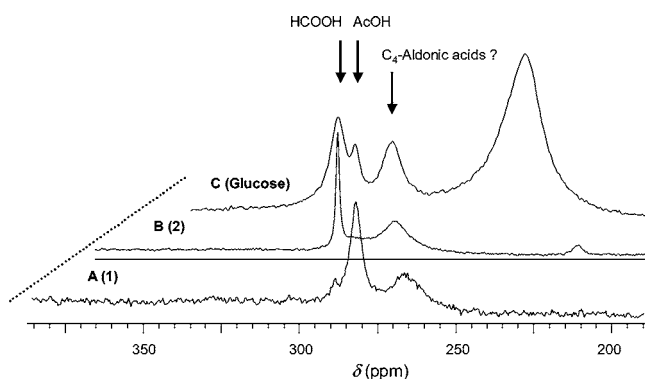


Figure 3. ¹⁷O NMR spectra obtained by heating (90 °C, 180 min, pH 10) in ¹⁷O-enriched water: (A) 1-deoxy-D-erythro-2,3-hexodiulose (1), (B) 3-deoxy-D-erythro-hexos-2-ulose (2), and (C) glucose with glycine. HCOOH, formic acid; AcOH, acetic acid.

degradation mechanism. Signals were neither detected in the carbonyl range (+545 to +625 ppm) nor in the alcohol range (-50 to +70 ppm). These data indicate that ¹⁷O did not exchange with the initial functional groups of the starting materials and that the oxygen from water was not incorporated into the alcohol or carbonyl groups.

Spiking experiments with acetic acid revealed the signal at δ 282 ppm as ¹⁷O-labeled acetic acid generated from **1** in sample A (18). Similarly, the signal at δ 289 ppm in sample B could be confirmed as formic acid formed by decomposition of **2**. Both signals were also observed in sample C obtained by glycine-induced degradation of glucose under the same reaction conditions. Additional signals were found, e.g., at δ 265 ppm, which may correspond to aldonic acids, as suggested by the data reported in **Table 2**. Spiking experiments with aldonic acids showed only weak ¹⁷O NMR signals (data not shown); however, their presence was already confirmed by HPAEC (**Figure 2**) and GC-MS after TMS derivatization (**Table 2**). On the other hand, the ¹⁷O NMR data provided unequivocal evidence for the incorporation of oxygen from water into the Maillard reaction products acetic and formic acid, which were at least partially labeled.

The role of molecular oxygen and water was further studied by GC-MS using ¹⁸O₂ and/or ¹⁸O-enriched water. Heating

glycine and glucose (90 °C, 15 h, pH 6) in water under ¹⁸O₂ resulted in a mixture of unlabeled and mono-¹⁸O-labeled acetic acid, as indicated by the molecular ions at *m/z* 60 (¹⁶O-M)⁺ and *m/z* 62 (¹⁸O-M)⁺: *m/z* 43 (100), 45 (95), 47 (40), 60 (70), and 62 (35), thus suggesting the incorporation of one oxygen atom into acetic acid. On the other side, reacting glycine and glucose in ¹⁸O-enriched water under ¹⁸O₂ also gave rise to a mixture of unlabeled and mono-¹⁸O-labeled acetic acid. Thus, independently of the reaction conditions, only monooxygen-labeled acetic acid was found in this study, suggesting the incorporation of only one ¹⁸O atom into acetic acid.

These data confirmed the necessity of molecular oxygen to initiate the oxidative α -dicarbonyl cleavage mechanism. The experimental findings can be explained by the hypothetical mechanism shown in **Figure 4**. Depending on the attack of dioxygen at the C-3 or C-2 carbonyl group of **1**, the two alkoxyradicals **3a** and **3b** can be assumed, both containing ¹⁸O isotopes. Single electron transfer reactions may then lead to the corresponding hydroperoxide anions **4a** and **4b**, which rearrange to a mixed acid anhydride **5**. The result of this Baeyer–Villiger type rearrangement is an asymmetric acid anhydride (**5**) bearing one ¹⁸O atom, while the second ¹⁸O atom of molecular oxygen is released as a hydroxyl anion. Hydrolysis of the monooxygen-labeled acid anhydride **5** in the presence of unlabeled water should give rise to a mixture of unlabeled and monooxygen-labeled acetic acid and unlabeled and monooxygen-labeled C₄-aldonic acids.

