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Review

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Fragmentation Pathways during Maillard-Induced Carbohydrate Degradation

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ABSTRACT: The Maillard reaction network with focus on the chemistry of dicarbonyl structures causes considerable interest of research groups in food chemistry and medical science, respectively. Dicarbonyl compounds are well established as the central intermediates in the nonenzymatic browning reaction and have been verified to be responsible for advanced glycation endproduct (AGE) formation. A multitude of Maillard dicarbonyls covering the range of the intact carbon backbone down to C_3 and C_2 fragments were detected in several carbohydrate systems, for example, in glucose, maltose, or ascorbic acid reactions. By definition, dicarbonyls with a C_2−C_5 carbon backbone must originate by fission of the original carbon skeleton. The present review deals with the five major mechanisms reported in the literature for dicarbonyl decomposition: (i) retro-aldol fragmentation, (ii) hydrolytic α-dicarbonyl cleavage, (iii) oxidative α-dicarbonyl cleavage, (iv) hydrolytic β-dicarbonyl cleavage, and (v) amine-induced β-dicarbonyl cleavage.

KEYWORDS: *dicarbonyl compounds, retro-aldol fragmentation, hydrolytic α-dicarbonyl cleavage, oxidative α-dicarbonyl cleavage, β-dicarbonyl cleavage*

INTRODUCTION

In the course of the Maillard reaction carbohydrates are subject to degradation processes resulting in lower molecular weight compounds.[1] Among these products carbonyl structures[2] and carboxylic acids[3] occur in the highest amounts. Furthermore, a class of highly reactive diketones, namely, α-dicarbonyl compounds, is present in Maillard reaction systems.[2,4] Although 1,2-dicarbonyls were detected only in small quantities, they have drawn major attention to food chemistry research regarding their pivotal role as color, flavor, and aroma precursors and also to medical sciences because their participation in advanced glycation endproduct (AGE) formation in vivo was established.[5]

Reactive α-dicarbonyl compounds were identified in various foods, for example, in cookies,[6] soy sauces,[7] milk products,[8] honey,[9] coffee,[10] beer,[11] wine,[12] and sweetened beverages (with high-fructose corn syrup as the major source),[13] and also in peritoneal fluids[14] as a medical device. Moreover, α-dicarbonyl compounds were found to be produced in vivo, where they can mediate chronic and age-related diseases such as atherosclerosis,[15] diabetes,[16] uremia,[17] and Alzheimer's disease.[18] Recently, in our working group the spectrum of α-dicarbonyl compounds present in the human body was extended by the structures 2-glucosulose (glucosone), 1-deoxy-2,3-glucodiulose (1-deoxyglucosone), and 3,4-dihydroxy-2-oxobutanal (threosone), among others, which are of considerably higher relevance than, for instance, the more stable 3-deoxy-2-glucosulose (3-deoxyglucosone) as the most frequently detected dicarbonyl in vivo besides glyoxal and methylglyoxal.[19]

However, as highly reactive and, thus, unstable Maillard intermediates, α-dicarbonyl compounds play a key role in carbohydrate decomposition.[5] Knowledge about the underlying fragmentation pathways is the basic prerequisite to understand changes occurring during storage and processing of food as well as during adverse alterations in vivo. Due to their high reactivity α-dicarbonyl compounds easily degrade, resulting in complex mixtures of reaction products, for example, again in short-chained α-dicarbonyls.[20] In the literature five major pathways exist, operating as mechanistic explanations for carbohydrate fragmentation reactions: (i) retro-aldol fragmentation,[1] (ii) hydrolytic α-dicarbonyl cleavage,[21] (iii) oxidative α-dicarbonyl cleavage,[22] (iv) hydrolytic β-dicarbonyl cleavage,[23] and (v) amine-induced β-dicarbonyl cleavage.[24] This review summarizes the current knowledge about chemical fragmentation reactions in carbohydrate Maillard chemistry.

APPROACHES TO INVESTIGATE FRAGMENTATION PATHWAYS

Published fragmentation pathways from early Maillard chemistry were more of a hypothetical nature than experimentally proven and also more oriented toward the formation of only one compound without identification of the postulated fragmentation counterpart or the suggested precursor structure. Investigations were limited by analytical techniques and procedures and, therefore, restricted to stable Maillard reaction products such as carboxylic acids that are easily detectable.[1]

It was a long time before investigators were able to identify reactive intermediates such as dicarbonyl compounds.[25] The use of trapping reagents such as *O*-alkyl hydroxylamines,[26] cysteamine,[27] aminoguanidine,[28] and diaminobenzene deriva-

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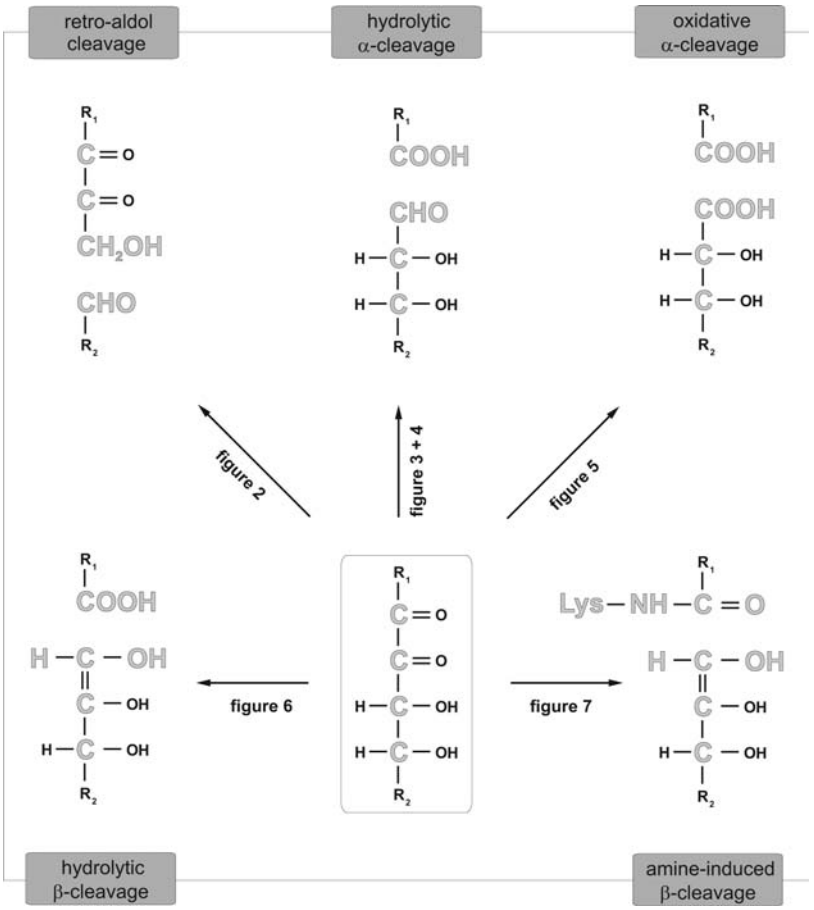


