JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 8293–8299 8293
DOI:10.1021/jf1008988

Investigation of Reactive α-Dicarbonyl Compounds Generated from the Maillard Reactions of L-Methionine with Reducing Sugars via Their Stable Quinoxaline Derivatives

Yvonne V. Pfeifer and Lothar W. Kroh*

Institute of Food Technology and Food Chemistry, Technical University of Berlin, Gustav-Meyer-Allee 25, 13355 Berlin, Germany

The formation of α-dicarbonyl compounds was investigated in methionine-catalyzed (Maillard reaction) thermal degradation of D-glucose, maltose, and dextrin 10 at three different pH values (3, 5, and 8). The α-dicarbonyl compounds were trapped as quinoxalines and could be quantified by HPLC and GC-MS. Formation of 1,4-dideoxypentodiulose from hexoses and disaccharides was evidenced for the first time. The use of L-methionine as the amino compound for the formation of 1,4-dideoxypentodiulose in model systems is explained. Furthermore, it could be shown that methionine has great effect on the formation of specific α-dicarbonyl compounds. At various pH values and also by application of mono-, di-, and oligosaccharides in all model reactions, 3-deoxyhexosulose and 1,4-dideoxypentodiulose were generated preferentially.

KEYWORDS: L-Methioninemethional; aroma; Maillard reaction; α-dicarbonyl compounds; 1,4-dideoxypentodiulose

INTRODUCTION

L-Methionine is an essential, proteinogenic amino acid for humans (*1*). L-Methionine is contained in many different foods; mainly it can be found in fish, meat, vegetables, egg, whole-grain bread, and rice. Furthermore, L-methionine is used in the flavor industry to produce aroma compounds, which smell like cooked potatoes, coffee, or roasted meat (*2*, *3*). The contribution of L-methionine to flavoring proceeds predominantly through thermal degradation or as a consequence of thermal interactions with other food ingredients such as reducing sugars. Investigations about reactions between amino acids and α-dicarbonyl compounds showed that oxidative degradation of the amino acid occurs and the so-called Strecker aldehydes are generated (*4*). The Strecker aldehydes of specific amino acids especially from L-leucine, L-phenylalanine, and L-methionine, but also from L-valine, L-isoleucine, and L-alanine, are well-known for their significant odors. These aldehydes are formed in food also from reactions between α-dicarbonyl compounds and amino acids (*5*). In recent years investigations have corroborated that the Strecker aldehydes of these three amino acids, L-leucine, L-phenylalanine, and L-methionine, are key aromas of many thermally treated foods (*6*−*8*). Reactions between L-methionine and monosaccharides were studied by Tressl et al. (*3*) and Rijke et al. (*9*). Both groups identified 3-(methylthio)propylamine, methional, methanethiol, and acrolein as reactive intermediates that are responsible for most of the end-products occurring in the model systems. Yu and Ho analyzed the formation of various heterocycles, for example, pyrazines generated from the thermal reactions of L-methionine and L-methionine sulfoxide with or without D-glucose (*10*). Yaylayan and Keyhani accomplished research about carbohydrate and amino acid degradation pathways in L-methionine/D-glucose model systems. They showed that D-glucose and L-methionine are precursors of thiofurans, thiopyrroles, and thiopyrazines, important compounds of meat aroma (*11*).

Although many investigations about the reaction of L-methionine with carbohydrates in thermally treated food and also in model systems were carried out, the role of specific α-dicarbonyl compounds is still unknown. Furthermore, it is ambiguous which of the multitude of reactive compounds arising from L-methionine in combination with carbohydrates generate the α-dicarbonyl compounds. The aim of this work is to gain deeper insights into the reaction between L-methionine and carbohydrates with respect to the formation of specific α-dicarbonyl compounds.

MATERIALS AND METHODS

Chemicals. The following chemicals were obtained commercially: D-glucose, D-fructose, maltose, sucrose, dextrin 10, L-methionine, 3-pentyn-1-ol, and ruthenium(IV)oxide hydrate were purchased from Fluka (Neu-Ulm, Germany); maltotriose, sodium periodate, 1,2-diaminobenzene, L-phenylalanine, L-alanine, γ-aminobutyric acid, L-leucine, L-cysteine, L-asparagine, and [$^{13}C_1$]-D-glucose were purchased from Aldrich (Steinheim, Germany). 1,2-Diaminobenzene was recrystallized twice from methanol.

Synthesis. *(2S,3R)-1-(2-Quinoxalinyl)-2,3,4-trihydroxybutane.* 3-Deoxy-D-*erythro*-2-hexosulose (3-DG) was synthesized largely according to the method of Madson and Feather (*12*). Interception of 3-deoxy-D-*erythro*-2-hexosulose as stable *(2S,3R)-1-(2-quinoxalinyl)-2,3,4-trihydroxybutane* was carried out according to the method of Glomb and Tschirnich (*13*).

GC-MS (3-DG-quinoxaline after acetylation): t_R, 27.99 min; *m*/*z* 360 (0.2%, M^+), 301 (7), 300 (9), 258 (12), 241 (49), 199 (33), 181 (24), 157 (34), 144 (100), 102 (11), 43 (89).

*Corresponding author (e-mail lothar.kroh@tu-berlin.de; phone +49 30 314 72 583; fax +49 30 314 72 585).

Published on Web 06/24/2010

(*1S,2R*)-*1*-(*3-Methyl-2-quinoxalinyl*)-*1,2,3-propanetriol* (1-DG-quinoxaline) was prepared largely according to the method of Beck and Ledl (*14*) via the Amadori compounds.

GC-MS (1-DG-quinoxaline after acetylation): t_R, 24.38 min; *m*/*z* 360 (0.2%, M^+), 301 (4), 300 (4), 241 (18), 199 (51), 198 (12), 174 (100), 157 (5), 143 (23), 107 (22), 103 (9), 43 (96).

(*1R,2S,3R*)-*1*-(*2-Quinoxalinyl*)-*1,2,3,4-tetrahydroxybutane* (glucosone-quinoxaline) was carried out according to the method of Morita et al. (*15*).

