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Reaction of amino sugars with malondialdehyde*†

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ABSTRACT

2-Amino-2-deoxy-D-glucose hydrochloride reacted with the sodium salts of malondialdehyde (**7**) and methylmalondialdehyde (**8**) to give 2-deoxy-2-[(3-oxo-1-propen-1-yl)amino]-α,β-D-glucopyranose (**12**) and 2-deoxy-2-[(2-methyl-3-oxo-1-propen-1-yl)amino]-α,β-D-glucopyranose (**13**), respectively, in good yields. Likewise, β-D-glucopyranosylamine gave 3-(β-D-glucopyranosylamino)-2-propenal (**14**) and 3-(β-D-glucopyranosylamino)-2-methyl-2-propenal (**15**). 2-Amino-2-deoxy-β-D-glucopyranose and **7** reacted at pH ~9 to give a mixture of 3-pyrrolecarbaldehyde (**19**), 5-(D-*arabino*-tetritol-1-yl)-3-pyrrolecarbaldehyde (**21**), and 5-(α,β-D-erythrofuranosyl)-3-pyrrolecarbaldehyde (**20αβ**). The Na salt of crude **1** is usually contaminated with 2,4-diformyl-3-(2,2-dimethoxyethyl)glutaraldehyde (**9**) and 2,4-diformyl-3-methylglutaraldehyde (**10**), so that, in its reactions with 2-amino-2-deoxy-D-glucose hydrochloride, 2-deoxy-2-[3,5-diformyl-4-(2,2-dimethoxyethyl)-1,4-dihydropyridin-1-yl]-α,β-D-glucopyranose (**16**) and 2-deoxy-2-(3,5-diformyl-4-methyl-1,4-dihydropyridin-1-yl)-α,β-D-glucopyranose (**17**) were produced in addition to **12**. Likewise, with β-D-glucopyranosylamine, it afforded **14** and 3,5-diformyl-1-β-D-glucopyranosyl-4-(2,2-dimethoxyethyl)-1,4-dihydropyridine (**18**). Compounds **16** and **17** were obtained also by the reaction of 2-amino-2-deoxy-D-glucose hydrochloride with **9** and **10**, respectively.

INTRODUCTION

Malondialdehyde (**1**), a product of the oxidative degradation of polyunsaturated lipids[1], has attracted attention because of its involvement in various processes such as cell-aging[2], cross-linking of biological molecules[3], and the deterioration of food[4]. The functional groups in **1** should facilitate reaction with the amino groups of proteins, and, in studies with model systems, it was found[5] that 3-aminoacroleins (**3**) and 1-amino-3-iminopropenes (**4**) are products of the reaction of **1** with primary amines and amino acids. Compounds **4** have fluorescence spectra similar to those of the cross-linking products of the reaction of **1** with proteins, which suggested that this process occurs through formation of 1-amino-3-iminopropene linkages[5b]. Furthermore, the lipofuscin pigments, which accumulate in aging organisms as a result of *in vivo* lipid peroxidation, also exhibit fluorescence similar to that of **4**; hence, they have been considered to be derived from the reaction of **1** with proteins[6].

Malondialdehyde (**1**) is an unstable compound, usually obtained by acid hydroly-

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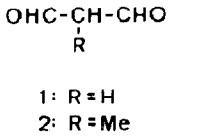
sis of 1,1,3,3-tetra-alkoxypropanes, during which partial polymerisation can occur[7] to give products that can react with amino compounds. The isolation of the 1,4-dihydropyridine-3,5-dicarbaldehydes **5** and **6**, after the reaction of hydrolysates of 1,1,3,3-tetramethoxypropane with amines and amino acids, can be understood in these terms. Compounds **5** and **6** have fluorescence spectra resembling those of the lipofuscin pigments[8].

The amino sugar components of biopolymers could also react *in vivo* with **1** with detrimental effects. Furthermore, glycosylamino acids and the products of their Amadori rearrangement can co-exist and react with **1** and highly reactive products derived therefrom, in partly oxidised, lipid-rich foods. The products might have light absorption properties that would contribute to the discoloration of food.

We now report on the reactions of 2-amino-2-deoxy-D-glucose and D-glucosylamine with **1** and methylmalondialdehyde (**2**) as model systems for more complex amino sugars. The products present in the hydrolysates of 1,1,3,3-tetra-alkoxypropane have been studied also. Although the reactions of β-diketones and β-ketoesters with amino sugars is well documented[9], there has been no report on the reactions with **1**.



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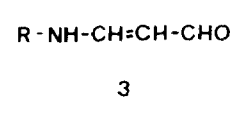
2: R = Me



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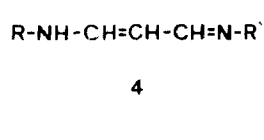

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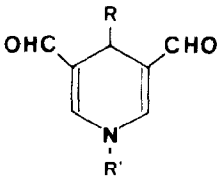
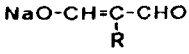
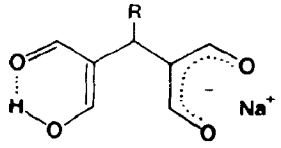


5: R = Me
6: R = $CH_2CH(OMe)_2$



7: R = H
8: R = Me



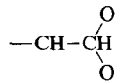
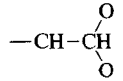
9: R = $CH_2CH(OMe)_2$
10: R = Me
11: R = ^{n}Pr

