Carbohydrate Research, 92 (1981) 37–49
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

ANALYSIS OF THE 220-MHz, P.M.R. SPECTRA OF SOME PRODUCTS OF THE AMADORI AND HEYNS REARRANGEMENTS

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(Received October 20th, 1978; accepted for publication in revised form, November 27th, 1980)

ABSTRACT

In order to establish whether p.m.r. spectroscopy is useful for identifying Amadori- and Heyns-rearrangement products, the p.m.r. spectra at 220 MHz of 16 rearrangement products derived from D-glucose or D-fructose and amino acids have been investigated. At pH 3, the protons of the NCH_2 group of *N*-substituted 1-amino-1-deoxy-D-fructoses (Amadori-rearrangement products) resonate at δ 3.25–3.60 in D_2O and are shifted upfield by 0.3–0.6 p.p.m. at pH 9. These protons exchange with deuterium. Also, in D_2O there is an equilibrium of the acyclic, furanose, and pyranose structures, the last being favoured. At pH $\geq$7, the equilibrium is completely shifted to the β-pyranose form, which adopts exclusively the $^2C_5$ conformation. At pH 3, the equilibrium favours the β-furanose form. At pH 3, H-1*e* and H-1*a* of *N*-substituted 2-amino-2-deoxy-D-glucoses (Heyns-rearrangement products) resonate at δ 5.55 and 5.04, respectively. At pH 9, the signal for H-2 is shifted upfield by 0.2–0.7 p.p.m. In D_2O solution, these compounds exist as an equilibrium of α- and β-pyranose forms in the $^4C_1$ conformation. The α anomer is stabilised by the amino acid group at position 2. At pH 3, the $\alpha\beta$-ratio is 2–4 : 1, and, at pH 9, 1.0–1.1 : 1.

INTRODUCTION

During dehydration, storage, and processing of foods, reactions between amino acids and sugars may give rise to Amadori-rearrangement products (ARP), usually formed from *N*-substituted aldosylamines. A number of ARP and Heyns-rearrangement products (HRP) have been isolated from natural products[1–5] and they are usually identified on the basis of i.r. and p.c. data.

Some ARP derived from D-glucose and primary or secondary amines can be identified[6] by p.m.r. spectroscopy, and acyclic, furanose, and pyranose forms can be distinguished. We now report an extension of this work to the rearrangement products derived from D-glucose and D-fructose and various amino acids.

