JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Milieu Dependence of Isomeric Composition of D-*arabino*-Hexo-2-ulose in Aqueous Solution Determined by High-Resolution NMR Spectroscopy

Martin Kaufmann,[†] Paul Timo Haase,*[,†] Clemens Mügge,[§] and Lothar Wilfried Kroh[†]

[†] Department of Food Chemistry and Food Analysis, Berlin Institute of Technology, Gustav-Meyer-Allee 25, TIB 4/3-1, D-13355 Berlin, Germany

[§]Department of Chemistry, Humboldt University of Berlin, Brook-Taylor-Straße 2, D-12489 Berlin, Germany

ABSTRACT: In this study, high-resolution ^{1}H NMR spectroscopy (600.03 MHz) and ^{13}C NMR spectroscopy (150.89 MHz) were used to elucidate the structures of equilibrating D-*arabino*-hexo-2-ulose (GLUC) (**1**) isomers in aqueous solution. Four isomers were formed from the investigated ketohexose, and their equilibrium is dependent on the pH value and temperature. Only hydrated GLUC (**1**) isomers were identified. The $^{2}C_{5}$-β-2,6-pyranoid and the β-2,5-furanoid GLUC (**1**) isomer were exclusively formed in aqueous solution. Thus, $^{4}C_{1}$-1,5-pyranoid isomers are predominating in the crystalline state. An increase in solution pH or temperature led to a pairwise conversion of configurative information. Thus, changing the measurement conditions permits control over the equilibrium's characteristic. Furthermore, all GLUC (**1**) isomers showed comparable reaction behavior regarding pH- and temperature-dependent degradation reactions.

KEYWORDS: *D-arabino-hexo-2-ulose, NMR spectroscopy, equilibration, isomeric composition, ketohexose*

INTRODUCTION

Nonenzymatic browning, first described in 1912 by Louis-Camille Maillard,[1] is a class of reactions between free amino functions and carbonyl carbons of reducing sugars.[2] Those reactions, also summarized under the term Maillard reaction, occur during physiological processes in vivo as well as in the course of technological food processing (heat treatment), where they possess both positive and negative aspects. Besides the formation of dyes, pigments, and flavorings, which influence the sensorial qualities of food, some Maillard products, such as D-*arabino*-hexo-2-ulose (GLUC) (**1**), exhibit reductone structures and thus have reducing properties, which inhibit the oxidative degeneration of food.[2] However, heat treatment of food promotes the genesis of mutagenic substances and cross-links essential amino acids such as lysine; thus, essential amino acids lose their physiological activities.[2,3] In in vivo reactions, Maillard products are associated with diabetes mellitus,[4] cataractogenesis,[5,6] morbus Alzheimer,[7] arteriosclerosis,[8] and the process of aging in general.[9]

Because of its reductone structure, GLUC (**1**) is one of the most important substances in the intermediate phase of the Maillard reaction.[10] It is built via a radical degradation of Amadori compounds in the presence of redox active metal ions such as Cu(II).[11] Thus, as a result of the predominating slightly oxidative reaction milieu, high levels of GLUC (**1**) were found in technical sugar juices at approximately 80 °C.[12] To improve the understanding of its biological activities and optimize the technological processing of food, it is essential to be aware of all possible reaction pathways of GLUC (**1**) in the course of the Maillard reaction, such as Strecker degradation, benzylic acid rearrangement, retro-aldol reaction, and α- and β-dicarbonyl cleavages.[2] These reaction pathways result in shorter chain products such as glyoxal. Via aldol additions and condensations and Michael additions, advanced glycation end products (AGEs),[13] melanoidins,[14,15] and heterocyclic aromatic amines (HCAs)[16] can be formed from such substances.

For the purpose of describing the characteristics of GLUC (**1**) in aqueous solution and to be able to derive relationships between its geometrical structures and chemical stability, its isomeric composition and equilibrium must be elucidated. However, Freimund et al. already described the isomeric composition of GLUC (**1**) in aqueous solution, but there are no studies dealing with the isomers' relative concentrations as a function of pH and temperature and thus of GLUC's (**1**) thermodynamic properties up to now.[17] Using high-resolution ^{1}H and ^{13}C NMR spectroscopy, we analyzed the qualitative and quantitative isomeric composition of GLUC (**1**) in aqueous solution as a function of time, temperature, and pH.

