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Structure–reactivity relationship of *Amadori* rearrangement products compared to related ketoses

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ABSTRACT

Structure-reactivity relationships of *Amadori* rearrangement products compared to their related ketoses were derived from multiple NMR spectroscopic techniques. Besides structure elucidation of six *Amadori* rearrangement products derived from D-glucose and D-galactose with L-alanine, L-phenylalanine and L-proline, especially quantitative ^{13}C selective saturation transfer NMR spectroscopy was applied to deduce information on isomeric systems. It could be shown exemplarily that the *Amadori* compound *N*-(1-deoxy-D-fructos-1-yl)-L-proline exhibits much higher isomerisation rates than D-fructose, which can be explained by C-1 substituent mediated intramolecular catalysis. In combination with a reduced carbonyl activity of *Amadori* compounds compared to their related ketoses which results in an increased acyclic keto isomer concentration, the results on isomerisation dynamics lead to a highly significant increased reactivity of *Amadori* compounds. This can be clearly seen, comparing approximated carbohydrate milieu stability time constants (*ACuSTiC*) which is 1 s for *N*-(1-deoxy-D-fructos-1-yl)-L-proline and 10 s for D-fructose at pD 4.20 ± 0.05 at 350 K. In addition, first NMR spectroscopic data are provided, which prove that α-pyranose of (amino acid substituted) D-fructose adopts both, $^{2}C_{5}$ and $^{5}C_{2}$ conformation.

1. Introduction

Since 1912, when Louis Camille Maillard observed that browning reactions occur while heating D-glucose in the presence of amino compounds, different *Maillard* reaction systems are constantly and thoroughly investigated until today.[1–12] Due to the high amount of different *Maillard* reaction products and their various possibilities of undergoing side reactions, quantitative description of *Maillard* reaction kinetics is challenging. Nevertheless, the initial phase of the *Maillard* reaction is well understood.[13] After a nucleophilic attack of an amino nitrogen at the carbonyl carbon of a reducing sugar, condensation and rearrangement leads to *Amadori* and *Heyns* products, respectively. Thereby, *Heyns* products originate from ketoses and thus, are aldose derivatives. For *Amadori* products, the inverse statement is true. According to Pilková et al., *Heyns* products show a much smaller browning rate than *Amadori* products in the absence of additional amino compounds.[14] In contrast, the presence of amino compounds causes their rearrangement to *Amadori* products.[15,16] In the following step of the *Maillard* reaction, *Amadori* compounds can enolise and eliminate the amino compound or become oxidised.[13,17,18] In this case, (deoxy-) osones are formed and amino compounds are released. Thus, amino compounds influence the first steps of the *Maillard* reaction acting as catalyst.[15] This in turn means that amino compounds will alter the degradation kinetics of sugars. Consequently, *Heyns* products will show higher reactivity than D-fructose. Since *Heyns* products show smaller browning rates than *Amadori* products in the absence of additional amino compounds,[14] it can be concluded that *Amadori* compounds show higher reactivity than D-fructose as well. In addition to those theoretical considerations, especially van Boekel et al.[19–21] formulated complex kinetic network models and provide valuable data on *Maillard* reaction kinetics. Analysing these data, it is striking that degradation kinetics of *Amadori* compounds indeed differ significantly from those of D-fructose, even though their molecular constitution only differs in C-1 substituent.

The aim of our work was to identify differences in the isomeric systems of ketoses and their *Amadori* derivatives that explain their different reactivities and that can be traced back to specific structural features. As model systems, we used D-fructose and D-tagatose as well as their related L-proline–, L-alanine– and L-phenylalanine–*Amadori* derivatives. In a first step, molecular conformation of all

Chemical compounds studied in this article: D-Fructose (PubChem CID: 5984); D-Tagatose (PubChem CID: 92092); D-Fructose + L-Proline (PubChem CID: 118797558); *N*-(1-deoxy-D-fructos-1-yl)-L-proline (PubChem CID: 71316983); *N*-(1-deoxy-D-fructos-1-yl)-L-alanine (PubChem CID: 71316979); *N*-(1-deoxy-D-fructos-1-yl)-L-phenylalanine (PubChem CID: 71316982)

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investigated substances was elucidated in aqueous solution. We subsequently quantified relative isomer concentrations in dependence on temperature and pD and measured thermodynamic activation parameters of ring opening using quantitative ^{13}C selective saturation transfer NMR (^{13}C SST qNMR) spectroscopy.