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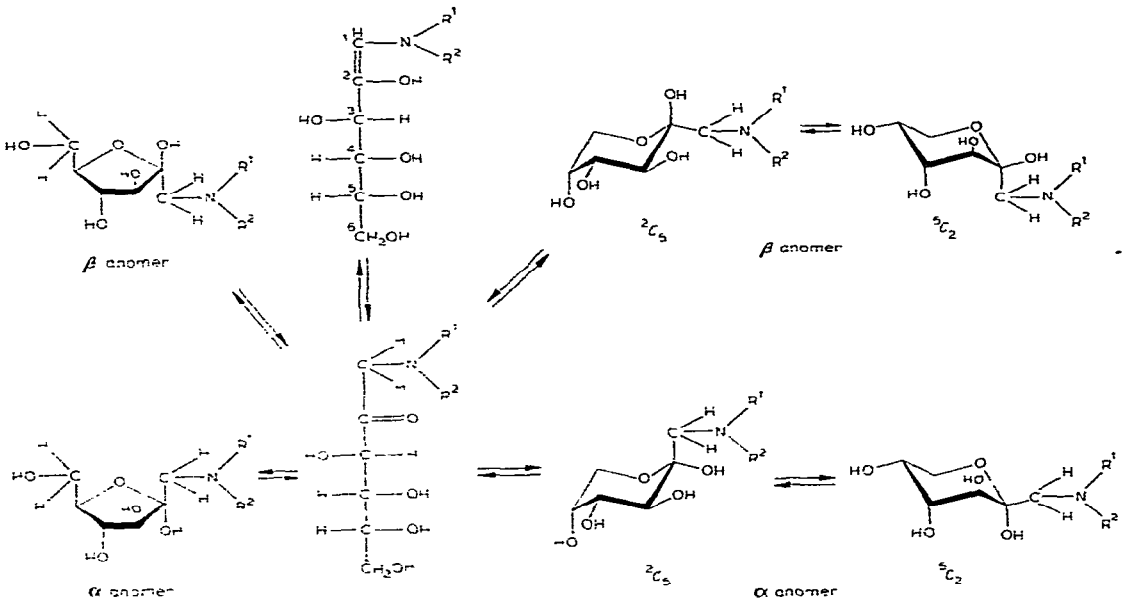
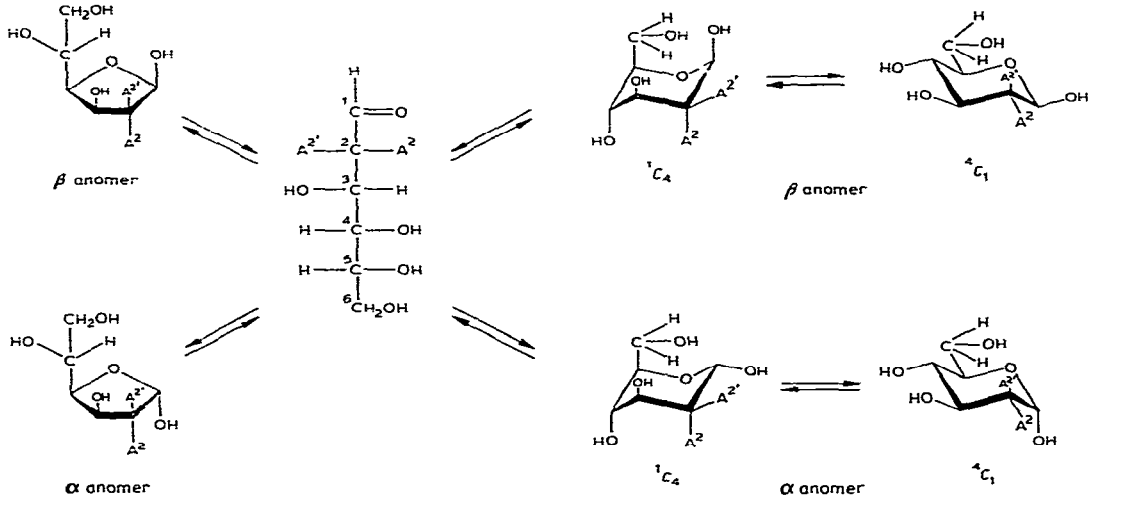


Scheme 1. Tautomeric equilibria of 1-amino-1-deoxy-D-*arabino*-hexuloses (ARP) in D_2O: **1** R^1 = H, R^2 = $-CH_2CO_2H$ (glycine)[7–12], **2** R^1 = H, R^2 = $-CH(Me)CO_2H$ (alanine)[7,8,11], **3** R^1 = H, R^2 = $-CH(Et)CO_2H$ (DL-2-aminobutyric acid), **4** R^1 = H, R^2 = $-(CH_2)_3CO_2H$ (4-aminobutyric acid)[8,13], **5** R^1 = H, R^2 = $-CH(CH_2CHMe_2)CO_2H$ (leucine)[7,8], **6** R^1 = H, R^2 = $-CH(CHMe_2)CO_2H$ (valine)[7,13], **7** $R^1 + R^2$ = $-CH(CO_2H)(CH_2)_3-$ (proline)[14,15], **8** $R^1 + R^2$ = $-CH(CO_2H)CH_2CH_2CO-$ (pyroglutamic acid), **9** R^1 = H, R^2 = $-CH(CO_2H)CH_2CH_2CO_2H$ (glutamic acid)[7,8,13], **10** R^1 = H, R^2 = $-CH(CO_2H)CH_2CO_2H$ (aspartic acid)[7,8,11,13], and **11** R^1 = H, R^2 = $-CH(CO_2H)CH_2CONH_2$ (asparagine)[8].



Scheme 2. Tautomeric equilibria of 2-amino-2-deoxy-D-glucose/mannose derivatives (HRP) in D_2O: **12** A^2, $A^{2'}$ = H, $-NHCH_2CO_2H$ (glycine)[7–12], **13** A^2, $A^{2'}$ = H, $-NHCH(Me)CO_2H$ (alanine)[7,8,11], **14** A^2, $A^{2'}$ = H, $-NH(CH_2)_3CO_2H$ (4-aminobutyric acid)[8,13], **15** A^2, $A^{2'}$ = H, $-NHCH(CO_2H)CH_2CH_2CO_2H$ (glutamic acid)[7,8,13], and **16** A^2, $A^{2'}$ = H, $-NHCH(CO_2H)CH_2CO_2H$ (aspartic acid)[7,8,11,13].