GC-MS (glucosone-quinoxaline after acetylation): t_R, 30.66 min; *m*/*z* 418 (0.2%, M^+), 359 (2), 358 (2), 299 (63), 257 (11), 215 (14), 213 (9), 202 (50), 196 (8), 160 (100), 143 (4), 129 (10), 115 (12), 102 (7), 43 (97).

(*1S,2R*)-*1*-(*3-Methyl-2-quinoxalinyl*)-*2,3-propanediol* (1,4-DDH-quinoxaline) was prepared largely according to the method of Morita et al. (*15*).

GC-MS (1,4-DDH-quinoxaline after acetylation): t_R, 22.31 min; *m*/*z* 302 (0.2%, M^+), 243 (16), 242 (7), 183 (100), 171 (40), 158 (77), 143 (7), 117 (9), 102 (9).

(*2S,3R*)-*1*-(*2-Quinoxalinyl*)-*2,3dihydroxypropane* (*3-DP-quinoxaline*). This synthesis was performed as described by Hollnagel and Kroh (*16*).

GC-MS (3-DP-quinoxaline after acetylation): t_R, 21.28 min; *m*/*z* 288 (0.3%, M^+), 229 (5), 228 (4), 169 (42), 157 (24), 144 (37), 129 (3), 117 (4), 43 (100).

1,4-Dideoxypentodiulose (17, 19, 20). To a solution of 3-pentyn-1-ol (2 mL, 26 mmol) in $CHCl_3$ (60 mL) and MeCN (60 mL) was added $NaIO_4$ (12.84 g, 60 mmol) in H_2O (120 mL). The mixture was vigorously stirred, and $RuO_2 \cdot H_2O$ (120 mg, 0.79 mmol) was added. Stirring was continued for 15 min in the presence of air at ambient temperature. Then the reaction mixture was filtered through a pad of silica and rinsed with CH_2Cl_2 as the eluent. The solution was dried with Na_2SO_4 and concentrated in vacuo to give a yellow oil. Purification via flash chromatography on silica (EtOAc/pentane, 1:1) afforded pure 1,4-dideoxypentodiulose (2.02 g) in 67% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 2.5, $-CH_3$) + 2.34 (s, 0.5, $-CH_3$), 2.60 (m, 1.6, $-CH_2$) + 2.99 (m, 0.4, $-CH_2$), 3.92 (m, 0.4, $-CH_2$) + 4.24 (m, 1.6, $-CH_2$).

^{13}C NMR (75 MHz, $CDCl_3$): δ 21.3, 23.5, 33.6, 38.4, 57.3, 61.9, 96.5, 197.0, 198.4, 209.9.

(*1S,2R*)-*1*-(*3-Methyl-2-quinoxalinyl*)-*2-ethyl Alcohol*. 1,4-Dideoxypentodiulose (200 mg, 1.72 mmol) and 1,2-diaminobenzene (167 mg, 1.55 mmol) were stirred for 30 min at 100 °C in an evacuated 5 mL flask. Purification via flash chromatography on silica (EtOAc/pentane, 1:1, EtOAc) afforded pure (1*S*,2*R*)-1-(3-methyl-2-quinoxalinyl)-2-ethyl alcohol (313 mg) in 98% yield as a bright orange solid.

^{1}H NMR (400 MHz, D_2O): δ 2.62 (s, 3, $-CH_3$), 3.12 (t, 2, J = 6.8 Hz, $-CH_2$), 3.98 (t, 2, J = 6.8 Hz, $-CH_2$), 7.70 (m, 2, Ar−H), 7.77 (m, 2, Ar−H).

^{13}C NMR (75 MHz, D_2O): δ 21.5, 37.2, 60.0, 126.7, 127.0, 129.7, 129.9, 139.3, 139.6, 154.3, 154.5.

GC-MS (1,4-DDP-quinoxaline after acetylation): t_R, 16.06 min; *m*/*z* 230 (0.2%, M^+), 188 (13), 187 (100), 169 (100), 159 (44), 158 (36), 144 (8), 117 (9), 77 (7).

Model Reactions. In a typical experiment an aqueous solution of carbohydrate (1 mmol) and amino acid (1 mmol) (L-methionine, L-phenylalanine, L-alanine, γ-aminobutyric acid, L-leucine, L-cysteine, L-asparagine) in 10 mL of H_2O was adjusted to a pH of 3 or 5 with 3 N HCl or to a pH of 8 with 3 N NaOH.

The model solutions were heated in sealed ampules at 100 ± 1 or 130 ± 1 °C for up to 300 min in a thermo block (Behr Labor Technik, behrotest ET2). After a defined reaction time, 500 μL of the samples was trapped with 500 μL of 0.05 M 1,2-diaminobenzene solution to intercept the α-dicarbonyls as quinoxalines. After 3 h at 25 °C, quinoxalines were analyzed by HPLC-DAD and GC-MS after acetylation. Values were expressed as the means of at least three independent determinations.

Model Reaction Diacetyl/Formaldehyde. In a typical experiment an aqueous solution of diacetyl (1 mmol) and formaldehyde (37%) (1 mmol) in 1.00 mL of H_2O was adjusted to a pH of 8. The model solution was stirred for 10 min at ambient temperature and then trapped with 1.00 mL of 0.05 M 1,2-diaminobenzene solution to intercept the α-dicarbonyls as quinoxalines.

Labeling Experiments. An aqueous solution of [$^{13}C_1$]-D-glucose (1 mmol) or [^{13}C]-D-glucose (1 mmol) and L-methionine (1 mmol) in 10 mL of H_2O was adjusted to a pH of 5. The model solution was heated in sealed ampules at 130±1 °C for up to 120 min in a thermo block (Behr Labor Technik, behrotest ET2). Afterward, 500 μL of the sample was trapped with 500 μL of 0.05 M 1,2-diaminobenzene solution to intercept the α-dicarbonyls as quinoxalines. After 3 h at 25 °C, quinoxalines were analyzed by HPLC-DAD and GC-MS after acetylation. The molecular ions (MS-EI) of labeled and unlabeled acetylated (1*S*,2*R*)-1-(3-methyl-2-quinoxalinyl)-2-ethyl alcohol [230 (10, [M^+])/231 (10, [M^+])] were used for the quantification.

