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Reactivity of 1-Deoxy-D-*erythro*-hexo-2,3-diulose: A Key Intermediate in the Maillard Chemistry of Hexoses

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Degradation of 1-deoxyhexo-2,3-diulose, a key intermediate in Maillard chemistry, in the presence of L-alanine under moderate conditions (37 and 50 °C) was investigated. Different analytical strategies were accomplished to cover the broad range of products formed and their differing chemical properties. These involved GC-MS analysis of trimethylsilyl and *O*-benzyloxime trimethylsilyl derivatives (after reaction with *O*-benzylhydroxylamine and *N*,*O*-bis(trimethylsilyl)acetamide), GC-FID analysis of the decyl ester of acetic acid (after reaction with decyl chloroformate), and HPLC-UV analysis of quinoxaline derivatives (after reaction with *o*-phenylenediamine). Among the compounds identified were carboxylic acids (glyceric acid and acetic acid) that can be seen as stable Maillard end-products. However, the formation of dicarbonyls (3,4-dihydroxy-2-oxobutanal, 1-hydroxybutane-2,3-dione, and 4-hydroxy-2-oxobutanal) and of hydroxycarbonyls (acetol) was verified presenting unstable, reactive Maillard intermediates. Results confirmed that β-dicarbonyl cleavage is a very important pathway within the degradation of 1-deoxyhexo-2,3-diulose. Other reactions taking place include enolization, water elimination, and oxidation.

KEYWORDS: Maillard reaction; carbohydrate degradation; 1-deoxyhexo-2,3-diulose; 1-deoxyglucosone; α-dicarbonyl compounds; dicarbonyl cleavage; acetic acid

INTRODUCTION

The complex of reactions between reducing sugars and compounds bearing an amino group, also referred to as the Maillard reaction or nonenzymatic browning, results in its early stage in the Amadori compound as a first analytically reachable intermediate (*1*). Later, reactive intermediates are formed, which are responsible for the formation of taste- and odor-active as well as colored compounds in processed food (*2*). Among these intermediates, dicarbonyls are of major importance. For example the Amadori compound of glucose delivers 3-deoxyglucosone (3-DG) via 1,2-enolization (*3*, *4*). This well-investigated compound reacts to, for example, 5-(hydroxymethyl)furan-2-carbaldehyde, an indicator for heat treatment of processed food. Glomb and Tschirnich showed that 1-deoxyhexo-2,3-diulose **1** [1-deoxyglucosone, 1-DG, (4*R*,5*R*)-4,5,6-trihydroxyhexane-2,3-dione] is of much higher reactivity in comparison to that of 3-DG (*5*). 1-DG is alternatively formed from the Amadori compound via 2,3-enolization. Numerous postulated reaction pathways in Maillard chemistry use 1-DG to explain the formation of well-known Maillard products (see **Figure 1**) such as 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one **2** (γ-pyranone) (*6*−*8*). This compound can be found in yields up to the g/kg range in heated food indicating the occurrence of the Maillard reaction. Its formation is explained by dehydration of the pyranoic hemiketal form of 1-DG. Analogous reactions of the furanoic form of **1** lead to 2,4-dihydroxy-2,5-dimethylfuran-3(2*H*)-one **3** (acetylformoin) (*9*) or the furanones **4** and **5**, already established earlier in Maillard model systems and processed food (*6*, *10*, *11*). Despite the obvious importance of 1-DG, there are, to the best of our knowledge, only three studies so far investigating the reactions of 1-DG from the native authentic compound (*12*−*14*), although an independent synthesis was already developed several years ago (*15*). In these studies, Davidek and co-workers proved that 1-DG is a precursor of acetic acid under cooking conditions. The formation of acetic acid was explained by a hydrolytic β-dicarbonyl cleavage of the isomeric 1-deoxyhexo-2,4-diulose. It was also found that to a minor extent 1-DG can undergo an oxidative α-dicarbonyl cleavage leading to other carboxylic acids (*13*).

In our study, we succeeded to expand the knowledge on the reactions of 1-DG and the products formed from it. Formation of organic acids as well as the formation of dicarbonyls and hydroxycarbonyls was established. Several different analytical methods were accomplished to obtain quantitative results, which allowed insights into the reaction mechanisms behind 1-DG degradation.

