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The preparation and characterization of some Amadori compounds (1-amino-1-deoxy-D-fructose derivatives) derived from a series of aliphatic ω-amino acids †

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Abstract

Amadori compounds (1-amino-1-deoxy-D-fructose derivatives) were prepared by reacting D-glucose with a series of aliphatic amino acids. These include Amadori compounds derived from glycine (**1**), β-alanine (**2**), γ-amino butyric acid (**3**), δ-aminovaleric acid (**4**), ε-aminocaproic acid (**5**) and N^{α}-formyl-L-lysine (**6**). In the FAB mass spectra, molecular-ion clusters as well as fragment ions corresponding to loss of water or CO_2 molecules were observed. The ^{13}C NMR spectra indicate that all the compounds are conformationally unstable, but that the predominant form present in solution (D_2O) is the β-pyranose form. The ^{1}H NMR spectra of **1** and **2** indicate a slow rotation around the C-1–C-2 bond, possibly as a result of an intramolecular hydrogen bond involving the carboxyl group. The pK_a's of all compounds were measured by pH-potentiometric titration in 0.2 M KNO_3 solution at 25°C. All compounds showed a decrease in the basicity of their amino groups (in the order of ~ 1.5 of the K_a value), and **1** and **2** showed a decrease in the basicity of their carboxyl groups (in the order of ~ 0.2) in comparison with that of parent amino acids.

Keywords: Amadori compounds; Maillard products; Synthesis; 1-Deoxy-D-fructopyranos-1-ylamines

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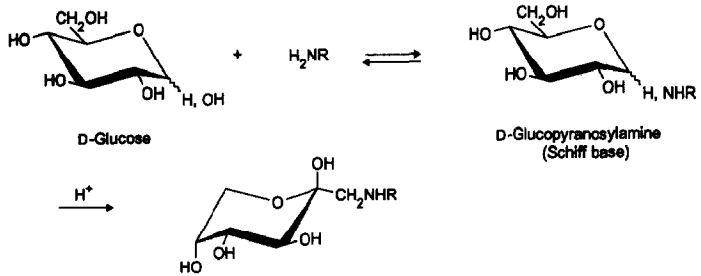
1. Introduction

1-Amino-1-deoxy-D-fructose derivatives (Amadori compounds) represent the initial compounds produced during the Maillard reaction, a complex degradative reaction involving the decomposition of both sugars and amino acids to give UV absorbing materials, polymeric pigments (Maillard polymers), dicarbonyl intermediates (derived from the reducing sugar) as well as a variety of dehydration and fragmentation products, derived largely from carbon atoms of the reducing sugar [1]. Amadori compounds are thought to be formed via the direct reaction of an amino group with a sugar to give a glycosylamine (Schiff base), which undergoes rapid rearrangment to the 1-amino-1-deoxy-D-fructose derivative (an Amadori compound), as shown in Scheme 1.

Evidence has recently been published which implicates the Maillard reaction in in vivo reactions, giving rise to "glycated proteins" [2]. The latter are proteins in which some of their available amino groups, usually the side chains of lysine residues [3], have undergone such reactions to give Amadori compounds. Such glycated proteins would be expected to undergo the usual degradative reactions over a period of time and would be expected to participate in further protein modification (crosslinking), the production of fluorescent materials, as well as a variety of other reactions that might affect the properties of the protein molecule. It has been suggested that the glycation of proteins and their subsequent decomposition may contribute to some of the pathophysiologies associated with a variety of diseases including diabetes [4], the formation of cataracts [5], and aging in general [6]. In addition, amino acids and short peptides are also involved in in vivo nonenzymatic glycation, and the corresponding Amadori compounds or degrada-



Scheme 1.

tion products derived from them have been reported to possess the ability to affect the adhesion and aggregation properties of cancer cells [7].

Most vegetables, fruit, cereal, and meat products contain free reducing sugars [8] and amino acids [8c,9], and the formation of the corresponding Amadori compounds occur during the storage and processing of food [10]. At least 60–70% of Amadori compounds taken orally transit into the blood and are excreted in the urine [11], suggesting that the ingestion of processed food represents another source of Amadori compounds for humans. ω-Amino acids such as γ-aminobutyric acid or β-alanine, as well as Amadori compounds derived from them, are present in foodstuffs in substantional amounts [9,10]. They can also be considered as more simple models for peptide bound lysine [12].