MATERIALS AND METHODS

Chemicals. Deuterium oxide (D_2O) 99.98 atom % D was from Chemotrade Chemiehandelsgesellschaft mbH (Leipzig, Germany); sodium deuteroxide 40% in D_2O, 99 atom % D, was from Sigma-Aldrich (Steinheim, Germany); D-glucose, pyranose-2-oxidase, and catalase were purchased from Sigma-Aldrich; Dowex 50W X8 was from Fluka (Steinheim, Germany); phenylhydrazine, benzaldehyde, and acetic acid were purchased from Merck (Darmstadt, Germany); ethanol was purchased from Berkel AHK (Berlin, Germany). Water with 0.055 μS/cm at 25 °C was used.

Standards. D-*arabino*-Hexo-2-ulose (GLUC) (**1**) ≥98% (TLC) was purchased from Sigma-Aldrich.

Special Issue: ISMR11 - 100 Years of the Maillard Reaction

Received: December 11, 2012
Revised: June 12, 2013
Accepted: July 3, 2013
Published: July 3, 2013

Materials and Equipment. NMR sample tubes, 5 mm o.d./178 mm length, round-bottom, were from Schott Economic (Mainz, Germany); the pH electrode was a pH electrode − spintrode, electrode glass shaft length = 180 mm, OD = 3 mm from Rototec-Spintec GmbH (Griesheim, Germany); the pH meter was a Five Easy FE20 from Mettler-Toledo AG (Schwerzenbach, Switzerland).

Synthesis. D-*arabino*-Hexo-2-ulose (GLUC) (**1**) was synthesized using the method proposed by Freimund et al. as well as using the proposed synthesis of Hellwig et al.[18,19]

Experimental Procedure. For structural assignment, solutions of GLUC (**1**) were equilibrated at room temperature for several hours. The relative concentration of each isomer was quantified by integrating its respective anomeric ^{1}H singlet. For pH and temperature dependence experiments, NMR spectra were recorded after an equilibration time of exactly 20 min. The error bars indicate the pH value before the beginning of the measurements (higher pH value) and before a new pH value had been set (lower pH value). The time dependence experiments were begun immediately after GLUC (**1**) had been dissolved. The time of solvent addition was set as zero. The *x*-coordinates of the data points shown in the corresponding figure represent the average of the starting and finishing times of each measurement.

Nuclear Magnetic Resonance Spectroscopy. NMR data were recorded on a Bruker Avance 600 spectrometer at 600.03 MHz for ^{1}H and at 150.89 MHz for ^{13}C at different temperatures adjusted with a Bruker BVT 3000 Digital and a Bruker B-CU 05, applying standard 1D and 2D spectroscopy (^{1}H, ^{13}C, COSY, TOCSY, NOESY, ROESY, HSQC, HMBC, HMQC, JRES) analyses. Chemical shifts are referenced to the internal standard acetone ($\delta(^1\text{H})$ = 2.04 ppm; $\delta(^{13}\text{C})$ = 30.5 ppm). TopSpin 1.3 was used as acquisition software. All results were evaluated using TopSpin 2.1.

RESULTS

The isomeric composition of GLUC (**1**) in aqueous solution was determined by NMR spectroscopy. Spin systems and ^3J(H,H) coupling constants were deduced from correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), and *J*-resolved (JRES) experiments. Proton−carbon connectivities were identified in heteronuclear single-quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC) spectra. ^1J(C,H) coupling constants were obtained from a heteronuclear multiple-quantum coherence (HMQC) spectrum. For structural elucidation, methylene units were used as anchor groups. Thus, the identification of particular spin systems on the basis of the information obtained from COSY, TOCSY, and JRES spectra was straightforward in all cases. The assignment of the carbonyl-C-2 carbons, as well as the identification of the types of ring structures, was performed using correlation peaks in the HMBC spectrum. Furthermore, chemical shifts of carbonyl-C-1 and carbonyl-C-2 provided information on hydration or hemiacetal binding of the carbonyl functionalities. Vicinal H,H-coupling constants were used to determine ring conformations in hexopyranoses. Direct C,H-coupling constants provided information on the configuration of anomeric protons in 1,5-pyranoid isomers (Table 1). In the case of the 2,6-pyranoid and the 2,5-furanoid isomer, nuclear Overhauser effect spectroscopy (NOESY) and rotating-frame nuclear Overhauser effect spectroscopy (ROESY) spectra provided configurational information.