MATERIALS AND METHODS

Materials. The following chemicals of analytical grade were commercially available: acetol (Acros Organics, Beerse, Belgium), *N*,*O*-bis(trimethylsilyl)acetamide with 5% trimethylchlorosilane, acetic acid, L-alanine, D-erythronic acid γ-lactone, D-(−)-erythrose, L(+)-erythrulose, *o*-phenylenediamine (OPD), heptafluorobutyric acid, 2,3,5-triphenyltetrazolium chloride (TTC) (Fluka/Sigma-Aldrich, Seelze, Germany), dipotassium hydrogen phosphate trihydrate, potassium dihydrogen phosphate

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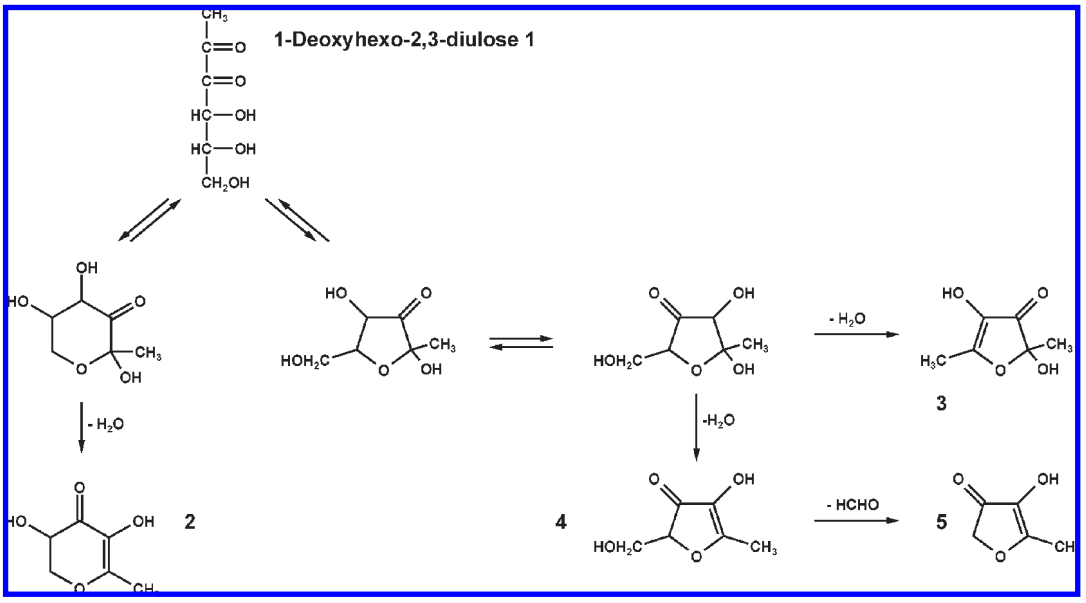

Figure 1. Formation of well-established Maillard products related to 1-deoxyhexo-2,3-diulose **1**: 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (γ-pyranone) **2**, 2,4-dihydroxy-2,5-dimethylfuran-3(2*H*)-one (acetylformoin) **3**, 4-hydroxy-2-(hydroxymethyl)-5-methylfuran-3(2*H*)-one **4**, and 4-hydroxy-5-methylfuran-3(2*H*)-one **5**.

(Merck, Darmstadt, Germany), calcium sulfate, diethyl ether, ethyl acetate (EtOAC) (Roth, Karlsruhe, Germany), diethylenetriaminepentaacetic acid, D(−)-threose (Sigma-Aldrich, Taufkirchen, Germany), D-glyceric acid calcium salt, 2-methylquinoxaline (Aldrich/Sigma-Aldrich, Steinheim, Germany), and Dowex 50W × 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-deoxy-D-*erythro*-hexo-2,3-diulose were synthesized as described in ref (*15*). Quinoxalines of 3,4-dihydroxy-2-oxobutanal (threosone), 1-hydroxybutane-2,3-dione (1-deoxythreosone), and 4-hydroxy-2-oxobutanal (3-deoxythreosone) were isolated from glucose−lysine−OPD reaction mixtures by multilayer counter current chromatography (data not shown). Their identity and purity were confirmed by NMR and high resolution mass spectrometry in accordance with ref (*16*).