For *gluco* series (a), A^2 = NR^1R^2, $A^{2'}$ = H. For *manno* series (b), A^2 = H, $A^{2'}$ = NR^1R^2.

RESULTS AND DISCUSSION

ARP and HRP may be prepared by heating the reactants with ~20% of their weight of water, or heating a dilute, methanolic solution. The first method generally gave better results, although, as a result of extensive browning, repeated purification was usually necessary. Addition of acid did not increase the product yield.

The ARP **1–11** and HRP **12–16** were synthesised (see Schemes 1 and 2). For the preparation of **8**, **9**, and **10**, it was necessary to neutralise one of the carboxyl groups of the amino acids. The synthesis of the new compounds **3** and **8** is reported. Compounds **1**, **2**, **6**, **7**, **10**, **12**, **13**, and **14** were obtained crystalline. Compound **9** could not be obtained in a pure state, because it was converted into **8** during isolation. Heyns and Paulsen[11] mentioned that compound **8** was formed when a solution of **9** was heated, but the pure compounds were not described.

The p.m.r. spectra of the ARP **1–11**, recorded for solutions in D_2O at pH 3, showed that each was a mixture of at least two components. The first-order chemical shifts and coupling constants of the major components for **1–11** at pH 3 and 9.5 are given in Tables I and II. Each ARP can exist (*cf.* ref. 6) in acyclic, pyranose, and furanose forms (see Scheme 1).

The AB doublets at δ 3.00–3.50 (spacings 13.0–15.0 Hz) can be assigned to the methylene protons (H-1,1′) of the pyranose and furanose structures. The complete exchangeability of these protons at pH 9.5 with deuterium confirms this assignment and the equilibria shown in Scheme 1.

From the good correspondence between the data for **1–11** (Tables I and II) with those for 1-deoxy-1-piperidino- and -1-morpholino-β-D-fructose[6], we conclude that the major component in **1–11** has the β-pyranoid structure and exists exclusively in the $^2C_5$ conformation. Thus, the minor component in **1–11** has the α-furanoid structure. The doublet at δ 4.20 $\pm$0.02 (spacing 4.5 Hz) is tentatively assigned to H-3 of the β-furanoid structure on account of the similarity to the chemical shift of H-3 of methyl β-D-fructofuranoside (see Table III).

Changing the pH from 3 to 9.5 results in upfield shifts of the signals for all protons from the amino acid residue (Table I), the α-aminomethine protons being shifted the most (0.3–0.6 p.p.m.); H-3,4,5,6,6′ of the sugar residue are influenced only slightly or not at all. Due to the very rapid exchange of H-1,1′ with deuterium at pH 9.5, the upfield shifts of their signals had to be recorded as follows. The solution of the ARP in water at pH 9.5 was freeze-dried and the p.m.r. spectrum was recorded as quickly as possible on a solution of the residue in D_2O.

In **8**, the signal for H-1 is shifted upfield (~0.2 p.p.m.), whereas that for H-1′ is shifted downfield (~0.5 p.p.m.), in comparison with the corresponding signals of **1–7** and **9–11** (pH 3.5). This effect is probably caused by the diamagnetic anisotropy of the carbonyl group from pyroglutamic acid. The ring of the amino acid residue evidently adopts a conformation such that H-1′ resides longer in the plane of the carbonyl group than does H-1.

The difference (0.08→0.24 p.p.m.) in chemical shift for H-1 and H-1′ of **10**

TABLE I

CHEMICAL SHIFTS (δ) OF THE PROTONS OF THE ARP's **1–11** IN D_2O SOLUTIONS AT pH 3 AND 9.5 (IN PARENTHESES) FOR MUTAROTATION MIXTURES

