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N.M.R. SPECTROSCOPY OF *N*-(1-DEOXY-D-FRUCTOS-1-YL)-L-AMINO ACIDS ("FRUCTOSE–AMINO ACIDS")

HARALD RÖPER, SIYKA RÖPER, KURT HEYNS,
Institut für Organische Chemie und Biochemie der Universität Hamburg, Martin-Luther-King-Platz 6, D-2900 Hamburg 13 (West Germany)

AND BERND MEYER
Fachbereich Chemie, Universität Oldenburg, Ammerländer Heerstr. 67–99, D-2900 Oldenburg (West Germany)

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ABSTRACT

High-resolution, ^{1}H- (360 and 400 MHz) and ^{13}C-n.m.r. (90.52 and 100.57 MHz) spectra of the mutarotated *N*-(1-deoxy-D-fructos-1-yl)-L-amino acids ("fructose–amino acids") **1–14** in D_2O are reported. The ^{1}H spectra allow unambiguous assignment of the signals of the major constituents (β-pyranose forms). Signals of the other forms are not well resolved and therefore not interpreted. The ^{13}C spectra of **1–14** show ~64% of β-pyranose, ~15% of α-furanose, ~15% of β-furanose, and ~6% of α-pyranose forms. For *N*-(1-deoxy-D-fructos-1-yl)-L-alanine (**2**), 2% of the keto form is present. In solution in D_2O, H-1 of the fructose moieties undergoes a slow H/D-exchange, which is strongly accelerated at more-basic pH values. Compound **2** is stable over the pH range 0.7–11.9, as revealed by ^{13}C-n.m.r. spectroscopy. The ^{13}C-shift changes caused by protonation/deprotonation are given. There is no significant change in the ratios of the various forms with change in pH.

INTRODUCTION

N-(1-Deoxy-D-fructos-1-yl)-L-amino acids (fructose–amino acids) are key substances of the non-enzymic browning (Maillard) reaction[1]. They are obtained by the reaction of D-glucose with L-amino acids and Amadori rearrangement of the resulting glucosylamine derivatives[2].

In connection with studies[3–5] of the preparation of nitrosamine derivatives of sugars and their biological action, n.m.r. spectroscopy has been applied to several fructose–amino acids that can exist in solution as mixtures of up to five isomers. Aliphatic and aromatic glycosylamines and their Amadori-rearrangement products have been investigated by ^{13}C-n.m.r. spectroscopy[6], and some fructose–amino acids (**1–4** and **9–10**) by ^{1}H-n.m.r. spectroscopy[7]. We now present ^{1}H- and ^{13}C-n.m.r. data for the Amadori products **1–14**.

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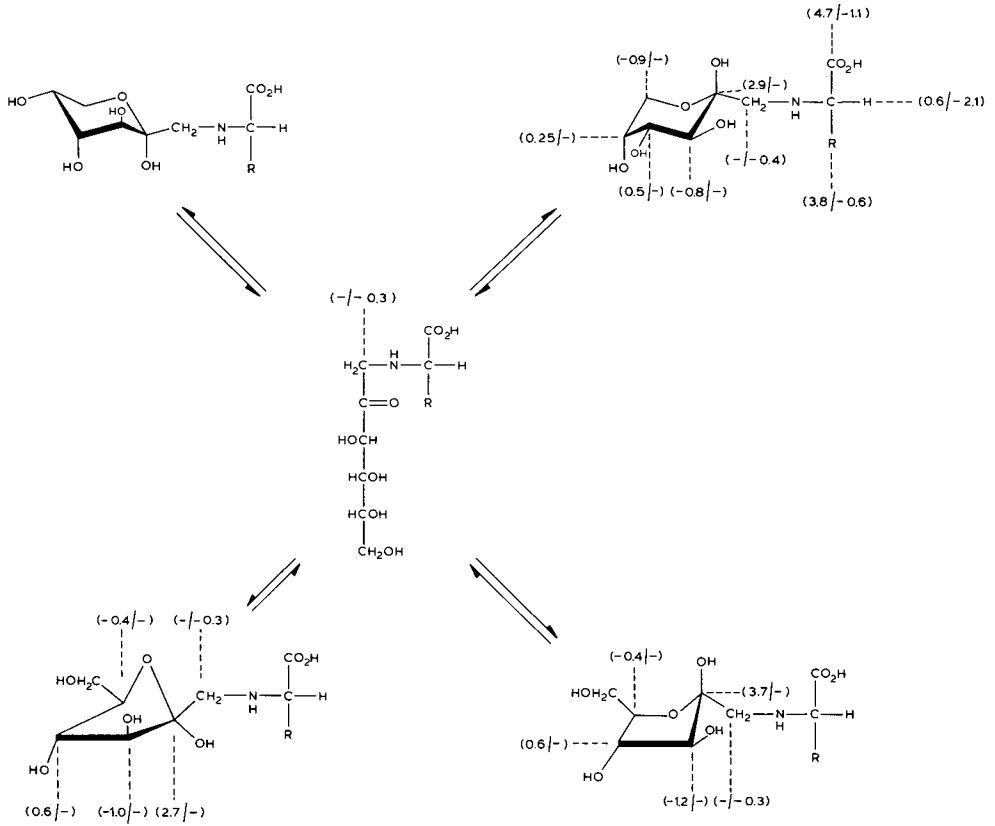
TABLE I