RESULTS AND DISCUSSION

Hydrolysis of 1,1,3,3-tetramethoxypropane catalysed by Dowex 50W-X8 (H^+) resin furnished an amorphous mixture of salts ("crude" NaMDA) that contained the sodium salt (**7**) of malondialdehyde (**1**) as the main component together with the mono-sodium salts of 2,4-diformyl-3-(2,2-dimethoxyethyl)glutaraldehyde (**9**) and 2,4-diformyl-3-methylglutaraldehyde (**10**). Crystallisation of this mixture yielded 75% of the known[10] monohydrate of **7**.The content of **9** increased (t.l.c.) when a shorter (0.5 h) reaction time was used. Column chromatography of the resulting "crude" NaMDA

TABLE I

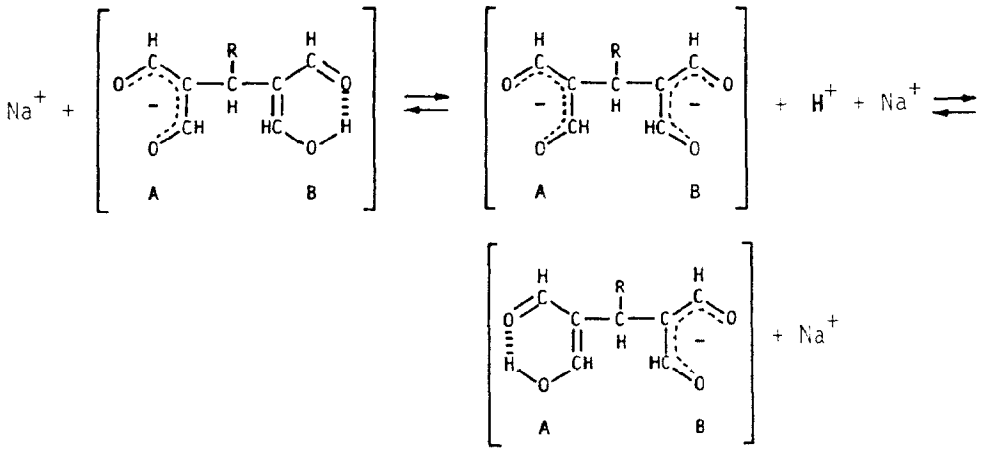
N.m.r. data (D_2O) (δ in p.p.m., J in Hz) for **9–11**

Nuclei	*Compound*		
	9	**10**	**11**
H-1,1′,1″,5	8.16s (4 H)	8.24s (4 H)	8.31s (4 H)
H-3	4.05t (J 8.3)	4.15q (J 7.6)	3.84t (J 8.3)
$-CH_2-$	1.95dd (J 6.0, 8.3)		
	4.16t (J 6.0)		
OMe	3.21s (6 H)		
CMe		1.25d (3 H)	
^{n}Pr			1.68m (2 H) 1.13m (2 H) 0.82m (3 H)
C-1,1′,1″,5	188.6	188.8	188.9
C-2, C-4	124.3	126.6	125.4
C-3	33.1	20.0	32.1
$-CH_2-$	21.5		
	105.1		
OMe	54.3		
CMe		16.8	
^{n}Pr			25.1, 25.4, 13.9

afforded 6% of a monohydrate of **9**, and preparative t.l.c. of fractions that contained **7** + **10** gave 0.5% of the dihydrate of **10**. Compound **10** could be prepared (41%) readily by condensation of 7 with acetaldehyde, and the similar reaction using butanal afforded 73% of a hydrate of the sodium salt of 2,4-diformyl-3-propylglutaraldehyde (**11**).

Evidence for the structures of **9–11** was provided by elemental analyses, spectral data, and the reactions described below. The n.m.r. spectra (Table I) showed that, in D_2O, the anion of these compounds had a symmetrical structure as shown in Scheme 1. The anions of **9–11** are acids in aqueous solution and the enolic proton exchanges rapidly (on the n.m.r. time-scale) between the units A and B through an intermediate dianion.

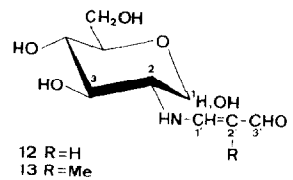
2-Amino-2-deoxy-D-glucose hydrochloride reacted with 1 equiv. of **7** or **8** in water at room temperature to afford **12** (70%) or **13** (65%). Compound **13** was obtained as a mixture of two crystalline forms (m.p. 195–196° and 202–203°) which probably correspond to the anomeric forms. Similar reactions with β-D-glucopyranosylamine furnished low yields of the 3-(β-D-glucopyranosylamino)acroleins **14** and **15**, but **15** could be obtained ($\sim$40%) more conveniently by reaction of β-D-glucopyranosylamine with 3-ethoxy-2-methylpropenal.



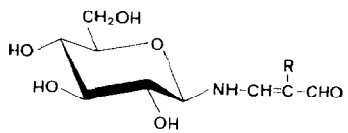
Scheme 1. Prototropic equilibrium of compounds **9**–**11** in aqueous solution.