Compound	*Sugar residue*							*Amino acid residue*								*Others*[b]
	H-1 d	*H-1'* d	*H-3* d	*H-4* dd	*H-5* m	*H-6* dd	*H-6'* dd	*αCH*	*αCH₂*	*βCH*	*βCH₂*	*βCH₃*	*γCH*	*γCH₂*	*γCH₃*	*δCH₃*	
1	3.33 (2.95[a])	3.38	3.75	3.88	4.00	3.76	4.01	—	3.73s (3.47s)	—	—	—	—	—	—	—	4.19d
2	3.30[a] (2.78[a]	3.32[a] 2.85[a]	3.75 3.75	3.88 3.87	4.01 3.99	3.76 3.70	4.02 4.02)	3.77q (3.24q)	—	—	—	1.51d (1.35d)	—	—	—	—	4.20d
3[c]	3.29 (2.90s[a])	3.33	3.76	3.90	4.01	3.75	4.05	3.70t (3.20t)	—	—	1.94 (1.70m)	—	—	—	0.98t (0.92t)	—	4.22d
4	3.31[a] (e		3.73 3.71	3.90 3.85	4.02 3.97	3.77 3.69	4.03 3.99)	—	3.15t (2.84t)	—	2.41t (2.26t)	—	—	1.98m (1.83m)	—	—	4.20d
5	3.26 (e	3.32	3.73 3.75	3.87 3.87	3.99 3.98	3.74 3.68	4.01 4.02)	3.70t (3.19t)	—	—	1.6–1.9m (1.62m)	—	1.6–1.9m (1.43m)	—	—	0.97d (0.91d; 0.92d)	4.18d
6	3.30 (2.77[a]	3.33 2.85[a]	3.77 3.76	3.88 3.86	3.99 3.97	3.76 3.68	4.02 4.01)	3.65d (2.98d)	—	2.28m (1.86m)	—	—	—	—	1.00d; 1.07d (0.92d; 0.95d)	—	4.22d
7	3.43; 3.43[d]	3.60; 3.55[d]	3.76	3.87	3.98	3.73	4.01	4.12dd	4.00m 3.29m	—	2.43m 1.93m	—	—	—	—	—	4.18d
	(3.10	3.35	3.66	3.84	3.97	3.68	4.00)	(3.68)	(3.78m; 2.92m)		(2.32m; 1.96m)						

8	3.18; 3.35[d]	3.98; 4.03[d]	3.60	3.82	3.93	3.63	3.93	4.64m	—	—	2.48m; 2.44m; 2.10m	—	—	—	—	—	4.02d; 3.70m
	([e])							(4.40m)			(2.45m; 2.39m; 1.96m)						
9	3.27; 3.32[d]	3.32; 3.35[d]	3.75	3.88	3.99	3.76	4.01	3.73t	—	—	2.15m	—	—	2.58t	—	—	4.20d
	(2.82s[a])							(3.23t)			(1.7–2.7m)			(1.7–2.7m)			
10	3.31; 3.36[d]	3.45; 3.41[d]	3.77	3.88	4.00	3.75	4.03	3.98dd	—	—	2.90dd; 2.98dd	—	—	—	—	—	4.19d
	3.38[d]	3.43[d]						(3.56dd)			(2.38dd; 2.62dd)						
11	(2.90[a]	2.94[a]	3.76	3.88	3.97	3.69	4.03)										
	3.33; 3.35[d]	3.48; 3.44[d]	3.76	3.90	4.00	3.78	4.03	4.04dd	—	—	2.96dd; 3.02dd	—	—	—	—	—	4.22d
	3.39[d]	3.45[d]															
	(2.99[a]	3.09[a]	3.72	3.83	3.95	3.66	3.98)	(3.66t)			(2.64dd; 2.76dd)						

[a]Partly exchanged with deuterium. [b]Signals arising from minor component, $J_{3,4}$ 4.5 Hz. [c]Diastereoisomeric mixture. [d]Signals arising from minor component, $J_{1,1'}$ 13.0–15.0 Hz. [e]Completely exchanged with deuterium.