^{1}H-N.M.R. CHEMICAL SHIFTS[a] FOR **5–8** AND **11–13**

	5	**6**	**7**	**8**[b]	**11**[c]	**12**[d]	**13**[e]	**14**[f]
H-1A	3.18(d)	3.23(m)	3.20	3.19m	3.09	2.89	3.00	3.03
H-1B	3.23(d)	3.28	3.26		3.17	3.01	3.08	3.19
H-3	3.57(d)	3.66	3.63	3.62	3.57	3.54	3.59	3.53
H-4	3.69(dd)	3.77	3.75	3.74	3.71	3.68	3.71	3.65
H-5	3.82(m)	3.88	3.86	3.86	3.84	3.82	3.83	3.79
H-6A	3.56(dd)	3.64	3.61	3.62	3.58	3.54	3.56	3.47
H-6B	3.84(dd)	3.87	3.88	3.88	3.85	3.80	3.83	3.73
H-2′	3.52(d)	3.75(t)	3.48(d)	3.72(t)	3.87(t)	3.63(t)	3.56(t)	3.91(dd)
H-3′A	1.79(m)	3.88(dd)	3.96(dq)	2.07(m)	3.12(d)	2.93(m)	3.05(m)	3.26(dd)
H-3′B	—	3.94(dd)	—					3.36(dd)
H-4′A	1.13(dq)	—	1.21(d,Me)	2.50(m)	—	—	—	—
H-4′B	1.35(dq)	—	—		—	—	—	—
Me-5′	0.78(d)	—	—	—	—	—	—	—
Me-6′	0.73(t)	—	—	—	—	—	—	—

[a]For the β-D-pyranose forms at 360 and 400 MHz [3–7% solutions in D_2O, internal acetone (δ 2.08)]. [b]S-Me δ 1.99(s). [c]Aromatic protons δ 7.26(m). [d]H-5′,9′, δ 6.71(dd); H-6′,8′, 7.02(dd). [e]Imidazolyl: H-2, δ 8.00(s); H-5, 7.03(s). [f]Indolyl: H-2, δ 7.19(s); H-4, 7.57(d); H-5, 7.05(dd); H-6, 7.13(dd); H-7, 7.39(d).



	R	L-Amino acid
1	H	Glycine[9,11-13]
2	$-\overset{3'}{Me}$	Alanine[9,10,13]
3	$-\overset{3'}{C}H\overset{4'}{Me}_2\overset{5'}{}$	Valine[9,13,14]
4	$-\overset{3'}{C}H_2\overset{4'}{C}H\overset{5'}{Me}_2\overset{6'}{}$	Leucine[9,10]
5	$-\overset{3'}{C}H(\overset{5'}{Me})\overset{4'}{C}H_2\overset{6'}{Me}$	Isoleucine
6	$\overset{3'}{C}H_2OH$	Serine[9,13]
7	$\overset{3'}{C}H(OH)\overset{4'}{Me}$	Threonine[9,13]
8	$\overset{3'}{C}H_2\overset{4'}{C}H_2S\overset{5'}{Me}$	Methionine
9	$\overset{3'}{C}H_2\overset{4'}{C}O_2H$	Aspartic acid[9,10,13]
10	$-\overset{3'}{C}H_2\overset{4'}{C}H_2\overset{5'}{C}O$	Pyroglutamic acid[7,13]
11	Phenylmethyl	Phenylalanine[9,14]
12	*p*-Hydroxyphenylmethyl	Tyrosine
13	Imidazolylmethyl	Histidine[15,16]
14	Indolylmethyl	Tryptophan[16]

Fig. 1. Equilibria for the D-fructose–amino acids **1–14** in D_2O solution. The probable conformations are depicted; only the $^2C_5$ chair of the β-pyranose form was established by ^{1}H-n.m.r. spectroscopy. The values in parenthesis give the chemical shifts induced at the basic and the acidic inflection-points, respectively, for **2** (see Fig. 2).

TABLE II

COUPLING CONSTANTS (Hz) FOR **5–8** AND **11–14**

	5	**6**	**7**	**8**	**11**	**12**[b]	**13**	**14**[c]
$J_{1A,1B}$	−12.8	—	−12.5	—	−12.8	−12.8	−12.8	−12.8
$J_{3,4}$	10.0	9.8	9.8	9.8	9.9	9.8	10.0	9.8
$J_{4,5}$	3.4	3.6	3.6	3.4	3.4	3.4	3.6	3.4
$J_{5,6A}$	2.0	2.0	2.2	2.4	2.2	[a]	2.0	2.0
$J_{5,6B}$	[a]	[a]	[a]	1.4	1.0	[a]	1.0	1.2
$J_{6A,6B}$	−12.4	−12.4	−12.7	−12.4	−13.1	−12.5	−12.8	−12.7
$J_{2',3'}$	3.6	4.0	7.0	6.0	6.4	7.0	6.4	7.0 (3'A), 5.6 (3'B)
$J_{3'A,3'B}$	—	−14.4	—	[a]	[a]	[a]	[a]	−15.5
$J_{3',4'}$	6.6	—	6.4	[a]	—	—	—	—
$J_{4',5'}$	7.4	—	—	—	—	—	—	—

[a]Not resolved. [b]J_{ortho} 7.5, J_{meta} 0.9 Hz. [c]Indolyl residue: $J_{4,5} = J_{6,7} = 8.0$, $J_{5,6}$ 7.0, $J_{4,6}$ 1.2, and $J_{5,7}$ 0.7 Hz.