Model Reaction Food Items. In a typical experiment 5.00 g of food sample was milled and roasted at 150/170 °C. After a defined time, the samples were derivatized for 30 min with 10 mL of 0.05 M 1,2-diaminobenzene solution, filtered, centrifuged at 6000 U, and analyzed by HPLC-DAD and GC-MS after acetylation.

Chromatography. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). Preparative column chromatography was performed on silica gel 60, 40−63 μm (Merck). All solvents were of chromatographic grade.

Nuclear Magnetic Resonance Spectroscopy (NMR). NMR spectra were recorded on a Bruker AC 400 instrument (Rheinstetten, Germany). Chemical shifts are given in parts per million relative to residual nondeuterated solvent as an internal reference.

High-Performance Liquid Chromatography (HPLC-DAD) Instrumentation: degasser, Gegasys DG-13000 (Knauer); pump, Shimadzu LC-10 AT; thermostat, 30 °C, Shimadzu CT0-6A; guard column, Nucleosil 120-5 C18 Macherey-Nagel; column, Nucleosil 5 C18 (250 mm × 4.6 mm); injection volume, 40 μL; eluent, methanol/water gradient, 1 mL/min.

Gas Chromatography (GC-MS). The samples were extracted with *n*-butanol. The solvent was dried off, the residue was dissolved in toluene, and acetic anhydride was added (*18*). Gas chromatography instrumentation: Finnigan GCQ; capillary column, BPX 5 (SGE, 30 m, 0.25 mm i.d., 0.5 μm film thickness); carrier gas, helium 4.6; detector, Finnigan Ion Trap Mass Analyzer GCQ; injection temperature, 270 °C; split 1:10; temperature program, initial temperature, 95 °C, held for 1 min, raised from 95 to 200 °C at 15 °C/min, 200 °C held for 1 min, raised from 200 to 280 °C at 3 °C/min, 280 °C held for 5 min, raised from 280 to 300 °C at 5 °C/min, 300 °C held for 5 min. Column effluents were analyzed by selected ion monitoring (SIM). Quantification was carried out by comparison of peak areas obtained in the TIC with those of standard solutions containing amounts of pure authentic reference compounds.

RESULTS AND DISCUSSION

Various studies concerning the relevance of L-methionine in thermally treated food have been performed to date. It is well-known that by thermal treatment of L-methionine both in the absence or in the presence of carbohydrate/dicarbonyl compound one of the main products is methional (*10*). Many investigations have been accomplished, although only limited information about the reaction pathways of the carbohydrate compounds in these model systems is available. Does the formation of specific α-dicarbonyl compounds depend on the presence of L-methionine, or is there no specific influence by the amino acid? Furthermore, the interest is directed toward the use of different carbohydrates. D-Glucose was used as a typical monosaccharide in food, maltose as an agent for disaccharides, and dextrin 10 as an agent for oligosaccharides, which showed in previous works that reaction behavior is different compared to monosaccharides (*16*).

Within this work various model reactions with different carbohydrates (mono-, di-, and oligosaccharides) and the amino acid L-methionine were performed in aqueous solutions (starting with pH 3, 5, and 8). The samples were heated in sealed ampules at 130 °C for a defined time and then treated with 0.5 M 1,2-diaminobenzene solution to trap the arising α-dicarbonyl compounds as stable, measurable quinoxalines (*16*).

In line with our investigations with L-methionine as amino component, an unidentified peak could be found in the HPLC chromatogram, which showed the typical UV spectrum of a quinoxaline (**Figure 1**).

Remarkably, this unknown component is only generated in the model system with L-methionine as the amino compound and

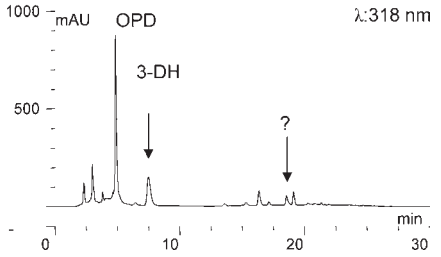

Figure 1. HPLC chromatogramm of a glucose/L-methionine model system.

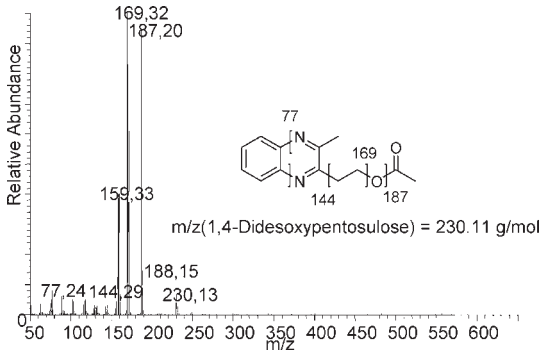

Figure 2. GC-MS spectrum (SIM-MS) of the acetylated quinoxaline of 1,4-dideoxypentodiulose.

with no other amino acid, that is, L-phenylalanine, L-leucine, L-asparagine, L-cysteine, or glycine.

It is a crucial point why this unknown α-dicarbonyl compound is formed only in the presence of L-methionine and what the structure is about. When the chromatographical retention time of this quinoxaline is compared with the established quinoxalines, it becomes clear that this component must be more nonpolar than the quinoxaline of 3-deoxyhexosulose and more polar than the quinoxaline of methylglyoxal. To obtain more information about the structure, the samples were acetylated and then measured as acetylated quinoxalines by GC-MS.