Degradation of 1-Deoxyhexo-2,3-diulose. A solution of 1-deoxy-4,5-*O*-isopropylidene-D-*erythro*-hexo-2,3-diulose (0.2 mmol) in water (2 mL) was stirred with Dowex 50W × 8 (H^+-form, 50−100 mesh, 2 mL) under argon atmosphere. Completeness of deprotection was checked after 3 h with TLC (TTC detection). The resin was filtered off and washed with MeOH. After evaporation of the combined solvents, the residue was dissolved in phosphate buffer (0.1 M, pH 7.4, 2.4 mL). Aliquots (150 μL) of this solution and a solution of L-alanine (0.2 mmol) in phosphate buffer (2.4 mL) were dispatched in screw-cap vials (1.5 mL) giving an incubation solution of 1-DG and L-alanine (42 mM, respectively). Incubation solutions were heated (37 and 50 °C), and samples were taken over time and analyzed by GC after derivatization. Values were expressed as the means of at least three independent determinations.

Deaerated Incubations. Degradation of 1-deoxy-D-*erythro*-hexo-2,3-diulose under deaerated conditions was carried out using phosphate buffer with 1 mM diethylenetriaminepentaacetic acid. Buffer was degassed with helium before samples were prepared; samples were deaerated with argon before incubation.

Derivatization Reactions. *Trimethylsilyl Derivatives.* Adopting the method described in ref (*5*), aliquots of the samples (50 μL) were dried and residues dissolved in pyridine (50 μL), and *N*,*O*-bis(trimethylsilyl) acetamide with 5% trimethylchlorosilane (50 μL) was added. Samples were kept 3 h at RT prior to injection into the GC-MS-system. Quantification was carried out by comparison of peak areas obtained in the TIC with those of standard solutions containing known amounts of the pure authentic reference compounds. Signals of target compounds were standardized using the signal of silylated phosphoric acid present in all samples. Data for silylated compounds obtained by GC-MS showed standard deviations < 10 mmol/mol 1-DG, resulting in coefficients of variation < 5%.

Decylchloroformate Derivative of Acetic Acid. Adopting the method described in ref (*17*), samples were spiked with chlorosuccinic acid (50 μg) dissolved in water as internal standard, and pyridine (40 μL) and decylchloroformate (50 μL) were added. The mixture was then sonicated for 10 min. Decyl esters were extracted with hexane, and the organic layer was analyzed by GC-FID. Quantitative results were obtained by internal calibration using commercially available acetic acid. (Data obtained with GC-FID showed standard deviations < 20 mmol/mol 1-DG, resulting in coefficients of variation < 3.5%. Coefficient of determination was always > 0.97.)

Quinoxaline Derivatives. Adopting the method described in ref (*5*), samples were spiked with *o*-phenylenediamine (4.95 μmol) dissolved in water (30 μL) and kept 5 h at RT prior to injection into the HPLC-UV-system (conversion of dicarbonyls to quinoxalines under these reactions conditions was almost 100% according to ref (*5*)). Quinoxalines were monitored at λ_M = 320 nm. Quantification was carried out by comparison of peak areas obtained at 320 nm with those of standard solutions containing known amounts of either commercially available reference compound (2-methylquinoxaline) or pure authentic quinoxalines isolated and elucidated from reaction mixtures in our working group. (Data obtained with HPLC-UV showed standard deviations < 1.5 mmol/mol 1-DG, resulting in coefficients of variation < 5%.)

O-Benzyloxime Trimethylsilyl Derivatives. Hydroxycarbonyl compounds were derivatized on the basis of ref (*18*) with some modifications. Samples were spiked with a solution of 6 μmol *O*-benzylhydroxylamine hydrochloride in water (300 μL) and reacted 3 h at 37 °C. The mixture was adjusted to pH 6 and extracted with 1 mL of diethyl ether. The organic layer was dried with calcium sulfate, evaporated with argon, and the residue silylated as described above. Quantitative results were obtained by external calibration using commercially available acetol. (Data obtained with GC-MS showed standard deviations < 3 mmol/mol 1-DG, resulting in coefficients of variation < 7.5%. Coefficient of determination was always > 0.98. Recovery was between 95 and 105%.)