When "crude" NaMDA reacted with 2-amino-2-deoxy-D-glucose hydrochloride, the 1,4-dihydropyridine-3,5-dicarbaldehydes **16** and **17** were produced in low yields in addition to **12**. Much better yields (43 and 47%, respectively) of **16** and **17** were obtained by treating the amino sugar hydrochloride with **9** and **10**, respectively. Similarly, the reaction of β-D-glucopyranosylamine with "crude" NaMDA afforded, in addition to **14**, 3,5-diformyl-1-β-D-glucopyranosyl-4-(2,2-dimethoxyethyl)-1,4-dihydropyridine (**18**, 5%, after chromatography).

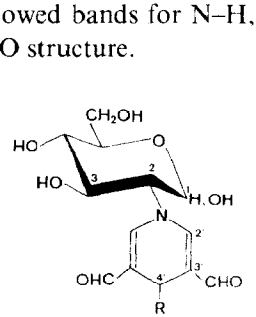
The structures of **12**–**15** were established on the basis of analytical and spectral data. The compounds exhibited u.v. spectra (see Experimental) similar to those described [5a,c,8b] for simple 3-(alkylamino)acroleins (**3**) and the analogous compounds derived from amino acids, and their i.r. spectra showed bands for N–H, C=O, and C=C + N–H expected[12] for the HN–C=C–CH=O structure.



12 R=H
13 R=Me



14 R=H
15 R=Me



16 R=$CH_2CH(OMe)_2$
17 R=Me

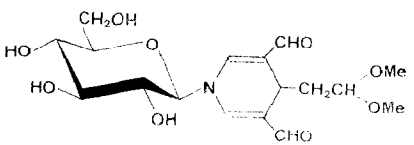


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TABLE II

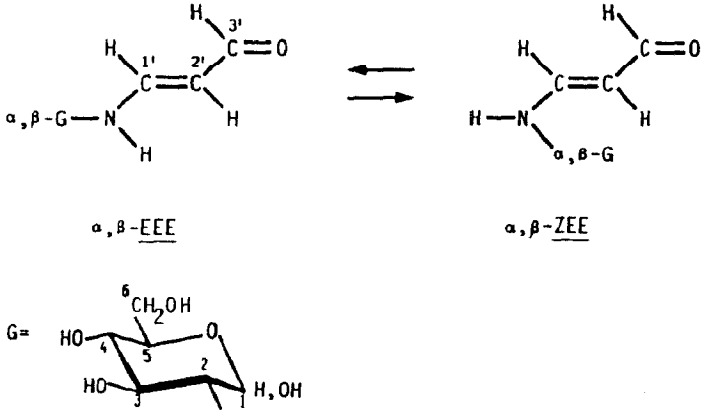
N.m.r. data (D_2O) (δ in p.p.m., J in Hz) for **12–15**

	12*α*		**12***β*		**13***α*	**13***β*	**14***β*	**15***β*
Atom	EEE	ZEE	EEE	ZEE				
H-1	5.19d ($J_{1,2}$ 3.5)		4.67d ($J_{1,2}$ 8.3)	4.69d ($J_{1,2}$ 8.3)	5.19d ($J_{1,2}$ 3.5)	4.70d ($J_{1,2}$ 8.4)	4.64d	4.50d ($J_{1,2}$ 8.3)
H-2	3.28dd ($J_{2,1}$ 3.5, $J_{2,3}$ 10.1)		2.98dd ($J_{2,1}$ 8.3, $J_{2,3}$ 10.1)		3.34dd ($J_{2,3}$ 10.0)	3.02dd ($J_{2,3}$ 10.1)		
H-3							3.6–3.4 m	3.6–3.4 m
H-4					3.7–3.4 m			
H-5	3.9–3.4 m		3.9–3.4 m					
H-6					3.81dd ($J_{6,5}$ 2.4, $J_{6,6'}$ 10.0)	3.82dd ($J_{6,5}$ 1.7, $J_{6,6'}$ 10.0)	3.90dd ($J_{6,5}$ 1.7, $J_{6,6'}$ 12.4)	3.79dd ($J_{6,5}$ 1.7, $J_{6,6'}$ 12.3)
H-6′						3.71dd ($J_{6',5}$ 3.7)	3.73dd ($J_{6',5}$ 4.8)	3.65dd ($J_{6',5}$ 4.3)
H-1′	7.43d ($J_{1',2'}$ 12.3)	7.50d ($J_{1',2'}$ 12.7)	7.42 ($J_{1',2'}$ 12.3)	7.55d ($J_{1',2'}$ 12.7)	7.21s	7.62d ($J_{1',2'}$ 13.0)	7.29s	
H-2′	5.39dd	5.44dd	5.39dd	5.47dd		5.56dd		
H-3′	8.62d ($J_{3',2'}$ 9.6	2 8.68d ($J_{3',2'}$ 9.1)	8.65d ($J_{3',2'}$ 9.6)	8.68d ($J_{3',2'}$ 9.1)	8.48s	8.50s	8.89d ($J_{3',2'}$ 9.1)	8.66s
Me					1.53s	1.53s		1.55s (3 H)
C-1	92.9	91.6	96.1	97.3	93.1	96.1	90.7	90.8
C-2	65.1	59.3	67.7	62.3	65.0	68.1	74.8	74.7
C-3	72.7[a]	73.1[a]	75.2	75.9	72.4	75.2	80.1	80.2
C-4	71.2[b]	71.2[b]	71.1[b]	71.1[b]	71.3	71.1	71.8	71.7
C-5	73.1[a]	73.1[a]	77.4	77.4	73.0	77.4	78.8	78.8
C-6	61.9[c]	61.9[c]	62.1[c]	62.1[c]	61.9	62.1	63.1	63.1
C-1′	167.3[d]	163.8	166.9[d]	164.6	164.5	165.1	166.4	163.6
C-2′	103.8[e]	101.7	103.6[e]	102.2	111.5	111.6	106.8	114.8
C-3′	193.6[f]	194.6	193.4[f]	194.7	192.3	192.2	196.4	195.5
Me					7.3	7.4		8.6

[a–f] Assignments could be interchanged.