RESULTS AND DISCUSSION

The fructose–amino acids **1–14** are depicted in Fig. 1, in terms of the structures present after mutarotation in aqueous solution. The ^1H spectra of these compounds were complex, the signals for the ring protons of the various structures appearing within a narrow range of ~0.3 p.p.m. (Table I). Consequently, only the signals of the major (β-pyranoid) form in each equilibrium mixture could be assigned.

The signal intensity for H-1 decreased on prolonged storage of solutions in D_2O due to a slow H/D exchange, presumably *via* an enamine form. The pH of solutions of **1–8** and **11–12** was ~3.8, that of **9** and **10** was ~1.8, and that of **13** and **14** was ~5.1. The observed shifts and coupling constants of the β-pyranose forms of **5–8** and **11–14** were as expected, and accorded with those[7] of **1–4, 9,** and **10.** The H-1 signals of the β-fructopyranose forms usually appeared as an AB-system at δ ~3.1, and the chemical shifts were not markedly affected by the pH of the solutions. The conformation for each compound was $^2C_5$, as indicated by the coupling constants (Table II); the *trans* disposition of H-3,4 gives rise to the large (9.8–10.0 Hz) $J_{3,4}$ values. Therefore, there is no effect on the conformation by variation of the amino acid substituents. The sub-spectra of the amino acid residues were as expected.

^{13}C-N.m.r. spectroscopy was used to determine the purity of the fructose–amino acids and the proportions of the various ring structures. It was important to check the structure of each preparation, because a slight variation in the concentration or in the work-up procedure may yield completely different products. For **6** and **13**, these by-products were obtained in high yield, but not identified. The ^{13}C-n.m.r. spectra (D_2O, internal acetone) of **1–14** generally contained two major groups of signals at δ 69.0–70.5 and 74.0–83.0 attributable to C-3,4,5 of the β-pyranose and the α- and β-furanose forms, respectively (Table III). For **3, 5,** and **7,** the C-2′ resonance of the amino acid moiety was found in the range of the β-pyranose signals. The assignment of the signals for C-1,2,6 was straightforward. The ^{13}C signals of the amino acid moieties were not altered significantly by the fructose moieties, except for that of C-2′ which was shifted downfield by ~7 p.p.m. upon fructosylation. The assignment of C-3,4,5 for the three major ring-forms (β-pyranose, α- and β-furanose) in equilibrated solutions was more difficult. The β-pyranose resonances were assigned by using selective decoupling, and the order of the C-3,4,5 signals was changed compared to that for D-fructose[18]. Because the ^1H spectra of these compounds did not allow a separate observation of the signals of the anomeric furanose forms, selective decoupling did not allow the assignment of these signals. In order to obtain this information, advantage was taken of the pH dependence of the chemical shifts in basic and acidic compounds. The ^{13}C-n.m.r. spectra of **2** were recorded in the pH range 0.7–11.9 for solutions in D_2O–DCl or D_2O–NaOD. The compound was stable in this pH range for several hours. With increasing concentration of salt in the sample, it was necessary to use a power gating

TABLE III

^{13}C-N.M.R. CHEMICAL SHIFTS[a,b] FOR D-FRUCTOSE–L-AMINO ACIDS **1–14** AND D-FRUCTOSE[d]

	1				**2**					**3**				**4**			
	β-p	*β-f*	*α-f*	*α-p*	*β-p*	*β-f*	*α-f*	*α-p*	*o*	*β-p*	*β-f*	*α-f*	*α-p*	*β-p*	*β-f*	*α-f*	*α-p*
C-1	53.39	51.44	52.48	—	52.22	50.21	51.40	48.37	52.74	53.49	53.00	53.19	—	52.54	51.94	52.18	48.64
C-2	95.47	99.01	101.92	—	95.75	99.33	102.27	96.27	207.35	95.38	98.22	101.68	—	95.41	98.95	101.83	95.86
C-3	70.15	78.04	82.83	70.52	70.42	78.48	83.15	70.87	76.24	70.27	78.25	82.75	70.53	69.98	78.08	82.66	70.58
C-4	69.51	74.33	76.22	72.18	69.83	74.81	76.68	72.20	—	69.48	74.10	76.09	72.13	69.44	74.23	76.15	71.92
C-5	69.07	81.14	82.59	65.94	69.36	81.59	82.96	65.78	—	69.02	81.16	82.62	65.61	69.04	81.11	82.66	65.31
C-6	64.11	62.09	61.08	62.98	64.35	62.41	61.39	62.74	63.23	64.01	62.01	60.91	62.67	64.02	61.99	60.95	62.83
C-1′	171.27	—	—	—	174.25	—	—	—	—	172.25	172.15	—	—	174.04	—	—	—
C-2′	49.89	49.72	49.80	—	58.67	58.50	57.81	—	—	69.02	68.75	—	—	62.26	—	—	—
C-3′					15.17	15.01	—	—	—	29.13	29.02	—	—	39.03	38.93	—	—
C-4′										18.66	18.50	18.34	—	24.67	—	—	—
C-5′										17.13	17.29	17.30	—	22.24	22.17	—	—
C-6′														21.50	22.04	—	—

