5. Mechanistic Pathways of Non-Enzymatic Flavor Formation

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This chapter focusses on the formation of flavor active structures by mechanisms based on the degradation of reducing carbohydrates in the presence of amines. As model reactions have led to the elucidation of a confusing diversity of compounds, special attention is given to the understanding of the basic reaction pathways explaining the evolution of the most abundant odorants predominately shaping the aroma profile of most foods.

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In 1912, the French chemist Louis Camille Maillard reported on chemical changes in amino acid sugar reaction mixtures with respect to browning and release of carbon dioxide. Since then the reaction of reducing sugars with amines is termed nonenzymatic browning or Maillard reaction [5.1, 2]. The colored high molecular weight products of the late reaction stages are called melanoidines. Although intermediates are similar in their basic reactive structure, the reaction has to be clearly differentiated from enzymatic browning processes. Here in contrast, the initial phase of the reaction of phenolic educts is catalyzed by enzymes, while nonenzymatic follow-up reactions then lead to late stage products like the browned high molecular weight melanines.

5.1 Maillard Reaction – General Considerations

Thus, in the historic sense the word Maillard reaction describes the reaction of two main constituents of foods. Not only reducing sugars glucose, fructose, maltose, and lactose, but also oligosaccharides are of importance. Pentoses are of interest for the processing of meat and meat products (Chap. 10), but can also be released from hemicelluloses, for example, under roasting conditions. Amino components are widely abundant in foods, most relevant are free amino acids, peptides, and proteins. The impact of the Maillard reaction on foods during processing, storage, and retail is of significant and very diverse importance. Other than browning, taste and aroma are changed, and not always just to yield better products but also to lead to off-flavors. However, these changes define our traditional perception of a specific food. Protein modifications not only lead to an irreversible loss of essential amino acids, but also to desired functional physical alterations. Not only some of the intermediates, but also late stage Maillard products are of significant antioxidative capacity and can prolong the shelf life of foods. On the other hand especially at very high temperatures, structures with strong carcinogenic and mutagenic properties are formed, for example, acrylamide and aromatic amines.

Reducing sugars in aqueous solutions are relatively stable within a pH range of 5–7. However, in the presence of amines this changes drastically. Amino components will degrade sugars by acid–base catalysis and by nucleophilic carbonyl reactions. For free amino acids the primary amine functions react, for peptides and proteins other than the N-termini the side chains of lysine and arginine are mainly targeted. Within the course of Maillard reaction α-hydroxycarbonyl and, most importantly, α-dicarbonyl structures are the key intermediates. Most, if not all follow-up structures can be mechanistically related to the latter group. Figure 5.1 shows established compounds relevant for the degradation of glucose [5.3, 4]. Exceptions are 1-amino-1,4-dideoxyglucosone and 1,4-dideoxyglucosone. Although these dicarbonyls show the typical C_6-carbon

backbone, they are formed in higher amounts specifically during the degradation of disaccharides. It is important to note that compared to the starting sugar, the dicarbonyls with the intact carbon backbone are of much higher reactivity. Other than these, the complete array of fragmentation from C_5- to C_2-products is found.

The understanding that α-dicarbonyl compounds are of central importance has changed the historic definition of the Maillard reaction. In the modern view this term is related to any amine-dicarbonyl reaction. This means that in addition to the classic degradation starting from intact sugars, the intermediate dicarbonyls can also stem from, for example, fat autoxidation, oxidation of phenolic structures, or from fermentative processes.

It is important to realize that the term Maillard reaction is not meant in the sense of a single reaction, for example, found in organic synthesis starting from educts A and B leading to products C and D. Instead, it is an extremely complex reaction cascade with multiple simultaneous processes. The main aspects can be described by cyclization, elimination, fragmentation, condensation, hydrolysis, rearrangement, and redox reactions, including both ionic and radical mechanisms. Owed to this complexity, today many Maillard products can still not be described by conclusive mechanistic pathways. The situation is even more difficult because many products are only formed in negligible amounts in the ppm range or even much lower. However, especially relevant for the aroma of foods, it is exactly these structures which due to their extremely low odor and taste threshold levels are of central interest to food industry.

In 2014, *Dunkel* et al. published a review on the chemical signatures of food odors and their relevance to human olfaction and to food biotechnology [5.5]. They screened literature from 1980 to 2013 for reports on aroma profiles of foods using very stringent inclusion/exclusion criteria. These were a bioactivity-directed approach based on coupled gas chromatography-olfactory analyses using the flavor dilution approach, unequivocal mass spectrometric, or retention index verification based on authentic reference standards, and a reliable quantitation by, for example, stable isotope dilution analyses. As a result, they selected 119 publications describing the key food odorants of 227 foods across the complete market basket with alcoholic beverages, meat products, fish and sea food, cereal and bakery products, dairy products, fats and oil seeds, fruits, vegetables, mushrooms, spices and herbs, cocoa and chocolate, coffee, tea, and many others. Surprisingly, the authors identified only 226 key odorants sufficient to describe the odor space of these foods. The authors even extrapolated from this observation that less than 230 main odorants, out of a total of about 10 000 volatiles assumed to be present in foods, might be enough to describe the total odorant space present in our foods and beverages. Out of the 226 odorants, the authors categorized three classes, namely 16 generalists with an abundance of more than 25% in the 227 food samples, 57 intermediaries with less than 25% but more than 5% abundance, and 151 individualists with less than 5% abundance. However most interestingly, many representatives of the generalist and also of the intermediaries can be related to the degradation of carbohydrates and amino acids, based on sole or alternative nonenzymatic pathways or a combination of both. This is especially true for the class of the generalists, where 10 out of 16 structures can be explained by Maillard reaction pathways. Table 5.1 lists these structures, and their abundances. Some of the compounds can evolve from both metabolic–enzymatic and nonenzymatic pathways. However, this chapter focuses

Table 5.1 Key odor structures in foods and beverages are formed by nonenzymatic pathways

Odorant *generalists*	**Abundance (%)**
3-(Methylsulfanyl)-propanal (methional)	54
2-/3-Methylbutanal	51
Butan-2,3-dione (diacetyl)	42
4-Hydroxy-2,5-dimethyl-3(*2H*)-furanone (furaneol)	41
3-Hydroxy-4,5-dimethyl-2(*5H*)-furanone (sotolone)	36
Acetic acid	29
Acetaldehyde	29
Ethyl-2/3-methylbutanoate	28
2-Acetyl-1-pyrroline	26
2-/3-Methylbutanoic acid	26
Odorant *intermediaries*	**Abundance (%)**
2-Methylpropanal	24
Phenylacetaldehyde	23
Ethyl-2-methylpropanoate	23
2-Ethyl-3,5-dimethylpyrazine	19
Dimethyltrisulfide	19
Phenylacetic acid	18
2,3-Diethyl-5-methylpyrazine	17
Furan-2-ylmethanethiol (2-furfurylthiol)	15
2-Methoxy-3-(propan-2-yl)pyrazine	14
Dimethylsulfide	14
Methanethiol	13
2-Acetyl-2-thiazoline	9
Propanal	7
2-Methylfuran-3-thiol	6
2-Ethyl-4-hydroxy-5-methyl-3(*2H*)-furanone (homofuraneol)	6
1,1-Diethoxyethane	6
Methyl-2/3-methylbutanoate	5

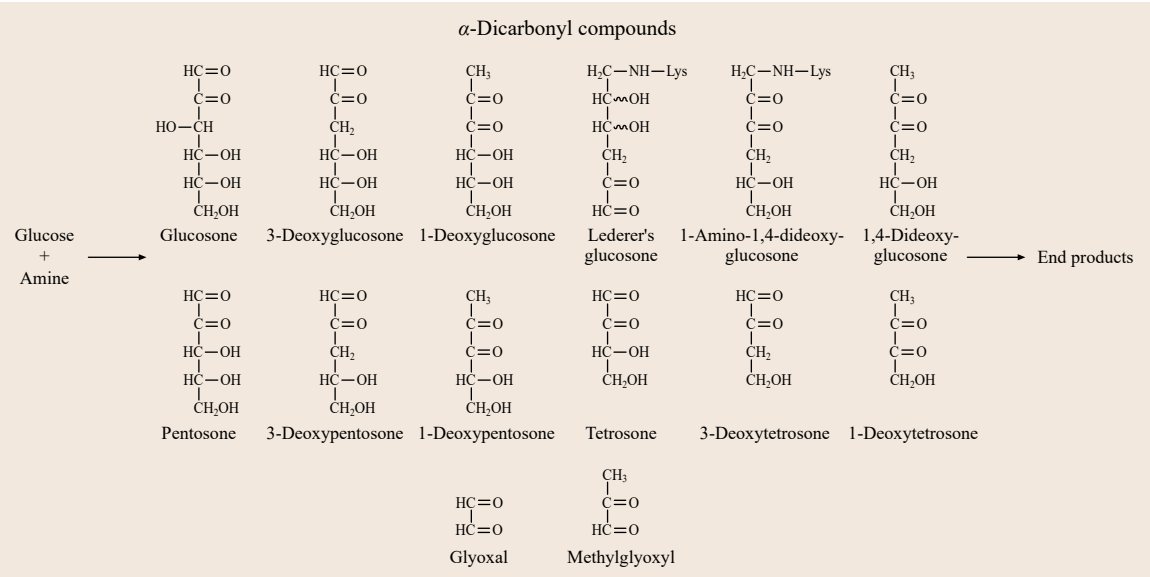

Fig. 5.1 Dicarbonyl compounds are the central Maillard intermediates

on the mechanisms explaining explicitly their nonenzymatic formation. Wherever possible, the following discussions are based on publications using isotopic labeling as the carbon module labeling (CAMOLA) approach [5.6] or using authentic intermediates for verification.