In an effort to better understand the role that Amadori compounds play in the Maillard reaction, as well as their role in biologically relevant interactions, we have synthesized a number of such compounds for use as models for biological studies. In this paper, we report the preparation of some Amadori compounds derived from a series of aliphatic ω-amino acids, as well as some data on their chemical constitution and acid–base properties.

2. Experimental

General methods.—Melting points were determined using a Thomas–Hoover melting point apparatus in open capillary tubes and are uncorrected. Optical rotations were measured at 25°C using a Perkin–Elmer Model 241 MC automatic polarimeter. ^{13}C NMR spectra (D_2O) were recorded at 125.8 MHz using 1,4-dioxane as the external standard ($\delta = 67.4$ ppm) and ^{1}H NMR spectra (D_2O) were obtained at 500.1 and 250.1 MHz with TSPS as internal standard ($\delta = 0.00$ ppm) using Bruker AMX 500 and ARX 250 instruments. Mass spectra were obtained on an AUTOSPEC-Q tandem hybrid mass spectrometer (VG Analytical Ltd., Manchester, UK) equipped with an OPUS data system. Fast-atom bombardment (FAB) mass spectrometry experiments were performed using a cesium gun operated at 20 keV energy and 2 μA emission. Samples, in water, were added to glycerol as the matrix. Exact-mass FAB experiments were carried out at 1 : 10 000 resolution using linear voltage scans under a data system control and collecting continuum data. Polyethyene glycol (PEG 300) ions served as the bracketing calibrant ions.

The potentiometric titration experiments were performed as follows. All solutions were made up using MilliQ HPLC Grade water (Millipore). A stock electrolyte solution was prepared from crystalline potassium nitrate (Mallinckrodt, AR) to give a 0.2 M concentration. Titrant solutions were prepared by diluting concd KOH solution (Fisher, certified for content $K_2CO_3 < 0.2\%$) or HCl (Fisher) in water and were kept under N_2. Their concentrations were checked titrimetrically against potassium hydrogen phthalate or TRIS, respectively. The electrodes used were as follows: a glass Fisher Full Range/High pH electrode (No. 13-620-295) and a calomel reference electrode (Fisher No. 13-620-52) in conjunction with a Fisher Accumet 915 pH Meter. The system was calibrated with standard buffers,

followed by titration of the HCl in 0.2 M KNO_3 with the alkali over the range pH 2–11.5. For the relationship (1) which expresses pH-meter readings as a function of hydrogen-ion concentration,

$$pH_{read} = C + e \cdot \log[H^+] \quad (1)$$

this procedure afforded values of efficiency $e \approx -0.995$ and $C \approx 0.05$ from acidic (pH 2–3) and $pK_w = 13.79 \pm 0.01$ from basic (pH 10.8–11.5) portions of the titration curves. The influence of the liquid junction potential on the linearity of the glass electrode function was not substantional within the pH region of these experiments (pH 2–11) and was not taken into account. Normally, at least two series of titration curves were obtained at different concentrations (2–10 mmol · dm^{-3}) of the amino acids and compounds **1–6**. Titers of the compounds were refined during the calculations of protonation constants by a simplex method. The stability of the Amadori compounds during titration was confirmed by repeated titrations of the same solutions. All titrations were performed at 25.0 ±0.1°C. Water-saturated purified N_2 was bubbled through the solutions in order to prevent CO_2 influence on the titration data at high pH.

A computer program, PSEQUAD [13], was used to calculate protonation constants in form of overall formation constants (β) of equilibrium (2):

$$nH^+ + L^- \overset{\beta_{n1}}{\rightleftharpoons} H_nL^{n-1} \quad (2)$$

where n = 1 or 2 from which more ordinary acid dissociation constants may be derived:

$$pK_{a2} = \log \beta_{11} \quad (3)$$

$$pK_{a1} = \log \beta_{21} - \log \beta_{11} \quad (4)$$

The reliability of the data obtained was supported according to IUPAC recommendations [14] by the comparison of our glycine and β-alanine dissociation constants values with recommended statistical values [15]: Glycine $pK_{a1} = 2.33 \pm 0.01$ (our), 2.37 ±0.07 (rec); $pK_{a2} = 9.60 \pm 0.01$ (our), 9.60 ± 0.05 (rec); β-Alanine $pK_{a1} = 3.57 \pm 0.01$ (our), 3.57 (rec); $pK_{a2} = 10.10 \pm 0.01$ (our), 10.14 (rec).

TLC was performed on Silica Gel G-60 glass plates using the following irrigants (v/v): A, 4:1:1 *n*-butanol–acetic acid–water and B, 2:3:1 *n*-butanol–pyridine–water. Plates were sprayed with 0.2% ninhydrin in acetone (for detection of amino acid derivatives as well as sugars), followed by heating at 120°C for 2–5 min. TLC R_f values are reported relative to the parent amino acids.