Figure 1. Fragmentation pathways for α-dicarbonyl compounds reported in Maillard literature.

tives[29] allowed a reliable detection of the various dicarbonyl compounds in Maillard reaction systems and, thus, displayed a major step forward to understand mechanistic relationships in fragmentation processes. *o*-Phenylenediamine has prevailed as the generally accepted and most often used derivatization reagent in dicarbonyl analyses.[30]

Modern approaches to verify fragmentation pathways in model systems are based mainly on three procedures. (i) Kinetic studies of model incubation systems conducted under aerobic and anaerobic conditions monitor the formation and degradation rates of Maillard reaction products. This method provides information about mechanistically related compounds and also allows identification of precursor structures.[31,32] (ii) In most cases fragmentation pathways are examined by degradation experiments starting from the carbohydrate. Of course, more detailed results regarding the formation of fragmentation products can be achieved by decomposition studies of the direct precursor compound. This implies syntheses of reactive intermediates. For example, 3-deoxyglucosone,[33−35] 4-*O*-α-glucopyranosyl-2-glucosulose (maltosone), and 1,4-dideoxy-2,3-glucodiulose (1,4-dideoxyglucosone)[32] were successfully synthesized for such investigations. The synthesis of the key intermediate in hexose chemistry, namely, 1-deoxyglucosone,[36] has to be considered as a milestone in this field.[37] (iii) An elegant tool to elucidate underlying reaction mechanisms in general is the use of isotopically labeled reactants in incubation systems. Thus, atoms in Maillard reaction products can be easily traced back to their original position in the precursor carbohydrate. The working groups of Tressl[38] and Yaylayan[39] have pioneered this procedure. However, this method is hampered by the high cost of labeled precursors and by the fact that many of the desirable carbohydrates or carbohydrate intermediates are not commercially available. Therefore, in most studies, carbohydrates labeled only at position C-1 were used.[40,41] As a consequence, many approaches with single-labeled sugars to investigate the origin of fragmentation products smaller than C_6 result in inconclusive data.[42] Nevertheless, the use of ^{13}C-enriched carbohydrates, if carefully planned and performed, delivers comprehensive results regarding fragmentation reactions.[43] A cost-effective method to realize labeling studies was developed by Yaylayan. He conducted the degradation of labeled sugars in a microreactor of a coupled pyrolysis−gas chromatography−mass spectrometry system.[44] Furthermore, ^{13}C-labeling applications, namely, CAMOLA experiments (carbon module labeling)[45,46] and the CBL technique (carbon bond labeling),[47] were established to clarify the mechanistic pathways of Maillard reaction products.

FRAGMENTATION PATHWAYS IN THE MAILLARD REACTION

Investigations of Weenen in 1998[20] and of Tressl and Rewicki in 1999[48] basically took three fragmentation pathways of α-dicarbonyl compounds into consideration within the Maillard reaction cascade: retro-aldolization, α-dicarbonyl cleavage, and β-dicarbonyl cleavage. From today's perspective this mechanistic framework has to be refined and extended (Figure 1) in view of two aspects: First, the α-dicarbonyl cleavage phenomenon has to be differentiated into a hydrolytic[21] and

an oxidative α-dicarbonyl cleavage. The latter was verified as a new sugar fragmentation pathway by Davidek et al. to occur under oxidative conditions.[22] Second, besides the hydrolytic β-dicarbonyl cleavage, recently an amine-induced β-dicarbonyl cleavage was established by ourselves leading to amide-AGEs directly.[24] Figure 1 presents all five pathways described for carbohydrate decomposition in an overview scheme. Each fragmentation pathway (Figures 2−7) is discussed extensively on the basis of one of several examples selected from the literature summarized in Tables 1−5.

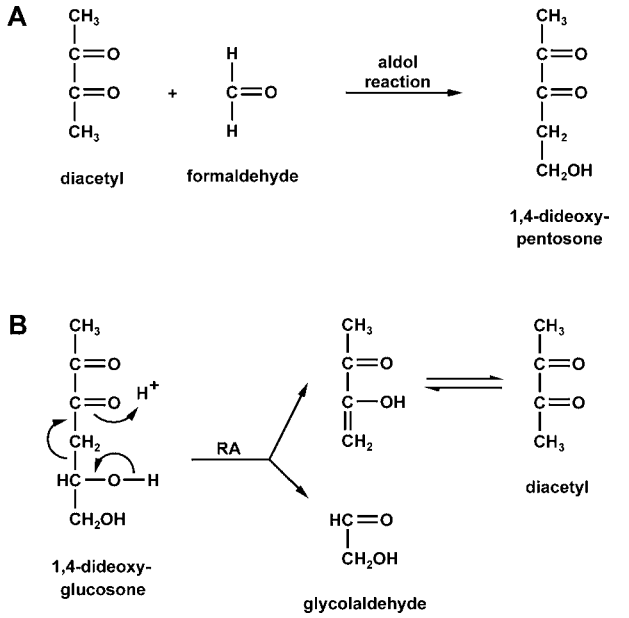


Figure 2. Aldol reaction of diacetyl and formaldehyde (A) and retro-aldolization (RA) of 1,4-dideoxy-2,3-hexodiulose (B), adapted from Pfeifer and Kroh.[50]