In general, both sugars and amino acids are too polar to contribute directly to the odor profile. This means that polar functionalities must get lost during the degradation process, which in most cases can be related to dehydration, elimination, fragmentation, and decarboxylation reactions.

5.2 α-Dicarbonyl Compounds

The early stage of the Maillard reaction is determined by the reactivity of the carbonyl function of the original sugar. In Fig. 5.2, glucose was chosen as a model compound. The structure is drawn in the open-chained form showing the native carbonyl function for simplification, although in aqueous systems carbonyl compounds exist mainly as hydrates or as half-acetalic structures. However, it is the availability of the free carbonyl function which determines the reactivity of the respective sugar. Reaction with the amine and dehydration leads to an imine, also called Schiff base or aldoimine. Keto-enol-tautomerism then gives an aminoketose via an 1,2-enaminol. This isomerization is also called Amadori rearrangement, the aminoketose termed Amadori product. The acid–base catalysis of the amino compounds basically allows the enolization to proceed along the complete carbon backbone of the sugar, which is prerequisite for the elimination of water or the amine. Starting from the 1,2-enaminol 3-deoxy-2-glucosulose (3-deoxyglucosone) is formed, the 2,3-enediol gives 1-deoxy-2,3-glucodiulose (1-deoxyglucosone). Substantial amounts of Lederer's glucosone verify that the enolization indeed proceeds to a 5,6-enediol intermediate [5.7]. In contrast, the formation of 2-glucosulose (glucosone) can only be explained by oxidation, that is, the amine catalyzed autoxidation of glucose, but mainly of the intermediate imine and of the Amadori product.

After enolization, 1-deoxyglucosone and glucosone show an α-oxoenediol motive. This means that these compounds are reductone structures, which are highly redox active and are easily degraded to give lower molecular weight fragments of even higher reactivity. Both, nonenzymatic reduction and oxidation in Maillard reaction systems can be explained by these intermediates. Oxidation leads to a 1,2,3-tricarbonyl compound. Thus, within the Maillard reaction it is exactly these structures with reductone character which direct the further course of reaction.

In Maillard literature, four main mechanisms of fragmentation have been proposed [5.8]:

1. Hydrolytic α-dicarbonyl cleavage
2. Retro-aldol cleavage

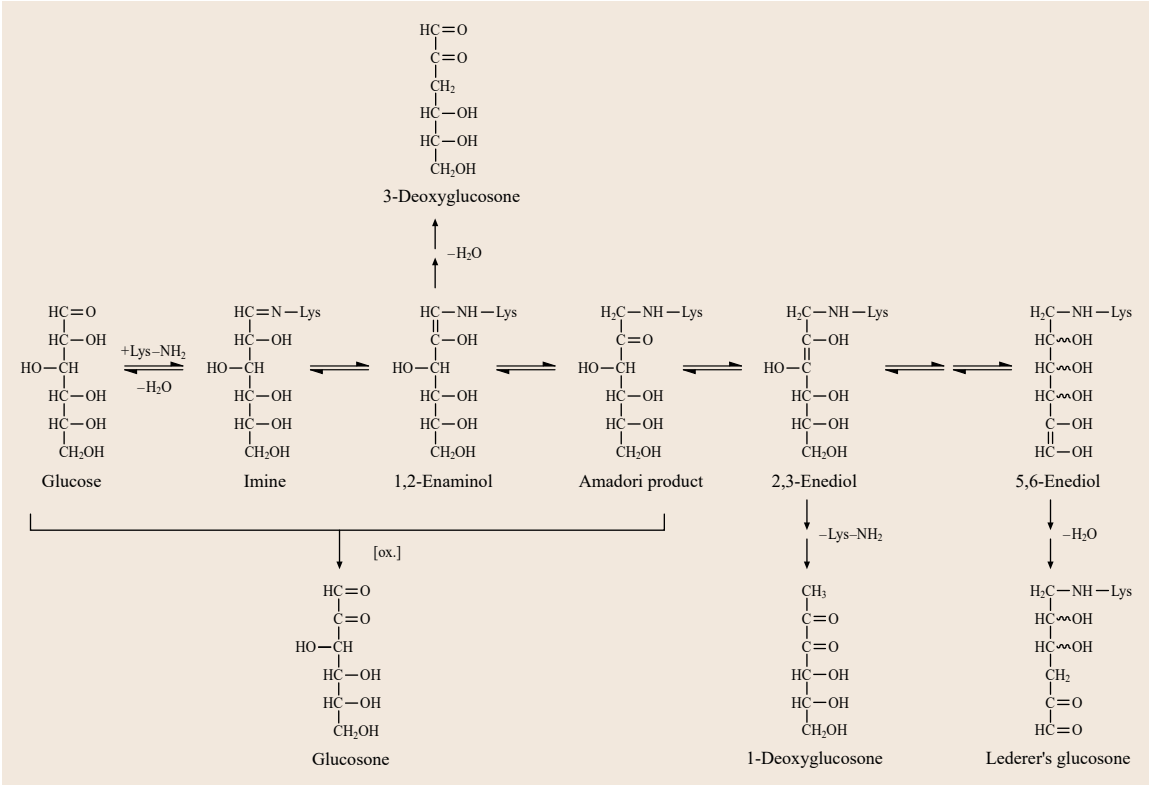

Fig. 5.2 Initial stage of Maillard reaction

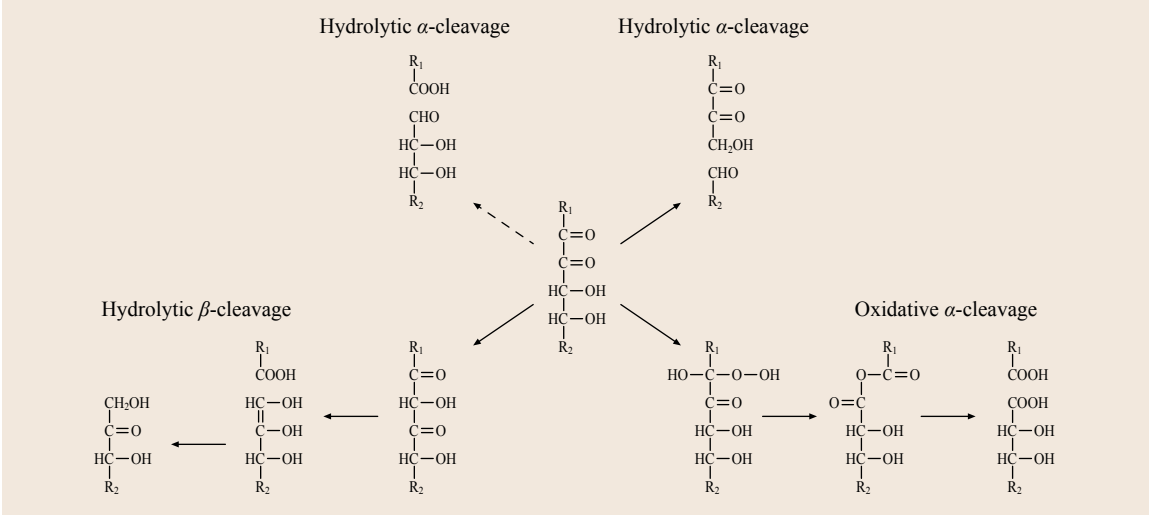

Fig. 5.3 Mechanisms of sugar fragmentation

3. Hydrolytic β-dicarbonyl cleavage
4. Oxidative α-dicarbonyl cleavage (Fig. 5.3).

Recently, the latter two have been refined by an additional amine-induced alternative route each.

The hydrolytic α-dicarbonyl cleavage describes the nonoxidative scission of an α-dicarbonyl motive to give a carbonyl and a carboxylic compound. It has been used very frequently especially in the older Maillard literature to explain the parallel formation of the two