	5				**6**				**7**				**8**			
	β-p	*β-f*	*α-f*	*α-p*	*β-p*	*β-f*	*α-f*	*α-p*	*β-p*	*β-f*	*α-f*	*α-p*	*β-p*	*β-f*	*α-f*	*α-p*
C-1	53.58	51.50	51.65	50.15	51.94	51.54	51.64	—	53.22	51.30	52.18	—	52.75	50.73	51.84	48.54
C-2	95.48	99.11	101.82	95.77	95.37	98.07	101.89	—	95.50	98.95	101.59	—	95.43	98.94	102.03	—
C-3	70.49	78.52	82.84	70.68	70.29	78.08	82.69	71.01	70.42	78.34	82.86	71.26	70.08	78.10	82.79	70.48
C-4	69.62	74.47	76.40	71.98	69.48	74.23	76.09	71.97	69.51	74.36	76.22	72.20	69.47	74.26	76.22	72.07
C-5	69.10	81.56	82.84	65.18	69.04	81.18	82.69	65.97	69.07	81.41	82.66	65.46	69.04	81.17	82.62	65.80
C-6	64.09	62.23	61.10	63.10	64.02	62.03	60.98	62.94	64.18	61.99	61.05	63.07	64.05	62.25	61.05	62.83
C-1′	171.91	—	—	—	171.41	—	—	—	171.25	—	—	—	172.67	—	—	—
C-2′	68.02	67.78	—	—	64.02	63.81	63.81	—	69.51	69.07	69.07	—	62.46	61.99	—	—
C-3′	35.87	35.70	—	—	59.23	59.03	59.03	—	66.14	66.03	—	—	29:22	28.57	—	—
C-4′	26.14	25.86	—	—					20.05	19.75	17.82	—	28.85		—	—
C-5′	14.47	—	—	—									14.32	—	—	—
C-6′	11.65	—	—	—												

	9				**10**			**11**				**12**			
	β-p	*β-f*	*α-f*	*α-p*	*β-p*	*β-f*	*α-f*	*β-p*	*β-f*	*α-f*	*α-p*	*β-p*	*β-f*	*α-f*	*α-p*
C-1	53.09	51.98	52.82	—	47.99	47.79	47.35	53.12	52.58	52.82	—	63.23	52.40	52.94	—
C-2	95.31	98.95	101.88	96.15	98.47	101.26	—	95.31	—	—	—	95.50	—	—	—
C-3	70.46	78.11	82.83	70.49	69.77	77.66	82.34	70.46	78.24	82.86	70.73	70.56	78.54	83.11	70.75
C-4	69.51	74.27	76.29	72.14	69.77	74.83	76.28	69.51	74.27	76.22	72.17	69.72	74.78	76.71	72.15
C-5	69.01	81.18	82.59	65.90	69.33	80.63	82.11	69.07	81.25	82.69	65.36	69.14	81.69	82.93	64.95
C-6	64.12	61.96	61.08	62.97	63.50	62.69	61.11	64.08	62.03	61.08	—	64.18	62.11	61.33	—
C-1′	171.88	—	—	—	176.49	175.98	176.50	172.46	—	—	—	172.69	172.59	—	—
C-2′	58.55	—	—	—	62.25	—	—	64.25	—	—	—	64.75	64.54	64.54	—
C-3′	33.80	—	—	—	22.97	23.51	22.97	35.83	—	—	—	35.21	—	—	—
C-4′	174.37	174.68	—	—	29.38	—	—	134.89	—	—	—	126.97	—	—	—
C-5′					180.36	180.13	179.8	129.36	—	—	—	130.94	—	—	—
C-6′								129.33	—	—	—	116.15	116.27	116.27	—
C-7′								127.98	—	—	—	155.32	—	—	—