In accordance with Freimund et al. and Köpper and Freimund,[17,20] we detected four isomers in aqueous solution, and the structural information could elucidate the constitution, configuration, and conformation of all of them (**1a**−**1d**). Of the four identified GLUC (**1**) isomers, two were hydrated $^4C_1$-1,5-pyranoses (**1a** and **1b**), one was the hydrated $^2C_5$-*β*-2,6-pyranose (**1c**), and one was the hydrated *β*-2,5-furanose (**1d**) (Figure 1).

Table 1. NMR Data of GLUC (1) Isomers in D_2O[a]

	isomer			
NMR data	**1a**	**1b**	**1c**	**1d**
δ(C-1)	94.8	95.3	89.6	90.2
δ(C-2)	93.8	93.2	97.6	101.0
δ(C-3)	73.8	76.6	68.0	76.0
δ(C-4)	69.0	68.9	70.1	74.7
δ(C-5)	72.2	76.3	69.4	80.7
δ(C-6)	61.2	61.2	63.7	62.2
^1J(C-1,H-1)	168.8	162.1		
δ(H-1)	4.76	4.51	4.86	4.75
δ(H-3)	3.56	3.34	3.73	4.00
δ(H-4)	3.32	3.26	3.68	3.88
δ(H-5)	3.69	3.28	3.80	3.62
δ(H′-6)	3.547	3.52	3.83	3.61
δ(H″-6)	3.67	3.71	3.553	3.48
^3J(H-3,H-4)	9.3	9.4	10.0	8.1
^3J(H-4,H-5)	10.3	8.6	3.4	8.3
^3J(H-5,H′-6)	6.3	6.1	1.4	3.1
^3J(H-5,H″-6)	2.3	2.0	2.0	5.8
^2J(H′-6,H″-6)	12.8	12.2	12.8	12.4

[a]Measurement conditions: pH 3.1; T = 296 K; ^{1}H NMR → 600 MHz; ^{13}C NMR → 150 MHz; all chemical shifts (δ) are given in ppm; all coupling constants (J) are given in hertz; 5% solutions in D_2O; internal acetone ($\delta(^1\text{H})$ = 2.04 ppm; $\delta(^{13}\text{C})$ = 30.5 ppm).

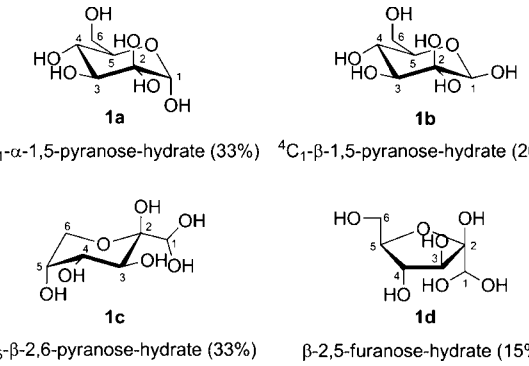

Figure 1. Isomeric composition and equilibrium of GLUC (**1**) isomers **1a**−**1d** in aqueous solution determined by NMR spectroscopy (pH 3.1; T = 296 K).

The plotted equilibration kinetic data at pH 3.1 and T = 296 K show that isomers **1a** and **1b** decreased during the first 70 min after GLUC (**1**) had been dissolved, whereas isomers **1c** and **1d** were formed in solution (Figure 2) until an equilibration condition was attained. The relative quantities of isomers **1a**−**1d** were 33, 20, 33, and 15%, respectively (Figure 1). These values are comparable to those published by Freimund et al. and those by Köpper and Freimund, respectively.[17,20] However, existing differences presumably result from slightly different measurement conditions. Interestingly, the time-dependent GLUC (**1**) isomerization differs from those reported for common sugars, where pyranose−furanose interconversion (fast mutarotation) is found to be faster than pyranose−pyranose interconversion (slow mutarotation).[21] In the case of GLUC (**1**), formation of **1c** is slightly faster than that of **1d**.