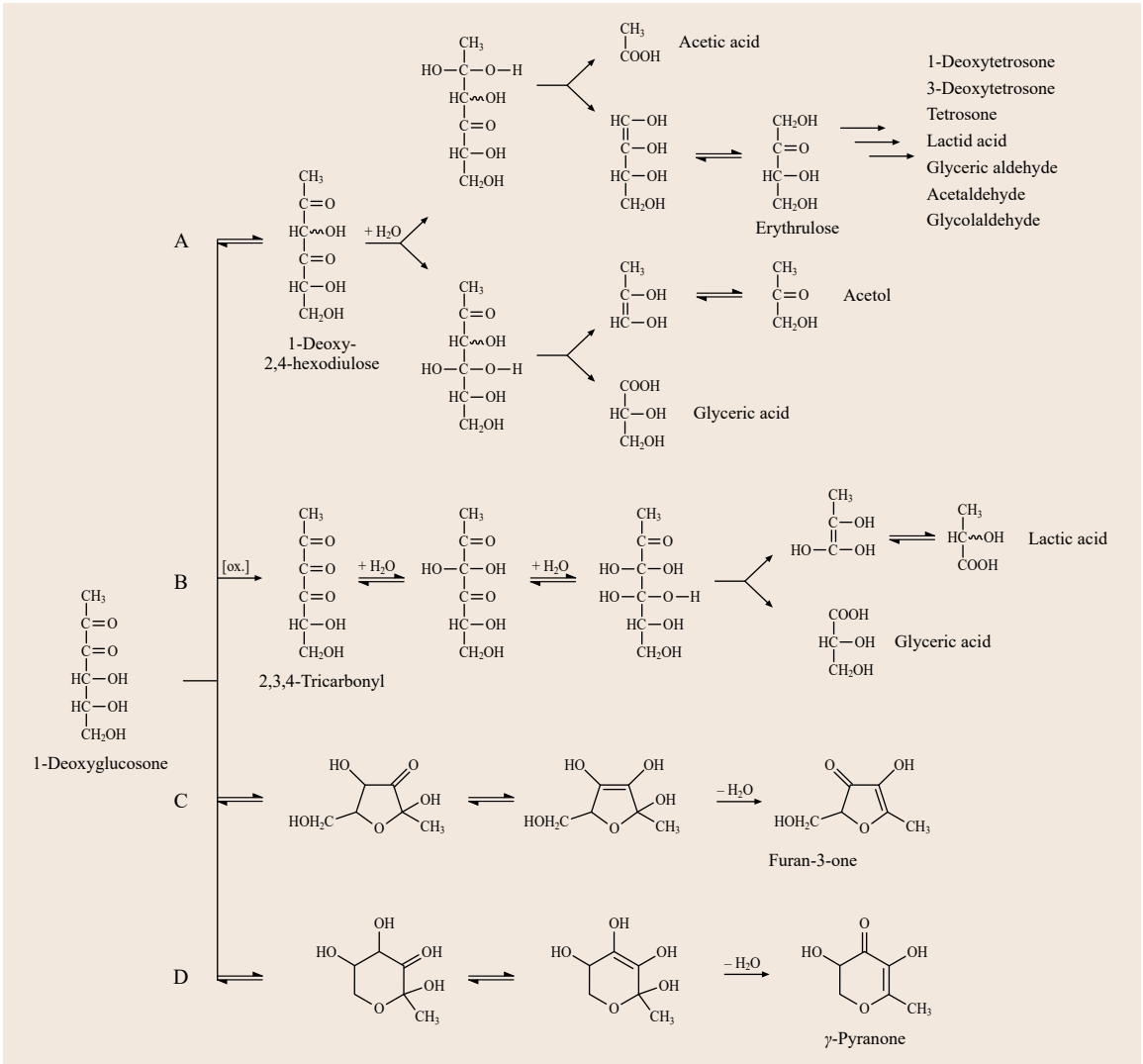

Fig. 5.4 1-Deoxyglucosone is the central intermediate of hexose Maillard chemistry: (A + B) β-dicarbonyl cleavage, (C + D) cyclization

molecule classes. However, recent investigations and also a comprehensive literature review have come to the conclusion that there is no experimental ground for this hypothetical mechanism and that its proposal is merely based on the coincidential, but non quantitative coexistence of structures with fitting complementary carbon backbone. Thus, this mechanism has to be excluded as void.

Retro-aldol cleavage is another mechanism that has been challenged recently. Any starting sugar shows the prerequisite β-hydroxycarbonyl moiety leading to two carbonyl fragments. Specifically from α-dicarbonyls, the mechanism leads to one α-dicarbonyl and one carbonyl molecule. This type of cleavage is commonly used to explain the generation of short-chained α-dicarbonyl compounds which are odor active as such, or are important intermediates involved in the formation of potent odor active compounds, that is, especially butane-2,3-dione (diacetyl), methylglyoxal, and 1-hydroxy-2-propanone (acetol). Aldol condensations are the reverse reaction and are frequently used in organic synthesis strategies under nonaqueous conditions. They have been also shown to proceed in aqueous systems at moderate temperatures, for example, leading from diacetyl and formaldehyde to 1,4-dideoxy-2,3-pentodiulose (1,4-dideoxypentosone) [5.9]. However, retro-aldol cleavages have been almost outruled for Maillard systems operated at moderate temper-

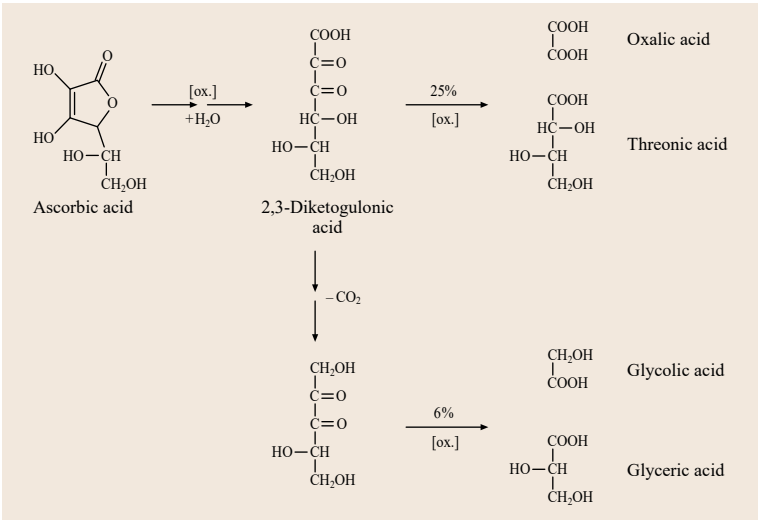

Fig. 5.5 Oxidative α-dicarbonyl cleavage is a major fragmentation mechanism during degradation of ascorbic acid

atures up to 50 °C. There was no scission of independently synthesized 1,4-dideoxy-2,3-hexodiulose (1,4-dideoxyglucosone) to give diacetyl and glycolaldehyde [5.4]. Instead, the educt was shown to be rather stable. In addition, incubations (< 50 °C) of authentic 1-deoxyglucosone and 3-deoxyglucosone both gave only negligible amounts of methylglyoxal [5.10, 11]. However, it is exactly these dicarbonyls which are frequently quoted as the main methylglyoxal precursors. Obviously, retro-aldol reactions are not favored at low temperatures and might require higher energy input found, for example, at boiling or roasting conditions.

β-Dicarbonyl cleavage reactions have evolved to one of the major fragmentation reactions already working at moderate temperatures. Although already mentioned in the earlier Maillard literature this mechanism has been studied first in detail by *Davidek* et al. in model systems using simple α- and β-dicarbonyl short-chained alkanes [5.12]. Later, these results were transferred by this and other groups to Maillard intermediates with a reductone motive. This allows an α-moiety to isomerize to a β-configuration, which is prerequisite for the β-scission. Figure 5.4 highlights reactions of 1-deoxyglucosone, which has to be evaluated as the major central intermediate of hexose chemistry with an exceptional high reactivity (e.g., half-life $\leq$ 30 min at 37 °C) [5.13]. Most important follow-up products can be rerouted to this structure. Detailed mechanistic studies were facilitated by a successful independent synthesis of 1-deoxyglucosone and allowed to explain about 70–80% of its total degradation under Maillard conditions. Path A starts from the isomeric 1-deoxy-2,4-hexodiulose. Hydration at the C_2-carbonyl function leads to acetic acid and an 1,2-enediol giving erythrulose. The yields of acetic acid were about 60% at 50 °C (up to 85% at 100 °C) independent of the presence of oxygen. This means that in hexose reactions this mechanism explains most if not all the acetic acid being formed. If the hydration proceeds at the C_4-carbonyl function glyceric acid and acetol result. While carboxylic acids have to be evaluated as stable Maillard endproducts the cleavage counterparts are α-hydroxycarbonyl intermediates which are prone to various follow-up reactions to give further carboxylic acids and dicarbonyls, but also important carbonyl structures as active odorants as such or as intermediates leading to odorants, for example, acetaldehyde, glycolaldehyde and glyceric aldehyde. Path B of Fig. 5.4 shows that β-dicarbonyl cleavage can also be facilitated from a double hydrated oxidation product of 1-deoxyglucosone (C_3 and C_4) to give lactic acid and as the counterpart glyceric acid in substantial yields. The putative cleavage starting from a C_2,C_3-dihydrate to give acetic acid and erythronic acid was experimentally disproven to be of importance. This also excludes a major alternative oxidative α-dicarbonyl cleavage reaction of 1-deoxyglucosone.

A forth cleavage reaction has been established only lately for Maillard systems, the oxidative α-dicarbonyl fragmentation. It was first evidenced from incubations of 2,3-pentandione to give acetic acid and propanoic acid in equimolar concentrations. The mechanism requires activated molecular oxygen stemming, for example, from UV irradiation or hydroperoxides. Incorporation at one of the carbonyl functions gives alkoxyradicals and after single-electron transfer reactions hydroperoxides. These rearrange via a Bayer–Villiger-type reaction to mixed asymmetric anhydride species, which are readily hydrolysed to two carboxylic acids (Fig. 5.3). Obviously, singlet oxygen is prerequisite

for the initial attack at the carbonyl function; however, if present, the reaction will also proceed at moderate conditions. This also must be the reason why during degradation of 1-deoxyglucosone this type of reaction is only of negligible importance. On the other hand, ascorbic acid is known to produce significant amounts of activated oxygen species. Consequently, *Smuda* and *Glomb* were able to verify 31% initiated by oxidative α-dicarbonyl cleavage out of a total of 75% of degradation related to fragmentation (Fig. 5.5) [5.14]. The remainder was attributed to decarboxylation and hydrolytic β-dicarbonyl cleavage. However, given the polarity of the resulting acids and the lack of carbonyl activity of the carboxylic acid function, it must be assumed that most products of the oxidative α-dicarbonyl fragmentation will not contribute to the odor profile.