All amino acids were purchased from Aldrich Chemical Co. N^{α}-formyl-L-lysine was prepared according to Hoffmann et al. [16]; L-lysine hydrochloride was the starting material instead of the formate salt used in the original work. δ-Aminovaleric acid was recrystallized twice from aq ethanol. Other amino acids were pure, as evidenced by TLC, and were used without purification.

General procedure for the preparation of Amadori compounds.—The procedures for the synthesis of compounds **1–6** involved the following procedure (see Scheme

1): A suspension of 36 g (0.2 mol) of anhyd D-glucose and 2.0 g sodium bisulfite in 60 mL of methanol and 30 mL of glycerol was refluxed for 30 min, followed by the addition of 0.07–0.09 mol of amino acid and 8 mL of acetic acid. This solution was refluxed until ~ 80% of the amino acid had reacted, as evidenced by TLC. The resulting brown, syrupy solution was diluted with 1 vol of water, placed on a 2 × 30 cm column of Amberlite IRN-77 (H^+) ion-exchange resin, and the column was eluted with 500 mL of water, followed by 0.2 N ammonium hydroxide or, in some cases, a buffer (0.2 M in pyridine and 0.4 M in acetic acid). Fractions of ~ 25 mL were collected. Early fractions contained D-glucose, uncharged pigments and D-glucose-derived degradation products. The Amadori compound, along with unreacted amino acid, usually eluted near the end of the water wash and at the beginning of the ammonium hydroxide wash. The combined fractions, which contained Amadori compounds were evaporated to 100 mL in vacuo and decolorized with charcoal (2.0 g). This solution was placed on a second 2 × 30 cm column of Amberlite IRN-77 (pyridinium form, pretreated with 10 mL of acetic acid). The column was eluted with water and 25-mL fractions were collected. The Amadori compounds usually eluted almost immediately. Fractions containing the desired products were evaporated in vacuo at 30°C to syrups.

Isolation of N-*(1-deoxy-D-fructos-1-yl)-glycine* (**1**).—Methanol was added to syrupy **1** to turbidity, and the resulting mixture was allowed to stand at room temperature for 4 days during which time crystallization (colorless prisms) occurred. The crystals were placed on a filter and washed with 3:1 methanol–water and dried in vacuo over calcium chloride. An additional crop of crystals was obtained from the mother liquor. Yield 3.4 g (22%, based on starting glycine): mp 146.5–147.5°C (dec) (lit. [17] mp 145°C, dec); $[\alpha]_D^{25}$ −66° (*c* 1.0, H_2O) (lit. [17] −67°); R_{Gly} 0.64 (*A*) and 1.49 (*B*). Exact mass of $[M+H]^+$ ion. Calcd for $C_8H_{16}NO_7$: 238.0927; found: 238.0932. See Results and Discussion section (Tables 1–4) for FABMS and NMR data. Anal. Calcd for $C_8H_{15}NO_7$: C, 40.51; H, 6.33; N, 5.91. Found: C, 40.18; H, 6.70; N, 5.58.

Isolation of N-*(1-deoxy-D-fructos-1-yl)-β-alanine* (**2**).—Methanol was added to syrupy **2** to turbidity, and the solution was allowed to stand at room temperature for 1 day, during which time crystallization occurred. After filtering, the crystals were washed with 3:1 methanol–water and then dried in vacuo over calcium chloride. Additional quantities of **2** were obtained from the mother liquor. Yield 9.9 g (44% based on starting β-alanine) of white microcrystalline powder: mp 179–180°C (dec) [lit. [18] mp 153°C (dec)]; $[\alpha]_D^{25}$ −49° (*c* 1.0, H_2O) (lit. [18] −56.9°); $R_{\beta Ala}$ 0.62 (*A*) and 1.48 (B). Exact mass of $[M+H]^+$ ion. Calcd for $C_9H_{18}NO_7$: 252.1083; found: 252.1088. See Results and Discussion section (Tables 1–4) for FABMS and NMR data. Anal. Calcd for $C_9H_{17}NO_7$: C, 43.03; H, 6.77; N, 5.58. Found: C, 42.70; H, 6.85; N, 5.20.