TABLE II

FIRST-ORDER COUPLING CONSTANTS (J, Hz) OF THE PROTONS OF THE ARP's **1–11**

Compound	*Sugar residue*						*Amino acid residue*				
	$J_{1,1'}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	$J_{\alpha\beta}$	$J_{\alpha\beta'}$	$J_{\beta\beta'}$	$J_{\beta\gamma}$	$J_{\gamma\delta}$
1	13.0	10.0	3.0	2.0	1.0	13.0					
2	12.5	10.0	3.5	2.0	1.0	13.0	7.5				
3	13.0	10.0	3.0	2.0	1.0	12.0	6.5			7.2	
4	—	10.0	3.5	2.0	1.5	13.0	7.0			8.0	
5	13.0	10.0	3.5	2.0	1.5	12.5	6.0			5.0	7.0
6	13.0	10.0	3.0	2.0	1.0	12.5	6.0			7.0	
7	13.5	10.0	3.5	2.0	1.5	13.0	5.0	9.0			
8	15.0	10.0	3.0	2.0	1.0	13.0					
9	12.0	10.0	3.0	2.0	1.5	12.5	6.0			6.0	
10	13.0	10.0	3.5	2.0	1.0	12.5	7.0 (9.5)[a]	5.0 (4.0)[a]	18.0 (15.5)[a]		
11	13.0	10.0	3.5	2.0	2.0	13.0	7.0	5.0	18.0		

[a]Measured at pH 9.5.

changes more markedly when the pH is raised from 3 to 9.5 than for **11** (0.06 → 0.12 p.p.m.). This effect may be caused by dissociation of the primary CO_2H group of **10** at pH 9.5.

The equilibrium compositions of **1–11** can be determined from the relative peak areas in the p.m.r. spectra (see Table IV). Where the signals overlap, the proportions of α-furanose form and acyclic forms are combined. The estimates for the acyclic form are questionable, as separate signals were not detected; they are obtained by subtracting the percentages for the furanoid and pyranoid structures from 100%. Doddrell and Allerhand[17] have shown that the ^{13}C-chemical shifts of the quaternary carbon atom (*i.e.*, C-2) of αβ-D-fructo-furanose and -pyranose can easily be distinguished; for the acyclic structure (see Scheme 1), C-2 may be expected to resonate at much lower field. Funcke and Klemer[23] showed that, for ARP derived from D-glucose and some amines, the different tautomeric forms could easily be determined by ^{13}C-n.m.r. spectroscopy.

As can be seen from the data in Table IV, for each ARP, except **11**, the pyranoid structure is favoured at pH 3; at pH ≥ 7, the equilibrium for most of the compounds is shifted to the α-pyranose form, which is substantiated by the disappearance of the signal at δ 4.20. Protonation of the amino groups shifts the equilibrium to the α-furanoid structure. This assumption could be confirmed for 1-deoxy-1-piperidino- and -1-morpholino-β-D-fructose[6]. In solution in D_2O (pH 9.5), these compounds occur exclusively in the α-pyranoid structure. On acidification to pH 1, signals for the furanoid structure were observed at δ 4.18 (d, J 4.5 Hz).

D-Fructose reacts with amino acids to give either two (**12a–16a** and **12b–16b** C-2 epimers, see Fig. 2) or one group of HRP (**1**, **2**, **4**, **9**, and **10**, see Scheme 1).

TABLE III

N.M.R. DATA FOR THE PROTONS OF METHYL α-D- AND β-D-FRUCTOFURANOSIDE IN D_2O SOLUTION

Compound	*Chemical shifts (δ)*							*Coupling constants (Hz)*					
	H-1 *d*	*H-1'* *d*	*H-3* *d*	*H-4* *dd*	*H-5* *m*	*H-6* *dd*	*H-6'* *dd*	$J_{1,1'}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
Methyl α-D-fructofuranoside	3.68	3.80	3.96	4.10	3.97	3.69	3.83	12.0	1.0[a]	3.0[a]	6.0	3.0	12.5
Methyl β-D-fructofuranoside	3.65	3.73	4.18	4.06	3.96	3.64	3.81	12.5	8.0	7.5	7.0	3.0	13.0

[a] $J_{3,4}$ and $J_{4,5}$ may be reversed.

TABLE IV

ESTIMATION OF THE EQUILIBRIUM COMPOSITION (%) OF THE ARP'S **1–11** IN D_2O SOLUTION (0.2M) AT 30° AND pH 3 AND 9.5 (IN PARENTHESES)

Compound	*Acyclic form (I)*	*Furanose form*		*Pyranose form (IIIβ)*
		IIα	*IIβ*	
1	⩾5		10	⩽85 (100)
2	⩾5		15	⩽80 (100)
3	⩾5		15	⩽80 (n.d.)[a]
4	⩾5		10	⩽85 (100)
5	⩾5		15	⩽80 (100)
6	⩾5	10	14	⩽70 (100)
7	⩾5		20	⩽75 (100)
8	⩾5	12	24	⩽60 (100)
9	⩾5		15	⩽80 (n.d.)
10	⩾5	18	18	⩽60 (100)
11	⩾5	22 (13)	22 (13)	⩽50 (<68)

[a]n.d., not determined.