The n.m.r. data (Table II) for **12** in D_2O revealed an equilibrium mixture of four isomeric forms, and the $J_{1',2'}$ (12.3–12.7 Hz) and $J_{2',3'}$ (9.1–9.6 Hz) values, by analogy with those[5c] for **3** derived from amino acids, indicated that the 3-oxo-2-propenyl group had the *EE* structure. Consequently, the isomers differed in anomeric configuration and in the *E* or *Z* configuration around the formal N–C-1′ bond (Scheme 2). The *α* anomers with the *EEE* and *ZEE* geometries had the same δ values for the sugar protons, and the *α* configuration was deduced from the $J_{1,2}$ value of 3.5 Hz. On the other hand, the *β* anomers with the *EEE* and *ZEE* geometries had $J_{1,2}$ 8.3 Hz. The ^{13}C-n.m.r. spectrum

contained four signals for anomeric carbons, the δ values of which also indicated **12** to be a mixture of two $\alpha\beta$-pairs. Integration of the appropriate ^{13}C and ^{1}H signals indicated the ratios ~4:3:4:2 for α-*EEE*, α-*ZEE*, β-*EEE*, and β-*ZEE* at equilibrium.



Scheme 2. Isomeric equilibria of compound **12** in aqueous solution. The symbols indicate, in the order shown, the alignments around the N–C-1′, C-1′–C-2′, and C-2′–C-3′ bonds in the delocalised system $N^{+}\text{----}C^{1'}\text{----}C^{2'}\text{----}C^{3'}\text{----}O^{-}$.

The n.m.r. spectra of **13** contained signals for the α and β anomers in the ratio ~2:3, with probably the same geometry in the *N*-substituent. It is reasonable to assume, by analogy with **12**, that the 3-oxo-2-propenyl moiety has the *EE* geometry.

The spectra of the glucosylamines **14** and **15** indicated them to be β anomers ($J_{1,2}$ ~8 Hz, δ ~91 for C-1). The $J_{1',2'}$ (13.0 Hz) and $J_{2',3'}$ (9.1 Hz) values for **14** indicated the *EE* alignment around the C-1′–C-2′ and C-2′–C-3′ bonds.

The 1,4-dihydropyridines **16**–**18** showed u.v. absorption (see Experimental) and fluorescence properties (λ^{Ex}_{max} 380–390, λ^{Em}_{max} 440–460 nm) similar to those[8] of **5** and **6**, and their i.r. spectra exhibited bands at ~1666 and ~1575 cm^{-1} attributable to the N–C=C–CH=O group. The ^{1}H and ^{13}C resonances for **16** and **17** (Table III) indicated $\alpha\beta$-mixtures in the ratios 2:3 and 1:2, respectively, whereas the data for **18** were consistent with the β anomer.

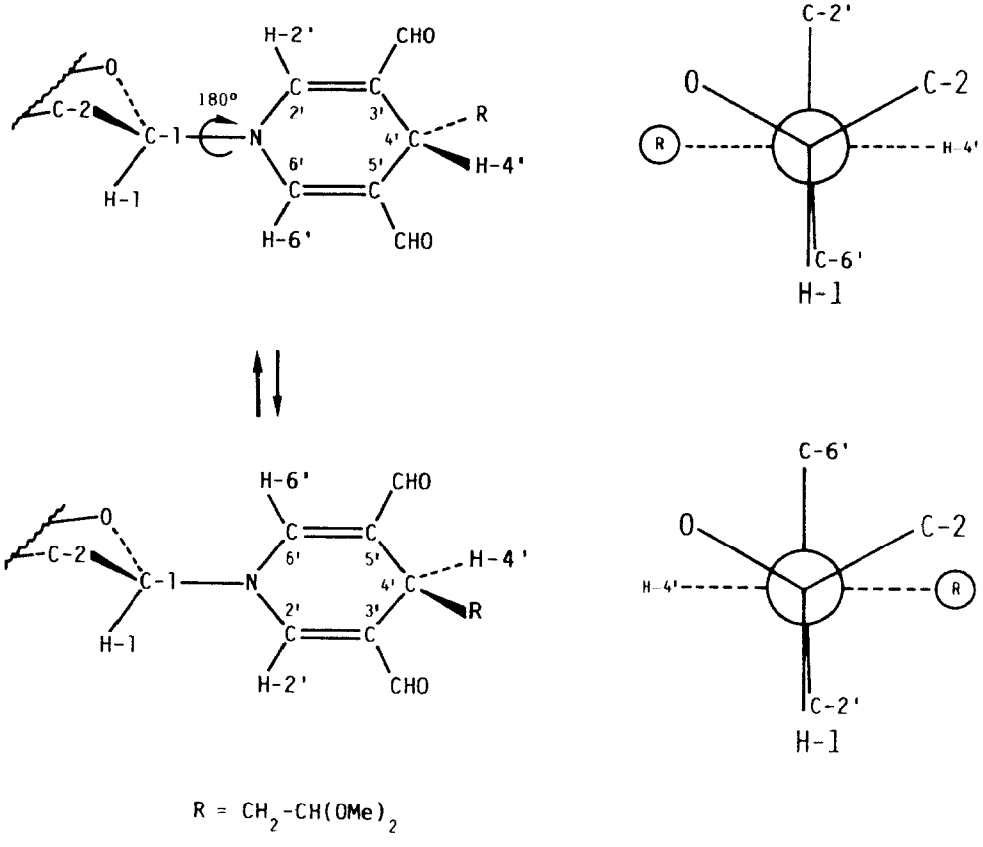
The ^{1}H-n.m.r. spectra of the dihydropyridine moieties of **16**–**18** contained slightly separated signals for H-2′ and H-6′, and for the CHO protons at positions 3′ and 5′. Similarly, the ^{13}C-n.m.r. spectra contained separate signals for the pairs C-2′,6′ and C-3′,5′, and also the CHO groups. The magnetic non-equivalence of these pairs of nuclei, which are symmetrical with respect to the plane of symmetry of the 1,4-dihydropyridine ring, probably reflects the presence of the chiral sugar substituent at the nitrogen and the Me (or dimethoxyethyl) substituent at position 4′. In the different conformations around the N–C-1 (or N–C-2) bond, the nuclei of each pair are always non-equivalent. Scheme 3 illustrates the situation for **18**.