5.3 Strecker Degradation

The Strecker degradation is by far the aspect of the Maillard reaction which accounts for most of the structures within the group of odor generalists and intermediaries listed in Table 5.1. 13 out of the 27 compounds can be explained directly from this mechanism, 7 by direct and indirect follow-up reactions. Basically, the term Strecker degradation describes the interaction of α-dicarbonyl compounds with amino acids including a decarboxylation step. This again underpins the importance of α-dicarbonyls as the central intermediates of Maillard reactions. After nucleophilic attack of the amino substituent at one of the carbonyl moieties and formation of an imine, the electron drawing second carbonyl function triggers the decarboxylation leading to an α,β-unsaturated imine, which hydrolyses to give the Strecker aldehyde and an eneaminol (Fig. 5.6, pathway A). This explains not only the formation of methional, 2-/3-methylbutanal, acetaldehyde, 2-methylpropanal, and phenyl acetaldehyde, but also formaldehyde from methionine, leucine/isoleucine, alanine, valine, phenylalanine, and glycine, respectively. Besides being aroma active with very low odor thresholds as such, these unpolar low molecular carbonyl structures are highly reactive and can perform, for example, aldol-type reactions to give further important aroma compounds. This also explains the spontaneous formation of 1,2-diethoxyethane from acetaldehyde in, for example, beer or wine. From the mechanistic point of view, the Strecker reaction represents a redox reaction. The amino acid is oxidized under decarboxylation, while the α-dicarbonyl gets reduced. This can be immediately realized from the comparison of the starting α-dicarbonyl to the α-hydroxycarbonyl structure, which is formed after tautomerism and hydrolysis of the resulting α-hydroxyimine to release ammonia already under moderate conditions (pathway B). If the tautomerism proceeds to an α-aminocarbonyl structure, condensation leads to dihydropyrazines, which spontaneously gets oxidized to give pyrazines (pathway C). These heteroaromatic structures contribute especially to the aroma profiles of baked and roasted foods. The basic reactions (A–C) have been continuously extended and have evolved today to a whole mechanistic cascade within the complex complete Maillard scheme. This is especially due to the fact that (I) many Strecker intermediates are very reactive, and that (II) the electronic isomerization reactions are reversible. In food systems of low water activity the intermediate hemiaminal prior to the release of the Strecker aldehyde cannot be formed (A) [5.15]. Instead, the α,β-unsaturated imine proceeds via ring formation to a 5-membered 4-oxazoline, which exists in an equilibrium to an 3-oxazoline (pathway D). Authentic synthesized oxazolines readily released the Strecker aldehyde upon addition of water (up to 56% at 37 °C). Other than by water content, the rate was strongly affected by pH and temperature. The existence of oxazoline derivatives was indeed verified in foods, for example, chocolate. Taken together, this alternative pathway explains the observation why especially from heat processed foods such as bread, snacks, and chocolates major amounts of odor active substances are formed during mastication and thereby add significantly to the retronasal perception.

In contrast to the α,β-unsaturated imine, the intermediate hemiaminal of pathway A represents an electron-rich eneaminol structure, which can be easily oxidized to give a heteroanalog α-dicarbonyl. Again, the electron-pulling carbonyl function initiates the intramolecular redox reaction to give the Strecker acid and the eneaminol after hydrolysis (pathway E) [5.16]. Thus, the ratio of formation of Strecker aldehyde to Strecker acid strongly depends on the reaction conditions. In-depth model reactions have indeed shown that in the presence of oxygen the ratio changed from about 1 : 1 under deaeration to 1 : 3–4 under aeration, that is, the formation of the Strecker acids represents a major mechanism for the processing of foods. The authors also excluded the notion that the formation of the acids might be an artifact of direct aldehyde oxidation by the use of stable isotopically labeled derivatives. Pathway E, therefore, explains the formation of the impor-

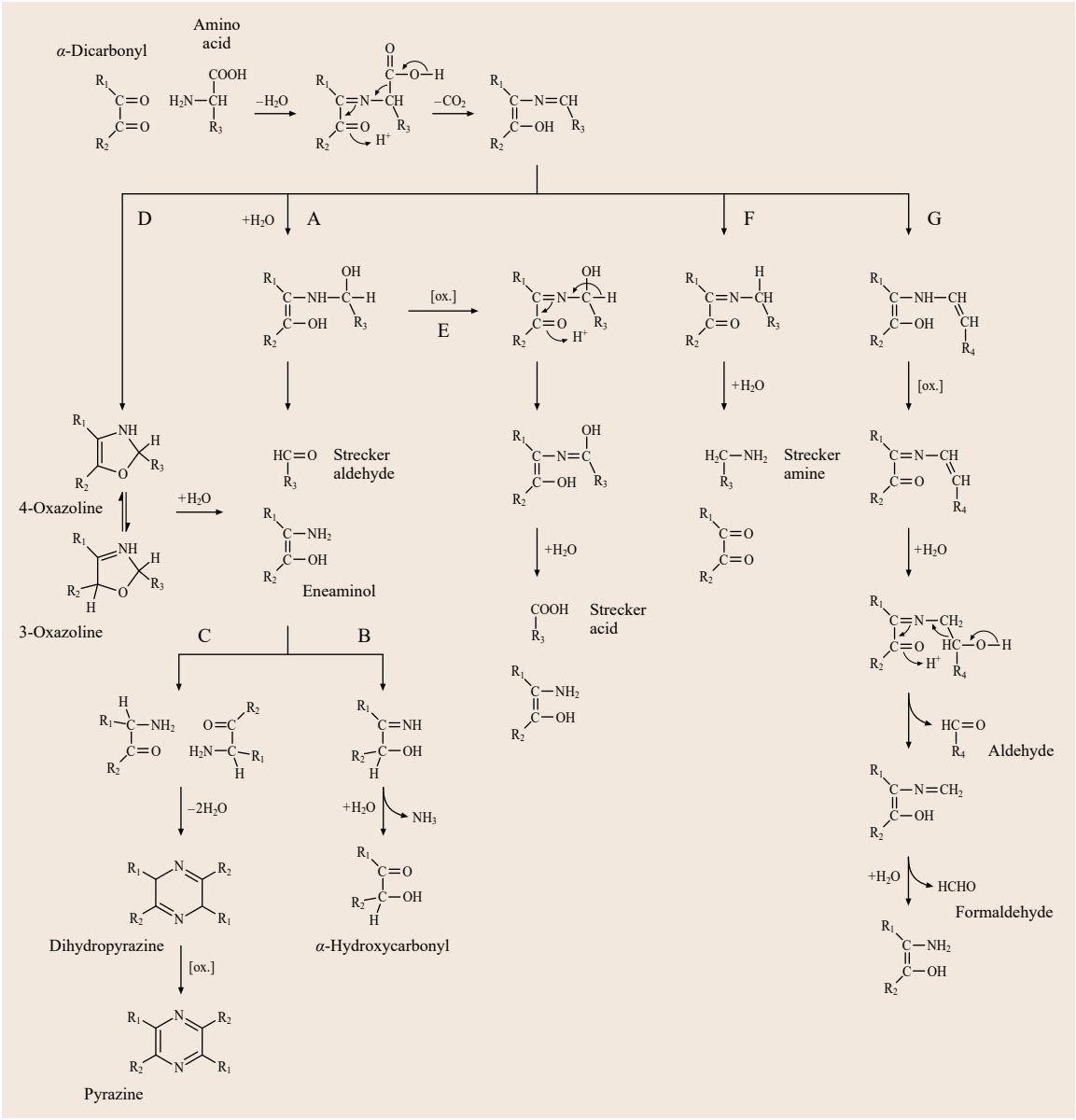

Fig. 5.6 The Strecker reaction cascade

tant odorants acetic acid, 2-/3-methylbutanoic acid, 2-methylpropanoic acid, phenylacetic acid from alanine, leucine/isoleucine, valine and phenylalanine, respectively. The acid formation also explains indirectly the detection of related methyl and ethyl esters in alcoholic beverages due to spontaneous esterification or transesterification from pectines induced, for example, under roasting conditions (ethyl-2,3-methylbutanoate, ethyl-2-methylpropanoate, methyl-2/3-methylbutanoate).

The reversibility of the above mentioned isomerization based on electronic rearrangement reactions is best envisioned by the detection of Strecker amines in amino acid/α-dicarbonyl mixtures (pathway F) [5.17]. This is obviously an alternative pathway to the pyridoxal phosphate-driven enzymatic generation of biogenic amines. However, the ratio of Strecker aldehydes to the respective amines ranged from about 1 : 100 (phenylalanine) to 1 : 1000 (other unpolar amino acids) in aqueous model systems. This and also the relatively high-odor thresholds might explain why Strecker amines are not found in above Table 5.1. The ratio might be related to the presence of electron pulling versus pushing groups,