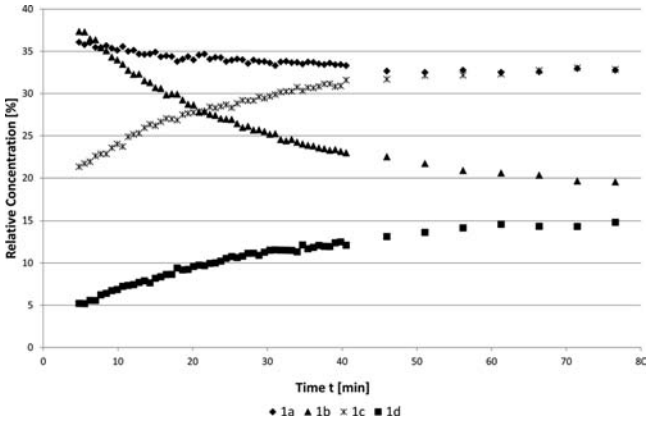

Figure 2. Time dependence of the equilibrium of GLUC (**1**) isomers **1a**−**1d** (time range, 4.8−76.6 min; pH 3.1; T = 296 K).

To obtain more information on the characteristics of GLUC (**1**) in aqueous solution, its isomeric equilibrium was determined as a function of pH and temperature. The pH value was varied from 3.1 to 8.1. These variations influenced the relative isomer concentrations in the pH range of 3.1−7.0 (Figure 3). In this interval, the pH did not drift notably to more

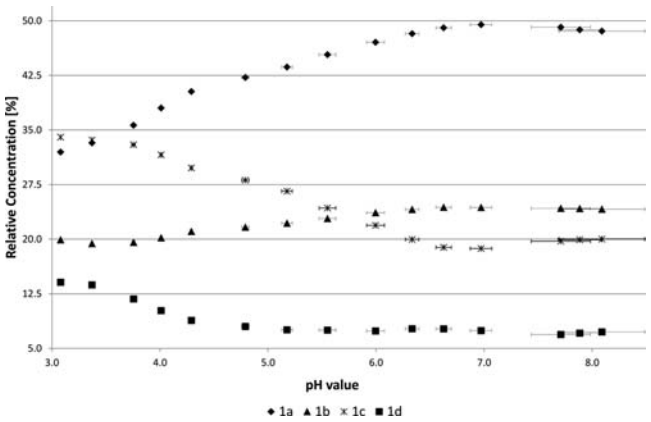

Figure 3. pH dependence of the equilibrium of GLUC (**1**) isomers **1a**−**1d** (pH range, 3.1−8.1; T = 296 K).

acidic values during the measurements. Thus, in comparison to the observed reaction behavior of 1-deoxy-D-*erythro*-hexo-2,3-diulose (1-DH), degradation of GLUC (**1**) is first initiated at higher pH values (pH >7) at room temperature. This behavior is presumably attributed to the hydrated carbonyl functionalities that seem to be more stable in weakly acidic and neutral conditions than nonhydrated carbonyl compounds.[22] With regard to the pairwise symmetrical properties of the rows of data in Figure 3 and suggesting that only little GLUC (**1**) is degraded in the pH range between 3.1 and 7.0, the observed shifts in the relative isomer concentrations presumably result from pairwise conversions of configurative information. The addition of the relative concentrations of isomers **1a** + **1c** and **1b** + **1d**, respectively, led to constant and thus pH-independent relative sum concentrations (Figure 4). A probable explanation for this observation is that an increase in pH results in a conversion of isomers **1c** and **1d** to isomers **1a** and **1b**. These results suggest that in the case of GLUC (**1**) isomers with exocyclic hydrated carbonyl functionalities are less stable in weakly acidic, neutral, and mildly basic conditions than their