The mechanism is in good agreement with the experimental data obtained. This dioxygen-triggered degradation pathway explains well (i) the ¹⁷O NMR signals obtained with **1**, (ii) the labeling pattern of the TMS derivatives of erythronic and threonic acids, and (iii) the formation of acetic and propanoic acids from 2,3-pentanedione. The fact that only one labeled oxygen atom was found in acetic acid, while the other oxygen atom originated from the sugar, indirectly proves the mechanism proposed. However, this mechanism is most likely a minor pathway, as compared to the hydrolytic β -dicarbonyl cleavage, leading to acetic acid and carbonyl type sugar degradation products.

Formation of Other Acids. The silylation experiments also revealed the presence of small amounts of lactic and glycolic acids (data not shown), which have previously been reported

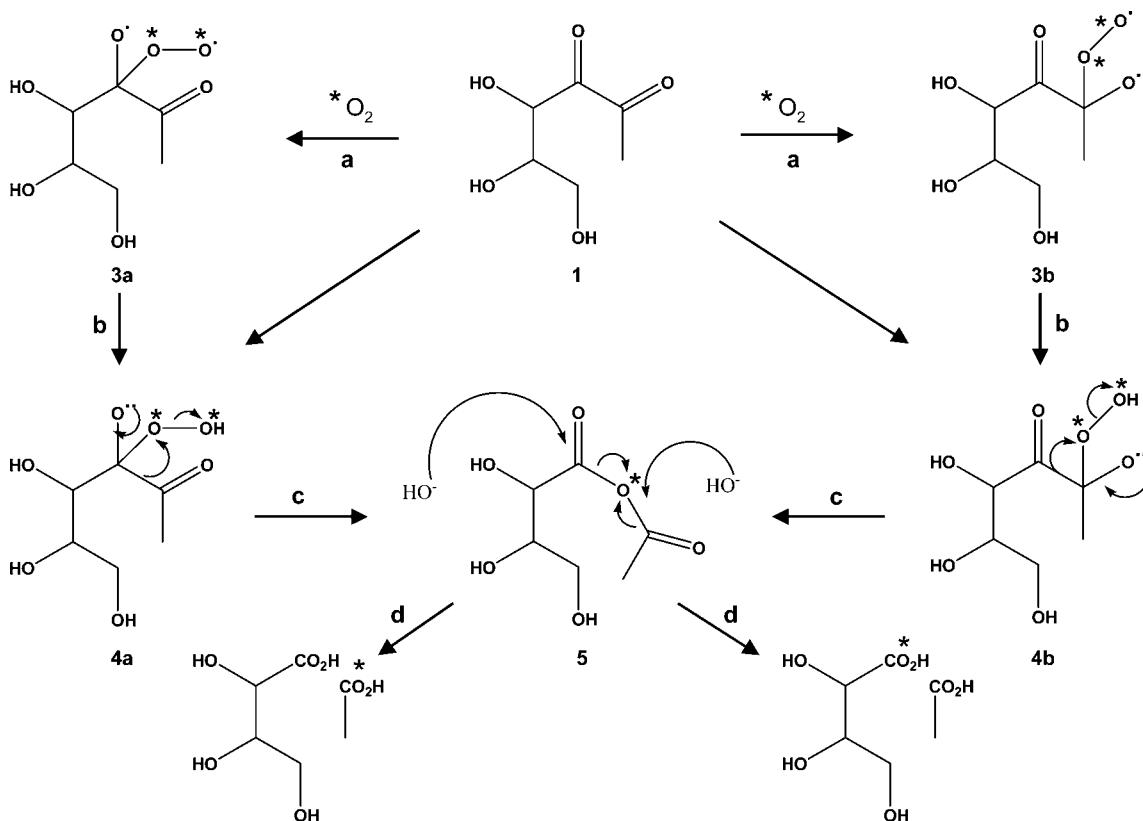


Figure 4. Hypothetical mechanism explaining the formation of acetic acid and C₄-aldonic acids by decomposition of 1-deoxy-2,3-hexodiulose (1). Key: a, incorporation of molecular oxygen; b, single electron transfer reaction; c, Baeyer–Villiger type rearrangement; and d, hydrolysis. An asterisk indicates labeling with ¹⁷O or ¹⁸O from ¹⁷O-enriched water and ¹⁸O₂, respectively.