Retro-aldol Cleavage (Table 1; Figure 2). Compounds being prone to retro-aldol reactions must contain a β-hydroxy carbonyl moiety as a structural feature. Sugars and Amadori and Heyns rearrangement products and α-dicarbonyl compounds are reported in the literature to undergo retro-aldolization.[5,20] The carbon−carbon bond cleavage takes place between C-α and C-β next to the carbonyl functionality, resulting in shorter-chained hydroxy ketones, hydroxy aldehydes, and dicarbonyl compounds.[2] In this paper we will focus on the retro-aldol reactions described for α-dicarbonyl fragmentation.

Aldol condensation and retro-aldol reactions can be assigned to topics of the early Maillard literature; for example, acetic acid was suggested to be formed as a follow-up product of glycolaldehyde by retro-aldol fragmentation of 1-deoxyglucosone.[1] Up to now retro-aldolization is by far the most accepted fragmentation pathway, for example, used to explain the formation of 1-hydroxy-2-propanone (acetol), methylglyoxal, and glyceraldehyde from deoxyosones generated during degradation of glucose.[20,42,49] Table 1 reveals selected examples of different α-dicarbonyl precursors that are postulated to be split into the given fragmentation products via retro-aldolization. Various efforts have been made to substantiate the proposed fragmentation mechanism.

The formation of methylglyoxal and glyceraldehyde via retro-aldol fragmentation of 1-deoxyglucosone was postulated by Weenen based on a degradation experiment of 1-^{13}C glucose resulting in labeled methylglyoxal and unlabeled glyceraldehyde.[20] In contrast, research by Voigt et al. has shown that degradation incubations of independently synthesized 1-deoxyglucosone led to extremely mismatched amounts of methylglyoxal and glyceraldehyde.[43] Thus, under the tested reaction conditions retro-aldolization seems to be very unlikely to explain the formation of methylglyoxal from 1-deoxyglucosone.

Thornalley et al. synthesized 3-deoxyglucosone to demonstrate in an incubation experiment that methylglyoxal is generated from this precursor compound via retro-aldol fragmentation. Although methylglyoxal could be detected in that model experiment concentrations reached only 0.09 μM when starting from 50 μM 3-deoxyglucosone. Investigations of Thornalley et al. did not include the detection and quantification of glyceraldehyde as the expected counterpart which had to be generated in similar concentrations to confirm the hypothetical retro-aldol cleavage.[49] In addition, in our opinion, a yield of 0.18 mol % methylglyoxal rejects the retro-aldolization route as an important fragmentation pathway to form methylglyoxal.

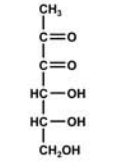
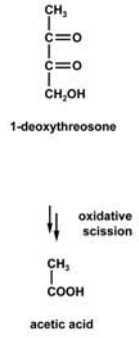
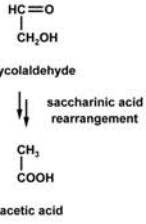
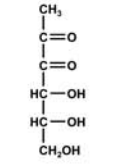
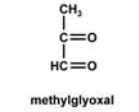
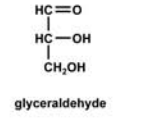
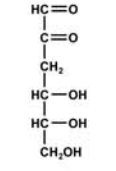
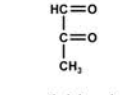
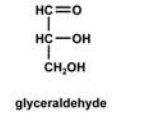
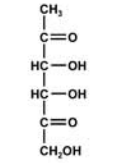
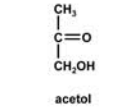
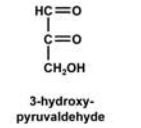
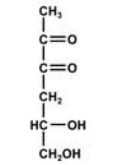
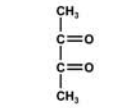
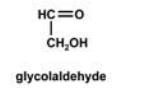
Yaylayan and Keyhani conducted labeling studies with all possible ^{13}C-glucose isotopomers. As a result, acetol was formed by 70% from former positions C1−C2−C3 of glucose. The expected counterpart, 3-hydroxypyruvaldehyde, was detected as its pyrazine derivative containing carbon atoms C4−C5−C6. On the basis of these results retro-aldol fragmentation of the proposed 2,5-tautomer of 1-deoxyglucosone was postulated as the major pathway to yield acetol.[42] Even though the labeling studies were in full support of the retro-aldolization, the authors did not quantify acetol and 3-hydroxypyruvaldehyde in experiments with the direct precursor 1-deoxyglucosone to confirm the suggested mechanism.