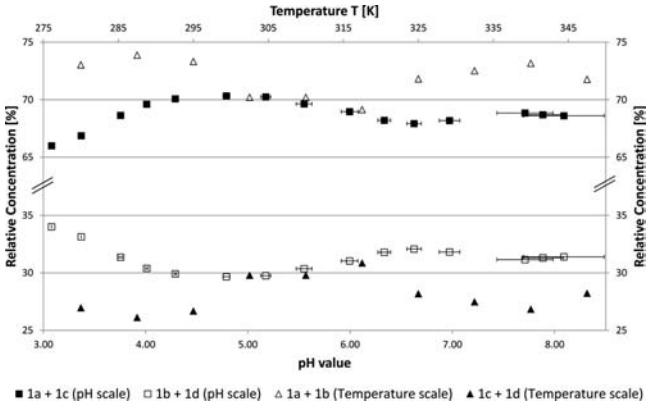

Figure 4. pH and temperature independence of the relative sum concentrations of GLUC (**1**) isomers **1a** + **1c** and **1b** + **1d** (pH range, 3.1−8.1; T = 296 K) and **1a** + **1b** and **1c** + **1d** (temperature range, 280.0−347.5 K; pH 5.2).

endocyclic equivalents and that hydronium as well as hydroxide ions do not catalyze isomerization, but influence the macroscopic equilibrium itself (Figure 5).

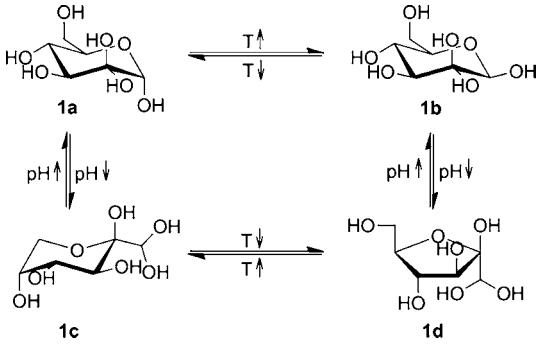

Figure 5. Proposed pH-dependent (temperature-dependent) conversion of GLUC (**1**) isomers **1a** + **1c** and **1b** + **1d** (**1a** + **1b** and **1c** + **1d**).

In the interval from pH 7.0 to 8.1 the pH drifted notably to more acidic values during the measurements (Figure 3). This indicates an initiation of hydroxyl buffering degradation reactions such as benzylic acid rearrangements and α- and β-dicarbonyl cleavages. However, the isomeric equilibrium remained constant; thus, all GLUC (**1**) isomers were degraded in comparable amounts in relation to their absolute concentrations.

Next, the influence of temperature on the isomeric equilibrium of GLUC (**1**) was observed over the temperature range of 280.0−347.5 K at pH 5.2. These variations influenced the relative isomer concentrations in the temperature range 295.0−347.5 K. In analogy to variations in the pH values, rows of data obtained from temperature variations show symmetrical properties (Figure 6). Thus, the observed shifts in the relative isomer concentrations presumably result from pairwise conversions of configurative information. In this case, the addition of the relative concentrations of isomers **1a** + **1b** and **1c** + **1d**, respectively, led to constant and therefore temperature-independent relative sum concentrations (Figure 4). A probable explanation for this observation is that an increase in temperature results in a conversion of isomers **1a** and **1c** to isomers **1b** and **1d** (Figure 5). Plotting all equilibrium constants as a function of temperature, thermodynamic properties of the

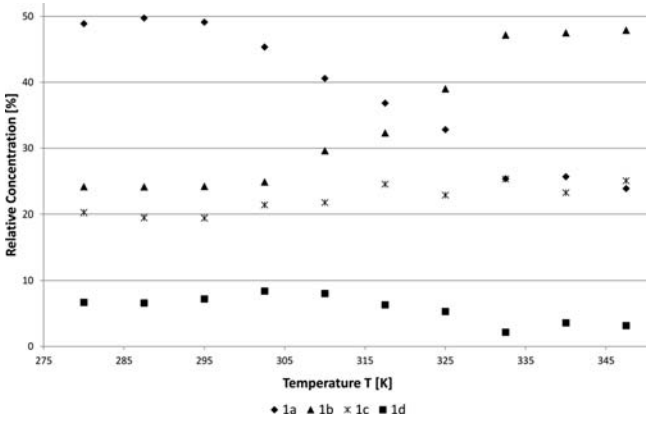

Figure 6. Temperature dependence of the equilibrium of GLUC (**1**) isomers **1a**−**1d** (temperature range, 280.0−347.5 K; pH 5.2).