Isolation of N-*(1-deoxy-D-fructos-1-yl)-γ-aminobutyric acid* (**3**).—Syrupy **3** was dissolved in a minimum amount of methanol, and propanol (~ 10 mL) was added dropwise to turbidity with vigorous stirring. This solution was then carefully poured into 150 mL of propanol with vigorous stirring to precipitate **3**, which was then allowed to stand for 24 h in the refrigerator. The microcrystalline product was

filtered, washed with propanol, and dried in vacuo over calcium chloride. Yield 9.7 g (34% based on starting γ-aminobutyric acid) of slightly hygroscopic, yellowish, microcrystalline powder: mp 73–76°C, $[\alpha]_D^{25}$ −49° (*c* 1.2, H_2O); $R_{\gamma Abu}$ 0.62 (*A*) and 1.50 (*B*). Exact mass of $[M+H]^+$ ion. Calcd for $C_{10}H_{20}NO_7$: 266.1240. Found: 266.1253. See Results and Discussion section (Tables 1–4) for FABMS and NMR data. Anal. Calcd for $C_{10}H_{19}NO_7 \cdot (0.5\ H_2O + 0.25\ CH_3OH + 0.25\ C_3H_8OH)$: C, 44.43; H, 7.80; N, 4.71. Found: C, 44.10; H, 7.70; N, 4.33.

Isolation of N-*(1-deoxy-*D*-fructos-1-yl)-δ-aminovaleric acid* (**4**).—Compound **4** was eluted from the second ion-exchange column using a the pyridine–acetic acid buffer described above. In this case, the syrup was precipitated using 2-propanol, rather than *n*-propanol. Yield 6.0 g (42% based on starting δ-aminovaleric acid) of slightly hygroscopic, yellowish, microcrystalline powder: mp 70–77°C; $[\alpha]_D^{25}$ −44° (*c* 1.2, H_2O); $R_{\delta Ava}$ 0.68 (*A*) and 1.22 (*B*). Exact mass of $[M+H]^+$ ion. Calcd for $C_{11}H_{22}NO_7$: 280.1396. Found: 280.1401. See Results and Discussion section (Tables 1–4) for FABMS and NMR data. Anal. Calcd for $C_{11}H_{21}NO_7 \cdot (0.5\ C_3H_8OH + 0.75\ H_2O)$: C, 46.50; H, 8.27; N, 4.34. Found: C, 46.49; H, 8.17; N, 4.25.

Isolation of N-*(1-deoxy-*D*-fructos-1-yl)-ε-aminocaproic acid* (**5**).—Compound **5** was initially obtained as a yellowish syrup, which was dissolved in an equal volume of methanol and poured into 1 L of propanol with vigorous stirring. After standing for 1 h at room temperature, the white precipitate was filtered, washed with propanol, and dried in vacuo over calcium chloride at 4°C. Yield 6.6 g (22%, based on starting ε-aminocaproic acid) of hygroscopic, yellowish, microcrystalline powder which is unstable at room temperature: mp 52–57°C; $[\alpha]_D^{25}$ −38° (*c* 1.4, H_2O) (lit. [19] −47°); $R_{\epsilon Aca}$ 0.73 (*A*) and 1.24 (*B*). Exact mass of $(M+H)^+$ ion. Calcd for $C_{12}H_{24}NO_7$: 294.1553. Found: 294.1557. See Results and Discussion section (Tables 1–4) for FABMS and NMR data. Anal. Calcd for $C_{12}H_{23}NO_7 \cdot (0.33\ C_3H_8OH + H_2O)$: C, 47.22; H, 8.38; N, 4.23. Found: C, 47.57; H, 8.24; N, 4.11.

Isolation of N-*(1-deoxy-*D*-fructos-1-yl)-*N$^{\alpha}$*-formyl-*L*-lysine* (**6**).—This compound was prepared using N^{α}-formyl-L-lysine as the starting material. The compounds were separated in pure form in one chromatographic run using an Amberlite IRN-77 column (4 × 50 cm) charged in the pyridinium form, and eluted first with water (500 mL) and then 1500 mL of buffer (0.1 M in pyridine and 0.3 M in acetic acid). Compound **6** eluted during the end of the water wash and the start of the suffer wash. The evaporated fractions gave a slightly yellow syrup to which methanol was added to turbidity. This mixture was allowed to stand at room temperature for three days, during which time crystallization occurred (dense, colorless rosettes). The pure crystals were triturated with 1:1 water–methanol and then washed with methanol in a filter and dried in vacuo over calcium chloride. Yield 6.1 g (41%, based on starting amino acid): mp 115°C (dec) (lit. [20] mp 107–109.5°); $[\alpha]_D^{25}$ −34° (*c* 1.4, H_2O); $R_{N^{\alpha}\text{-}formylLys}$ 0.73 (*A*) and 0.93 (*B*). Exact mass of $[M+H]^+$ ion. Calcd for $C_{13}H_{25}N_2O_8$: 337.1611. Found: 337.1608. See Results and Discussion section (Tables 1–4) for FABMS and NMR data. Anal. Calcd for $C_{13}H_{24}N_2O_8 \cdot H_2O$: C, 44.07; H, 7.37; N, 7.90. Found: C, 44.35; H, 7.55; N, 7.72.