	13			**14**[c]			D-Fructose[d]				
	β-p	*β-f*	*α-f*	*β-p*	*β-f*	*α-f*	*β-p*	*β-f*	*α-f*	*α-p*	*o*
C-1	52.79	50.54	—	52.65	50.32	51.67	65.6	64.7	64.5	63.2[e]	67.3
C-2	95.27	99.07	—	94.89	—	—	99.1	102.8	105.7	99.0	214.2
C-3	70.33	78.31	83.09	70.05	77.98	82.32	69.3	77.5	83.4	71.8[e]	—
C-4	69.75	74.89	76.75	69.20	73.92	76.12	71.1	76.3	77.9	72.1[e]	72.8[e]
C-5	69.19	81.49	82.84	68.56	80.80	82.32	70.4	82.1	83.0	66.2	71.5[e]
C-6	64.13	62.0	61.37	63.54	61.31	61.01	64.6	63.7	62.7	62.2[e]	64.2[e]
C-1′	173.33	—	—	172.91	—	—					
C-2′	62.73	62.55	62.55	63.10	62.83	63.00					
C-3′	26.54	—	—	25.65	25.85	—					
C-4′	130.46	—	—								
C-5′	117.21	—	—								
C-6′	134.90	—	—								

[a]Measured at 90.52 and 100.57 MHz for 15–30% solutions in D_2O; internal acetone (δ 30.5). [b]Signals whose chemical shifts are not given were usually obscured by the parent β-pyranose signal, or by others; or, for the quaternary carbon atoms, were of too low intensity. [c]Indolyl resonances: C-2, 124.76; C-3, 107.17; C-4, 118.29; C-5, 121.93; C-6, 119.27; C-7, 111.71; C-8, 126.48; C-9, 136.25 p.p.m. [d]Data from the literature[18] for purposes of comparison, measured at 80° in D_2O at 67.8 MHz. [e]Uncertain assignments.

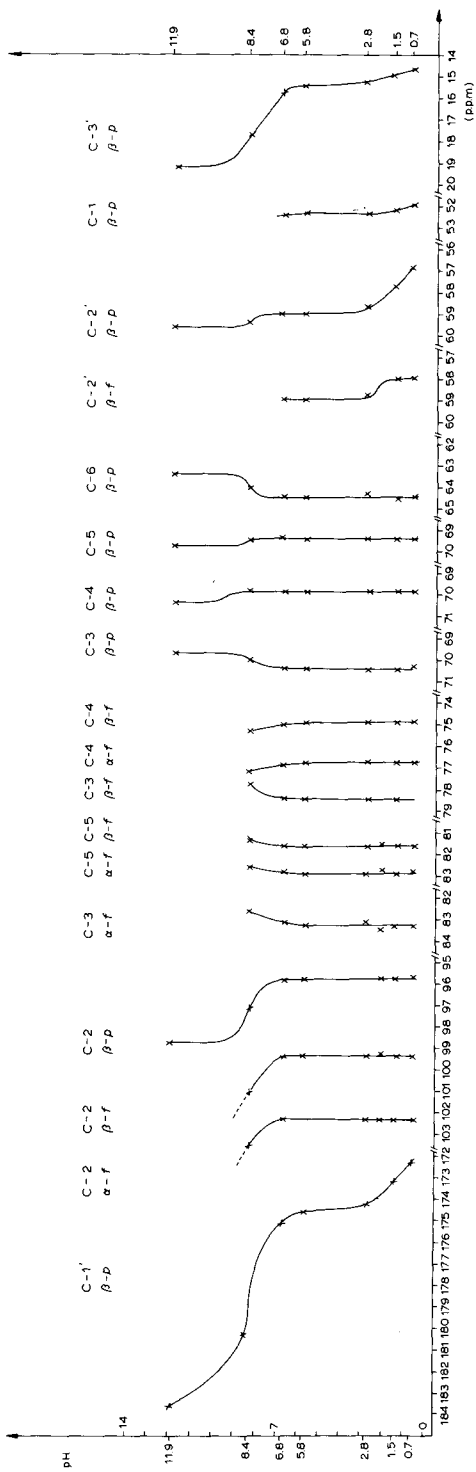


Fig. 2. ^{13}C-N.m.r. chemical shifts for **2** as a function of pH.

technique for the decoupler in order to avoid a temperature gradient in the sample although 5-mm tubes were already used to minimise this effect[8].

The pK_a values for fructose–alanine (**2**) obtained by this method are 1.7 and 8.5, respectively. Because of the limited number of points on the titration curves (Fig. 2), these estimates were not very precise, but they showed the variation in the pK value of the amino group compared with that (9.69) of alanine; the acidic pK is not very much different from that (2.35) of alanine. The positions of the furanose signals could not be determined with the required accuracy at pH values higher than that of the inflection point because of severe broadening of the resonances. This was not accompanied by a decrease in the total ratio of furanoses to pyranoses. In contrast, Tjan *et al.*[7] stated that, at pH ≥ 7, only the β-pyranose was found in the equilibria for **1–4** and **9–10**. The broadening of the furanose signals increased with increase in pH and there was a change in the line positions. At pH 11.9, only three broad humps were visible, probably because of fast mutarotation in alkaline solution between the furanose anomers. It is likely that the open-chain form is an intermediate in this process, although this could not be determined. The β-pyranose form did not participate in this fast interconversion, because its signals remained sharp. The mutarotation of **2** was catalysed only by bases, and not by acids as for fructose. The initial mutarotation required several hours to reach equilibrium after dissolving the compounds in water, so that, initially, a greater proportion of the β-pyranose form (up to 80%) was detected together with ~10% of the α- and β-furanose forms.