GC-MS. Samples were analyzed on a Thermo Finnigan Trace GC Ultra coupled to a Thermo Finnigan Trace DSQ (both from Thermo Fisher Scientific GmbH, Bremen, Germany). The GC column was HP-5 (30 m × 0.32 mm, film thickness 0.25 μm; Agilent Technologies, Palo Alto, CA). Injector, 220 °C; split ratio, 1:30; transfer line, 250 °C. The oven temperature program was as follows: 100 °C (0 min), 5 °C/min to 200 °C (0 min), and 10 °C/min to 270 °C (10 min). Helium 5.0 was used as carrier gas in constant flow mode (linear velocity 28 cm/s, flow 1 mL/min). Mass spectra were obtained with EI at 70 eV (source: 210 °C) in Full scan mode (mass range *m*/*z* 50−650).

GC-FID. Samples were analyzed on a HP 6890N chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector. The column was HP-5 (30 m × 0.32 mm, film thickness 0.25 μm; Agilent Technologies, Palo Alto, CA). Injector, 250 °C; split ratio, 1:10; detector, 270 °C. Helium 4.6 was used as carrier gas in constant flow mode (linear velocity 28 cm/s, flow 1.6 mL/min).

HPLC-UV. A Jasco PU-2089 Plus quaternary gradient pump with degasser was used combined with a Jasco AS-2055 Plus autosampler. Elution of materials 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 × 4.6 mm) by Knauer (Berlin, Germany) using a flow rate of 1 mL/min. The mobile phase used was water (solvent A) and water/MeOH 3:7 (v/v, solvent B). Heptafluorobutyric acid (0.6 mL/L) was added to both solvents. Samples were injected at 20% B, gradient then changed linear to 30% B in 35 min, to 40% B in 5 min, then to 70% B in 15 min (held 5 min), then changed to 100% B in 30 min (held 10 min), and then changed to 20% B in 5 min (held 15 min).

RESULTS

Carboxylic Acids. Degradation of 1-deoxyhexo-2,3-diulose **1** in the presence of amine lead to several peaks, which could be analyzed by GC-MS after silylation. **Figure 2** shows the total ion chromatogram of a derivatized sample (after 12 h, at 37 °C under deaerated conditions). Important signals that could be identified were assigned to lactic acid **6**, glyceric acid **9**, 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one **2**, erythrulose **10**, and 4-hydroxy-2-(hydroxymethyl)-5-methylfuran-3(2*H*)-one **4**. As expected, silylated L-alanine **7** and phosphoric acid **8** gave the largest signals.

Formation of glyceric acid is shown in **Figure 3**. Obviously, it was formed rather fast within the first hour. Later on, concentrations increased at a smaller rate, nearly linear over the whole period of time monitored. Formation was significantly favored under aerated conditions, for example, 171 mmol/mol 1-DG of glyceric acid was formed after 12 h at 37 °C under aerated conditions (vs 40 mmol/mol 1-DG under deaerated conditions).

Another carboxylic acid found was acetic acid. **Figure 4** shows a GC-FID-chromatogram of the decylester formed after derivatization with decyl chloroformate (chlorosuccinic acid was used as internal standard). Acetic acid was a major product formed from **1** even under the less rigorous conditions used in the present work. Formation was slightly preferred under deaerated conditions (up to 650 mmol/mol 1-DG was obtained after 24 h at 37 °C under deaerated conditions vs 550 mmol/mol 1-DG under aerated conditions).