All quinoxalines show the same typical mass fragments of *m*/*z* 144, 117, and 77, which are caused by the quinoxaline skeleton (**Figure 2**). Thus, the task consisted of searching for more substances in the GC spectrum that show these typical fragments and that have retention indices in accord with the results from the HPLC. Such a substance, eluting on the GC column at 16.06 min, could be found with a *m*/*z* ratio from 230. With regard to the appropriated fragmentation and its retention indices, the unknown dicarbonyl was posited to be 1,4-dideoxypentodiulose. For confirmation of the proposed structure 1,4-dideoxypentodiulose was synthesized in one step. Various syntheses have been described by de Kimpe et al. (*19*, *20*), but they are all more complicated.

The oxidation of 3-pentyn-1-ol with $NaIO_4$ and $RuO_2 \cdot H_2O$ as catalyst afforded 1,4-dideoxypentodiulose in pure form in 67% yield. In a next step the α-dicarbonyl was trapped with 1,2-diaminobenzene to yield the stable, measurable quinoxaline in nearly quantitative yield of 98% (**Figure 3**).

Comparison of the synthesized dicarbonyl compound with the model systems revealed that the new compound is actually 1,4-dideoxypentodiulose (**Figure 4**).

To investigate the differences in reaction between mono- and oligosaccharides, the reaction behavior of D-glucose with L-methionine was investigated thoroughly. The reactions were performed at various pH values (3, 5, and 8). Noticeably, independent from the pH value the formation of 3-deoxyhexosulose and 1,4-dideoxypentodiulose

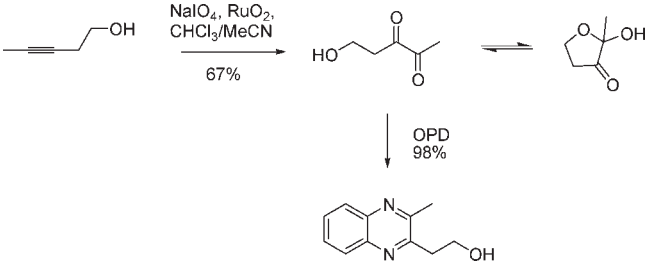

Figure 3. Synthesis of 1,4-dideoxypentodiulose and its quinoxaline.

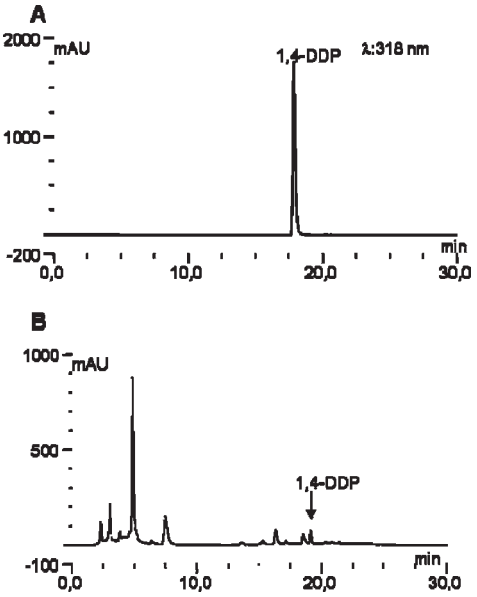

Figure 4. HPLC chromatogram of the synthesized new quinoxaline (**A**) and of the model system D-glucose/L-methionine, at 130 °C and with 60 min of thermal treatment (**B**).

Table 1. Influence of Carbohydrate and pH Value on the Formation of α-Dicarbonyl Compounds (Milligrams per Liter) in Model Systems from L-Methionine/Carbohydrate at 130 °C after 180 min

	D-glucose			maltose			dextrin10		
	pH 3	pH 5	pH 8	pH 3	pH 5	pH 8	pH 3	pH 5	pH 8
glucoson	0	0	0	0	0	0	0	0	0
1-DH	0	0	1.9	0	6.9	36.7	0	0	7.5
3-DH	40.1	32.8	27.3	8.2	15.6	19.6	10.3	15.3	15.9
3-DP	0	0	0	0	0	4.4	0	0	0
1,4-DDH	0	0	0	0	0	0	0.5	0.8	0
glyoxal	0.2	0.7	0.5	0	0.3	0.4	0.1	0	0
1,4-DDP	6.8	7.4	2.4	0.1	4.3	4.7	0.7	7.4	3.9
methylglyoxal	0	0	2.0	0	0	0.4	0	0	1.9
diacetyl	0	0	1.1	0	0	0.9	0	0	1.1

is predominant (**Table 1**). That is not the case when using other amino compounds (*16*, *21*).

The higher amount of 3-deoxyhexosulose at acidic pH values is in accordance with the literature, whereby the formation of 3-deoxyhexosulose is preferred (**Figure 5**) (*5*).

At a pH of 8 1-deoxy-2,3-hexodiulose, glyoxal, methylglyoxal, and diacetyl were formed in addition. 1,4-Dideoxypentodiulose arose just like 3-deoxyhexosulose at an acidic pH value in higher amounts than in alkaline values (**Figure 6**).

From the given standard reference quinoxalines, 3-deoxyhexosulose and 1,4-dideoxypentodiulose were also dominant α-dicarbonyl compounds in model reactions with maltose. Using maltose