The first-order chemical shifts and coupling constants for **12–16** in solution in D_2O at pH 3 and 9 are given in Tables V and VI. The relative intensities of the two low-field doublets at δ 5.55 $\pm$0.02 (J 3.5 Hz) and 5.04 $\pm$0.02 (J 8.0 Hz) were pH-dependent, suggesting an anomerisation, and are assigned to H-1*e* and H-1*a*, respectively, in pyranoid structures. Consequently, H-2 is axial. At pH 3, the signals for H-2 can easily be recognised by their splitting patterns: J 3.5 and 10.5 Hz for δ 3.30 $\pm$0.04, and J 8.0 and 10.5 Hz for δ 3.06 $\pm$0.04. Thus, H-3 is axial and the magnitudes of $J_{3,4}$ (9.5 Hz) and $J_{4,5}$ (9.5 Hz) indicate that H-4 and H-5 are also axial.

The data in Table V show that an increase of pH from 3 to 9.5 causes a marked upfield shift of the signal for H-2 (0.2–0.7 p.p.m.), and smaller ones for H-1 (0.05–0.4 p.p.m.) and H-3 (0.08–0.15 p.p.m.), indicating that the amino group is located at C-2. Substantial evidence for this conclusion is obtained by comparison of δ H-1, δ H-2, and δ H-3 for **12–16** with those of the corresponding protons in α- and β-D-glucose[19] and of similar compounds[20–22]. For **12–16**, H-2 resonates at higher field than H-2 of D-glucose; this is due to the difference in electronegativity between nitrogen and oxygen. The δ values for H-1 of the hydrochlorides and free bases of **12–16** are slightly greater (0.08–0.25 p.p.m.) than those of the hydrochloride salt (H-1α, δ 5.47; H-1β, δ 4.97) and free base (H-1α, δ 5.22; H-1β, δ 4.60) of 2-amino-2-deoxy-D-glucose[10,11]. This deshielding effect is probably caused by the carboxyl group of the amino acid residue in **12–16**.

The two extra signals at δ 5.23 (d, J 1.5 Hz) and 2.84 (dd, J 1.5 and 4.5 Hz) in the p.m.r. spectrum of **13** at pH 9 are probably due to H-1 and H-2, respectively, of 2-alanino-2-deoxy-D-mannose (**13b**). The HRP **15** and **16** are contaminated with

CHEMICAL SHIFTS (δ) OF THE PROTONS OF THE HRP's **12a–16a** IN D_2O SOLUTION AT pH 3 AND 9 (IN PARENTHESES) FOR MUTAROTATION MIXTURES AND OF α- AND β-D-GLUCOPYRANOSE[a]

Compound	*Sugar residue*							*Amino acid residue*					*Others*
	H-1 *d*	*H-2* *dd*	*H-3* *dd*	*H-4* *dd*	*H-5* *m*	*H-6* *dd*	*H-6'* *dd*	*αCH*	*αCH₂*	*βCH₃*	*βCH₂*	*γCH₂*	
α-D-Glucopyranose	5.27	3.56	3.75	3.44	3.81	4.05	4.12						
β-D-Glucopyranose	4.67	3.29	3.59	3.45	3.52	3.91	4.22						
12a(α) 12α	5.55	3.31	3.99	3.48	3.88	3.76	3.85		3.75s				
	(5.37)	(2.95)	(3.81)	(3.44)	(3.8)	(3.77)			(3.48dd; 3.54dd)				
12a(β) 12β	5.05	3.09			3.50								
	(4.70)	(2.50)			(3.4)	(3.71)			(3.42dd; 3.49dd)				
13a(α) 13α	5.56	3.28	3.92	3.46	3.86	3.78	3.84	4.18q		1.51d			5.24d[a]
	(5.30)	(2.59)	(3.80)	(3.40)				(3.63q)		(1.25d)			(5.23d[a]; 2.84dd[a])
13a(β) 13β	5.04	3.11			3.50	3.70				1.53d			
	(4.62)	(2.45)					(3.89)			(1.23d)			
14a(α) 14α	5.56	3.29	3.93	3.47	3.88	3.77	3.86		3.22t		1.98m	2.43t	
	(5.51)	(3.10)	(3.85)	(3.45)		(3.76)	(3.85)		(3.03)		(1.83m)	(2.27t)	
14a(β) 14β	5.06	3.03			3.49								
	(4.77)	(2.63)				(3.71)	(3.88)		(3.06t)		(1.90m)	(2.32t)	
15a(α) 15α	5.53	3.25	4.05	3.45				4.20dd			2.17m	2.57m	
15a(β) 15β	5.00	3.04			← 3.6–3.9 →								
16a(α) 16α	5.56	3.36	4.00	3.47									
	(5.34)	(2.75)		(3.43)	(← 3.6–3.9 →)			4.17t	2.8–3.1m				
16a(β) 16β	5.06	3.14						(3.68t)	(2.35dd; 2.49dd)				
	(4.65)	(2.47)						4.30t					