Hydroxycarbonyl and Dicarbonyl Compounds. Besides carboxylic acids, we also detected several hydroxycarbonyl and dicarbonyl compounds. Erythrulose was analyzed after silylation via GC-MS. Formation of this C4-sugar was considerably favored under deaerated conditions. An almost 4-fold higher amount was obtained after 16 h at 37 °C (162 mmol/mol 1-DG vs 43 mmol/mol 1-DG). Levels reached a maximum at about 16 h and decreased in later samples. This pattern of a peak concentration with subsequent degradation was also found in the case of the dicarbonyl compounds detected in the incubation mixture, i.e., threosone (3,4-dihydroxy-2-oxobutanal), 1-deoxythreosone (1-hydroxybutane-2,3-dione), and 3-deoxythreosone (4-hydroxy-2-oxobutanal). Formation of these dicarbonyls reached a peak level after about 1 h at 50 °C. The initial rate of formation was slightly lower at 37 °C; maximum levels were reached after 2 h at this incubation temperature. In addition, amounts of dicarbonyls detected were generally much lower than those of carboxylic acids or erythrulose. Formation of 1-deoxythreosone and 3-deoxythreosone was favored under deaerated conditions, while threosone formation was preferred in presence of oxygen. Highest

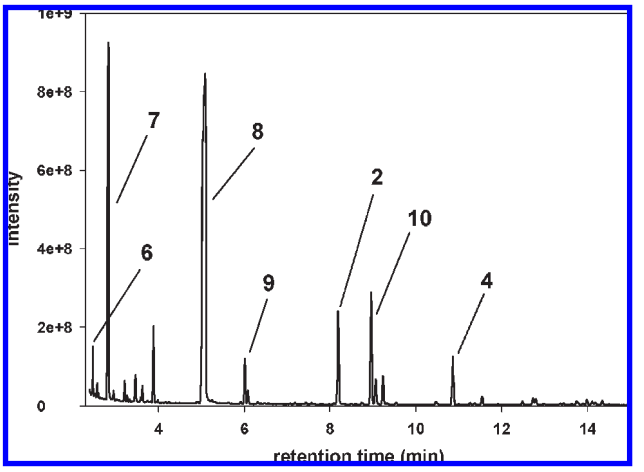

Figure 2. Total ion chromatogram of a 1-DG/L-alanine incubation mixture (42 mM in 0.1 M phosphate buffer, pH 7.4, after 24 h at 37 °C under deaerated conditions). Signals are trimethylsilyl derivatives of lactic acid **6**, L-alanine **7**, phosphoric acid **8**, glyceric acid **9**, γ-pyranone **2**, erythrulose **10**, and 4-hydroxy-2-(hydroxymethyl)-5-methylfuran-3(2*H*)-one **4**.

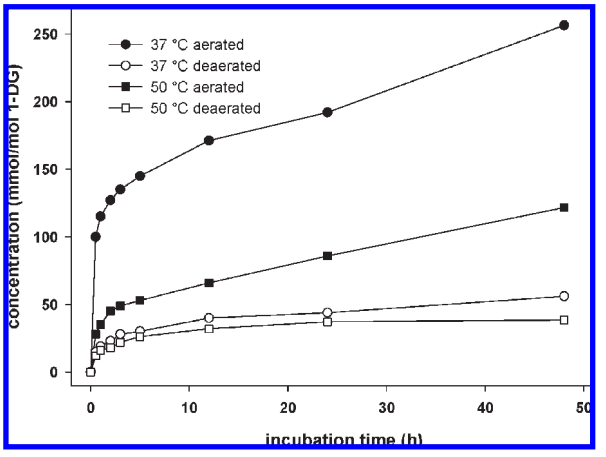

Figure 3. Formation of glyceric acid (1-DG/L-alanine, 42 mM in 0.1 M phosphate buffer, pH 7.4).