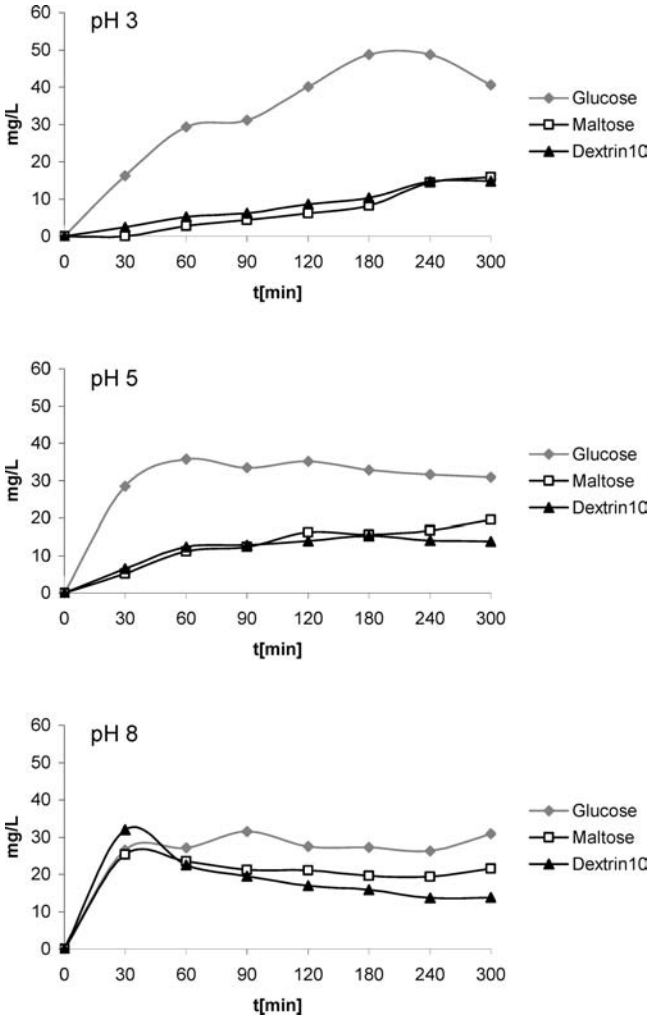

Figure 5. Formation of 3-deoxyhexosulose (3-DH) from D-glucose, maltose, and dextrin10 with L-methionine at pH 3, 5, and 8 and 130 °C over a 300 min time period.

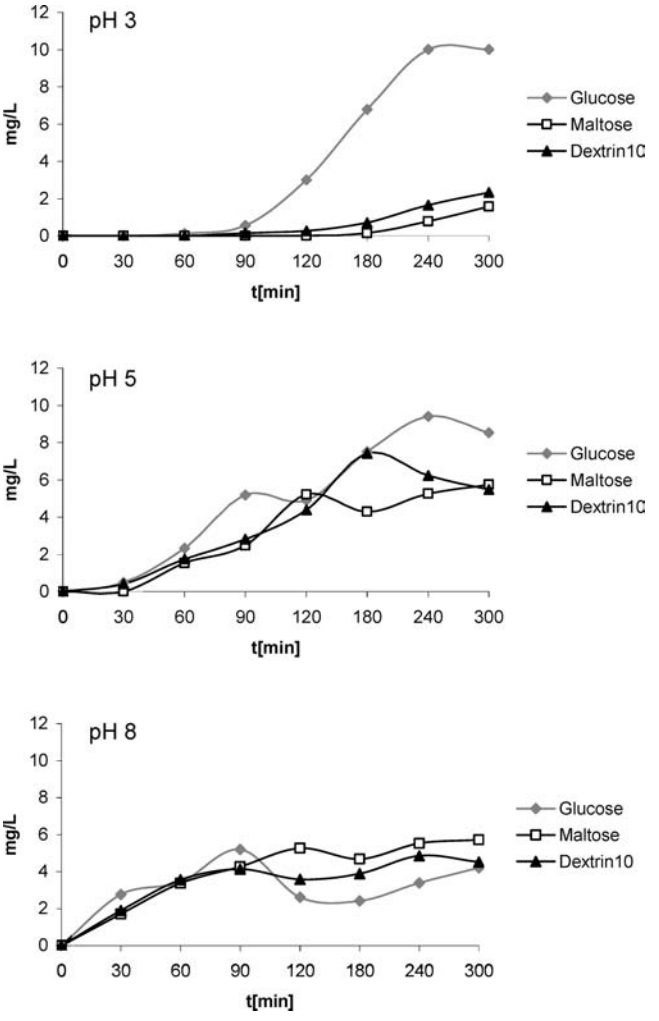

Figure 6. Formation of 1,4-dideoxypentodiulose (1,4-DDP) from D-glucose, maltose, and dextrin 10 with L-methionine at pH 3, 5, and 8 and 130 °C over a 300 min time period.

as carbohydrate compound the percentage of these two α-dicarbonyl compounds differs from D-glucose model systems under various pH values (see **Figures 5** and **6**). Whereas at a pH of 3 after 300 min 17 mg/L 3-deoxyhexosulose was produced, at a pH of 8 25 mg/L was generated. Thus, in this case formation of 3-deoxyhexosulose is preferred at alkaline pH values. Furthermore, 1-deoxy-2,3-hexodiulose (the dominant α-dicarbonyl compound in this reaction), 3-deoxypentodiulose, 1,4-dideoxyhexodiulose, glyoxal, and diacetyl were formed at a pH of 8. 1,4-Dideoxypentodiulose arose from maltose at a pH of 3 in low concentrations compared to D-glucose. At a pH of 8 the ratio is reversed; maltose forms 6 mg/L 1,4-dideoxypentodiulose, D-glucose only 4 mg/L. The behavior of dextrin 10 in model systems with L-methionine is similar to that of the disaccharide maltose (see **Figures 5** and **6**). 3-Deoxyhexosulose is generated at a pH of 3, 5, and also 8 in nearly equal concentrations. After 300 min of thermal treatment, about 12 mg/L of 3-deoxyhexosulose could be detected. At pH values of 3 and 5 1,4-dideoxy-2,3-hexodiulose is formed and additionally 3-deoxyhexosulose and 1,4-dideoxypentodiulose are formed in small amounts. At a pH of 8 1-deoxy-2,3-hexodiulose, 3-deoxypentodiulose, glyoxal, methylglyoxal, and diacetyl were produced as well.

1,4-Dideoxypentodiulose shows the same behavior in its formation as in the model system maltose/L-methionine. At a pH of 3, 2 mg/L was produced after 300 min; at a pH of 5 or 8, 5 mg/L was formed.

By comparison of the formation of the α-dicarbonyl compounds in dependence on the carbohydrates, it becomes clear that by using D-glucose the formation of 3-deoxyhexosulose and 1,4-dideoxypentodiulose is preferred. Using the disaccharide maltose or oligosaccharides such as dextrin 10 no pH dependence of the formation of 3-deoxyhexosulose can be detected (*22*). The generation of 1,4-dideoxypentodiulose is favored at pH values of 5 and 8.