From a mechanistic point of view, one of the selected examples is illustrated in Figure 2 and will be discussed in more detail. Pfeifer and Kroh postulated the formation of 1,4-dideoxyglucosone via a "peeling-off" mechanism from oligosaccharides followed by degradation into 2,3-butanedione (diacetyl) and glycolaldehyde via retro-aldolization. To elucidate this fragmentation pathway, an inverse experiment was conducted. In a model incubation (aqueous solution, pH 8) diacetyl and formaldehyde were reacted for 10 min at ambient temperature to give 1,4-dideoxypentosulose (1,4-dideoxypentosone) detected as its quinoxaline derivative. Obviously as expected, 1,4-dideoxypentosone was generated as the aldol condensation product. This finding led to the assumption that if diacetyl is operating as an educt of aldol condensation, it must be also a product of retro-aldolization.[50] To test this hypothesis, we established the successful synthesis of 1,4-dideoxyglucosone in our working group for direct degradation experiments of the suggested C6-precursor structure. Neither diacetyl nor glycolaldehyde as the counterpart was detected in incubations of 1,4-dideoxyglucosone conducted under similar conditions to Pfeifers inverse experiment. Thus, retro-aldolization has to be ruled out as the formation pathway of diacetyl from 1,4-dideoxyglucosone.[32]

In conclusion, there is, to the best of our knowledge, no persuasive experimental proof for the retro-aldol reaction of dicarbonyls. Thus, fragmentation routes based on this mechanism remain largely hypothetical.

Hydrolytic α-Dicarbonyl Cleavage (Table 2; Figures 3 and 4). Second to retro-aldol reactions, the hydrolytic

Table 1. Retro-aldol Fragmentation of Different α-Dicarbonyl Compounds Reported in the Literature

α-dicarbonyl precursor	fragmentation product	expected counterpart	experimental basis	comments	ref
 1-deoxyglucosone	 1-deoxythreosone; oxidative scission; acetic acid	 glycolaldehyde; saccharinic acid rearrangement; acetic acid	identification of acetic acid	hypothetical mechanism to explain acetic acid formation	(1)
 1-deoxyglucosone	 methylglyoxal	 glyceraldehyde	labeling studies with 1-^{13}C glucose	results disproven by Voigt (43)	(20)
 3-deoxyglucosone	 methylglyoxal	 glyceraldehyde	model incubations with synthesized 3-deoxyglucosone	degradation of 3-deoxyglucosone yielded only 0.18 mol-% methylglyoxal	(49)
 2,5-tautomer of 1-deoxyglucosone	 acetol	 3-hydroxy-pyruvaldehyde	labeling studies with 1-^{13}C, 2-^{13}C, 3-^{13}C, 4-^{13}C, 5-^{13}C, 6-^{13}C glucose	results of labeling experiments for both counterparts in support of retro-aldol-reaction quantification of counterparts not performed	(42)
 1,4-dideoxyglucosone	 diacetyl	 glycolaldehyde	inverse experiment: aldol condensation of diacetyl and formaldehyde	results disproven by incubation of 1,4-dideoxyglucosone, diacetyl was not detected (32)	(50)

α-dicarbonyl cleavage is the most frequently reported dicarbonyl fission reaction in the Maillard reaction network.[21,51−53] The hydrolytic α-dicarbonyl fragmentation reaction is understood as the breakage of the carbon−carbon bond between both carbonyl moieties toward a carboxylic acid and an aldehyde. From a mechanistic view this reaction must be based on an intramolecular disproportionation without changing the stereochemistry given by the precursor. Table 2 lists selected examples of α-dicarbonyl precursors suggested in the literature to trigger the α-dicarbonyl fragmentation route.

2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyrane-4-one (γ-pyranone) is an established important intermediate in Maillard hexose chemistry resulting from 1-deoxyglucosone.[54,55] Kim and Baltes synthesized 1-^{13}C-γ-pyranone for mechanistic oriented degradation studies. After hydrolytic ring-opening of the cyclic γ-pyranone, the 3,4-tautomer of

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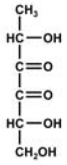
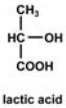
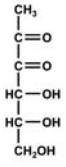
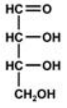
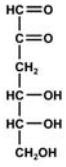
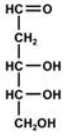
Table 2. Hydrolytic α-Dicarbonyl Cleavage of Different α-Dicarbonyl Compounds Reported in the Literature

α-dicarbonyl precursor	fragmentation product	expected counterpart	experimental basis	comments	ref
 3,4-tautomer of 1-deoxyglucosone	 lactic acid	 glyceraldehyde	model incubations with synthesized 1-^{13}C γ-pyranone	results of labeling experiments for both counterparts in support of a hydrolytic α-dicarbonyl cleavage quantification of counterparts not performed	(21)
 1-deoxyglucosone	 acetic acid	 erythrose	labeling studies with 1-^{13}C, 2-^{13}C, 3-^{13}C glucose	results of labeling experiments (labeled acetic acid) in support of a hydrolytic α-dicarbonyl cleavage detection of erythrose not performed	(51)
 3-deoxyglucosone	HCOOH formic acid	 2-deoxypentose	model incubations of glucose and fructose quantification of formic acid and furfurylalcohol	2-deoxypentose not detected, but furfurylalcohol as a possible follow-up product mismatched amounts of formic acid and furfurylalcohol	(52)

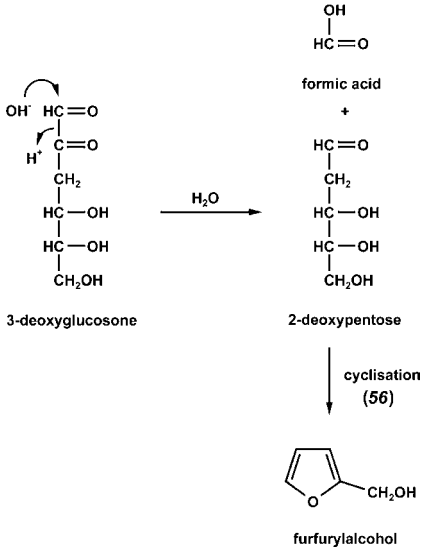


Figure 3. Hydrolytic α-dicarbonyl cleavage reaction of 3-deoxyglucosone, adapted from Brands and van Boekel.[52]

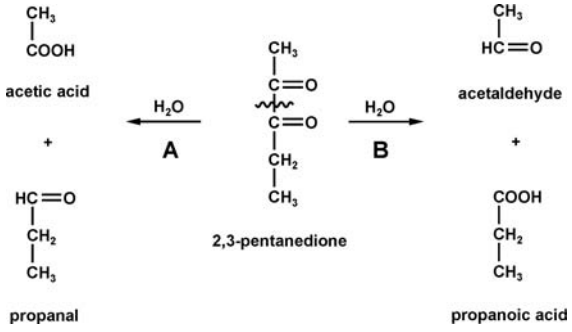


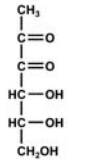
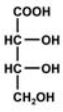
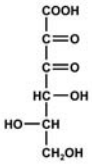
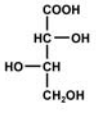
Figure 4. Hypothetical hydrolytic α-dicarbonyl cleavage of 2,3-pentanedione leads to acetic acid and propanal (A) or acetaldehyde and propanoic acid (B).