Table 3. Characteristic Mass Spectra (MS) Fragments of TMS Derivatives^a of Glyceric Acid Formed from Glucose and Selected ¹³C-Labeled Glucose Isotopomers under Air or ¹⁸O-Dioxygen

reaction systems ^b	characteristic fragments, <i>m/z</i> (%) ^c					
	[M – 15] ⁺	[a + TMS] ⁺ ^d	[c] ⁺	[M – 15 – 118] ⁺ ^e	[c + H – OTMS] ⁺	[b] ⁺
[1,2- ¹³ C ₂]glucose/glycine/air	307 (5)	292 (39)	205 (16)	189 (40)	117 (9)	103 (18)
[3- ¹³ C]glucose/glycine/air	307 (7)	292 (45)	205 (19)	189 (55)	117 (13)	103 (21)
[6- ¹³ C]glucose/glycine/air	308 (6)	292 (40)	206 (17)	190 (48)	118 (9)	104 (18)
D-glucose/glycine/ ¹⁸ O ₂	309 (3)	307 (6)	294 (18)	292 (40)	205 (18)	189 (55)

^a The carboxylic group (C-1) of the glyceric acid TMS derivatives corresponds to the C-4 of glucose. ^b Glycine (0.3 mmol) and D-glucose or various ¹³C-labeled glucose isotopomers (0.3 mmol) were reacted in a phosphate buffer (3 mL, 0.2 mol/L, pH 8; 15 h, 90 °C). ^c Only major and characteristic ions are shown. Characteristic signals marked in bold are discussed in the text. ^d McLafferty rearrangement products. ^e Most probably formed from fragment [M – 15]⁺ by elimination of CO and TMSOH (24).

(10). In addition, glyceric acid was identified in our samples, the relative amounts of which were comparable to those of C₄ acids based on peak areas. The formation of glyceric acid has been suggested to proceed via β-cleavage of 1-deoxy-2,4-hexodiulose (15, 16). However, it may also be generated by oxidative α-dicarbonyl cleavage, as indicated by our experimental data. Both unlabeled and ¹⁸O-labeled glyceric acids were formed in the presence of ¹⁸O-dioxygen (Table 3). The ion at *m/z* 294, formed by a McLafferty type rearrangement involving the β-TMS group, clearly indicated that ¹⁸O was incorporated into the carboxyl group. The ratio between labeled and unlabeled glyceric acid was 1/3 to 2/3, respectively. The [6-¹³C]glucose isotopomer formed glyceric acid labeled at the terminal hydroxymethyl group, as indicated by the ion at *m/z* 206 formed

from vicinal diol end groups. Only unlabeled glyceric acid was formed from the [1,2-¹³C₂]glucose and [3-¹³C]glucose isotopomers. Thus, glyceric acid was exclusively formed from C-4, C-5, and C-6 atoms of glucose (Table 3). Similarly to the formation of acetic acid and C₄-aldonic acids, the levels of lactic acid were much lower than those of glyceric acid (data not shown). A small part of lactic acid (about 20%) was ¹⁸O-labeled.

MECHANISTIC CONSIDERATIONS

The experimental data shown in this study and in our previous paper (17) suggest at least two competing pathways leading to short chain acids in the course of the Maillard reaction cascade, i.e., (i) a minor oxidative α-dicarbonyl splitting and (ii) a major

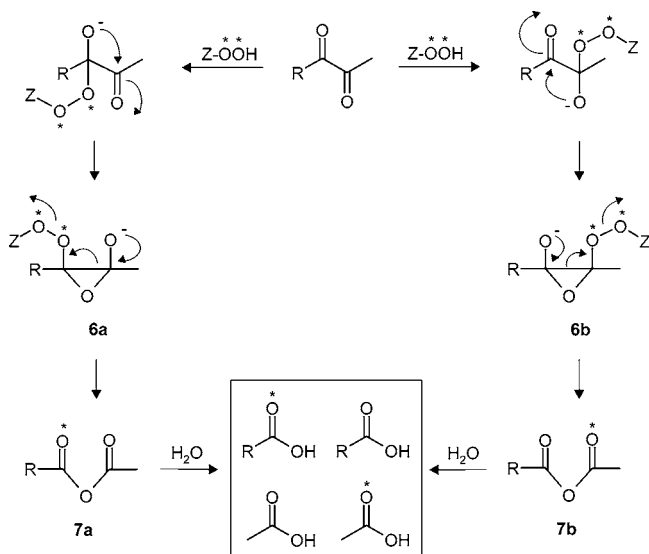


Figure 5. Alternative hypothetical mechanism for the formation of acetic acid and C_4 -aldonic acids ($R = \text{CHOH-CHOH-H}_2\text{OH}$) through oxidation by hydroperoxides. For an explanation, see the text. An asterisk indicates labeling with ^{18}O -labeled oxygen from $^{18}\text{O}_2$.