The experiments also show that there is a difference between the formation of 3-deoxyhexosulose and the formation of 1,4-dideoxypentodiulose. Whereas the concentration of 3-deoxyhexosulose arises directly, 1,4-dideoxypentodiulose is formed in higher amounts not before 90 min.

Now the question arises: which role does 1,4-dideoxypentodiulose play in methionine-containing food? Does it influence the aroma properties itself, or does it generate resulting products, which form intensive, characteristic aroma compounds? In real food samples 1,4-dideoxypentodiulose was generated: sunflower seeds, roasted for 15 min at 170 °C, yielded 1.76 mg/kg, and in cashews, 1.4 mg/kg was formed (**Figure 7**).

The well-known aroma compound 2,3-pentanedione could be formed from 1,4-dideoxypentodiulose by water elimination as reported by Weenen et al. (*23*). 2,3-Pentanedione has aroma properties similar to those of diacetyl and provides a buttery aroma. It is formed in addition to diacetyl, for example, during

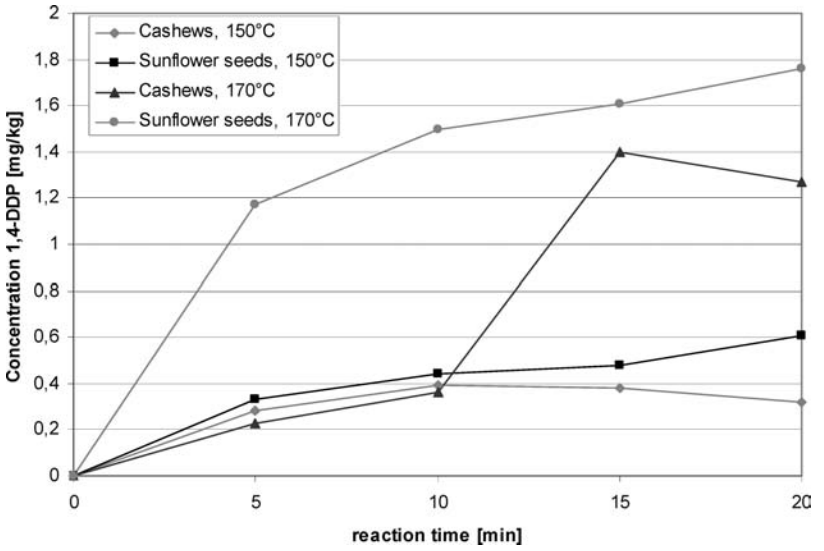

Figure 7. Formation 1,4-dideoxypentodiulose (1,4-DDP) from cashew nuts and sunflower seeds in roasted model systems at 150/170 °C over a 20 min time period.

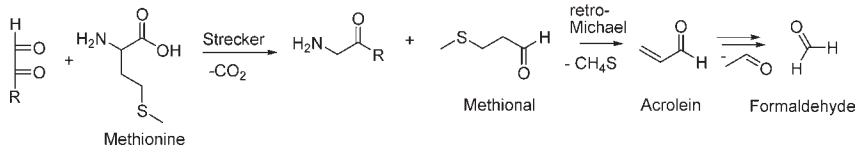

Figure 8. Generation of formaldehyde from methionine via Strecker degradation and retro-Michael reaction.

beer aging (*24*). Furthermore, it is likewise that 1,4-dideoxypentodiulose yields 2-methyl-3-furanone by β-elimination. 2-Methyl-3-furanone has a sweet aroma and could be found in baked potatoes and chips as an aroma compound (*25*).

Formation of 3-Deoxyhexosulose. On the basis of the investigations of various model systems with different carbohydrates and diverse pH values it can be deduced that the amino acid L-methionine produces 3-deoxyhexosulose and 1,4-dideoxypentodiulose in all model systems independently from the carbohydrate present. The formation of 3-deoxyhexosulose in the model system D-glucose/L-methionine at acidic pH values is well-known. At alkaline pH ranges other α-dicarbonyl compounds can be detected as well. An explanation is the catalysis of enolization, rearrangement, and fragmentation reactions in the course of the Maillard reaction at alkaline conditions. Therefore, the low concentration of 3-deoxyhexosulose at this pH value can be elucidated. Considering the total α-dicarbonyl concentration at different pH ranges, the amount is higher at alkaline values, in accordance with the literature (*5*).

By the use of the disaccharide maltose or the oligosaccharide dextrin 10 initially the glycosidic bond must be cleaved to obtain reducing sugars. Therewith, the lower amount of 3-deoxyhexosulose in comparison to D-glucose at acidic pH values can be explained. At a pH of 8 there is only a slight difference in the 3-deoxyhexosulose concentration of various carbohydrates, because the actual concentration of glucose is lower. In this pH regimen color and melanoidin formation from D-glucose is faster. The approach of postderivatization allows the determination of the actual concentration of the α-dicarbonyl compounds, so that the formed 3-deoxyhexosulose reacts to melanoidins or decomposition products and cannot be detected as quinoxaline any more.

Formation of 1,4-Dideoxypentodiulose. 1,4-Dideoxypentodiulose could be detected only in model systems with pentoses as carbohydrate compound up to now (*26*). Presumably it is generated via a formation pathway similar to that of the production of 1,4-dideoxy-2,3-hexodiulose from hexoses. First, 1-deoxy-2,3-hexodiulose is formed via the 2,3-endiol of the Amadori product by β-elimination. Keto−enol tautomerization and a second β-elimination yield 1,4-dideoxy-2,3-hexodiulose.

The key question is whether 1,4-dideoxypentodiulose can be generated from hexoses and what role L-methionine plays in the formation of this α-dicarbonyl compound. The importance of this amino acid becomes apparent when the results are compared with those obtained from investigations with other amino acids. The first hypothesis was that the formation of 1,4-dideoxypentodiulose was conditional on the sulfur of L-methionine, but model reactions with L-cysteine as amino compound could not approve this assumption. Also, the use of L-phenylalanine, L-asparagine, γ-aminobutyric acid, L-leucine, glycine, and glutamine did not yield 1,4-dideoxypentodiulose.