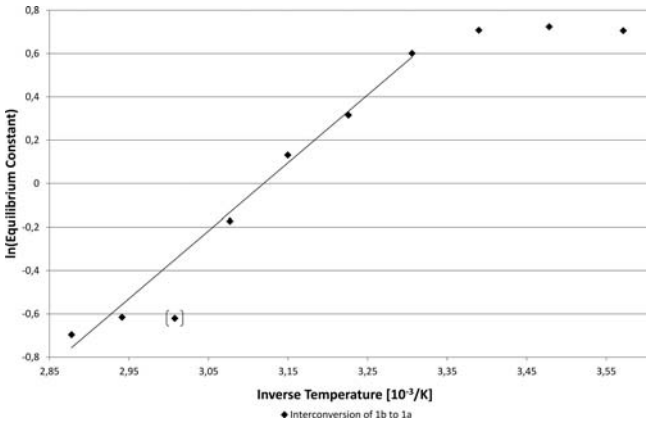

Figure 7. Temperature dependence of the equilibrium constant of interconverting isomers **1b** and **1a** (temperature range, 280.0−347.5 K; pH 5.2).

observed isomerization can be derived (Figure 7).[23] In that context it is noteworthy that GLUC (**1**) obviously does not behave in the same manner as common sugars do. The concentration of the furanoid GLUC (**1**) isomer **1d** does not increase with increasing temperature, as has been expected with regard to the behavior of other furanoses.[24−26] That means that in the case of GLUC (**1**), the furanoid isomer **1d** is not the high-enthalpy isomer, but is entropically least stable (Table 2).

Table 2. Thermodynamics of Equilibrating GLUC (1) Isomers

reaction	$\Delta G°_{298}$ (J/mol)	$\Delta H°$ (J/mol)	$\Delta S°$ (J/K mol)
1a ⇌ **1b**	1820 ± 88	26017 ± 1157	81 ± 4
1a ⇌ **1c**	2076 ± 61	15215 ± 809	44 ± 3
1a ⇌ **1d**	3910 ± 86	−7596 ± 1131	−39 ± 4

Our results show that the additional carbonyl or rather *gem*-diol functionality exceedingly alters the enthalpic and entropic properties of GLUC (**1**) compared to common hexoses. Unfortunately, we are not able to provide any explanation for these observations up to now.

A pH shift from 5.2 to 3.7 during the whole series of temperature-dependent measurement indicated occurring degradation reactions. However, the temperature dependence of the equilibrium constants shows a linear correlation in the temperature interval from 302.5 to 347.5 K; thus, all GLUC (**1**) isomers were degraded in comparable amounts in relation to their absolute concentrations. The nonlinear correlation of temperature and equilibrium constants in the temperature interval from 280.0 to 302.5 K is a result of the chosen equilibration time of 20 min that obviously did not suffice to reach an equilibration state at such low temperatures (Figure 7).