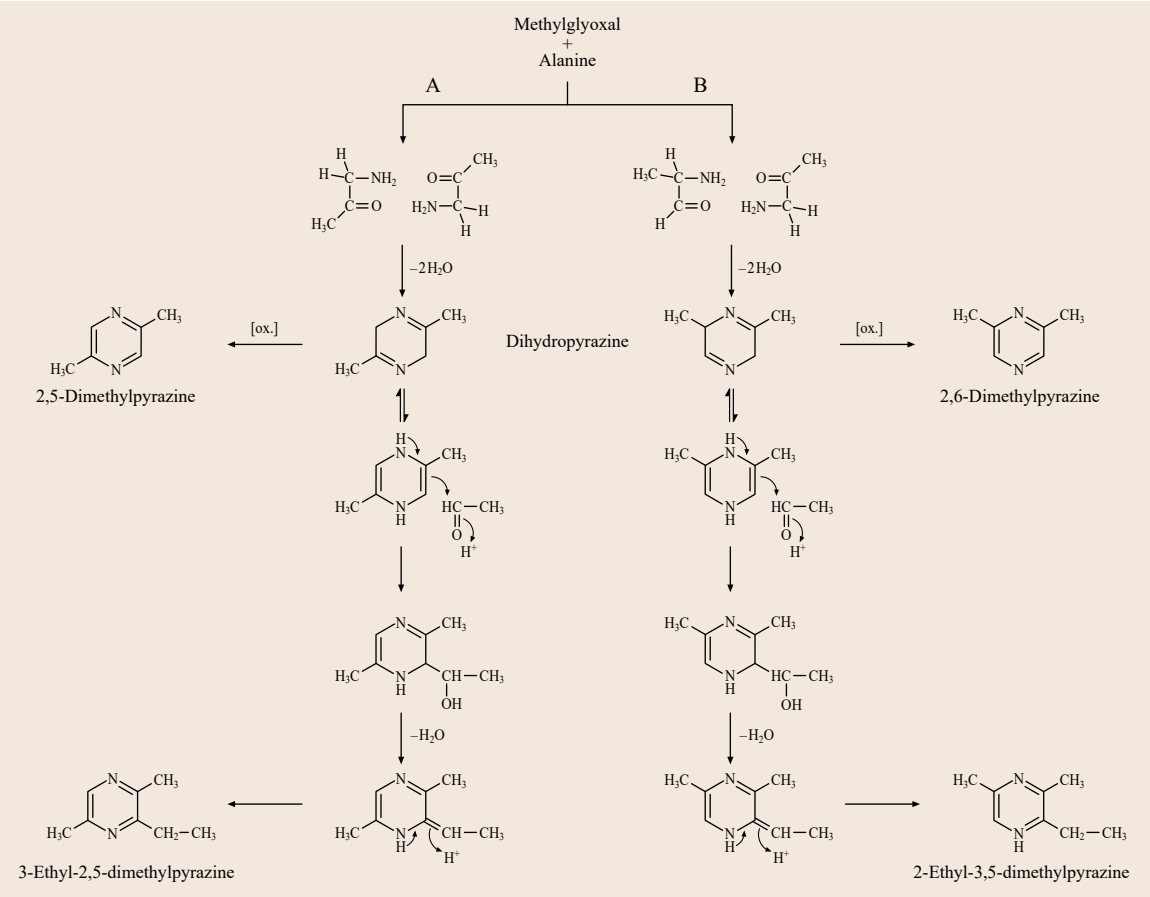

Fig. 5.7 Oxidative and nonoxidative formation of pyrazines

and also to a possible extension of unsaturation. This notion and also the reversibility were enlightened by the detection of substantial amounts of benzaldehyde (up to 7%) in reactions of 2-phenylethylamine in presence of methylglyoxal besides very small amounts of the respective Strecker aldehyde. Here (pathway G) after oxidation and addition of water, an intermediate half acetal was proposed to cleave off first benzaldehyde in a retro-aldol-type reaction and then formaldehyde to result again in the central eneaminol structure. Obviously, the cleavage of the half-acetal is strongly facilitated by the electron drawing dicarbonyl moiety.

It has to be mentioned that the Strecker pathways shown in Fig. 5.6 have been further extended today. One facet is that Strecker aldehydes have been detected in model systems of Amadori products [5.18]. This is expected, as Amadori products are transient intermediates in any Maillard system starting from carbohydrates leading to α-dicarbonyl compounds. However, the authors suggested a direct oxidative degradation route to explain different ratios of Strecker aldehydes to acids depending on the particular carbohydrate. On the other hand, Strecker-type reactions were identified in pyrolysis systems mimicking roasting processes [5.19] and in the interaction with fat autoxidation intermediates [5.20]. In contrast to earlier discussed reactions with a central eneaminol intermediate, here in both cases ylide intermediates were suggested as the important pivotal structures.

The oxidation of the dihydropyrazines to the aromatic derivatives in Fig. 5.6 occurs spontaneously in presence of atmospheric oxygen. In Fig. 5.7, this reaction is depicted in more detail for reactions with methylglyoxal and leads to 2,5-dimethylpyrazines in pathway A. Alternatively to 1-aminopropan-2-one, in pathway B condensation can also include 2-aminopropanal. However, this molecule is less abundant because the aldehyde function of methylglyoxal is more reactive than the keto moiety. After oxidation 2,6-dimethylpyrazine is formed. Thus in this oxidative pathway, the ring substituents must stem from the α-dicarbonyl compounds involved in the formation of the

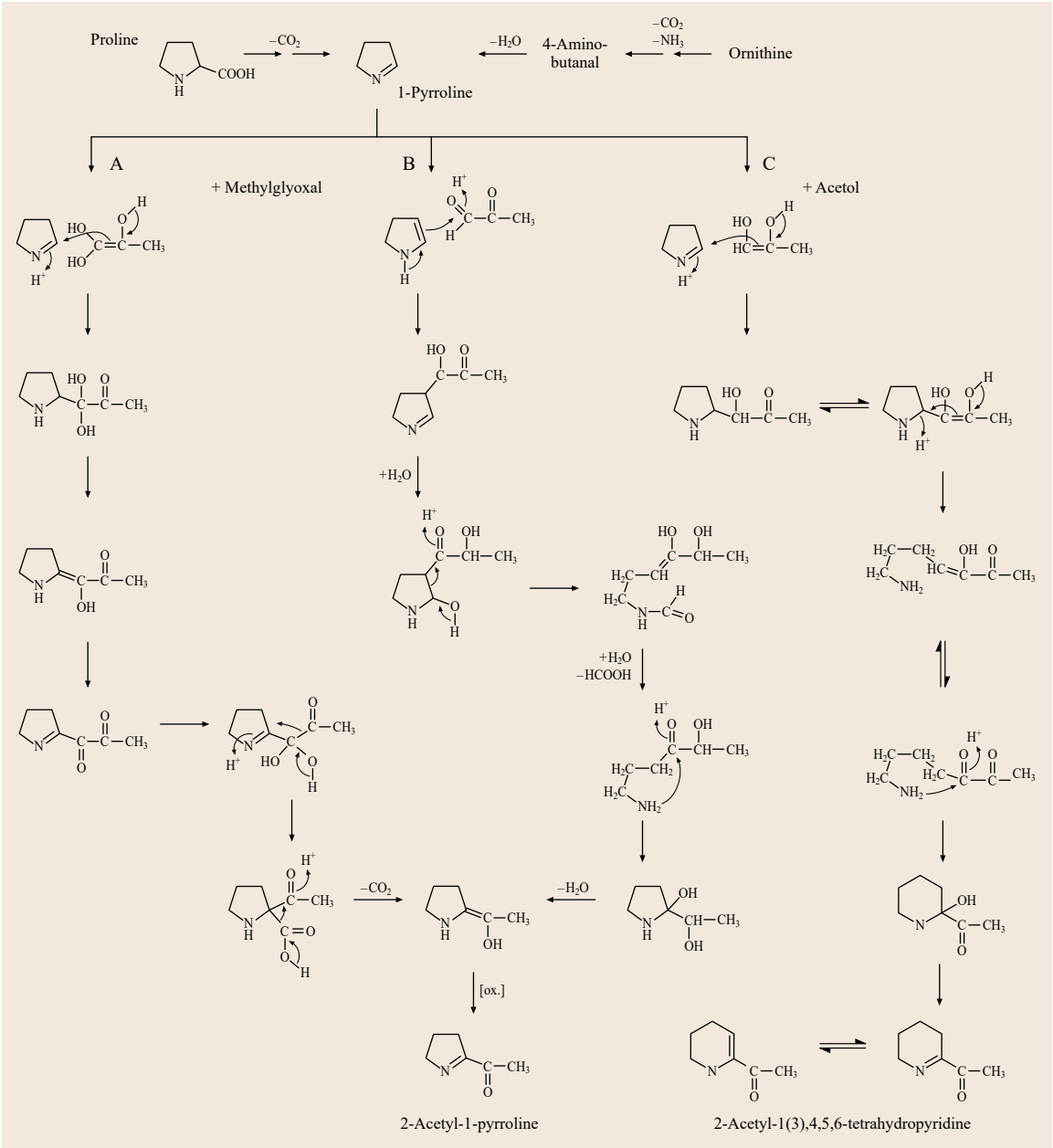

Fig. 5.8 Main routes of formation of 2-acetyl-1-pyrroline and 2-acetyl-1(3),4,5,6-tetrahydropyridine by Strecker degradation of proline with methylglyoxal

intermediate eneaminol [5.21]. As pyrazines are important agents of aroma profiles of baked, roasted, or fried foods with earthy odor qualities their mechanism of formation has been studied in detail and revealed additional nonoxidative pathways [5.22]. Here, the substitution pattern is argued by condensation of carbonyl structures to the electron rich dihydropyrazine ring system. As an example, the reaction with acetaldehyde is shown, which is the Strecker aldehyde of alanine, but may also originate from fermentative processes. Dehydration and tautomerization gives 3-ethyl-2,5-dimethyl- and 2-ethyl-3,5-dimethylpyrazine, respectively, without the need of an oxidative step. If the condensation reaction occurs with an α-dicarbonyl like methylglyoxal, the formation of acylated pyrazine derivatives can be explained.