So what differentiates the properties of L-methionine from the other amino acids? Considering the amino acids in thermally treated model reactions with carbohydrates, it is remarkable that L-methionine indeed like all other amino acids forms the corresponding Strecker aldehyde (**Figure 8**). Methional is an unstable aldehyde, which decomposes very quickly into methanethiol, dimethyl disulfide, and acrolein via a retro-Michael reaction (*28*). From the produced acrolein formaldehyde is generated in the next step via hydration to 3-hydroxypropanal and subsequent retro-Aldol reaction.

It is conceivable that 1,4-dideoxypentodiulose is generated in an Aldol reaction from diacetyl with formaldehyde (**Figure 9**)

Diacetyl can be produced in the Maillard reaction via different pathways (see **Figure 9**). It can be formed via a retro-Aldol reaction from D-glucose. Thereby, the C_6-frame is cleaved into glycolaldehyde and D-erythrose. In following oxidation and elimination steps diacetyl is produced (*27*). Oligosaccharides

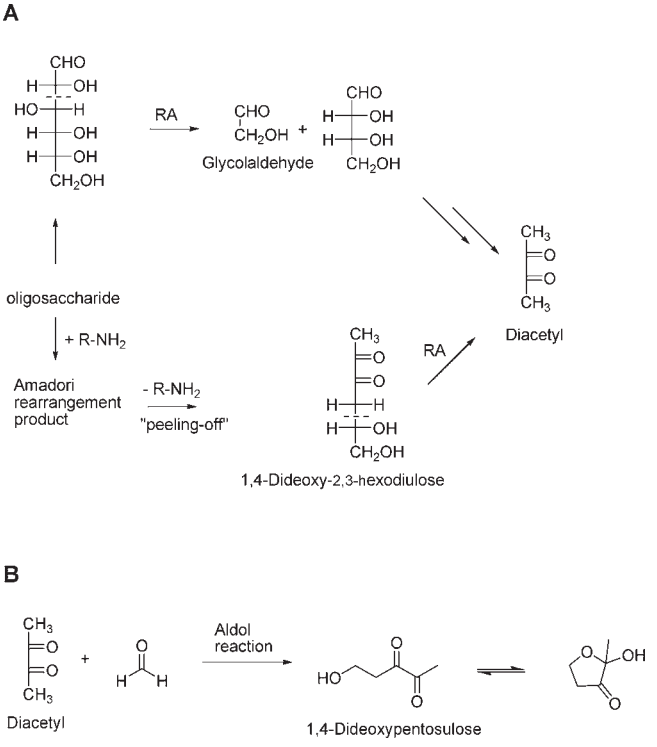

Figure 9. Generation of diacetyl from oligosaccharides via two different formation pathways: (**A**) pathway via glycosidic cleavage and retro-Aldol reaction of D-glucose; (**B**) pathway via the peeling-off mechanism and retro-Aldol reaction of 1,4-dideoxy-2,3-hexodiulose. Subsequent Aldol reaction of diacetyl with formaldehyde yields 1,4-dideoxypentodiulose (**B**).

Table 2. Amounts of 1,4-Dideoxypentodiulose Generated upon Heating of a Glucose/L-Methionine, [$^{13}C_1$]Glucose/L-Methionine, or [^{13}C]Glucose/L-Methionine Model System

α-dicarbonyl isotopomer	yield (mg/L)
1,4-dideoxypentodiulose	5.6
[$^{13}C_1$]-1,4-dideoxypentodiulose	not detectable
[$^{13}C_{1-4}$]-1,4-dideoxypentodiulose	5.4

can react in an alternative route to Amadori products, and via the peeling-off mechanism 1,4-dideoxy-2,3-hexodiulose is formed and hence, by retro-Aldol cleavage, diacetyl (*16*). For reassurance of the postulated mechanism diacetyl (1 mmol) and formaldehyde (1 mmol) were dissolved in water (1 mL) and stirred for 10 min at a pH of 8 at ambient temperature. After 10 min, the reaction mixture was trapped with 1 mL of 0.05 M 1,2-diaminobenzene solution to yield the quinoxaline of 1,4-dideoxypentodiulose.

To confirm these results, the following experiment was performed: Isotopically labeled [$^{13}C_1$]-D-glucose or [^{13}C]-D-glucose was added to an aqueous solution of L-methionine. The reaction was started and, after thermal treatment for 120 min, the amounts of unlabeled 1,4-dideoxypentodiulose, which was expected to be formed, as well as [$^{13}C_1$]-1,4-dideoxypentodiulose, formed only via another reaction pathway, and [$^{13}C_{1-4}$]-1,4-dideoxypentodiulose should quantified. As shown in **Table 2**, 1,4-dideoxypentodiulose and [$^{13}C_{1-4}$]-1,4-dideoxypentodiulose were formed in high amounts, whereas the $^{13}C_1$-labeled compound was not detectable. This result supports the hypothesis that 1,4-dideoxypentodiulose is formed via an Aldol reaction from diacetyl and formaldehyde during the thermal treatment of D-glucose/L-methionine model systems.

This work points out that L-methionine has a great effect on the formation of specific α-dicarbonyl compounds. At various pH values and also by the application of mono-, di-, and oligosaccharides in all model reactions 3-deoxyhexosulose and 1,4-dideoxypentodiulose were generated preferentially. The identity was unequivocally confirmed by chemical synthesis of 1,4-dideoxypentodiulose and its quinoxaline. Formation of 1,4-dideoxypentodiulose from hexoses was evidenced the first time and could be quantified. Furthermore, we could show a new reaction pathway for α-dicarbonyl compounds in the Maillard reaction via Aldol condensation.

ACKNOWLEDGMENT

We are indebted to Dr. Reinhard Zeisberg, Technical University Berlin, for NMR measurement.