TABLE III

N.m.r. data (δ in p.p.m., J in Hz) for **16–18**

Atom	**16***α*	**16***β*	**17***α*	**17***β*	**18***β*
H-1	5.40d ($J_{1,2}$ 3.2)	5.00d ($J_{1,2}$ 8.4)	5.33d ($J_{1,2}$ 3.2)	5.00d ($J_{1,2}$ 8.4)	4.84d
H-2		3.32dd ($J_{2,3}$ 10.8)		3.32dd ($J_{2,3}$ 10.8)	
H-3	4.14dd ($J_{3,2}$ 11.1, $J_{3,4}$ 8.8)		4.14dd ($J_{3,2}$ 11.1, $J_{3,4}$ 8.8)		
H-4					
H-5					4.0–3.5m
H-6	3.9–3.5m		3.9–3.5m		
H-6′					
H-2′	7.45d[a] ($J_{2,4'}$ 1.2)	7.38d[b]	7.36s[c]	7.28s[d]	7.57s(11 H)[e]
H-4′*					
H-6′	7.39d[a] ($J_{4,6'}$ 1.2)	7.34d[b] ($J_{4,6'}$ 1.2)	7.30s[c]	7.26s[d]	7.54s[e]
O / —CH \ O	1.66t (J 5.5)	1.66t			1.76t (2 H) (J 5.5)
	4.32t (J 5.5)	4.33t (J 5.5)			4.42t (1 H) (J 5.5)
OMe	3.17s	3.17s			3.27s (6 H)
CHO	9.11s (3 H) 9.07s (1 H)		9.09s (3 H) 9.07s (1 H)		9.23 (1 H)[f] 9.22s (1 H)[f]
Me			0.97d (3 H) (J 7.5)	0.96d (3 H) (J 7.5)	
C-1	92.8	94.8	92.7	94.8	91.7
C-2	69.8	68.5	69.8	68.5	69.7
C-3	72.0	73.1	71.9	73.0	79.2
C-4	71.6	71.4	71.5	71.4	71.9
C-5	72.9	77.3	73.0	77.3	76.9
C-6	61.9	62.0	61.8	61.9	61.3
C-2′	151.3[a]	152.7[b]	149.8[g]	151.8[g]	149.6[h]
C-6′	150.1[a]	151.0[b]	150.5[g]		148.2[h]
C-4′	25.6	25.5	23.6	23.8	25.1
C-3′	122.1[c]	124.7[e] 121.7	125.0[f]		122.0[i]
C-5′	122.0[c]		124.6[e]	124.9[f]	121.7[i]
CHO	194.2		194.6	194.4	193.8, 193.7
O / —CH \ O	37.7	38.1			37.3
	104.5	104.4			103.9
OMe	54.7[d] 54.3[d]	54.5[d] 54.3[d]			54.1 53.7
Me			22.5	22.7	

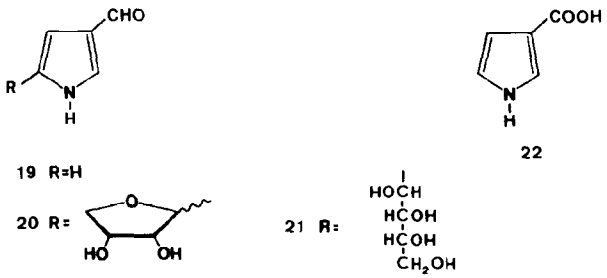
[a–i] Assignments could be interchanged; *overlapped in the sugar signals



Scheme 3. Partial perpective and Newman projection formulae for compound **18**, showing the magnetic non-equivalence of H-2',6', C-2',6', C-3',5', and the CHO groups. These nuclei exchange sites in a rotation of 180° around the N–C-1 bond, but do not have identical environments because of the presence of the substituent at C-4' (encircled in the background of the Newman projections).