The assignment of the signals for C-3,4,5 for the three major forms using the chemical shift information from the pH-dependent spectra (Fig. 2) was straightforward. For the β-fructopyranose form, the change in the chemical shifts at the basic inflection-point decreased with increase in the number of bonds between the amino/ammonium group at C-1 and the carbon atom observed. A rather large effect ($\Delta\delta$ −0.9) was found for C-6. These changes of the chemical shift accorded with the results from the selective-decoupling experiments. The furanose forms showed a similar behavior. Compared to the ^{13}C-n.m.r. spectrum of fructose[18], the signals of C-4 of the α-fructofuranose and C-3 of the β-fructofuranose changed their position. The δ values of the furanose forms given in Fig. 1 were extrapolated from the shift difference observed at the inflection point. The change of pH in the acidic medium affected mainly the amino acid residue, although a slight effect was observed at the C-1 of the fructose.

For aqueous solutions of the fructose–amino acids at equilibrium, there were ~61% of β-pyranose, ~16% of α-furanose ~15% of β-furanose, 6% of α-pyranose, and 2% of keto forms present, the latter being detectable only in >30% solutions. The proportions of the various components of the equilibrium were not significantly altered by change in pH, as shown for **2**, and were not dependent on the amino acid (Table IV).

TABLE IV

PERCENTAGES OF THE VARIOUS FORMS OF THE D-FRUCTOSE-L-AMINO ACIDS **1–14** IN D_2O SOLUTION, AS DETERMINED BY ^{13}C-N.M.R. SPECTROSCOPY[a]

	β-Pyranose	*β-Furanose*	*α-Furanose*	*α-Pyranose*	
1	66	14	15	5	
2	61	15	16	6[b]	
3	66	13	15	6	
4	65	14	16	5	
5	69	13	14	4	
6	65	15	16	4	
7	62	13	20	5	
8	66	15	15	4	
9	66	13	16	5	
10	65	15	20	n.d.[c]	
11	68	11	16	5	
12	63	10	17	10	
13	65	16	17	n.d.	
14	64	15	21	n.d.	

[a]Mutarotation equilibrium was reached after several hours at room temperature or in <6 h at 65°. [b]Keto form, 2%. [c]Not determined: the signal-to-noise ratio did not allow an accurate determination.

EXPERIMENTAL

Melting points were determined with a Leitz melting-point microscope and $[\alpha]_{589}$ values for aqueous solutions (1-dm path-length) with a Perkin–Elmer 243 polarimeter. N.m.r. spectra (^1H, ^{13}C; internal acetone) were recorded with Bruker WH 360 and WM 400 instruments operating at 360 (90.52) and 400 (100.57) MHz, respectively. ^1H-N.m.r. samples were subjected to a H/D-exchange by repeated dissolution/concentration with D_2O. pH Values were measured with a micro glass-electrode (Wilmad). All reactions and separations were monitored by t.l.c. on silica gel G 60 F_{254} (Merck) or cellulose foil F_{254} (Merck) with 1-butanol–acetic acid–water (2:1:1) or chloroform–methanol–conc. ammonia (3:3:1) and detection with conc. H_2SO_4, 0.3% naphthoresorcinol–ethanol–M H_2SO_4, or ethanolic 1% ninhydrin. Ion-exchange chromatography was performed on Dowex 50W X-8 (H^+) resin (100–200 mesh), and column chromatography on silica gel 60 (Merck) using water saturated with 1-butanol.

Preparation of N-*(1-deoxy-*D-*fructos-1-yl)-*L-*amino acids.* — The Amadori compounds **1–14** were prepared according to the procedure of Hodge and Fisher[17] for *N*-(1-deoxy-D-fructos-1-yl)glycine. A mixture of D-glucose, L-amino acid, and sodium pyrosulfite in the molar ratios 4:1:0.5 in water or methanol–water (1:1) was boiled under reflux with vigorous stirring. The monosodium salts of L-glutamic and L-aspartic acid, L-phenylalanine, L-tyrosine, L-histidine, and L-tryptophan were treated in ethanol–water (1:1) or methanol–water (4:1). The reaction mixtures were subjected to ion-exchange chromatography (columns, 2.6 × 26 cm). The excess of D-glucose and browning products were eluted with ethanol–water

(1:1) (10 column vol.) and subsequently with water (3 column vol.). The Amadori products were eluted with 0.1M ammonia, followed by unreacted amino acids. This procedure was satisfactory for **1–5**, whereas **6–10** were eluted partly by water (after D-glucose) and partly by 0.1M ammonia (together with the amino acids). Compounds **11–14**, which contain aromatic amino acid residues, were eluted with 0.1M ammonia, together with the corresponding amino acids. These mixtures were fractionated by chromatography on columns of silica gel. The combined fractions were freeze-dried, crystallised from ethanol, and dried over phosphorus pentaoxide.

The pH values given were calculated from the pD values measured according to the different ion products, using the equation pH = pD − 0.4.

The following compounds were prepared.