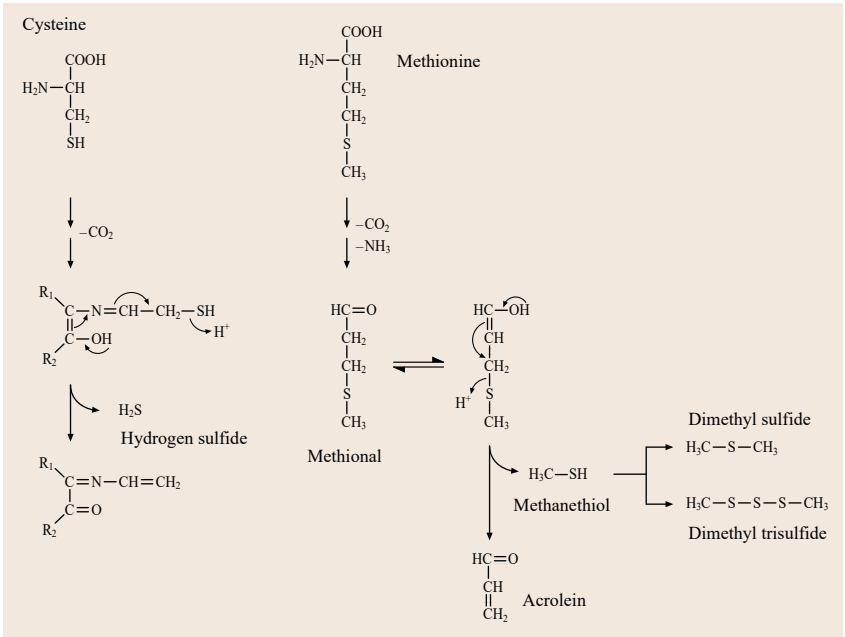

Fig. 5.9 Strecker degradation of cysteine and methionine leads to important odor active small nucleophiles

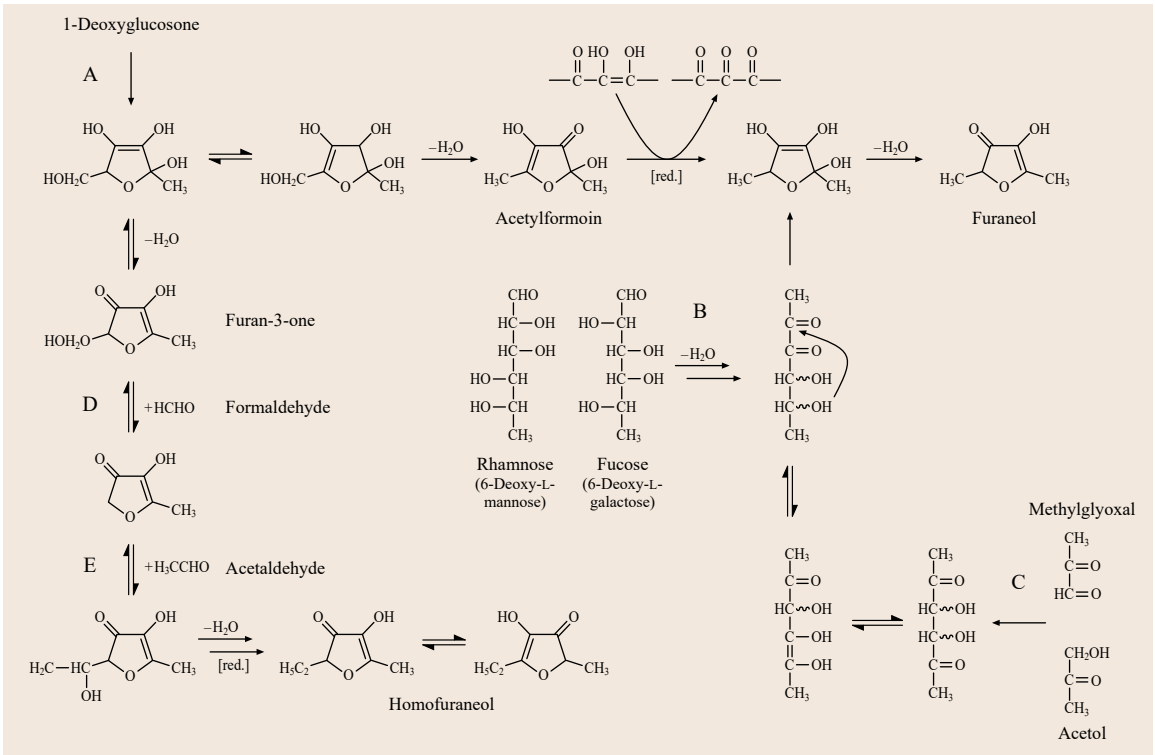

Fig. 5.10 Formation pathways of 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (furaneol)

2-Acetyl-1-pyrroline is the main representative of a class of N-containing heterocycles with roasty, popcorn-like odor notes and very low odor thresholds, especially for 2-acetyl-1-pyrroline which ranks 9 among the odorant generalists in Table 5.1 with 26% abundance. Central intermediate in the formation is 1-pyrroline,

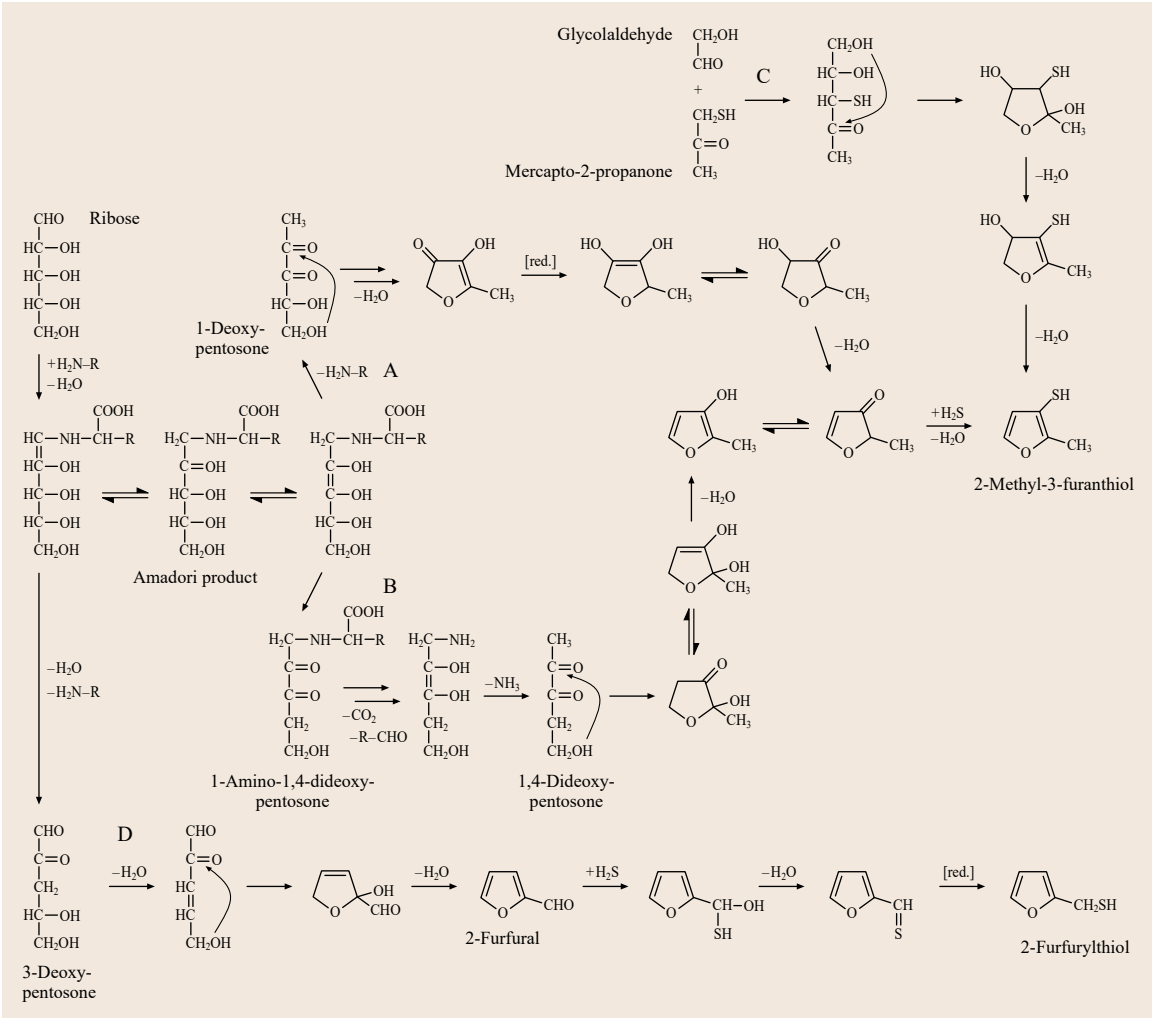

Fig. 5.11 Main formation mechanisms of 2-methyl-3-furanthiol and 2-furfurylthiol

which is the Strecker decarboxylation product of the amino acid proline [5.23]. Alternatively, but of less importance, 1-pyrroline can be formed by cyclization of the ornithine Strecker aldehyde 4-aminobutanal [5.24]. The reaction mechanism has been studied in-depth by the use of isotopically labeled educts elucidating two routes based on carbohydrate fragmentation C_3-products, that is, methylglyoxal and acetol (Fig. 5.8). The main course starts from the hydrate of methylglyoxal (pathway A) and thus allows a nucleophilic attack of the derivatized aldehyde function at 2-C of the pyrroline ring. Dehydration then leads to an N-analog reductone configuration, which can be easily oxidized to give a 1,2,3-tricarbonyl configuration. As known from other such configurations the central carbonyl function gets hydrated and allows the carbon backbone to rearrange to bring the carboxylic group in β-position to the carbonyl substituent of the acetyl group. Decarboxylation results in an electron rich eneaminol moiety that is oxidized to 2-acetyl-1-pyrroline. The loss of a C_1-fragment from the starting C_3-carbonyl has been independently verified by several authors using the CAMOLA approach to result predominately in the double labeled target compound [5.25]. However to a minor extent, a triple labeling was found too. This means that alternatively a second mechanism exists, which incorporates the intact C_3-carbon backbone of the starting carbonyl to the final pyrroline structure. The ratio of both pathways is 3/1 at pH 5.5 and changes to about 1/1 at pH 8.2, but is also influenced by other factors like the moisture content of the respective system.