When an aqueous solution of 2-amino-2-deoxy-D-glucose hydrochloride and **7** was heated, a mixture containing **12** and minor amounts of 3-pyrrolecarbaldehyde (**19**), 5-(*α,β*-D-erythrofuranosyl)-3-pyrrolecarbaldehyde (**20***αβ*), and 5-(D-*arabino*-tetritol-1-yl)-3-pyrrolecarbaldehyde (**21**) resulted. The yields of pyrroles increased with increasing pH, and the best yields were obtained (t.l.c. and ^{1}H-n.m.r. data) after 1 h at pH 9. ^{1}H-N.m.r. spectroscopy indicated that the transformation of **12** was complete and that **19**, **20***αβ*, and **21** were formed in the ratios ~3:1:2. These 3-pyrrolecarbaldehydes, particularly **19**, are unstable and turn brown during storage. Their isolation and purification could be achieved only by column chromatography followed by preparative t.l.c., and the yields were low.

The structures of **19**, **20***αβ*, and **21** were established on the basis of analytical and spectral data, and **19** was also characterised by conversion into 3-pyrrolecarboxylic acid[11] (**22**).



The formation of (alditol-1-yl)pyrroles from sugar enaminones similar to **12** is well documented, and mechanisms to explain the cleavage of the sugar chain have been proposed[9,13]. The dehydration of (D-*arabino*-tetritol-1-yl)pyrroles similar to **21**, to give (α,β-D-furanosyl)pyrroles, has also been investigated[9,13]; this reaction is catalysed by both acids and bases[13,14].

The above results show the ability of amino sugars to react with **1** and with such salts as **10**, derived therefrom, that might also be present among the products of lipid peroxidation. The resulting heterocyclic compounds could contribute to the browning of oxidised, lipid-rich foods. Further work using amino sugars more similar to those involved in the Maillard reaction is in progress.

EXPERIMENTAL

General methods. — Melting points were determined on a Reichert 222127 apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241MC polarimeter. U.v. spectra were recorded with a Hewlett–Packard 8450A spectrophotometer, fluorescence spectra (for solutions in MeOH) with a Perkin–Elmer LS-5 spectrophotometer [the relative molar intensity (RMI) was expressed against μM quinine sulfate in 0.05M sulphuric acid with excitation at 350 nm and emission at 450 nm], and i.r. spectra with a Perkin–Elmer 782 spectrometer. Elemental analyses were conducted at the Departamento de Química Analítica (University of Seville). N.m.r. spectra were recorded for solutions in D_2O with a Varian XL-200 spectrometer at 200 MHz for ^1H and 50.2 MHz for ^{13}C, using internal acetone (δ 2.17) and 1,4-dioxane (δ 67.4), respectively. Double resonance experiments were performed and proton-decoupled APT were obtained to assist in signal assignments. Mass spectra were obtained using a Kratos, MS-80RFA instrument. T.l.c. was performed on Silica Gel 60 F_{254} (Merck), with *A*, ethyl acetate–methanol (7:1), *B*, ethyl acetate–methanol–acetic acid–water (6:2:1:1); *C*, ethyl acetate–methanol–triethylamine–water (6:2:1:1); *D*, acetone–water (20:1); *E*, ethyl acetate–methanol (5:1); *F*, ether–ethanol (6:1); and *G*, ethyl acetate–methanol (3:1); and detection by u.v. light, iodine vapor, the Ehrlich reagent, and/or by charring with sulphuric acid. Preparative t.l.c. was performed on Silica Gel F_{254} (Merck) and column chromatography on Silica Gel 60 (230–400 mesh; Merck) and Aluminium Oxide 60 G (neutral, Merck) with solvents *D*; *H*, acetone–water (5:1); and *I*, acetone–water (10:1). Solutions were concentrated under diminished pressure at $< 30°$.

3-Ethoxy-2-methylpropenal and the sodium salt (**8**) of methylmalondialdehyde were prepared as described[5c].

Sodium salts of malondialdehyde[8c] (**7**), *2,4-diformyl-3-(2,2-dimethoxyethyl)glutaraldehyde* (**9**), *and 2,4-diformyl-3-methylglutaraldehyde* (**10**). — 1,1,3,3-Tetramethoxypropane (8.2 g, 50 mmol) and water (100 mL) were stirred with Dowex 50W-X8 (H^+) resin (40 g) for 1 h, when λ_{max} 267 nm had reached a maximum . The resin was removed, the pH of the filtrate was adjusted to **7** by the addition of 5M NaOH, and the solution was extracted with ethyl acetate (3 × 25 mL). The aqueous layer was concentrated to a thick syrup which was treated with acetone to give an orange solid ("crude" **7**). T.l.c. of this product revealed **7** (R_F 0.20, solvent *D*, main component), **9** (R_F 0.71), and **10** (R_F 0.0, R_F 0.40, solvent *A*, traces). Recrystallisation from acetone–water gave **7** monohydrate[5c,8c] (3.40 g, 75%), m.p. 240° (dec.), $\lambda_{max}^{H_2O}$ 266 nm (ε 30 000). ^{1}H-n.m.r. data (D_2O): δ 8.60 (d, 2 H, *J* 10.2 Hz) and 5.25 (t, 1 H, *J* 10.2 Hz).