1-deoxyglucosone was hypothesized as an open-chained intermediate to degrade in lactic acid and glyceraldehyde via a hydrolytic α-dicarbonyl fragmentation reaction. The hydrolytic carbonyl decomposition was based on the detection of lactic acid exclusively labeled at the methyl group and unlabeled glyceraldehyde as a byproduct.[21] A final convincing proof for the suggested mechanism by quantification of the respective counterparts or by experiments with complementary labeled precursors was not performed.

Ginz et al. conducted labeling experiments with different glucose isotopomers (1-^{13}C, 2-^{13}C, 3-^{13}C) to explain the formation of various carboxylic acids. For example, NMR results for acetic acid led to the assumption that the carboxylic acid is formed from 1-deoxyglucosone by hydrolytic α-dicarbonyl cleavage of the C-2−C-3 bond with erythrose as the expected counterpart. The final concluding link, the detection of erythrose, was not performed.[51] Thus, the proposed fragmentation mechanism has to be regarded as a mere assumption.

In another degradation study of glucose and fructose by Brands and van Boekel, the α-dicarbonyl cleavage route was considered as a self-evident fragmentation pathway to yield

Table 3. Oxidative α-Dicarbonyl Cleavage of Different α-Dicarbonyl Compounds Reported in the Literature

α-dicarbonyl precursor	fragmentation product	expected counterpart	experimental basis	comments	ref
 1-deoxyglucosone	 acetic acid	 erythronic acid	model incubations of synthesized 1-deoxyglucosone labeling studies with 1,2-^{13}C, 3-^{13}C, 6-^{13}C glucose model incubations of glucose performed under $^{18}O_2$-atmosphere	results of ^{13}C labeling and $^{18}O_2$ experiments in complete support of an oxidative α-dicarbonyl cleavage	(22)
 2,3-diketo-gulonic acid	 oxalic acid	 threonic acid	labeling studies with 1-^{13}C, 2-^{13}C, 3-^{13}C, 5-^{13}C, 6-^{13}C ascorbic acid quantification of both counterparts	results of labeling experiments and amounts of both counterparts in complete support of an oxidative α-dicarbonyl cleavage	(58)

formic acid. A C5 compound (2-deoxypentose) should be formed via a C-1−C-2 scission of 3-deoxyglucosone in parallel at similar concentrations (Figure 3). Unexpectedly, a 2-deoxypentose sugar was not detected in the model experiments. Instead, furfurylalcohol was found as a possible follow-up product known to be generated from 2-deoxypentose after cyclization and dehydration.[56] The authors mentioned that the amounts of furfuryl alcohol did not equal the concentration of formic acid (approximately factor 1000 difference) and, therefore, expressed doubts themselves concerning the accuracy of the underlying fragmentation pathway. They speculated the mismatched amounts to be explained by further unknown follow-up products formed from the 2-deoxypentose sugar.[52]

The unsatisfactory results from known mechanistic studies regarding the hydrolytic α-dicarbonyl cleavage reaction gave rise to in-depth investigations by Davidek and co-workers with focus on carboxylic acid formation. The validity of the hydrolytic α-dicarbonyl cleavage hypothesis was disproved by the authors in a simple model experiment with 2,3-pentanedione (Figure 4). The theory of hydrolytic α-dicarbonyl fragmentation calls for two possible sets of fragmentation counterparts: on the one hand, acetic acid and propanal and, on the other hand, propanoic acid and acetaldehyde. Neither propanal nor acetaldehyde was identified in Davidek's model experiment. Instead, the detection of acetic acid and propanoic acid at similar concentration levels rather pointed to an oxidative α-dicarbonyl cleavage.[57]

In view of the published data, the hydrolytic α-dicarbonyl cleavage has to be ruled out as a carbohydrate fragmentation mechanism.

Oxidative α-Dicarbonyl Cleavage (Table 3; Figure 5). As mentioned above, Davidek's experiments of 2,3-pentanedione clearly indicated that an alternative oxidative α-dicarbonyl cleavage reaction indeed occurs as a previously not ascertained sugar fragmentation pathway to result in carboxylic acid formation.[22] In support, recent data from our working group on ascorbic acid degradation provided strong evidence that threonic acid is also formed via an oxidative α-dicarbonyl cleavage from 2,3-diketogulonic acid.[58] Table 3 briefly summarizes the findings of Davidek et al. and of ourselves concerning the oxidative α-dicarbonyl cleavage reaction.