[a]Signals tentatively assigned to H-1 and H-2, respectively, of 2-amino-2-deoxymannose; $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 4.5 Hz.

TABLE VI

FIRST-ORDER COUPLING CONSTANTS (J, Hz) OF THE PROTONS OF HRP's **12a–16a** AND OF α- AND β-D-GLUCOPYRANOSE[a]

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	J_{gem}	αCH_2 $J_{\alpha\beta}$	$J_{\alpha\beta'}$	$J_{\beta\beta'}$	$J_{\beta\gamma}$
α-D-Glucopyranose	3.8	10.0	9.0	9.0	4.0	2.5	−12.0					
β-D-Glucopyranose	8.1	8.5	8.8	9.0	5.0	2.0	−12.2					
12α 12a(α)	3.5	10.0	10.0	9.5	5.0	2.0	12.5	16.0				
12β 12a(β)	8.0	9.5						17.0				
13α 13a(α)	3.5	10.5	9.5	9.5	5.5	2.0	12.0		6.2			
13β 13a(β)	8.0	10.5										
14α 14a(α)	3.5	10.5	9.5	9.5	5.5	2.5	12.5		7.0			8.0
14β 14a(β)	8.0	10.5			5.5	2.0	12.5					
15α 15a(α)	3.5	10.5	9.5	9.5	←	[a]	→		5.0	10.0		6.5
15β 15a(β)	8.0	10.0										
16α 16a(α)	3.5	10.5	9.5	9.5	←	[a]	→		4.0	4.0	20.0	
16β 16a(β)	8.0	10.5										

[a]Not determined.

TABLE VII

ANOMERIC COMPOSITIONS (%) OF THE HRP'S **12a–16a** AND OF D-GLUCOPYRANOSE IN D_2O (0.2M) AT 30°, AND pH 3 AND 9 (IN PARENTHESES) AT EQUILIBRIUM

Compound	*α Anomer*	*β Anomer*
12a	75 (53)	25 (47)
13a	80 (50)	20 (50)
14a	80 (53)	20 (47)
15a	75	25
16a	75 (52)	25 (48)
D-Glucopyranose	36	64

the isomeric 1-amino-1-deoxy-D-fructoses (**9**, **10**) and, as a result of the overlapping signals at δ 3.60–3.90, the signals for H-5,6,6′ could not be assigned. For **1–11**, signals for H-1,1′ are shifted mostly upfield (0.16–0.55 p.p.m.), in comparison with the other protons of the amino acid residue, when the pH is changed from 3 to 9.5.

Table VII gives the anomeric composition of **12–16** and of D-glucopyranose equilibria for solutions in D_2O at various pH values. Replacement of HO-2*e* by an amino group stabilises the α-D-pyranose form in the equilibrium mixture. Hydrogen bonding between the substituents at C-1 and C-2 affords a more stable, *cis*-fused, five-membered ring than the corresponding *trans*-fused ring; this probably accounts, at least in part, for this observation[21].

Thus, it is established that **12–16** are mainly derivatives of 2-amino-2-deoxy-D-glucose (**12a–16a**) which, in solution in D_2O, exist mainly as the α- and β-pyranoses in the $^4C_1$ conformation.