3. Results and discussion

The above study describes relatively straightforward procedures for the synthesis of Amadori compounds in reasonably good yields, and in pure form. The synthetic analogues are relatively stable and can be stored in the dry state in the freezer for periods of several months without loss of purity.

FABMS data on the compounds (Table 1) reveal protonated molecular ions, along with dimers, trimers, and dehydrated molecular ions. This suggests that the compounds may well be highly hydrogen bonded in solution as well as in the solid state [21].

Several other workers have reported data on the MS of Amadori compounds. Vernin et al. [22] reported FABMS data on Amadori compounds derived from valine, methionine, and proline. The most intense peaks corresponded to $[M + H^+]$ with less intense peaks corresponding to $[M - H_2O + H]^+$ as well as for $[M - 2H_2O + H]^+$ peaks. Surprisingly, they did not report peaks corresponding to solvated Amadori compounds and a cluster peak $[2M + H]^+$ was detected only for the proline derivative.

Yaylayan and Sporns [23] also studied the electron-impact mass spectra of some Amadori compounds and concluded that the most characteristic peaks arise from (*i*) loss of 1, 2, or 3 molecules of water, (*ii*) loss of CO_2 or H_2CO_2, or (*iii*) loss of the amino acid residue with or without the amino group. Almost all of these peaks are observed in FABMS as well (in addition to the molecular ions), particularly when the concentration of Amadori compound is high. This is probably due to the fact that a wide variety of excitation energies for molecules (0.1 eV or more) can be obtained under FAB conditions. The experiments described herein used dilute solutions of the Amadori compound in glycerol, which gave mainly molecular ions, clusters, and solvated ions. This is probably because of the probability that a fast atom or ion will impact the glycerol surface near an Amadori compound is very low. As a result, most of the Amadori compounds receive a low excitation energy and the MS contains largely molecular ions. Using higher concentrations of Amadori compounds increases this probability greatly, resulting in a greater abundance of fragment ions.

^{13}C and ^1H NMR spectra of various Amadori compounds in solutions were studied extensively [19,24,25]; thus, no complications arose when we assigned signals in both types of spectra of water solutions of **1–6** (Tables 2 and 3). All of them show predominance of the β-pyranose form (Table 2) as well as a constant distribution between four tautomeric forms in equilibrium. It supports the view [24] that the tautomeric equilibria of 1-amino-1-deoxy-D-fructoses are not affected by the amine. The conformation of the β-fructopyranose ring is the same for all Amadori compounds in the zwitterionic form and also for **1** in acidic or basic media. The value of the vicinal coupling constant ($J_{3,4}$ 9.8 Hz, Table 4) suggests a *trans* disposition of the corresponding axial protons and therefore a $^2C_5$ conformation of the ring.

An evident difference between the ^1H NMR spectra of **1–6** can be found in the shape of the H-1 proton signals. For **1** and **2** they appear to be nonequivalent. This

Table 1
Peaks in the FAB mass spectra (mass range, 100–1000 m/z) of Amadori compounds [a]

Compd.	$[M+H]^+$	$[2M+H]^+$	$[3M+H]^+$	$[M+G+H]^+$	$[M+2G+H]^+$	$[M-H_2O+H]^+$	$[M-H_2O+G+H]^+$	$[M-CO_2+H]^+$
1 [b]	238 (100)	475 (8.2)	712 (0.9)			220 (85)		194 (17)
2	252 (100)	503 (6.5)	754 (1.1)	344 (5.1)	436 (1.2)	234 (37)	326 (3.0)	208 (9.0)
3	266 (100)	531 (2.4)	796 (0.2)	358 (5.2)	450 (3.2)	248 (16)	340 (1.8)	222 (8.0)
4	280 (100)	559 (1.5)		372 (6.5)	464 (3.3)	262 (20)		236 (15)
5	294 (100)	587 (1.9)	880 (0.13)	386 (2.9)	478 (0.86)	276 (21)	368 (2.9)	250 (28)
6	337 (100)	673 (3.8)		429 (3.7)		319 (11)	411 (2.8)	293 (13)

[a] Signals of solvent glycerol clusters $[nG+H]^+$ are excluded. Relative intensities of ion peaks are given in parentheses.
[b] The spectrum of an aq solution saturated with the compound before an addition to the glycerol matrix. It contains also peaks corresponding to: $[M-CH_2COO]^+$, 180 (36); $[M-CH_2COO-H_2O]^+$, 162 (47); $[2M-CH_2COO]^+$, 417 (3.4); $[3M-CH_2COO]^+$, 654 (0.4); $[4M+H]^+$, 950 (0.43).