Thus in pathway B, to explain the complete integration of the C_3 carbonyl, the reaction with native methylglyoxal is suggested. This allows the C,H-acidic

3-C position of pyrroline to nucleophilic attack the aldehyde function. Tautomerism and hydration lead to a hemiaminal function in β-position to the keto group of the former α-dicarbonyl backbone to enable a retro-aldol-type fragmentation. This means that in contrast to pathway A the C_1-fragment can now be cleaved off as formic acid. Cyclization, dehydration and oxidation give 2-acetyl-1-pyrroline. If the reaction starts at the oxidation level of acetol, the direct condensation of the hydroxymethylene function with the 2-C position of the ring can be realized (pathway C). In this case, the ring opening is facilitated by β-elimination of the amine function, which leads to ring expansion to a piperidine derivative, which after dehydration gives the tautomers 2-acetyl-1(3),4,5,6-tetrahydropyridine. These compounds can be found ranked under the individualists with 2.6% abundance in the earlier mentioned review on the total odorant space of foods.

The α-hydroxy carbonyl compound acetol is the result of the reduction of methylglyoxal during the Strecker cascade. As both carbonyls are common carbohydrate fragmentation products it was expected that both 2-acetyl-1-pyrroline and 2-acetyl-1(3),4,5,6-tetrahydropyridine were also found in reaction mixtures of higher sugars [5.26], but especially during the degradation of 1-deoxyglucosone and its follow-up product acetylformoin [5.27]. As explained in Figs. 5.4 and 5.10, acetol and methylglyoxal are immediate fragmentation products of these important sugar degradation intermediates. It has to be added that the described mechanisms have further been underlined by the detection of the propionyl pyrroline and propionyl piperidine analogs in reaction mixtures of proline with diacetyl and 1-hydroxy-2-butanone, respectively [5.28].

It is important to understand that the Strecker degradation leads to the production of very reactive small nucleophiles already under moderate conditions. This can be seen from the above described release of ammonia from the resulting α-hydroxyimine structure. However, even more important as educts for follow-up reactions leading to important odor active structures is the release of small sulfur compounds (Fig. 5.9). Cysteine is decarboxylated to the transient α,β-unsaturated imine. This brings the thiol substituent in β-position and leads to elimination of hydrogen sulfide. Besides being aroma active itself this potent nucleophile can condense with other Maillard-derived structures to give important odorants with extremely low thresholds like 2-methyl-3-furanthiol or 2-furfurylthiol (Fig. 5.11). Methionine gives the Strecker aldehyde methional, which easily eliminates methanethiol [5.29]. Oxidation and disproportionation then explains the formation of dimethyl sulfide and dimethyl trisulfide.

5.4 Other Mechanisms

Figure 5.4 depicts the main routes of degradation for 1-deoxyglucosone. Besides fragmentation, cyclization (C + D) leads to furanoic and pyranoic ring structures. 4-Hydroxy-2-(hydroxymethyl)-5-methylfuran-3(*2H*)-one (furan-3-one) is the main compound and reaches up to yields of 50% under moderate conditions. However, it is a nonstable intermediate and is quickly degraded at later stages. 3,5-Dihydroxy-6-methyl-2,3-dihydro-*4H*-pyran-4-one (γ-pyranone) shows the same reaction pattern, but at much lower levels (up to 8%). The formation of γ-pyranone within the 1-deoxy-route is closely related to the formation of 3-hydroxy-2-methyl-*4H*-pyran-4-one (maltol) from disaccharides, ranked within the individualists with 0.9% abundance in above odor signature study.

If alternatively to the formation of furan-3-one water is eliminated at the hydroxymethyl substituent of the furanoic half-acetal 2,4-dihydroxy-2,5-dimethylfuran-3(*2H*)-one (acetylformoin) results (Fig. 5.10, route A). This compound can easily react with nucleophiles to give, for example, pyrrol derivatives, but is also very redox active [5.30]. Disproportionation or reaction with other reducing structures or Strecker-type reactions followed by elimination of water then gives 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (furaneol) [5.31], which is one of the key generalist odorants with 41% abundance. The reduction step is prerequisite and explains why 6-deoxysugars like rhamnose or fucose are much more effective in generating furaneol [5.32]. Here, the 1-deoxy-route (B) directly leads to the target compound by repetitive water elimination without the need for a reduction step. The same accounts for the detection of furaneol in methylglyoxal or methylglyoxal-acetol incubations [5.33]. Aldol condensation gives the C_6-carbon backbone of the 1,6-dideoxy-hexodiulose intermediate (C). Interestingly, furan-3-one was reported to fragment to 4-hydroxy-5-methyl-3(*2H*)-furanone (norfuraneol), which is the pendant from the pentose 1-deoxy-route, and formaldehyde. This represents a retro-aldol reaction. Furaneol was also detected in pentose glycine reaction mixtures. Here, formaldehyde stems mainly from the Strecker degradation of glycine. Obviously, this is an equilibrium reaction, that is, formaldehyde condenses with norfuranol to result in furaneol via

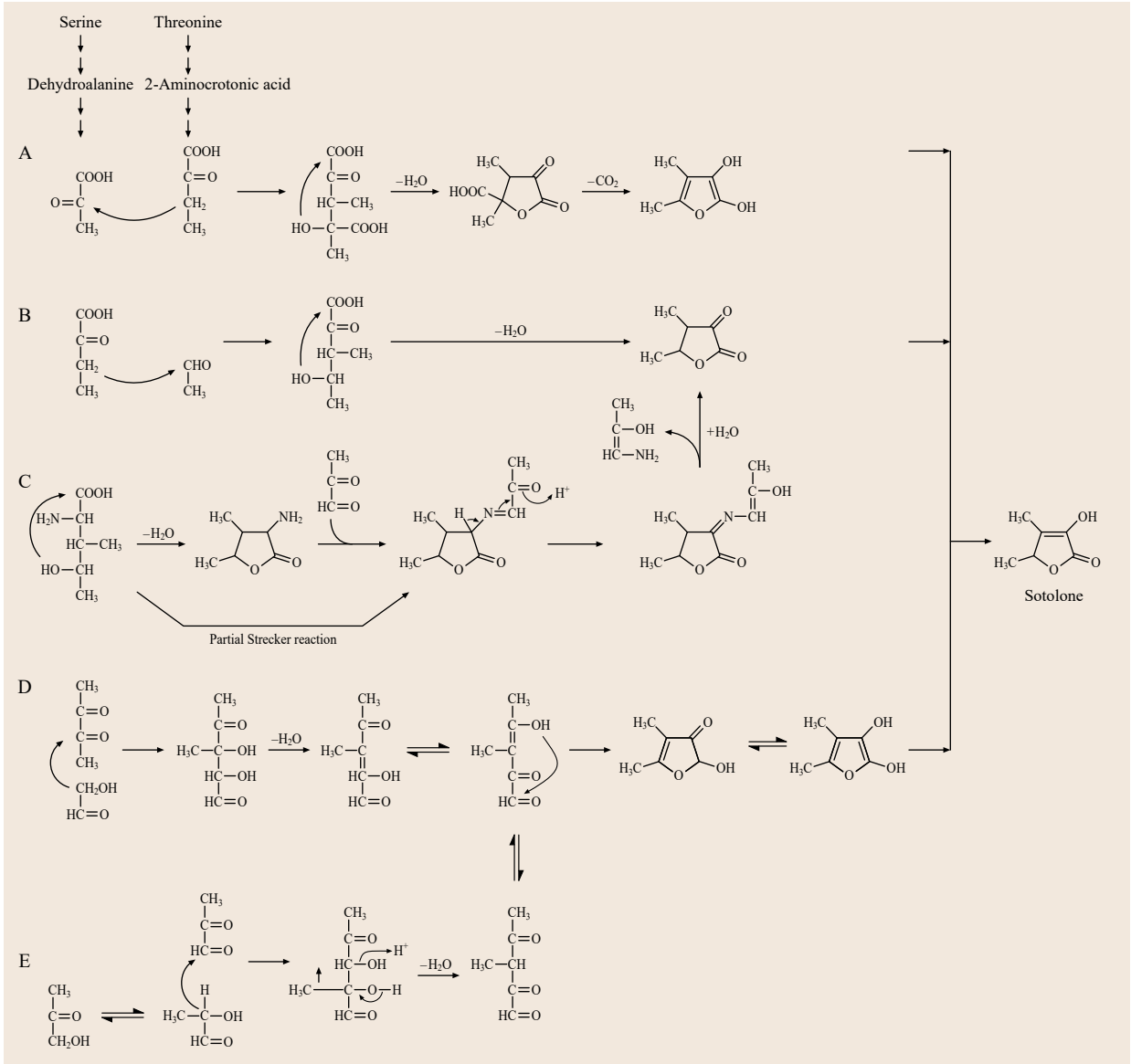

Fig. 5.12 Formation pathways leading to 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone (sotolone)

furan-3-one and acetylformoin (D) [5.34]. This aldol condensation can also occur with acetaldehyde and thus explains the formation of 4-hydroxy-2(5)-ethyl-5(2)-methyl-3(*2H*)-furanone (homofuraneol) in pentose alanine Maillard systems (E). This compound exists in two tautomeric forms in a ratio of 2 : 1.