Following the degradation mechanism of 2,3-pentanedione, an oxidative α-dicarbonyl cleavage of 1-deoxyglucosone should give rise to erythronic acid as the counterpart of acetic acid. Indeed, Davidek et al. identified erythronic acid and smaller amounts of threonic acid in 1-deoxyglucosone incubations. Labeling studies with several ^{13}C-glucose isotopomers have shown that both C_4-aldonic acids originate from the expected lower part of the original carbon backbone C3−C4−C5−C6. From in-depth investigations with isotopically labeled ^{18}O-dioxygen, Davidek and co-workers were able to establish the oxidative α-dicarbonyl cleavage of 1-deoxyglucosone as the most likely degradation pathway in complete support of their results (Figure 5). This pathway requires activated molecular oxygen generated by photooxidation processes or hydroperoxide species. The dioxygen-triggered mechanism starts with the incorporation of molecular oxygen by an attack at the C-2 or C-3 carbonyl moiety resulting in two alkoxyradicals. After a single-electron transfer reaction, the corresponding hydroperoxide anions occur, leading via a Baeyer−Villiger type rearrangement reaction to mixed asymmetric acid anhydrides with only one ^{18}O atom remaining in the molecule. The second ^{18}O atom was proposed to leave the hydroperoxide anion as a hydroxyl anion. Hydrolysis of the acid anhydride yields a mixture of monooxygen-labeled and unlabeled acetic acid and erythronic acid in a ratio of 1:1, respectively.[22]

In model incubations of different ^{13}C-ascorbic acid isotopomers, we found threonic acid to stem exclusively from C3−C4−C5−C6 of the original carbon backbone. The only C_2 compound labeled at C-1 and C-2 was detected in the incubation system as oxalic acid. Assuming oxalic acid as the

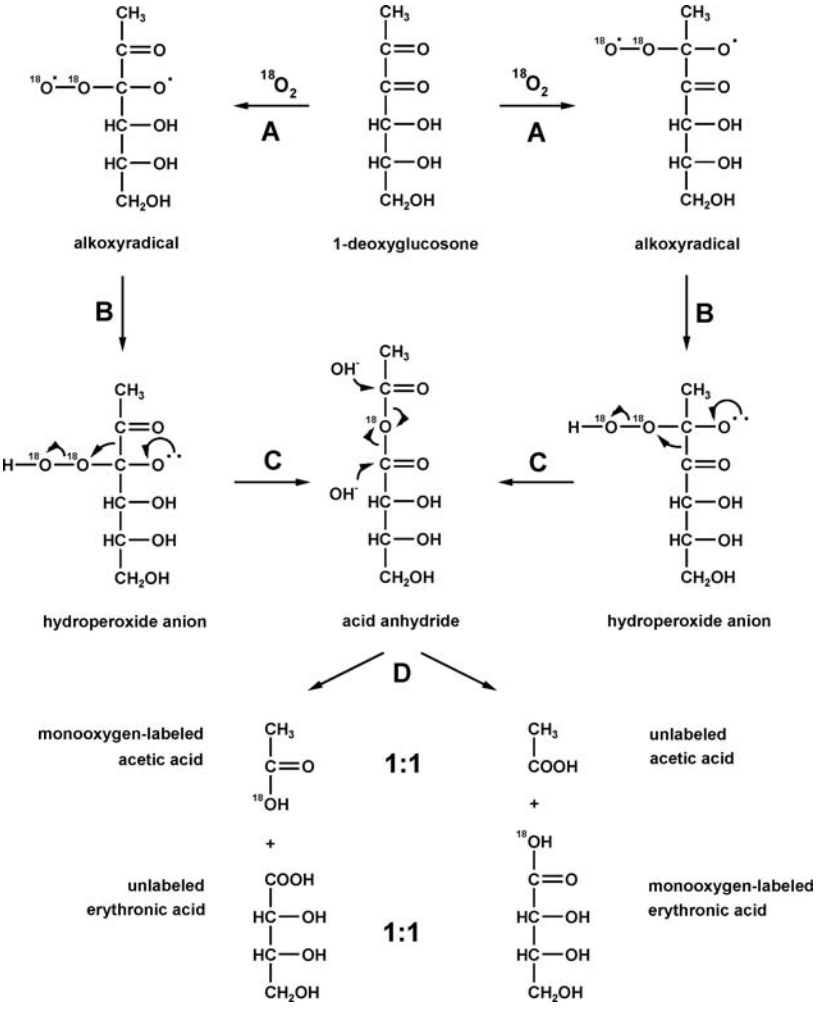


Figure 5. Oxidative α-dicarbonyl cleavage reaction of 1-deoxyglucosone, adapted from Davidek, with the following key steps: incorporation of molecular oxygen (A), single-electron transfer reaction (B), Baeyer–Villiger type rearrangement (C), and hydrolysis (D).[22]

fitting counterpart in an oxidative α-dicarbonyl cleavage reaction of 2,3-diketogulonic acid, threonic acid has to be generated in similar amounts. Taking into account that the 2,4-tautomer of 2,3-diketogulonic acid also releases oxalic acid via a hydrolytic β-dicarbonyl cleavage, the quantification results were in good agreement with the suggested mechanism.[58] Detailed information regarding the degradation pathways of ascorbic acid will be published elsewhere.

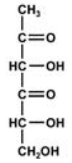
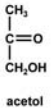
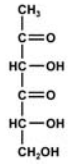
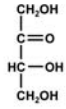
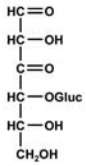
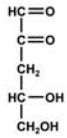
Hydrolytic β-Dicarbonyl Cleavage (Table 4; Figure 6). The hydrolytic β-dicarbonyl cleavage is known as a retro-Claisen reaction[59] and was first reported by Hayami as an extreme case of acyloin cleavage. During the decomposition reaction β-diketones (diacylcarbinols) are cleaved into α-hydroxy carbonyl compounds and carboxylic acids.[23]

Table 4 presents two typical examples of hydrolytic β-dicarbonyl cleavage[23,57] and another case resulting in the formation of a carboxylic acid and an α-dicarbonyl compound instead of the anticipated α-hydroxy carbonyl structure.[32] Hydrolytic splitting of β-dicarbonyl sugars was mentioned already in 1961 to explain the formation of acetol from glucose and fructose as an alternative fragmentation pathway to retro-aldol reactions.[23] This fragmentation route postulated by Hayami first seemed to be largely hypothetical, but the results were later confirmed by Weenen, who identified labeled acetol and unlabeled glyceric acid in 1-^{13}C-glucose degradation experiments.[20]