hydrolytic β -dicarbonyl cleavage. The minor oxidative pathway combines an O_2 -sensitive oxidation step of α -dicarbonyls leading to alkoxyradicals (25) with a base-catalyzed Baeyer–Villiger reaction of hydroperoxide anions (26), giving rise to a monolabeled mixed acid anhydride as the key intermediate. The oxidizing species may be molecular oxygen activated by melanoidins as sensitizers (photooxidation) or other oxidizing agents (e.g., hydroperoxides) derived via concomitant autoxidation of glucose catalyzed by traces of transition metals. A similar direct oxidative cleavage of α -dicarbonyls with peroxides (KHSO_5) has recently been reported in preparative organic synthesis (27), involving the intermediacy of peroxyhemiacetals, which upon Baeyer–Villiger type rearrangements give rise to an anhydride and finally to the carboxylic acid function.

Alternatively, the reaction may proceed via epoxiperoxide type intermediates (Figure 5). Depending on the carbonyl at which the attack of the hydroperoxide takes place, two epoxiperoxides (6a and 6b) can be formed, which react to the mixed anhydrides 7a and 7b, respectively. The electrophilic power generated by potential heterolysis of the oxygen–oxygen bond is the probable driving force for the peroxide cleavage (28). Hydrolysis of 7 gives rise to acids, which is also well in line with the labeling results obtained with $^{18}\text{O}_2$. The difference between both pathways lies in the labeling position of the intermediate, i.e., anhydride oxygen (Figure 4) and carbonyl oxygen (Figure 5). Thus, it is suggested that acid formation from α -dicarbonyls in the Maillard reaction goes via an oxidative mechanism, involving a Baeyer–Villiger type rearrangement and/or a peroxide cleavage of an epoxiperoxide type intermediate. In general, this type of reaction mechanisms is proposed for the first time in the context of the Maillard reaction cascade. However, the postulated intermediates, i.e., mixed acid anhydrides and epoxiperoxides, remain hypothetical for the time being.

In agreement with that, the reactions A1 and A2 (Figure 6) resulted in acetic and erythronic acids as well as glyceric and lactic acids, respectively. All four acids have unequivocally been identified in this study, thus corroborating the hypothesis of the oxidative α -dicarbonyl cleavage. A facile isomerization of 1-deoxy-2,3-hexodiulose to 1-deoxy-2,4-hexodiulose can be expected due to the well-known carbonyl mobility in Maillard

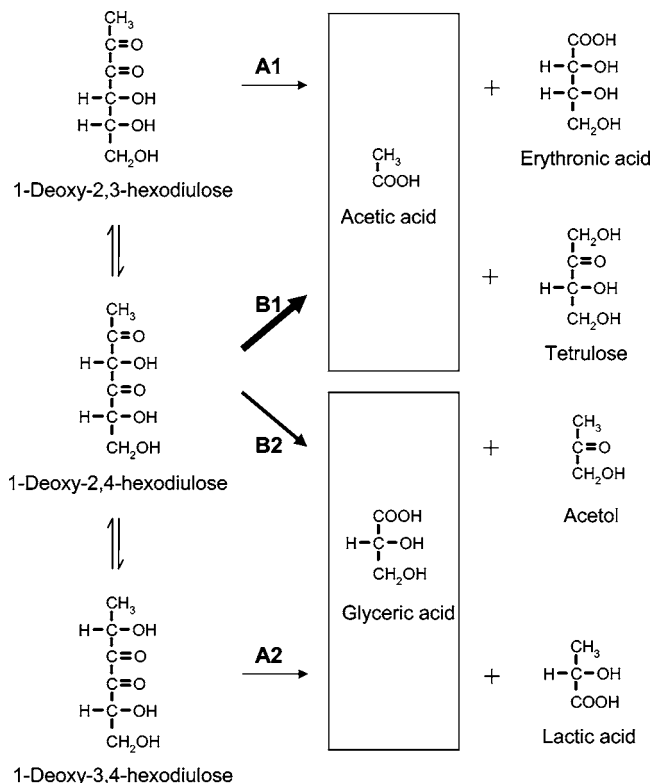


Figure 6. Scheme summarizing possible pathways of sugar fragmentation via 1-deoxyhexodiuloses. A1 and A2, oxidative α -dicarbonyl cleavage; B1 and B2, hydrolytic β -dicarbonyl cleavage by nucleophilic attack of OH^- at the C-2 and C-4 carbonyl groups, respectively.

intermediates described by Biemel et al. (29). Furthermore, the relative amounts obtained and the labeling studies using ^{13}C -labeled sugars, ^{18}O -dioxygen, and/or ^{17}O -enriched water confirmed the validity of this pathway.