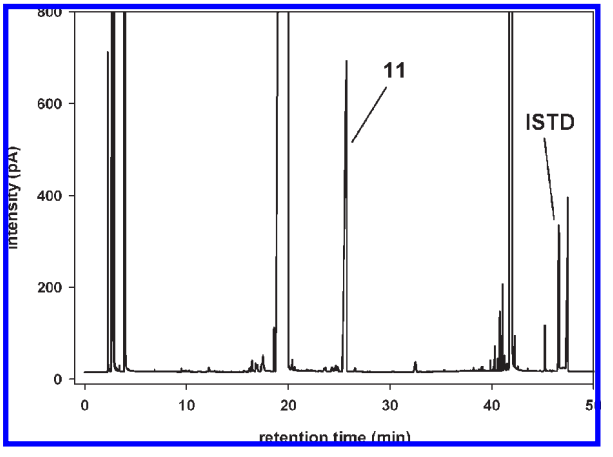

Figure 4. Identification of acetic acid by GC-FID (acetic acid decyl ester **11** and chlorosuccinic acid decyl ester **ISTD**).

levels were obtained for 1-deoxythreosone (**Figure 5**), which was formed up to 31 mmol/mol 1-DG under deaerated conditions. About half of this amount (14 mmol/mol 1-DG) was measured under aerated conditions. This 2:1 ratio changed to about 1.5:1 at 50 °C (28 vs 19 mmol/mol 1-DG). The other two dicarbonyls were

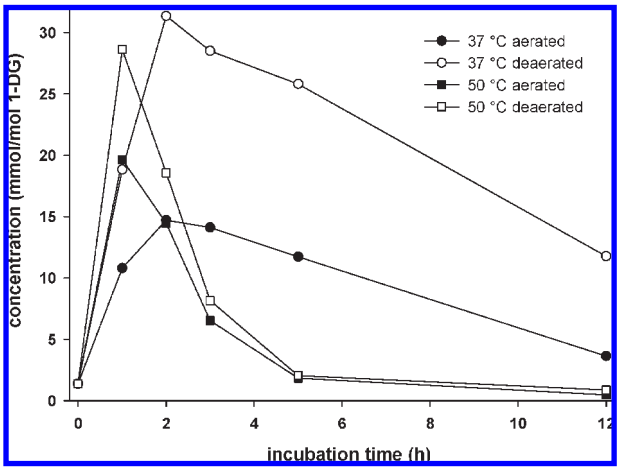

Figure 5. Formation of 1-deoxythreosone (1-DG/L-alanine, 42 mM in 0.1 M phosphate buffer, pH 7.4).

Table 1. Degradation of 1-Deoxyhexo-2,3-diulose Leads to C4-Dicarbonyls

	37 °C[a]		50 °C[b]	
	mmol/mol 1-DG			
	aerated	deaerated	aerated	deaerated
1-deoxythreosone	14	31	19	28
3-deoxythreosone	3	10	5	10
threosone[c]	14	5	14	10

[a] Maximum concentrations were measured after 2 h. [b] Maximum concentrations were measured after 1 h. [c] Maximum concentrations were measured after 30 min.

measured in lower amounts (**Table 1**). In the same range were the concentrations quantified for hydroxypropane-2-one (acetol). This hydroxycarbonyl compound was determined from the incubation by GC-MS analysis as its *O*-benzyloxime trimethylsilyl derivative. Slightly lower amounts were obtained for deaerated conditions, for example, 10 mmol/mol versus 18 mmol/mol 1-DG (after 12 h at 37 °C) and 28 mmol/mol versus 38 mmol/mol 1-DG (after 12 h at 50 °C).

DISCUSSION

Products formed during the degradation of **1** in the presence of amine can be divided into two groups. On the one hand, there are carboxylic acids representing stable degradation products that accumulated over time. On the other hand, reactive intermediates (dicarbonyl and hydroxycarbonyl compounds) were formed to a certain amount and immediately degraded once the precursor was exhausted.

Carboxylic acids have been described as products of sugar fragmentation very early in papers on Maillard reaction mechanisms. Hodge already postulated **1** being involved in acetic acid formation (*1*). He assumed the formation of glycolaldehyde from 1-DG via retro-aldolization followed by a saccharinic acid rearrangement to give acetic acid. Brands and van Boekel proposed acetic acid to be formed from **1** via a hydrolytic α-dicarbonyl cleavage (*19*). Davidek et al. recently showed that large amounts (up to 85 mol %) of acetic acid were indeed formed from **1** at 100 °C regardless of the presence of an amino acid or amine. However, their mechanistic study (*12*) suggested a hydrolytic β-dicarbonyl cleavage of the 2,4-tautomer of **1** as the major pathway for acetic acid formation (**Figure 6**). In addition, no evidence was found for the hydrolytic α-dicarbonyl cleavage that seemed well established in the literature. Instead, they reported