Again, Davidek and co-workers performed detailed studies regarding the hydrolytic β-dicarbonyl cleavage reaction. They unequivocally demonstrated that the β-dicarbonyl cleavage is the major pathway leading to the formation of acetic acid in aqueous hexose[54,60] and pentose-based Maillard reaction systems.[61] Supported by investigations of Voigt et al., the β-dicarbonyl cleavage route must be considered as the major carbohydrate fragmentation pathway in general.[36,43] The mechanism was proved by Davidek et al. in model degradation experiments of 2,4-pentanedione yielding almost equal amounts of acetic acid and acetone. The results were applicable to the degradation of 1-deoxyglucosone that is split into acetic acid and erythrulose. Exemplary for this case, mechanistic details for the β-dicarbonyl fragmentation are shown in Figure 6. 1-Deoxyglucosone isomerizes into the 2,4-tautomer 1-deoxy-2,4-hexodiulose, which can be nucleophilically attacked by a hydroxyl anion, resulting in acetic acid and a C4-enediol. The C4-enediol is subject to further isomerization reactions mainly leading to tetruloses. This is in contrast to the void mechanism of hydrolytic α-dicarbonyl cleavage, which does not allow for a change in the stereochemistry of a given precursor.

β-Dicarbonyl cleavage can also generate α-dicarbonyl compounds, which was demonstrated for a maltose Maillard

Table 4. Hydrolytic β-Dicarbonyl Cleavage of Different α-Dicarbonyl Compounds Reported in the Literature

α-dicarbonyl precursor	fragmentation product	expected counterpart	experimental basis	comments	ref
 2,4-tautomer of 1-deoxyglucosone	 acetol	 glyceric acid	identification of acetol in glucose and fructose incubation systems	postulated mechanism later confirmed by Weenen (20): 1-^{13}C glucose labeling studies yielded labeled acetol and unlabeled glyceric acid	(23)
 2,4-tautomer of 1-deoxyglucosone	 acetic acid	 erythrulose	model incubations with synthesized 1-deoxyglucosone	identification of both counterparts in 1-deoxy-glucosone incubations, results later confirmed by Voigt (43)	(57)
			degradation studies of 2,4-pentanedione as model molecule	amounts of counterparts in support of a hydrolytic β-dicarbonyl cleavage	
 1,3-tautomer of maltosone	formic acid	 3-deoxypentosone	model incubations with synthesized maltosone	degradation of maltosone yielded 16 mol-% 3-deoxy-pentosone	(32)
			kinetic studies of maltosone and 3-deoxypentosone in maltose incubation systems	kinetic studies in support of a hydrolytic β-dicarbonyl cleavage	

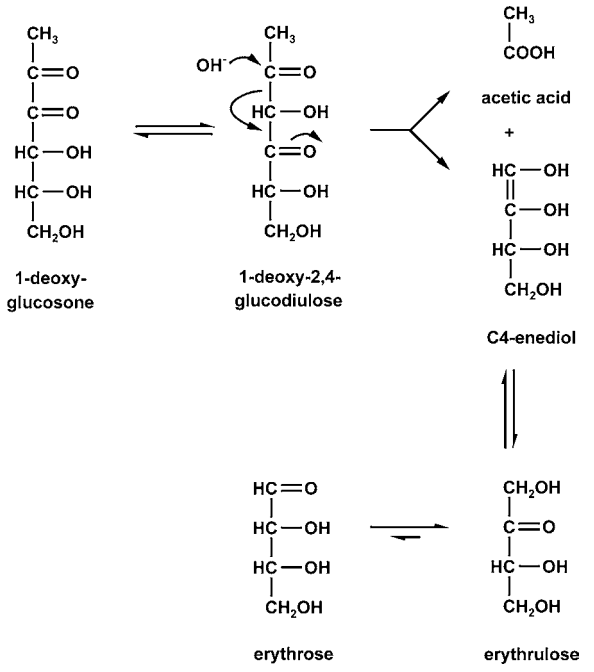


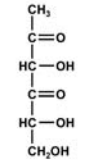
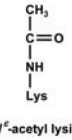
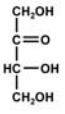
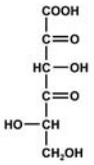
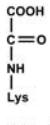
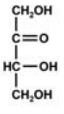
Figure 6. Hydrolytic β-dicarbonyl cleavage reaction of 1-deoxyglucosone, adapted from Davidek.[57]

reaction system. As a result of kinetic experiments performed in the presence and absence of oxygen, the formation of 3-deoxypentosone was assigned to oxidative conditions. The experiments clearly pointed out maltosone as the most likely precursor structure that fulfills the criteria of being formed via oxidation. For verification, maltosone was synthesized independently and incubated separately. Maltosone was transformed in an amount of 16 mol % into 3-deoxypentosone, which is in good support of the assumed β-dicarbonyl cleavage. Here, the glucose located at position C-3 of the intermediate C5-enediol immediately triggers β-elimination to result in 3-deoxypentosone.[32]

Amine-Induced β-Dicarbonyl Cleavage (Table 5; Figure 7). In 2010 a novel class of amides (amide-AGEs) formed during the degradation of the hexose key intermediate 1-deoxyglucosone was established in aqueous Maillard model systems[24] and later also in the human blood plasma.[62] It was found that in analogy to the hydrolytic β-dicarbonyl cleavage, an amine-induced β-dicarbonyl fragmentation reaction occurs, generating carboxylic acid amides and α-hydroxy carbonyl counterparts.[24]