The oxidative α -dicarbonyl cleavage suggested in this paper is in contradiction to the hydrolytic α -dicarbonyl mechanism frequently reported in the literature (5, 9–13). To the best of our knowledge, however, there are no experimental data published in the Maillard literature proving its validity. In general, 1,2-dicarbonyl compounds can undergo base-catalyzed reactions in three ways: (i) benzilic acid type rearrangements, (ii) fission of the carbon bond in the α -position to the dicarbonyl unit, and (iii) cleavage of the 1,2-dicarbonyl bond (30, 31). Electronic and stereochemical factors seem to play an important role. Moderately electron-withdrawing substituents facilitate the benzilic acid type rearrangement, which is by far the most often observed reaction taking place under alkaline conditions. Only fused bicyclo 1,2-dicarbonyl systems having a high degree of ring strain tend to react via base-catalyzed ring (α -dicarbonyl) fission to form an acid and carbonyl function, most likely because the stereochemical demands for a migration are not given to favor the benzilic acid type rearrangement. Overall, there is no indication in the organic chemistry literature for a facile hydrolytic α -dicarbonyl cleavage that might be expected to occur in the Maillard reaction cascade. Therefore, the frequently reported hydrolytic α -dicarbonyl cleavage of 1 can be ruled out as a pathway forming carboxylic acids under the conditions used in this work (diluted aqueous solutions, 90–120 $^\circ\text{C}$, pH 6–10). Additional work is required simulating dry-roasting conditions to be able to generalize this statement.

The thermally induced formation of acetic acid from alkaline sugar solutions has been described in the presence (32) and also the absence (33) of oxygen. Indeed, we found independently

of the reaction atmosphere about 30–33 mol % acetic acid generated from glucose at pH 8 (Table 1), of which only 1/3 was formed by the oxidative reaction pathway. Specially designed experiments using model compounds (e.g., 2,3- and 2,4-pentanedione, C₆-deoxy- α -dicarbonyl intermediates) confirmed our hypothesis of the hydrolytic β -dicarbonyl cleavage as the major pathway (B1 in Figure 6) generating acetic acid (17) and the oxidative α -dicarbonyl cleavage as the minor pathway (A1 in Figure 6). Similarly, both unlabeled and ¹⁸O-labeled glyceric acids were formed in the presence of ¹⁸O-dioxygen (Table 3) with a ratio of 1/3 to 2/3 between labeled and unlabeled glyceric acid. These data indicate that glyceric acid can also be generated by both pathways, with the hydrolytic β -elimination of 1-deoxy-2,4-hexodiulose being the dominating mechanism (B2 in Figure 6). However, the minor pathway via oxidative α -dicarbonyl cleavage of 1-deoxy-3,4-hexodiulose (A2 in Figure 6) induced by oxidizing species also contributes to the formation of this Maillard reaction product.

The data reported in this study provide strong evidence for the coexistence of at least two newly described reaction pathways (Figure 6) generating acetic and glyceric acids from hexose sugars. The oxidative α -dicarbonyl cleavage mechanism requires oxidizing species and goes via the intermediates 1-deoxy-2,3-hexodiulose and 1-deoxy-3,4-hexodiulose, resulting in acetic and C₄-aldonic acids (pathway A1) and glyceric and lactic acid (pathway A2), respectively. An alternative major pathway to acetic (glyceric) acid consists of a hydrolytic β -dicarbonyl cleavage mechanism by a nucleophilic attack of the hydroxyl ion at the C-2 (C-4) carbonyl group of 1-deoxy-2,4-hexodiulose leading to acetic (glyceric) acid by a retro-Claisen type β -cleavage reaction (pathways B1 and B2). The corresponding C₄ (C₃) carbonyl counterparts could be identified. In general, the formation of acetic acid represents a major pathway in sugar degradation and is thought to contribute much to the mass balance in Maillard samples.

In conclusion, our study indicates the multitude of possible reactions that may lead to sugar degradation products upon Maillard type reactions. It also shows the role of oxygen and water, which actively participate in the Maillard reaction cascade. The elucidation of the new pathways was possible thanks to the use of independent and complementary approaches (GC-MS, ¹⁷O NMR, and HPAEC) and analyzing both volatile and nonvolatile species, if required after appropriate derivatization. Also, the use of specific intermediates and labeled precursors helped to get a more precise insight into the reaction mechanisms, performed under conditions close to food processing. The type of research applied in this study resulted in new findings to better understand Maillard type reactions with the ultimate goal of favoring the formation of desirable compounds upon food processing.

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