DISCUSSION

This work points out that the equilibrium of GLUC (**1**) isomers highly depends on pH and temperature in the pH range 3.1−7.0 at T = 296 K and in the temperature interval from 295.0 to 347.5 K at pH 5.2. An increase in pH from 3.1 to 7.0 presumably led to a conversion of isomers **1c** and **1d** to isomers **1a** and **1b**. Furthermore, as the temperature increased from 295.0 to 347.5 K, isomers **1a** and **1c** presumably converted into isomers **1b** and **1d**. In addition, all GLUC (**1**) isomers showed comparable reaction behavior regarding pH- and temperature-dependent degradation reactions. These results show that changing the measurement conditions (pH and temperature) permits control over the equilibrium's characteristic and over the stability and reactivity of GLUC (**1**) isomers. Interestingly, GLUC's (**1**) (thermodynamic) properties alter from those reported for common monocarbonyl sugars. GLUC (**1**) exhibits complex mutarotation, whereby the pyranoid isomer **1c** forms more rapidly than the furanoid isomer **1d** does. Furthermore, changes of pH do not influence the observed system in a catalytic way, but alter the macroscopic equilibrium itself. Finally, increasing temperature does not favor the formation of the furanoid isomer **1d**. These points show that our state of knowledge about common carbohydrates cannot be extrapolated to related Maillard intermediates in all respects. For that reason it is necessary to determine the thermodynamic and kinetic properties of further α-dicarbonyl as well as Amadori and Heyns compounds to be able to derive relationships between geometrical structure and entropic and enthalpic stabilities. An increase in that knowledge is indispensable for a more detailed understanding of the mechanistics of Maillard reaction and thus for optimizing technological food processing as well as for an improvement of the understanding of Maillard products' biological activities.

AUTHOR INFORMATION

Corresponding Author

*(P.T.H.) Phone: +49-30-31472806. Fax: +49-30-31472585. E-mail: paul.haase@tu-berlin.de.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank A. Thiesies and H. Weißhoff for their skillful technical support.

ABBREVIATIONS USED

NMR, nuclear magnetic resonance; GLUC, glucosone, D-*arabino*-hexo-2-ulose; AGE, advanced glycation end product; HCA, heterocyclic aromatic amine; TLC, thin layer chromatography; COSY, correlation spectroscopy; TOCSY, total correlation spectroscopy; NOESY, nuclear Overhauser effect spectroscopy; ROESY, rotating-frame nuclear Overhauser effect spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond correlation; HMQC,

heteronuclear multiple quantum coherence; JRES, *J*-resolved; *J*, coupling constant; δ, chemical shift; 1-DH, 1-deoxy-D-*erythro*-hexo-2,3-diulose

REFERENCES

(1) Maillard, L.-C. Action des acides aminés sur les sucres; formation des mélanoïdines par voie méthodique. *C. R. Acad. Sci.* **1912**, *154*, 66−68.

(2) Belitz, H.-D.; Grosch, W.; Schieberle, P. *Lehrbuch der Lebensmittelchemie*, 6th ed.; Springer: Berlin, Germany, 2008.

(3) Jägerstad, M.; Laser Reuterswärd, A.; Olsson, R.; Grivas, S.; Nyhammar, T.; Olsson, K.; Dahlqvist, A. Creatin(in)e and Maillard reaction products as precursors of mutagenic compounds: effects of various amino acids. *Food Chem.* **1983**, *12*, 255−264.

(4) Schleicher, E. Die Bedeutung der Maillard-Reaktion in der menschlichen Physiologie. *Z. Ernaehrungswiss* **1991**, *30*, 18−28.

(5) Nagaraj, R. H.; Sell, D. R.; Prabhakaram, M.; Ortwerth, B. J.; Monnier, V. M. High correlation between pentosidine protein crosslinks and pigmentation implicates ascorbate oxidation in human lens senescence and catarctogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10257−10261.

(6) Lee, J. H.; Shin, D. H.; Lupovitch, A.; Shi, D. X. Glycosylation of lens proteins in senile cataract and diabetes mellitus. *Biochem. Biophys. Res. Commun.* **1984**, *123*, 888−893.

(7) Vitek, M. P.; Bhattachary, K.; Glendening, J. M.; Stopa, E.; Vlassara, H.; Bucala, R.; Manogue, K.; Cerami, A. Advanced glycation end products contribute to amyloidosis in Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4766−4770.

(8) Brownlee, M.; Vlassara, H.; Kooney, A.; Ulrich, P.; Cerami, A. Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science* **1986**, *232*, 1629−1632.

(9) Monnier, V. M.; Nagaraj, R. H.; Portero-Otin, M.; Glomb, M.; Elgawish, A.-H.; Sell, D. R.; Friedlander, M. A. Structure of advanced Maillard reaction products and their pathological role. *Nephrol., Dial., Transplant.* **1996**, *11*, 20−26.