Table 5 includes one example of amine-induced β-dicarbonyl fragmentation for 1-deoxyglucosone[24] and for 2,3-diketogulonic acid,[58] respectively. Carboxylic acid amides were already mentioned before to be formed in Maillard reaction systems, for example, N^{ϵ}-oxalyl-lysine was identified in human lens proteins[63] and N^{ϵ}-formyl-lysine was detected in glycated

Table 5. Amine-Induced β-Dicarbonyl Cleavage of Different α-Dicarbonyl Compounds Reported in the Literature

α-dicarbonyl precursor	fragmentation product	expected counterpart	experimental basis	comments	ref
 2,4-tautomer of 1-deoxyglucosone	 N^{ε}-acetyl lysine	 erythrulose	model incubations with synthesized 1-deoxyglucosone quantification of acetic acid/amide and erythrulose	formation of N^{ε}-acetyl lysine follows acetic acid formation at lower concentrations	(24)
 2,4-diketo-gulonic acid	 N^{ε}-oxalyl lysine	 erythrulose	labeling studies with 1-^{13}C, 2-^{13}C, 3-^{13}C, 5-^{13}C, 6-^{13}C ascorbic acid quantification of oxalic acid/amide and erythrulose	results of labeling experiments in support of a β-dicarbonyl cleavage formation of N^{ε}-oxalyl lysine follows acetic acid formation at lower concentrations	(58)

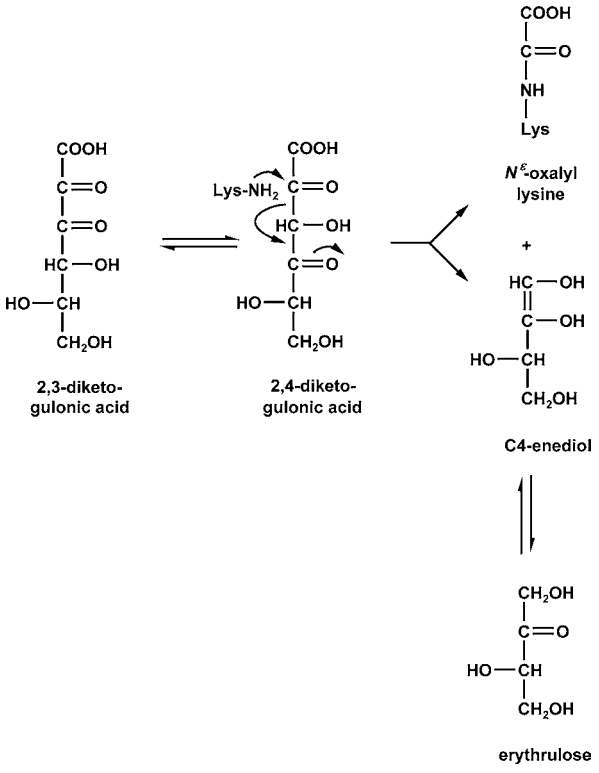


Figure 7. Amine-induced β-dicarbonyl cleavage reaction of 2,3-diketogulonic acid.

β-lactoglobulin.[64] However, the mechanism of formation was not studied. Amounts of carboxylic acid amides are generally low due to stoichiometric and stereochemical reasons and thus not appropriate as a single elucidation criteria. On the other hand, their corresponding carboxylic acids are operating as the quantitatively important counterparts to the α-hydroxy carbonyl compounds formed in the reaction systems. Thus, the identification of both, the amides in parallel to the corresponding acids, provides strong evidence for the underlying amine-induced β-dicarbonyl cleavage. Kinetic experiments indeed showed the same formation progress for both classes of stable Maillard endproducts. For example, N^{ε}-acetyl-lysine was formed in parallel to acetic acid with erythrulose as the counterpart from the 2,4-tautomer of 1-deoxyglucosone, in line with the amine-induced β-dicarbonyl fragmentation pathway.[24]

Mechanistically, the fragmentation reaction is working analogous to the hydrolytic β-dicarbonyl cleavage and is explained in detail for the formation of N^{ε}-oxalyl-lysine from 2,4-diketogulonic acid in Figure 7. Nucleophilic attack of the ε-amino function of lysine and subsequent β-cleavage release N^{ε}-oxalyl-lysine and a C4-enediol that isomerizes into erythrulose. This fragmentation pathway was proved indirectly by matching quantification of the corresponding oxalic acid and erythrulose. Conducted labeling studies were in complete support of the fragmentation pathway as erythrulose was found to originate at 100% from C3−C4−C5−C6 and oxalic acid and the amide approximately at 80% from C1−C2 of former ascorbic acid.[58] As both fragmentation pathways rely on the same mechanism, the parallel detection of corresponding carboxylic acids and amides is self-evident.

The present review of the literature toward fragmentation pathways of dicarbonyl compounds in the Maillard reaction cascade revealed a surprising fact regarding retro-aldol reactions. Retro-aldolization seemed to be well understood and is also most widely used in current Maillard literature to explain various fragmentation products without questioning its validity. However, to the best of our knowledge, no mechanistic study conclusively underpinned its existence for dicarbonyl degradation. Thus, the concept of retro-aldolization needs to be challenged and should be re-examined on a mechanistic basis and for its importance to Maillard reactions. In contrast, it must be stated explicitly that the frequently suggested hydrolytic α-dicarbonyl cleavage is nonexistent and has only been brought up on the basis of the adventitious, but nonquantitative, coexistence of matching counterparts. In view of the recent

research on Maillard fragmentations, the coexistence of hydroxy carbonyls and carboxylic acids has to be assigned to the β-dicarbonyl cleavage route. Quantitatively this mechanism proved to be the almost unrivaled fragmentation pathway, also explaining the formation of the novel class of amide-AGEs. A minor, but important, cleavage route is based on oxidative α-dicarbonyl scission to lead to matching carboxylic acid counterparts.

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Notes

The authors declare no competing financial interest.

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