EXPERIMENTAL*

Melting points were determined in capillary tubes, with short-stemmed thermometers, and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter. T.l.c. was performed on silica gel (Merck); isatin was present in the eluent. After elution, the plates were heated at 125°, sprayed with a solution of Cr_2O_3 in conc. sulphuric acid, and heated at 125°. P.m.r. spectra (220 MHz) were recorded with a Varian spectrometer. Sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)-propionate was used as the internal standard for solutions in D_2O. All samples were subjected to a preliminary deuterium-exchange by repeated treatment with fresh D_2O and freeze-drying. The pH was adjusted with either DCl or NaOD. I.r. spectra were recorded with a Perkin–Elmer 225 spectrometer.

*1-[(2L)-2-Carboxy-5-oxo-1-pyrrolidinyl]-1-deoxy-*D*-fructose* (**8**) *and 1-deoxy-1-[(1L)-1,3-dicarboxypropylamino]-*D*-fructose* (**9**). — A mixture of D-glucose (36 g,

*The i.r. and p.m.r. spectra of all compounds are available on request.

0.2 mol), monosodium L-glutamate (17.3 g, 92 mmol), and water (20 ml) was heated at 60° for 45 min. Methanol (150 ml) was added dropwise with stirring, and the solution was heated at 60° for 68 h and then concentrated to dryness at ~40°. The residual oil was eluted from a column (45 × 3 cm) of Bio-Rad AG50-X8 resin (100–200 mesh) with water (1.3 litre), giving glucose + **8**, and with 1.5% trichloroacetic acid (1.3 litre), giving **9**. The isolation was monitored by h.p.t.l.c. (chloroform–methanol–water–acetic acid, 4:4:1:1). The fractions which contained **9** were extracted with ether, and the aqueous solution was treated with charcoal and concentrated at 25°/~15 mmHg to a small volume. Freeze-drying then yielded a powder (8.7 g), which was dissolved in methanol and was precipitated with acetone (200 ml). The dried product (1.14 g) was very hygroscopic and had m.p. 120–130° (dec.). P.m.r. spectroscopy revealed that it was a mixture of **8** and **9**. During recrystallisation of **9** from various solvent mixtures, **9** was converted into **8**. Pure **8** (h.p.t.l.c., see above) was obtained by adding acetone–ether (1:1) to an ethanolic solution of **8**, and also from the eluate (see above) after fermentation of the D-glucose with *Saccharomyces cerevisiae* or by chromatography on Bio-Rad AG3-X4A resin. The white, crystalline **8** had m.p. 125–126.5° (dec.), $[\alpha]_D^{20}$ −52.0° (after heating at 50–52°/0.05 mmHg for 6 h; *c* 1.0, water).

Anal. Calc. for $C_{11}H_{17}NO_6$: C, 45.36; H, 5.88; N, 4.81. Found: C, 45.18; H, 6.04; N, 4.80.

*1-(1-Carboxypropylamino)-1-deoxy-*D*-fructose* (**3**). — A mixture of D-glucose (3.6 g, 20 mmol), DL-2-aminobutyric acid (2.06 g, 20 mmol), and methanol (50 ml) was boiled under reflux for 15 h and then concentrated under diminished pressure. The residual oil was eluted from a column of Bio-Rad AG50W-X8 resin (50–100 mesh) (75 ml) with water (0.5 litre) and then 4% trichloroacetic acid. The appropriate fractions (15 ml each) were combined, extracted with ether, and then concentrated at 35°/~15 mmHg to dryness, and the residue was recrystallised twice from methanol–ethanol. The dried, white crystals (0.66 g, 12.5%) had m.p. 80° (dec.), $[\alpha]_D^{20}$ −49.3° (*c* 1, water).

Anal. Calc. for $C_{10}H_{19}NO_7$: C, 45.29; H, 7.22; N, 5.28; Found: C, 45.65, 45.79; H, 7.93, 7.80; N, 4.79, 4.85.

ACKNOWLEDGMENTS

The authors thank Mr. W. J. Buis (Institute for Organic Chemistry TNO, Utrecht, The Netherlands) for elemental analyses, Mr. L. J. Hoogendoorn (Laboratory for Applied Scientific Research (TNO), Delft, The Netherlands) for recording the p.m.r. spectra, and Mr. D. J. Poll for skilful technical assistance.

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