Table 2
^{13}C NMR chemical shifts and estimated equilibrium composition of tautomeric forms of Amadori compounds (zwitterionic form) in D_2O solutions at 25°C

Carbon atom		Compound No.					
		1	2	3	4	5	6
C-1	β-pyr	54.11	53.69	53.75	53.50	53.67	53.65
	α-pyr	49.61	49.38	n.r. [a]	n.r. [a]	n.r. [a]	49.79
	β-fur	53.19	52.93	53.14	52.92	53.09	53.07
	α-fur	52.14	51.74	51.93	51.60	51.73	51.72
C-2	β-pyr	96.20	96.23	96.37	96.15	96.33	96.30
	α-pyr	97.03	96.87	97.02	96.81	96.88	96.91
	β-fur	99.66	99.63	99.72	99.51	99.72	99.67
	α-fur	102.65	102.67	102.81	102.55	102.74	102.70
C-3	β-pyr	70.84	70.70	70.48	70.29	70.51	70.46
	α-pyr	72.88	72.72	72.54	72.34	72.51	72.53
	β-fur	78.72	78.72	78.71	78.55	78.79	78.73
	α-fur	83.53	83.41	83.39	83.14	83.38	83.35
C-4	β-pyr	70.22	70.20	70.23	70.01	70.21	70.18
	α-pyr	71.21	71.21	71.23	71.02	71.23	71.20
	β-fur	75.01	75.00	75.05	74.81	75.04	74.98
	α-fur	76.93	76.96	76.86	76.64	76.93	76.86
C-5	β-pyr	69.81	69.77	69.83	69.63	69.82	69.79
	α-pyr	66.81	66.54	66.48	66.21	66.33	66.34
	β-fur	81.82	81.77	81.77	81.52	81.75	81.70
	α-fur	83.29	83.25	83.18	83.00	83.24	83.20
C-6	β-pyr	64.82	64.87	64.83	64.63	64.82	64.80
	α-pyr	63.88	63.56	63.46	63.46	n.r. [a]	63.35
	β-fur	62.75	62.69	62.74	62.51	62.71	62.67
	α-fur	61.77	61.72	61.66	61.42	61.66	61.63
COO^-		171.90	179.23	182.24	183.55	184.35	179.25
		171.85	179.33		183.62		179.53
C^{α}		50.56	32.62	35.53	37.39	38.05	54.36
		50.51	32.50	35.45	37.47		
		50.40					
		50.19					
C^{β}			46.18	22.77	23.27	25.77	31.98
			46.09	22.68	23.24	25.73	33.03
			45.94	22.64	23.30	25.60	
C^{γ}				49.37	24.46	26.05	22.92
				49.32			
C^{δ}					48.86	26.41	25.62
					48.75		25.45
					48.63		
C^{ε}						49.32	49.11
						49.20	48.99
						49.08	48.88

Table 2 (continued)

Carbon atom		Compound No.					
		1	2	3	4	5	6
C^{HCON}							164.56
Relative %							
	β-pyr	69	70	70	73	72	70
	α-pyr	5	4	5	4	5	4
	β-fur	13	11	13	11	11	13
	α-fur	13	15	12	12	12	13

[a] Not resolved (n.r.) because of overlapping or low intensity

has also been observed for other Amadori compounds derived from α-amino acids [19,24a,25a] because of two coupled (geminal $J_{1A,1B} \approx -13$ Hz) signals at ~3.1 ppm. In contrast, the spectra of aqueous solutions of **3–6** (Table 3 and refs 19 and 24a), as well as that of *N*-(1-deoxy-D-fructos-1-yl)-morpholine [25b], show only a singlet signal for the two H-1 protons. Other workers [25] explained the singlet as a result of partial exchange of one of the protons for deuterium. Indeed, in our spectra of **3–6**, values of integrals of the singlet peaks corresponded exactly to two hydrogen nuclei if the spectra were run immediately after dissolution of the substances in D_2O. Obviously, in Amadori compounds that have a carboxyl group located close to the amino group (α- or β-), a rotation around C-1 is staggered,