N-(1-Deoxy-D-fructos-1-yl)-L-leucine monohydrate[9,10] (**4**); from L-leucine (9.2 g, 0.07 mol), D-glucose (50.3 g, 0.28 mol), and sodium pyrosulfite (6.6 g, 0.04 mol) in refluxing methanol–water (80 mL, 1:1) for 3 h. A solution of the yellow-brown, syrupy product in ethanol–water (250 mL, 1:1) was filtered, and fractionated on Dowex 50W X-8, to give **4** (3.1 g, 14.2%) as white needles, m.p. 145° (dec.), $[\alpha]_D^{20}$ −37° (*c* 0.5, water).

Anal. Calc. for $C_{12}H_{23}NO_7 \cdot H_2O$: C, 46.30; H, 8.09; N, 4.50. Found: C, 46.08; H, 7.95; N, 4.37.

N-(1-Deoxy-D-fructos-1-yl)-L-isoleucine (**5**); from D-glucose hydrate (45.3 g, 0.23 mol), L-isoleucine (8 g, 0.06 mol), and sodium pyrosulfite (6 g, 0.03 mol) in refluxing methanol–water (40 mL, 1:1) for 4 h. A solution of the resulting syrup in ethanol–water (200 mL, 1:1) was filtered, and eluted from Dowex 50W X-8 with water, to give **5** (3.0 g, 16.8%) as a white powder, m.p. 120° (dec.), $[\alpha]_D^{20}$ −31° (*c* 1.6, water).

Anal. Calc. for $C_{12}H_{23}NO_7$: C, 49.14; H, 7.90; N, 4.78. Found: C, 48.91; H, 7.89; N, 4.68.

N-(1-Deoxy-D-fructos-1-yl)-L-serine monohydrate[9,13] (**6**); from L-serine (7.05 g, 0.07 mol), D-glucose (48.3 g, 0.27 mol), and sodium pyrosulfite (6.4 g, 0.03 mol) in refluxing methanol–water (60 mL, 2:1) for 3.5 h. A solution of the resulting syrup in ethanol–water (150 mL, 1:1) was filtered, and eluted from Dowex 50W X-8 with water, to give **6** (3.4 g, 17.8%) as white needles, m.p. 110° (dec.), $[\alpha]_D^{20}$ −46° (*c* 2.3, water); lit.[13] $[\alpha]_D^{20}$ −46° (*c* 2, water) for anhydrous **6**.

Anal. Calc. for $C_9H_{17}NO_8 \cdot H_2O$: C, 37.90; H, 6.71; N, 4.91. Found: C, 37.73; H, 6.55; N, 4.79.

N-(1-Deoxy-D-fructos-1-yl)-L-threonine monohydrate[9,13] (**7**); from D-glucose hydrate (35.6 g, 0.18 mol), L-threonine (6 g, 0.05 mol), and sodium pyrosulfite (4.8 g, 0.03 mol) in water (25 mL); boiling water-bath for 2 h. A solution of the resulting syrup in ethanol–water (200 mL, 1:1) was filtered, and eluted from Dowex 50W X-8 with water, to give **7** (4.3 g, 28.6%), m.p. 108° (dec.), $[\alpha]_D^{20}$ −47° (*c* 0.4, water); lit.[13] $[\alpha]_D^{20}$ −49.8° (*c* 2, water) for anhydrous **7**.

Anal. Calc. for $C_{10}H_{19}NO_8 \cdot H_2O$: C, 40.13; H, 7.07; N, 4.68. Found: C, 40.49; H, 7.20; N, 4.58.

N-(1-Deoxy-D-fructos-1-yl)-L-methionine (**8**); from L-methionine (9.1 g, 0.06 mol), D-glucose hydrate (43.9 g, 0.22 mol), and sodium pyrosulfite (5.8 g, 0.03 mol) in methanol–water (60 mL, 1:1); boiling water-bath for 3.5 h. A solution of the resulting syrup in ethanol–water (250 mL, 1:1) was eluted from Dowex 50W X-8 with water, to give **8** (4 g, 21%), m.p. 95° (dec.), $[\alpha]_D^{20}$ −34° (*c* 0.35, water).

Anal. Calc. for $C_{11}H_{21}NO_7S$: C, 42.43; H, 6.80; N, 4.50; S, 10.30. Found: C, 42.11; H, 6.90; N, 4.38; S, 10.01.

N-(1-Deoxy-D-fructos-1-yl)-L-pyroglutamic acid monohydrate[7,13] (**10**); from monosodium L-glutamate (12.8 g, 0.08 mol), D-glucose (62.7 g, 0.35 mol), and monosodium pyrosulfite (7.6 g, 0.04 mol) in refluxing water (80 mL) for 3 h. A solution of the resulting yellow syrup in ethanol–water (250 mL, 1:1) was eluted from Dowex 50W X-8 partly by water and partly by ammonia; the later fractions were contaminated by L-glutamic acid. Subsequent purification by chromatography on silica gel gave **10** (0.7 g, 3%) as white plates, m.p. 90–105° (dec.), $[\alpha]_D^{20}$ −39° (*c* 1.9, water); lit.[7] m.p. 125–126.5° (dec.), $[\alpha]_D^{20}$ −52° (*c* 1, water), for anhydrous **10**.