3-Hydroxy-4,5-dimethyl-2(*5H*)-furanone (sotolone) is another furane derivative, which is of major importance to the odor composition of many foods. Figure 5.12 summarizes nonenzymatic formation pathways that have been reported in the literature. Most mechanisms include an aldol condensation step to build up the branched six-membered carbon skeleton. Relevant to strong heating processes combined with acidic pH values found, for example, under roasting conditions or protein hydrolysis is the formation of pyruvic acid from serine via dehydroalanine, enolization, and loss of ammonia (A). Threonine gives 2-oxobutanoic acid via 2-aminocrotonic acid. Both carbonyl molecules condensate to give sotolone after cyclization, decarboxylation, and rearrangement reactions. This represents an aldol-driven C_3+C_4 route, where the carboxylic acid function at 5-C is then eliminated due to its β-position to the other ring carbonyl

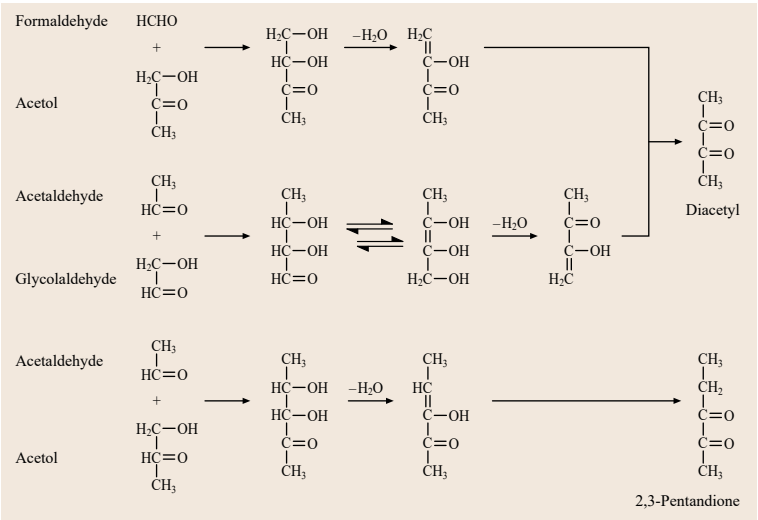

Fig. 5.13 Aldol condensation of short-chained carbonyl compounds explains the formation of diacetyl and 2,3-pentandione

functions. Alternatively, in pathway (B) for aged sake, a C_4+C_2 condensation product from 2-oxobutanoic acid and acetaldehyde was proposed to cyclize directly to the dimethyl substituted furanone [5.35]. Acetaldehyde may stem from acid degradation of threonine like the carboxylic acid or from alanine as the Strecker aldehyde. The positive correlation of acetaldehyde to the formation of sotolone was also reported for flor-sherry wines [5.36]. However, the most efficient pathway was reported for condiments as lovage and especially fenugreek (C) [5.37]. Here, the most abundant amino acid is 4-hydroxy-isoleucine, which already boasts the prerequisite functionalities. In-depth investigations of Blank et al. verified the intermediate 3-amino-lactone to be the most potent educt, to give sotolone in 40% yield at pH 6 in the presence of methylglyoxal. This unusual but very efficient deamination by a Strecker-type reaction can be argumented by the explicit C–H acidic position to give a conjugated 3-membered double bond system which is then hydrolyzed to give sotolone and the Strecker reaction typical eneaminol. On the other hand, incubations starting directly from 4-hydroxy-isoleucine were much less efficient, and a direct partial Strecker reaction was proposed eventually leading to the same ring intermediates in competition to the formation of high amounts of the Strecker aldehyde 3-hydroxy-2-methylbutanal. Alternatively, pathways D + E were proposed to explain the verified formation of sotolone in Maillard systems independent from the degradation of amino acids [5.38]. In (D), diacetyl (C_4) condenses with glycolaldehyde (C_2), dehydration then leads to a furanoic half-acetal to give sotolone after keto-enol-tautomerism. In case (E) of the condensation of acetol (C_3) and methylglyoxal (C_3) the methyl substitution pattern of the C_4-carbon backbone has first to rearrange to proceed via the same ring intermediates. In theory, this can be explained by a pinacol-pinacolone-type reaction. However to the best of our knowledge, such reactions have not yet been experimentally proven for food-relevant processing conditions.

It becomes obvious that aldol condensation reactions of short-chained Maillard intermediates play an important role in the generation of important flavor compounds. Alternatively to the above proposed retro-aldol fragmentation of 1,4-dideoxy-derivatives the formation of diacetyl was explained by the condensation of short-chained carbonyl structures, that is, formaldehyde or acetaldehyde with acetol and glycolaldehyde (Fig. 5.13) [5.28, 39]. This represents a C_1+C_3 and a C_2+C_2 route based on common sugar fragmentation products or Strecker aldehydes, respectively, to give the C_4 target structure after elimination of water and tautomerism. Aldol condensation of acetaldehyde (C_2) and acetol (C_3) also plausibly asserts the formation of 2,3-pentandione.

The Strecker degradation leads to the formation of very reactive small nucleophiles, that is, ammonia which can be released from the eneaminol intermediates, but especially hydrogen sulfide and methanethiol from the breakdown of cysteine and methionine, respectively. These nucleophiles can then easily react with carbonyl moieties to give odor intensive compounds of very low threshold, which are key agents of, for example, meaty aroma profiles. 2-Methyl-3-furanthiol was verified in incubations of 4-hydroxy-5-methyl-3(*2H*)-furanone, which is the main product of ribose via the 1-deoxypentosone route (Fig. 5.11, pathway A) [5.40]. However, this mechanism requires a reduction step to give a furan derivative of the ap-

propriate total redox level, which after elimination of water and substitution with hydrogen sulfide results in the aromatic target molecule. Although reduction steps in Maillard systems can be explained by the presence of reductone structures, this reasoning is a problem in this specific case, as the intermediate 4-hydroxy-5-methyl-3(*2H*)-furanone itself represents a cyclic reductone ether of explicit reducing capacity. Consequently, when *Cerny* and *Davidek* conducted an in-depth investigation based on the CAMOLA approach using ^{13}C-labeled sugars and authentic synthesized intermediates, they only observed very low amounts of 7% 2-methyl-3-furanthiol via this route in reaction mixtures of ribose, 4-hydroxy-5-methyl-3(*2H*)-furanone and cysteine at 95 °C and pH 5 [5.41]. Alternatively for the remaining 93%, they proposed that starting from the Amadori product the 2,3-enediol intermediate eliminates water at the position 4 to result in 1-amino-1,4-dideoxy-pentosone (pathway B). Although this α-dicarbonyl boasts an amino-reductone moiety, the prerequisite reduction step can in this case be explained by the Strecker reaction. Decarboxylation is here a strong driving force for the reduction to result in 1,4-dideoxypentosone after elimination of ammonia from the intermediate Strecker eneaminol [5.42]. Cyclization, water elimination, and incorporation of hydrogen sulfide then lead to 2-methyl-3-furanthiol. In their specific incubations starting from ribose the authors verified fragmentation and recombination reactions to be of negligible importance for the formation of the target furanthiol. However as stated earlier, sugar fragmentation is a general major aspect of Maillard sugar degradation. It is thus not unexpected that models with glycolaldehyde and mercapto-2-propanone lead to substantial amounts of 2-methyl-3-furanthiol (pathway C) [5.43]. Aldol condensation followed by cyclization and elimination of water directly leads to the target, while no reduction step is needed in this case. Mercapto-2-propanone represents an easily arguable acetol derivative. It has to be mentioned that in this study highest yields were obtained under extreme conditions of 180 °C in water-free systems.

A thiol furan structure of even higher importance to the odor space of foods is 2-furfurylthiol. For the pathway leading to this molecule above two studies were consistent with older literature [5.44], that is, the main precursor is 2-furfural, while fragmentation mechanisms were excluded. This means that starting from the *N*-glycoside of ribose the 1,2-eneaminol gives 3-deoxypentosone after elimination of water at the C-3 position and hydrolysis of the amine. Cyclization leads to 2-furfural, which condenses with hydrogen sulfide to result in 2-furfurylthiol after a reduction step (pathway D). To complete the discussion on both thiols, 2-methyl-3-furanthiol and 2-furfurylthiol, it has to be mentioned that minor pathways were reported to be valid in other reaction mixtures. This is owed to the fact, that (I) with, for example, thiamine or glutathione there are additional sources of sulfur than cysteine in foods [5.45], and (II) that hexoses and oligosaccharides can also generate substantial amounts of sugar intermediates with a five-membered carbon backbone following the fragmentation routes described earlier.

5.5 Conclusions

Major steps forward have been made in the elucidation of the complex Maillard reaction pathways resulting in nonenzymatic flavor formation. One major methodological driver was the use of stable isotopic labeled educts and intermediates to trace back their specific reaction pathways in the course of Maillard reaction. This has led to the characterization of 1-deoxyglucosone as the key reactive structure in hexose degradation systems based on insights into fundamental fragmentation mechanisms. Among others, short-chained molecules such as methylglyoxal, acetol, and acetaldehyde are thereby frequently found intermediates in the formation of major odorants. In this respect, hydrolytic β-dicarbonyl cleavage has evolved as the major scission reaction. In contrast, aldol and especially retro-aldol reactions were not substantiated to such an extent as would have been anticipated from the previous literature. Furthermore, it is important to note that the mechanistic degradation of higher sugars like disaccharides or oligosaccharides, which are much more relevant to many foods, is still not understood in full detail. On the other hand, the Strecker degradation as one aspect of the Maillard degradation of amino acids has been expanded from a simple decarboxylation and deamination reaction to a widely branched mechanism with all aspects of the general Maillard reaction scheme, including fragmentation, condensation, elimination, and redox reactions. Especially redox and rearrangement reactions are often important pieces in the puzzle of pathways explaining the formation of major odorants, but detailed insights into the underlaying mechanisms are still lacking and are awaiting to be uncovered.

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