(10) Gobert, J.; Glomb, M. A. Degradation of glucose: reinvestigation of reactive α-dicarbonyl compounds. *J. Agric. Food Chem.* **2009**, *57*, 8591−8597.

(11) Kawakishi, S.; Tsunehiro, J.; Uchida, K. Autoxidative degradation of Amadori compounds in the presence of copper ion. *Carbohydr. Res.* **1991**, *211*, 167−171.

(12) Kroh, L. W.; Fiedler, T.; Wagner, J. The formation of colored compounds in technical sucrose solutions − new knowledge about their molecular basics and molecular size distribution. *Zuckerindustrie (Berlin, Ger.)* **2007**, *132*, 25−32.

(13) Schalkwijk, C. G.; Posthuma, N.; ten Brink, H. J.; ter Wee, P. M.; Teerlink, T. Induction of 1,2-dicarbonyl compounds, intermediates in the formation of advanced glycation end-products, during heat-sterilization of glucose-based peritoneal dialysis fluids. *Peritoneal Dial. Int.* **1999**, *19*, 325−333.

(14) Cämmerer, B.; Kroh, L. W. Investigation of the influence of reaction conditions on the elementary composition of melanoidins. *Food Chem.* **1995**, *53*, 55−59.

(15) Kroh, L. W.; Fiedler, T.; Wagner, J. α-dicarbonyl compounds−key intermediates for the formation of carbohydrate-based melanoidins. *Ann. N. Y. Acad. Sci.* **2008**, *1126*, 210−215.

(16) Schuirmann, E.; Eichner, K. Bildung von IQ-Verbindungen in Fleisch und Fleischprodukten. *Z. Ernaehrungswiss* **1991**, *30*, 56−64.

(17) Freimund, S.; Baldes, L.; Huwig, A.; Giffhorn, F. Enzymatic synthesis of D-glucosone 6-phosphate (D-*arabino*-hexos-2-ulose 6-(dihydrogen phosphate)) and NMR analysis of its isomeric forms. *Carbohydr. Res.* **2002**, *337*, 1585−1587.

(18) Freimund, S.; Huwig, A.; Giffhorn, F.; Köpper, S. Rare keto-aldoses from enzymatic oxidation: substrates and oxidation products of pyranose 2-oxidase. *Chem.−Eur. J.* **1998**, *4*, 2442−2455.

(19) Hellwig, M.; Degen, J.; Henle, T. 3-Deoxygalactosone, a "new" 1,2-dicarbonyl compound in milk products. *J. Agric. Food Chem.* **2010**, *58*, 10752−10760.

(20) Köpper, S.; Freimund, S. The composition of keto aldoses in aqueous solution as determined by NMR Spectroscopy. *Helv. Chim. Acta* **2003**, *86*, 827−843.

(21) Angyal, S. J. The composition and conformation of sugars in solution. *Angew. Chem., Int. Ed.* **1969**, *8*, 157−226.

(22) Kaufmann, M.; Haase, P. T.; Mügge, C.; Kroh, L. W. Milieu dependence of 1-deoxy-D-*erythro*-hexo-2,3-diulose in aqueous solution determined by high-resolution NMR spectroscopy. *Carbohydr. Res.* **2012**, *364*, 15−21.

(23) Wertz, P. W.; Garver, J. C.; Anderson, L. Anatomy of a complex mutarotation. Kinetics of tautomerization of α-D-galactopyranose and β-D-galactopyranose in water. *J. Am. Chem. Soc.* **1981**, *103*, 3916−3922.

(24) Angyal, S. J. The composition of reducing sugars in solution. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 15−68.

(25) Jaseja, M.; Perlin, A. S. Two-dimensional NMR spectral study of the tautomeric equilibria of D-fructose and related compounds. *Magn. Reson. Chem.* **1990**, *28*, 283−289.

(26) Maple, S. R.; Allerhand, A. Detailed tautomeric equilibrium of aqueous D-glucose. Observation of six tautomers by ultrahigh resolution carbon-13 NMR. *J. Am. Chem. Soc.* **1987**, *109*, 3168−3169.

NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on July 15, 2013, with an error in Figure 7. The corrected version was reposted July 23, 2013.