1,1,3,3-Tetramethoxypropane (8.2 g) in water (100 mL) was treated with Dowex 50W-X8 (H^+) resin (30 g) for 0.5 h as described above. Column chromatography (silica gel, solvent *D*) of the resulting syrupy product gave **9** (0.8 g). Crystallisation and recrystallisation from methanol gave **9** as a hydrate, m.p. 140° (dec.), $\lambda_{max}^{H_2O}$ 249 nm (ε 21 327); υ_{max}^{KBr} 3470, 3223 (OH, enol), and 1597 cm^{-1} (C=C, enol). The n.m.r. data are given in Table I.

Anal. Calc. for $C_{11}H_{15}NaO_6 \cdot H_2O$: C, 46.69; H, 6.02. Found: C, 46.61; H, 5.78.

Eluted second was a mixture of **7** and **10**. Preparative t.l.c. (solvent *A*, 3 irrigations) yielded **10** (50 mg, 0.5%), m.p. 250° (dec., from acetone–water), $\lambda_{max}^{H_2O}$ 250 nm (ε 26 086); υ_{max}^{KBr} 3500, 3214 (OH, enol), and 1584 cm^{-1} (C=C, enol). The n.m.r. data are given in Table I.

Anal. Calc. for $C_8H_9NaO_4 \cdot 2.5H_2O$: C, 40.51; H, 5.94. Found: C, 40.28; H, 5.73.

Compound **10** was prepared more conveniently by storing a mixture of **7** (0.5 g, 5 mmol) and water (1 mL) with acetaldehyde (0.15 mL, 2.5 mmol) at room temperature overnight. The mixture was concentrated and the residue was treated with ethanol to give **10**, m.p. ~230° (dec.). Recrystallisation from acetone–water yielded the analytical sample (0.47 g, 41%), m.p. 250° (dec.).

The sodium salt of 2,4-diformyl-3-propylglutaraldehyde (**11**). — To a solution of **7** (188 mg, 1.68 mmol) in water (2 mL) was added butanal (72 mg, 1 mmol), and the solution was kept at room temperature. Monitoring of the reaction (u.v. and t.l.c.) indicated that the transformation of the reactants into **11** was complete in ~5 days. Concentration of the solution and treatment of the residue with ethanol gave a white powder (175 mg, 73%), recrystallisation of which from methanol afforded **11** as a hydrate; $\lambda_{max}^{H_2O}$ 250 nm (ε 26 297); υ_{max}^{KBr} 3438, 3200 (OH, enol), and 1602 cm^{-1} (C=C, enol). The n.m.r. data are given in Table I.

Anal. Calc. for $C_{10}H_{13}NaO_4 \cdot 0.25H_2O$: C, 53.45; H, 6.05. Found: C, 53.66; H, 6.19.

*2-Deoxy-2-[(3'-oxo-1'-propen-1'-yl)amino]-α,β-*D*-glucopyranose* (**12**). — To a solution of 2-amino-2-deoxy-D-glucose hydrochloride (1.0 g, 4.6 mmol) in water (4 mL) was added **7** (0.45 g, 4.0 mmol), and the solution was kept at room temperature overnight, then freeze-dried. The residual yellow thick syrup contained **12** (t.l.c.; R_F

0.54, solvent *B*) as the main component. Preparative t.l.c. (solvent *C*) of a portion (0.5 g) gave **12** (0.34 g, 70%), m.p. 179–181°, $[\alpha]_D^{15}$ −24° (*c* 0.3, water); $\lambda_{max}^{H_2O}$ 279 nm (ε 20643); υ_{max}^{KBr} 3395 (OH, NH), 1662 (C=O), and 1592 cm^{-1} (C=C–NH). The n.m.r. data are shown in Table II.

Anal. Calc. for $C_9H_{15}NO_6$: C, 46.55; H, 6.47; N, 6.00. Found: C, 46.47; H, 6.48; N, 5.72.

A smaller yield (15%) of **12** was obtained by column chromatography (solvent *H*) on neutral alumina.

*2-Deoxy-2-[(2'-methyl-3'-oxo-1'-propen-1'-yl)amino]-α,β-*D*-glucopyranose* (**13**). — To a solution of 2-amino-2-deoxy-D-glucose hydrochloride (2.0 g, 9.2 mmol) in water (4 mL) was added **8** (1.0 g, 9.2 mmol). The solution was kept at room temperature overnight, then freeze-dried. Column chromotography (neutral alumina, solvent *I*) of the syrupy residue afforded **13** (1.49 g, 65%), m.p. 188–189° and 195–196°. Recrystallisation from methanol gave the analytical sample, m.p. 195° and 202–203°, $[\alpha]_D^{15}$ −16° (*c* 0.5, water), R_F 0.62 (solvent *D*); $\lambda_{max}^{H_2O}$ 284 nm (ε 42470); υ_{max}^{KBr} 3500–3100 (OH, NH), 1658 (C=O), and 1598 cm^{-1} (C=C–NH). The n.m.r. data are given in Table II.