Anal. Calc. for $C_{11}H_{17}NO_8 \cdot H_2O$: C, 42.72; H, 6.19; N, 4.53. Found: C, 42.63; H, 6.10; N, 4.50.

N-(1-Deoxy-D-fructos-1-yl)-L-phenylalanine monohydrate[9,14] (**11**); from L-phenylalanine (8.4 g, 0.05 mol), D-glucose (36 g, 0.2 mol), and sodium pyrosulfite (4.8 g, 0.03 mol) in methanol–water (25 mL, 1:1); boiling water-bath for 2 h. A solution of the resulting syrupy product in ethanol–water (250 mL, 1:1) was eluted from Dowex 50W X-8 with 0.1M ammonia. The later fractions containing **11** were contaminated with L-phenylalanine and were purified by chromatography on silica gel, to give **11** (4.2 g, 24%) as a white powder. Recrystallisation from water saturated with 1-butanol gave material having m.p. 143° (dec.), $[\alpha]_D^{20}$ −34° (*c* 2.2, water); lit.[14] m.p. 170° (dec.; browning at 140°), $[\alpha]_D^{20}$ −33° (*c* 2, water), for anhydrous **11**.

Anal. Calc. for $C_{15}H_{21}NO_7 \cdot H_2O$: C, 52.17; H, 6.71; N, 4.05; Found: C, 52.28; H, 6.63; N, 3.98.

N-(1-Deoxy-D-fructos-1-yl)-L-tyrosine dihydrate (**12**); from L-tyrosine (6 g, 0.03 mol), D-glucose hydrate (36 g, 0.18 mol), and sodium pyrosulfite (4.7 g, 0.024 mol) in refluxing methanol–water (100 mL, 4:1) for 5.5 h. A solution of the resulting syrup in ethanol–water (200 mL, 1:1) was filtered, and eluted from Dowex 50W X-8 with 0.1M ammonia, to give **12** together with L-tyrosine. The mixture was fractionated by chromatography on silica gel with water-saturated 1-butanol. The fractionation was monitored by h.p.l.c. [30 cm μ-Bondapak C-18, 0.05M tetrabutylammonium phosphate (PIC A) in water, 1 mL/min; 500 p.s.i.; u.v. detector (254 nm); T_{Tyr} 4.65, T_{FruTyr} 5.5 min]. Compound **12** was obtained as a white powder (0.25 g, 2.0%), m.p. 115° (dec.), $[\alpha]_D^{20}$ −16° (*c* 0.5, dimethyl sulfoxide).

Anal. Calc. for $C_{15}H_{21}NO_8 \cdot 2\ H_2O$: C, 47.49; H, 6.64; N, 3.69. Found: C, 47.20; H, 6.71; N, 3.49.

N-(1-Deoxy-D-fructos-1-yl)-L-histidine dihydrate[15,16] (**13**); from L-histidine

(8.2 g, 0.053 mol), D-glucose hydrate (43.2 g, 0.22 mol), and sodium pyrosulfite (5.2 g, 0.027 mol) in refluxing methanol–water (100 mL, 4:1) for 1 h. A solution of the resulting syrup in ethanol–water (250 mL, 1:1) was eluted from Dowex 50W X-8 with 0.1M ammonia, to give **13** together with L-histidine. The fractionation was monitored by h.p.l.c. (see above); **13**, T_{His} 3.8, T_{FruHis} 4.3 min. A second fractionation on Dowex 50W X-8 yielded **13** (0.2 g, 1.1%), as a light-yellow powder, m.p. 130° (dec.), $[\alpha]_D^{20}$ −30.5° (*c* 0.3, water); lit.[15] m.p. 120–130° for anhydrous **13.**

Anal. Calc. for $C_{12}H_{19}N_3O_7 \cdot 2\ H_2O$: C, 40.79; H, 6.56; N, 11.89. Found: C, 40.55; H, 6.66; N, 11.68.

N-(1-Deoxy-D-fructos-1-yl)-L-tryptophan dihydrate[16] (**14**); from L-tryptophan (10.4 g, 0.05 mol), D-glucose (36.7 g, 0.2 mol), and sodium pyrosulfite (4.8 g, 0.03 mol) in refluxing methanol–water (100 mL, 3:1) for 2.5 h; the L-tryptophan did not dissolve completely. A solution of the resulting syrupy product in ethanol–water (250 mL, 1:1) was eluted from Dowex 50W X-8 with 0.1M ammonia, to give only a small proportion of pure **14**; the main fraction also contained L-tryptophan. Purification by chromatography on silica gel gave, after crystallisation from 1-butanol, **14** (1.1 g, 5.4%), m.p. 143° (dec.), $[\alpha]_D^{20}$ −40° (*c* 0.3, water); lit.[16] $[\alpha]_D^{20}$ −7.2° (*c* 2.6, 2M hydrochloric acid) for anhydrous **14.**

Anal. Calc. for $C_{17}H_{22}N_2O_7 \cdot 2\ H_2O$: C, 50.74; H, 6.51; N, 6.96. Found: C, 50.95; H, 6.59; N, 6.88.

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