Table 3
^{1}H NMR chemical shifts [a] (ppm) of major tautomeric forms of Amadori compounds (zwitterionic form) in D_2O solutions at 25°C

Hydrogen atom	Compound No.							
	1	1 [b]	1 [c]	2	3	4	5	6
H-1A	3.315(d)	3.356(d)	2.757(d)	3.323(m)	3.304(s)	3.301(s)	3.299(s)	3.300(s)
H-1B	3.392(d)	3.412(d)	2.882(d)					
H-3	3.772(d)	3.758(d)	3.731(d)	3.753(d)	3.734(d)	3.732(d)	3.728(d)	3.732(d)
H-4	3.903(dd)	3.892(dd)	3.872(dd)	3.898(ddd)	3.892(dd)	3.893(dd)	3.890(dd)	3.891(dd)
H-5	4.021(m)	4.012(m)	3.971(m)	4.016(m)	4.008(m)	4.010(m)	4.010(m)	4.014(m)
H-6A	3.776(dd)	3.769(dd)	3.664(d)	3.776(ddd)	3.767(dd)	3.761(dd)	3.758(dd)	3.787(dd)
H-6B	4.035(dd)	4.014(dd)	4.005(d)	4.023(dd)	4.014(dd)	4.016(dd)	4.011(dd)	4.007(dd)
H_α-A	3.663(d)	3.932(d)	3.250(d)	2.608(t)	2.334(t)	2.238(t)	2.201(t)	4.257(dd)
H_α-B	3.728(d)	3.970(d)	3.215(d)					
H_β				3.297(t)	1.939(m)	1.629(m)	1.583(m)	1.796(m)
H_γ					3.140(t)	1.739(m)	1.379(m)	1.422(m)
H_δ						3.128(t)	1.733(m)	1.746(m)
H_ε							3.118(t)	3.119(t)
H_{formyl}								8.094(s)

[a] Abbreviations: (s), singlet; (d), doublet; (dd), double doublet; (ddd), doublet of double doublets; (t), triplet; (m), multiplet.
[b] One equiv of DCl is added.
[c] One equiv of NaOD is added.

Table 4
First-order coupling constants (Hz) of protons of major tautomeric forms of Amadori compounds (zwitterionic forms) in D_2O solutions at 25°C

Coupling constant	Compound No.							
	1	1[a]	1[b]	2	3	4	5	6
$J_{1A,1B}$	−12.9	−12.9	−12.9	−13.2				
$J_{3,4}$	9.8	9.8	9.7	9.8	9.8	9.8	9.8	9.8
$J_{4,5}$	3.3	3.4	n.r.[c]	3.3	3.3	3.3	3.4	3.3
$J_{5,6A}$	2.1	2.0	n.r.	1.8	2.1	2.1	2.0	1.6
$J_{5,6B}$	1.3	n.r.	n.r.	1.2	1.1	1.2	n.r.	n.r.
$J_{6A,6B}$	−13.1	−13.1	−12.7	−12.9	−13.1	−12.9	−13.0	−12.9
$J_{\alpha A,\alpha B}$	−17.3	−17.0	−17.5					
$J_{\alpha B}$				6.5	7.0	6.9	7.3	
$J_{\alpha,\beta A}$								7.6
$J_{\alpha,\beta B}$								5.1
$J_{\beta,\gamma}$					7.4	n.r.	n.r.	n.r.
$J_{\gamma,\delta}$						7.5	n.r.	n.r.
$J_{\delta,\varepsilon}$							7.9	7.7

[a] One equivalent of DCl was added.
[b] One equivalent of NaOD was added.
[c] n.r., Not resolved.

and this leads to non-equivalency of the H-1 atoms. A possible reason for this assumption is that in crystalline **1** [21] the carboxyl oxygen, one of two ammonium protons, and O-3 are involved in a strong intramolecular three-centered hydrogen bonding and form two conjugated pseudocycles that are apparently planar. The conformation around the C-1–C-2 bond is of the *trans–gauche* type and staggered. Probably, in aqueous solutions the neighboring carboxyl groups also promote the formation of a hydrogen bond between O-3 and the ammonium proton. The participation of the carboxyl group in hydrogen bonding evidently restricts a rotation around the C^{α}–N bond and makes the two H^{α} atoms non-equivalent and coupled as well. The addition of equimolar quantities of either strong acid or strong base obviously does not affect the intramolecular hydrogen bonding and, consequently, non-equivalency of protons in the both methylene groups of **1** (Tables 3 and 4). Increasing the basicity of a solution of **1** caused a shift of the H-1 and H^{α} signals to higher field. Thus, the most probable conformation of the major tautomer in aqueous solutions of Amadori compounds of α- or β-amino acids must be as shown in Fig. 1. Probably, in less polar solvents the intramolecular hydrogen bonds form more readily in fructose–amines. In pyridine, which cannot