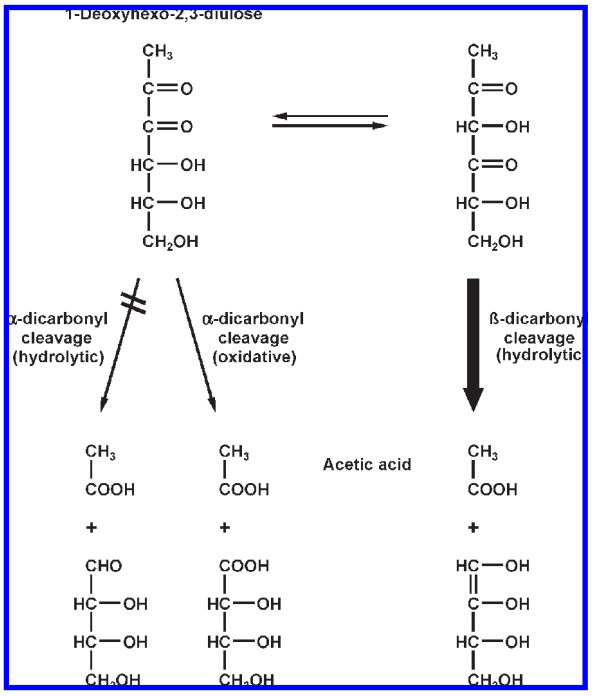

Figure 6. Mechanism of 1-deoxyhexo-2,3-diulose degradation via α- and β-dicarbonyl cleavage leading to acetic acid.

that nearly similar concentrations of acetic acid and propanoic acid were formed in a 2,3-dicarbonyl model system containing pentane-2,3-dione. However, yields were very low compared to that in β-dicarbonyl fragmentation. Therefore, a minor additional oxidative α-dicarbonyl cleavage was suggested and researched more closely in a later study (*13*). We also found large yields of acetic acid even under the far more moderate conditions used herein. The slightly higher amounts that were obtained under deaerated conditions may be explained by the greater stability of **1** under these conditions. Additional formation of acetic acid via an oxidative α-dicarbonyl cleavage should result in higher yields under aerated conditions, which could not be attested from our data. Furthermore, we were not able to identify erythronic acid, which would be the counterpart expected in that mechanism. Thus, oxidative α-dicarbonyl cleavage might indeed be only of minor relevance or even nonexistent under the reaction conditions herein.

The enediol intermediate occurring within the β-dicarbonyl cleavage is in our opinion a linchpin in the formation of the second group of compounds (reactive intermediates) investigated in this study. Tautomerization leads to erythrulose (see **Figure 7**), which was obtained in significant amounts in the incubation mixtures. It should be mentioned that the two possible isomeric aldoses erythrose and threose could not be identified in our samples. The 4-fold higher amount of erythrulose obtained under deaeration can be explained by the instability of its direct and intermediate precursors in the presence of oxygen, i.e., the C4-enediol and 1-DG. The oxidation product of the enediol is threosone, which was verified as a degradation product formed from **1** in our samples especially under aeration. This compound is rather known from nonenzymatic browning reactions involving ascorbic acid (*20*). Usui and co-workers recently reported threosone during the degradation of glucose and fructose (*16*). However, these authors explained its formation via glucosone and did not consider 1-DG as a possible precursor in threosone formation. Oxidation of an enediol is a well-established step, for example, oxidation of the 1,2-enediol of glucose giving D-*arabino*-hexos-2-ulose (glucosone).