LITERATURE CITED

(1) Meister, A. *The Role of Amino Acids in Nutrition*; Academic Press: New York, 1965; pp 201−230.
(2) Hodge, J. E. *Origin of Flavour in Food. Nonenzymatic Browning Reactions*; AVI Publishing: Wesport, CT, 1967.
(3) Tressl, R.; Helak, B.; Martin, N.; Kersten, E. *Formation of Amino Acid Specific Maillard Products and Their Contribution to Thermally Generated Aromas*; ACS Symposium Series; American Chemical Society: Washington, DC, 1989; pp 156−169.
(4) Hofmann, T.; Münch, P.; Schieberle, P. Quantitative model studies on the formation of aroma-active aldehydes and acids by Strecker-type reactions. *J. Agric. Food Chem.* **2000**, *48*, 434–440.
(5) Ledl, F.; Schleicher, E. The Maillard reaction in food and human body − new results in chemistry, biochemistry and medicine. *Angew. Chem.* **1992**, *102*, 597–626.
(6) Kerler, J.; Grosch, W. Character impact odorants of boiled chicken: changes during refrigerated storage and reheating. *Z. Lebensm. Unters. Forsch.* **1997**, *205*, 232–238.
(7) Blank, I.; Sen, A.; Grosch, W. Potent odorants of the roasted powder and brew of Arabica coffee. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 239–245.
(8) Wagner, R.; Grosch, W. Evaluation of potent odorants of french fries. *Lebensm. Wiss. Technol.* **1997**, *30*, 164–169.
(9) Rijke, D.; Dort, J. M.; Boelnes, H. *Shigematsu Variation of the Maillard Reaction*; de Gruyter: Berlin, Germany, 1981; pp 417−431.
(10) Yu, T.; Ho, C. T. Volatile compounds generated from thermal reaction of methionine and methionine sulfoxide with or without glucose. *J. Agric. Food Chem.* **1995**, *43*, 1641–1646.
(11) Yaylayan, V. A.; Keyhani, A. Carbohydrate and amino acid degradation pathways in L-methionine/D-[$_{13}$C]glucose model systems. *J. Agric. Food Chem.* **2001**, *49*, 800–803.
(12) Madson, M. A.; Feather, M. S. An improved preparation of 3-deoxy-D-erythro-2-hexosulose via the bis(benzoylhydrazone) and some related constitutional studies. *Carbohydr. Res.* **1981**, *94*, 183–197.
(13) Glomb, M. A.; Tschirnich, R. Detection of α-dicarbonyl compounds in Maillard reaction systems. *J. Agric. Food Chem.* **2001**, *49*, 5543–5550.
(14) Beck, J.; Ledl, F.; Severin, T. Formation of 1-deoxy-D-erythro-2,3-hexodiulose from Amadori compounds. *Carbohydr. Res.* **1988**, *177*, 240–243.
(15) Morita, N.; Inoue, K.; Takagi, M. Quinoxalines derived from D-glucose and *o*-phenylendiamine in a weakly acidic medium. *Agric. Biol. Chem.* **1981**, *45*, 2665–2888.
(16) Hollnagel, A.; Kroh, L. W. Degradation of oligosaccahrides in nonenzymatic browning by formation of α-dicarbonyl compounds via a "peeling off" mechanism. *J. Agric. Food Chem.* **2000**, *48*, 6219–6226.
(17) Semmelhack, M. F.; R. C., S.; Federle, M. J.; Bassler, B. L. An expeditious synthesis of DPD and boron binding studies. *Org. Lett.* **2005**, *7*, 569–572.
(18) Nedvidek, W.; Fischer, F. L. P. Detection of 5-hydroxymethyl-2-methyl-3(2*H*)-furanone and of α-dicarbonyl compounds in reaction mixtures of hexoses and pentoses with different amines. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 228–228.

(19) de Kimpe, N.; et al. Synthesis of laurencione, a labile dihydrofuranone derivative from red alga *Laurencia spectabilis*. *J. Org. Chem.* **1995**, *47*, 3280–3284.
(20) de Kimpe, N.; et al. New formal synthesis of laurencione, a labile dihydrofuranone derivative from red alga *Laurencia spectabilis*. *Synthesis* **1995**, *1995*, 1131–1134.
(21) Fiedler, T.; Moritz, T.; Kroh, L. W. Influence of α-dicarbonyl compounds to the molecular weight distribution of melanoidins in sucrose solutions: part 1. *Eur. Food Res. Technol.* **2006**, *223*, 837–842.
(22) Kroh, L. W.; Wagner, T. F. J. The formation of coloured compounds in technical sucorse solutions − new knowledge about their molecular basics and molecular size distribution. *Sugar Ind.* **2007**, *132*, 25–32.
(23) Weenen, H. Reactive intermediates and carbohydrate fragmentation in Maillard chemistry. *Food Chem.* **1998**, *62*, 393–401.
(24) Vanderhaegen, B.; Neven, H.; Coghe, S.; Verstrepen, K. J.; Verachtert, H.; et al. Evolution of chemical and sensory properties during aging of top-fermented beer. *J. Agric. Food Chem.* **2003**, *51*, 6782–6790.
(25) Oruna-Concha, M. J.; Duckham, S. C.; Ames, J. M. Comparison of volatile compounds isolated from skin and flesh of four potato cultivars after baking. *J. Agric. Food Chem.* **2001**, *49*, 2414–2421.
(26) Cerny, C.; Davidek, T. Formation of aroma compounds from ribose and cysteine during the Maillard reaction. *J. Agric. Food Chem.* **2003**, *51*, 2714–2721.
(27) Yaylayan, V. A.; Keyhani, A. Origin of 2,3-pentanedione and 2,3-butanedione in D-glucose/L-alanine Maillard model systems. *J. Agric. Food Chem.* **1999**, *47*, 3280–3284.
(28) Pripis-Nicolau, L. Formation of flavor components by the reaction of amino acid and carbonyl compound in mild conditions. *J. Agric. Food Chem.* **2000**, *48*, 3761–3766.

Received for review March 10, 2010. Revised manuscript received May 30, 2010. Accepted June 03, 2010.