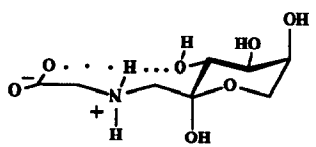


Fig. 1. A 1-amino-1-deoxy-D-fructopyranos-1-yl derivative (Amadori compound): probable conformation due to extensive intramolecular hydrogen bonding.

Table 5
Acid dissociation constants of carboxyl groups (K_{a1}) and amino groups (K_{a2}) of Amadori compounds in aqueous solutions [a]

Compound	pK_{a1}	$\log \beta_{11} = pK_{a2}$	$\log \beta_{21} = pK_{a1} + pK_{a2}$
1	2.20 (2.33)	8.18±0.01 (9.60)	10.39±0.02
2	3.35 (3.57)	8.84±0.01 (10.10)	12.19±0.01
3	4.03 (4.07)	8.93±0.01 (10.37)	12.96±0.02
4	4.25 (4.27)	9.16±0.02 (10.63)	13.41±0.02
5	4.44 (4.37)	9.25±0.02 (10.72)	13.69±0.02
6	3.08 (3.09)	9.02±0.01 (10.60)	12.10±0.01

[a] $T = 298 \pm 0.1$ K, $I = 0.2$ M (KNO_3). The corresponding constants of the parent amino acids are given in parentheses.

compete for hydrogen bonding like water, all Amadori compounds of aliphatic or aromatic amines showed two signals corresponding to coupled H-1 protons [25b].

The calculated protonation constants of **1–6**, together with the constants of the parent amino acids, are summarized in Table 5. Anet [17] and Röper [26] reported pK_a values for fructose–glycine, but unfortunately without any experimental details. Nevertheless, their values of pK_{a2} (8.4 and 8.21, correspondingly) are very close to that obtained by us.

The apparent lowering of amino acid protonation constants as a result of derivatization by the 1-deoxy-fructos-1-yl residue is evident from Table 5. The decrease in K_{a2} of the amino group is nearly the same for all Amadori compounds (in the order of ~ 1.5). The influence of the carbohydrate residue on K_{a1} of the carboxyl group in **1** and **2** is much less (in the order of ~ 0.2), and there is no evidence of this effect on the constant in **3–6**, where a long distance (4 atoms and more in the chain) between those groups diminishes the mutual influence. Two effects may be responsible for decreasing the basicity of Amadori compounds: (*i*) a negative inductive effect of electronegative carbonyl group of the carbohydrate [27] and (*ii*) cooperative solvation effect of four hydroxyl groups present in the deoxyfructosyl residue on protonation processes at the amino and carboxyl groups [28]. The second effect appears to be much more important as can be seen from

Table 6
Amino group acid dissociation constants (K_{a2}) [a] of some glycine *N*-derivatives, R–NH–CH_2–CO_2H, in water at 25°C

R	H–	CH_3–	HOOC–CH_2–	$(HOCH_2)_2CH$–	$(HOCH_2)_3C$–	1-deoxy-D-fructopyranos-1-yl
pK_a	9.60 [c] (0.2) 9.57 (0.1)	9.99 (0.5)	9.34 (0.1)	8.33 (0.1)	8.13 (0.1)	8.18 [c] (0.2)

[a] Ref 29.
[b] Values of ionic strengths are given in parentheses.
[c] This work.

comparison of the influence of glycine *N*-substituent structure on basicity of the secondary amino group (Table 6).

It is noteworthy that Baynes and co-workers [30] have also reported on the tautomeric composition for our compounds **1** and **6** as well as for Amadori compounds prepared from poly(lysine) and protein RNase A, and have found the same tautomeric composition for all compounds in aqueous solutions. Therefore, it appears that 1-deoxy-D-fructose residues contained in glycated proteins are quite close in conformational equilibria to low molecular weight Amadori compounds. It also follows that glycated proteins would be expected to have an isoelectric point at a slightly lower pH value (up to 0.8 pH units) because of the decreased basicity of the glycated amino groups.

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