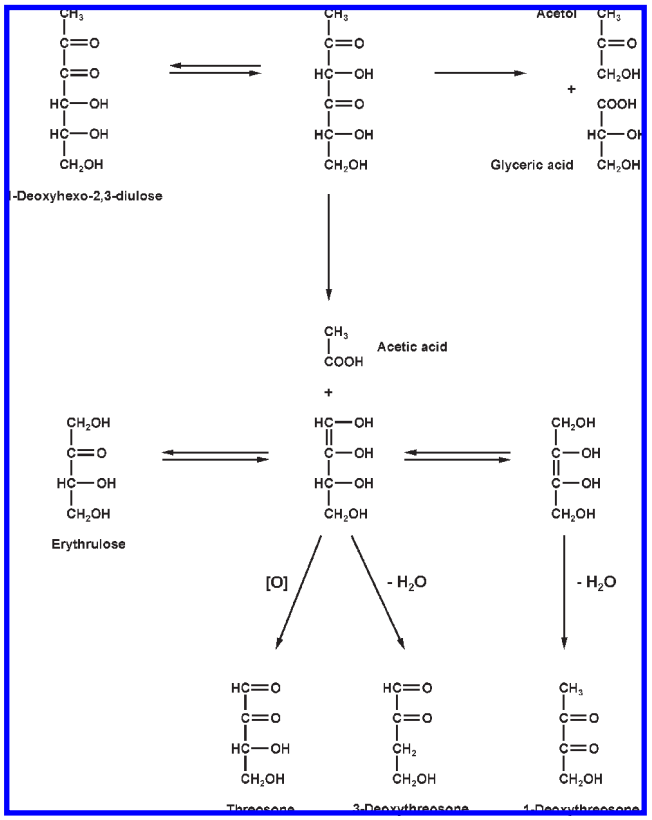

Figure 7. Degradation of 1-deoxyhexo-2,3-diulose leads to C2, C3, and C4 fragmentation.

The enediol may also be dehydrated at C-3 resulting in 3-deoxythreosone (4-hydroxy-2-oxobutanal), which we identified as its stable quinoxaline in the incubation mixtures. The isomeric 2,3-enediol may readily be formed from the described 1,2-enediol and should give rise to 1-deoxythreosone (1-hydroxybutane-2,3-dione) in an equivalent reaction. This compound was also verified as its quinoxaline in the degradation of **1**. Both dicarbonyls were originally reported by Nedvidek from glucose/amino acid model systems (*21*). Davidek identified the quinoxaline of 1-deoxythreosone by GC-MS analysis in glucose/glycine incubations and interpreted it as additional evidence of glucose degradation along the 1-DG road (*12*).

The nucleophilic attack of a hydroxyl anion, which has to be assumed within the β-dicarbonyl cleavage mechanism, can of course also happen at the C-4 carbonyl group of the 2,4-tautomer of **1**. This results in the formation of glyceric acid and 1-hydroxypropan-2-one (acetol). Acetol formation from hexoses was already investigated about 50 years ago, and Hayami proposed β-dicarbonyl cleavage to explain such results (*22*, *23*). Weenen later reported acetol formation from glucose and proposed **1** to be involved (*24*). Yaylayan carried out label incorporation analysis of D-glucose/amino acid systems under pyrolysis conditions (*25*, *26*). He concluded that 70% of the detected acetol was derived from 1-DG by retro-aldolization of the isomeric 1-deoxyhexo-2,5-diulose. However, β-dicarbonyl cleavage seems to be a more likely pathway or at least an additional pathway. Glyceric acid formation from glucose was also investigated by Davidek and co-workers. It was shown that thermal treatment of [1,2-$^{13}C_2$]glucose and [3-^{13}C]glucose isotopomers resulted in unlabeled glyceric acid only (*13*). This is in agreement with the β-dicarbonyl cleavage mechanism of the 2,4-isomer of **1**. The higher amounts of glyceric acid we obtained under aerated conditions may be explained by additional oxidative fragmentation pathways.

Interestingly, we measured only negligible amounts of 2-methylquinoxaline (data not shown), although we expected methylglyoxal to be an important compound formed from **1** via retro-aldolization. Thus, retro-aldol fragmentation may not play a major role in 1-DG chemistry under the conditions investigated in our experiments. The different pathways of the degradation of **1** resulting in the products investigated in the present work are summarized in **Figure 7**. The 2,4-tautomer of 1-deoxyhexo-2,3-diulose plays a pivotal role in the formation of fragmentation products with a C2, C3, and C4 carbon backbone.

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