2. Results and discussion

2.1. Structural assignments by NMR spectroscopy

Structure determination of *Amadori* rearrangement products was made on the basis of high-resolution NMR spectroscopy. Since ^{1}H NMR spectra are crowded especially in the saccharide region and *Amadori* compounds do not bear an anomeric proton, anomeric ^{13}C signals were chosen as anchor groups for structure elucidation. Anomeric carbons were fitted to their adjoining spin systems using heteronuclear multiple bond correlations (HMBC). Spin systems and geminal as well as vicinal coupling constants were deduced on the basis of correlation spectroscopy (COSY), selective total correlation spectroscopy (selTOCSY), heteronuclear single quantum coherence total correlation spectroscopy (HSQC-TOCSY) and homonuclear *J*-resolved (JRES) experiments. Proton-carbon connectivities were detected comparing heteronuclear single quantum correlations (HSQC) to HSQC–TOCSY correlations. Finally, the identification of ring structures was based on HMB correlations. Homonuclear coupling constants in combination with nuclear *Overhauser* effect spectroscopy (NOESY) spectra were used to determine ring conformation.

2.2. Isomeric composition of Amadori rearrangement products and ketoses in aqueous solution

All investigated *Amadori* compounds and ketoses (cf. Scheme 1) show four NMR signals in the anomeric region of ^{13}C spectra. Additionally there is an "anomeric" signal downfield from 200 ppm that belongs to the keto carbon of the acyclic isomer.

Anomeric carbon signals of furanoses (**c** and **d**) are low field shifted compared to pyranoses (**a** and **b**), whereas β-furanose (**d**) is at higher field than α-furanose (**c**) (for derivatives of D-fructose **1** and D-tagatose **2**).[22,23] Since coupling patterns of D-tagatose derived *Amadori* compounds **4**, **6** and **8** could be determined properly, structures of pyranoses of amino acid substituted D-tagatose (**a′** and **b**) could be elucidated without any doubt. α-Pyranoses (**a′**) adopt the $^{5}C_2$ chair which is in agreement with the crystal structure of α-D-tagatopyranose **2a′**,[24] whereas β-pyranoses of D-tagatose derivatives in analogy to β-pyranoses of D-fructose derivatives exist in the $^{2}C_5$ conformation (**b**). Thereby, β-pyranoses of D-fructose derivatives (**b**) exhibit the same structure in aqueous solution as could be elucidated in crystalline state.[25–32] Additionally, derivatives of D-fructose **1** as well as D-tagatose **2** form α- and β-furanoses (**c** and **d**) as well as a non-hydrated acyclic keto isomer (**e**). The only structures that have to be discussed in detail are the α-pyranoid ones of derivatives of D-fructose (**1a′** and **1a″**) since signal overlapping is too intense to extract vicinal coupling constants. Existing publications mostly do not provide any experimental hint for α-pyranose ring geometry of D-fructose **1** and D-fructose derived *Amadori* products in aqueous solution. That is why their respective structure is still under discussion. There are publications describing the conformation of α-pyranoses of D-fructose (derivatives) to be $^{2}C_5$ (**a″**) without providing (profound interpretations of) their experimental data.[33,34] Authors referring to those publications adopt the structure without critically discussing possible ring conformations.[35] This is remarkable because Barclay et al. provide a full ^{1}H NMR signal assignment for all D-fructose isomers (**1a**–**1e**) without presenting any coupling constants.[35] Nonetheless, figure 2 of their publication shows coupling patterns for every single proton. The qualitative examination of this figure shows that ^{3}J(H-C-3; H-C-4) for the α-pyranoid isomer **1a** equals that of the β-pyranoid one **1b**. However, a more precise graphical analysis of the coupling constants leads to an inconsistent result. That is, coupled protons in general need to have equal coupling constants which does not apply to the discussed case (cf. Table 1). Coupling patterns obviously do not fit together. Thus, a reliable interpretation of the presented data is not possible. In addition to the problem that spectral information for α-D-fructopyranose **1a** is rare, even theoretical work does not lead to satisfactory results.[36] French et al. showed that the conformational energy difference between $^{2}C_5$ (**a″**) and $^{5}C_2$ (**a′**) is about 2.55 kJ mol^{-1} and thus is equal to thermal energy at 34 °C. This should possibly result in a fast exchange between both α-pyranoses at ambient temperature so that NMR spectroscopy just provides the mean spectrum of both conformers. This idea is supported by the work of Polacek and Kaatze who detected a low frequency acoustical relaxation of D-fructose **1** in water and water–ethanol mixtures applying ultrasonic absorption spectroscopy that was assigned to $^{5}C_2 \leftrightarrow {}^{2}C_5$ ring inversion.[37,38] Furthermore, experimentally determined vicinal coupling constants of the α-pyranoid isomer of D-fructosamine hydrochloride in D_2O (^{3}J(H-C-5; H_a-C-6 = 3.1 Hz; ^{3}J(H-C-5; H_b-C-6 = 3.0 Hz) are too high for $^{2}C_5$ (**a″**) and too low for $^{5}C_2$ (**a′**) conformation.[36,39] If both conformers coexisted in similar amounts, linewidth of the detectable anomeric α-pyranoid ^{13}C NMR signal of D-fructose **1** and its derivatives **3**, **5** and **7** should increase with decreasing temperature or with increasing magnetic flux density. We thus measured linewidths of D-[2-^{13}C]fructose **1** and *N*-(1-deoxy-D-[2-^{13}C]fructos-1-yl)-L-proline **3** in dependence of temperature (cf. Table 2). As Table 2 shows, the mean of all anomeric linewidths except for that of α-pyranose is nearly temperature independent. Since anomeric carbon resonances of α- and β-D-fructopyranose **1a** and **1b** become more and more isochronal with increasing temperature, accurate linewidth determination of α-D-fructopyranose **1a** is not possible. Nevertheless, it can be qualitatively seen that linewidth increases with decreasing temperature. This is the first reliable NMR spectroscopic proof that derivatives of D-fructose **1** adopt both, $^{2}C_5$- and $^{5}C_2$-α-pyranoid conformation. In contrast to that, linewidth of the *Amadori* product's α-pyranose **3a** can be determined very precisely. Doing so, one can estimate activation enthalpy of $^{5}C_2 \leftrightarrow {}^{2}C_5$ ring inversion, extracting approximated factorised rate constants using equation (1).[40] This approach is valid under the condition that the ratio of the $^{5}C_2$- and $^{2}C_5$-conformer is constant in the examined temperature interval. Since this interval is small in the present case, this precondition can be regarded as to be met.

Table 1
Graphically determined coupling constants for α-D-fructopyranose **1a** extracted from figure 2 from Barclay et al.[35]

J-couplings [Hz]	H-C-3		H-C-4		H-C-5
H-C-3	-	≠	ca. 12.5		-
H-C-4	ca. 6.7		-	≠	ca. 3.4
H-C-5	-		ca. 5.8		-

$$k_A = \frac{4\pi p_A p_B{}^2 \delta\nu^2}{\Delta_e} \Leftrightarrow \frac{k_A}{4\pi p_A p_B{}^2 \delta\nu^2} = \frac{1}{\Delta_e} \Rightarrow const. \cdot k_A = \frac{1}{\Delta_e} \quad (1)$$

Thereby, k_A is the rate constant for $^{5}C_2 \leftrightarrow {}^{2}C_5$ ring inversion, $p_A(p_B)$ the mole fraction of $^{5}C_2$- ($^{2}C_5$-) conformer, $\delta\nu$ the relative chemical shift of their anomeric carbon resonances and Δ_e the exchange broadening of linewidth. Since $4\pi p_A p_B{}^2 \delta\nu^2$ is constant for narrow temperature intervals, k_A is inversely proportional to Δ_e. Regarding the *Eyring* equation, the factor of proportionality exclusively influences activation entropy. Thus, activation enthalpy can be approximated without further restrictions. Doing so, activation enthalpy