Anal. Calc. for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.92; N, 5.66. Found: C, 48.72; H, 6.86; N, 5.61.

*3-(β-*D*-Glucopyranosylamino)-2-propenal* (**14**). — To a solution of *β*-D-glucopyranosylamine (1 g, 5.6 mmol) in water (2 mL) were added **7** (0.6 g, 5.6 mmol) and M HCl (6 mL). The solution was kept at room temperature overnight, then freeze-dried. The residual syrup was treated with ethanol, the solid (0.41 g) was collected, and the filtrate was concentrated to dryness. The residue crystallised from methanol to give **14** (79 mg, 7%), m.p. 182–184°, $[\alpha]_D^{15}$ −61° (*c* 0.8, water), R_F 0.35 (solvent *C*); $\lambda_{max}^{H_2O}$ 279 nm (ε 19586); υ_{max}^{KBr} 3361 (OH, NH), 1615 (C=O), and 1550 cm^{-1} (C=C–NH). The n.m.r. data are given in Table II.

Anal. Calc. for $C_9H_{15}NO_6$: C, 46.35; H, 6.47; N, 6.00. Found: C, 46.17; H, 6.41; N, 5.95.

*3-(β-*D*-Glucopyranosylamino)-2-methyl-2-propenal* (**15**). — A solution of *β*-D-glucopyranosylamine (0.5 g, 2.8 mmol) in water (2 mL) was stirred overnight at room temperature with 3-ethoxy-2-methylpropenal (0.33 g, 3 mmol), then freeze-dried. Column chromatography (neutral alumina, solvent *H*) of the residue gave **15** (0.19 g, 30%), isolated as a syrup having R_F 0.42 (solvent *D*), $[\alpha]_D^{15}$ −12° (*c* 0.3, water); $\lambda_{max}^{H_2O}$ 287 nm (ε 34750); υ_{max}^{KBr} 3397 (OH, NH), 1660 (CHO), and 1607 cm^{-1} (C=C). The n.m.r. data are given in Table II.

Anal. Calc. for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.92; N, 5.66. Found: C, 48.58; H, 6.76; N, 5.79.

*2-Deoxy-2-[3',5'-diformyl-4'-(2,2-dimethoxyethyl)-1',4'-dihydropyridin-1'-yl]-α,β-*D*-glucopyranose* (**16**) *and 2-deoxy-2-(3',5'-diformyl-4'-methyl-1',4'-dihydropyridin-1'-yl)-α,β-*D*-glucopyranose* (**17**). — A solution of 2-amino-2-deoxy-D-glucose hydrochloride (1.0 g, 4.6 mmol) in water (2 mL) was treated with "crude" **7** (0.45 g, 4.0 mmol) as described above for the preparation of **12**. Column chromatography (neutral alumina, solvent *H*) of the freeze-dried reaction mixture afforded a fraction (0.1 g) containing

(t.l.c., n.m.r.) **16** (main component; R_F 0.78, solvent *B*) and **17** (R_F 0.60, solvent *E*). Preparative t.l.c. (solvent *A*, 2 irrigations) afforded **16** (68 mg) and **17** (5 mg).

Compound **16**, isolated as a syrup, had $[\alpha]_D^{15}$ +74° (*c* 1, water); λ_{max}^{MeOH} 237, 264, and 385 nm (ε 14 740, 9128, and 8915); λ_{max}^{Ex} 380 nm, λ_{max}^{Em} 465 nm (RMI, 0.68); υ_{max}^{KBr} 3391 (OH), 1666 (C=O), and 1575 cm^{-1} (C=C). The n.m.r. data appear in Table III.

Anal. Calc. for $C_{17}H_{25}NO_9 \cdot 0.5H_2O$: C, 51.41; H, 6.59; N, 3.53. Found: C, 51.01; H, 6,23; N, 3.56.

Compound **16** was also prepared by adding **9** (26 mg, 0.09 mmol) to an aqueous solution of 2-amino-2-deoxy-D-glucose hydrochloride (20 mg, 0.09 mmol) and isolated (15 mg, 43%) by preparative t.l.c. (solvent *D*).

Compound **17**, isolated as a syrup, had $[\alpha]_D^{15}$ +43° (*c* 0.3, water); λ_{max}^{MeOH} 237, 265, and 389nm (ε 15 603, 7220, and 8903); λ_{max}^{Ex} 389 nm, λ_{max}^{Em} 448 (RMI, 0.50); υ_{max}^{KBr} 3417 (OH), 1663 (C=O), and 1570 cm^{-1} (C=C). The n.m.r. data appear in Table III.

Anal. Calc. for $C_{14}H_{19}NO_7$: C, 53.67; H, 6.11; N, 4.47. Found: C, 53.88; H, 6.23; N, 4.50.

Compound **17** was obtained better by treating 2-amino-2-deoxy-D-glucose hydrochloride (0.1 g, 0.5 mmol) in water (2 mL) with **10** (0.11 g, 0.5 mmol) at room temperature overnight. The mixture was freeze-dried, and preparative t.l.c. (silica gel, solvent *E*) of the residue afforded **17** (82 mg, 47%).

3',5'-Diformyl-1'-β-D-glucopyranosyl-4'-(2,2-dimethoxyethyl)-1',4'-dihydropyridine (**18**). — A solution of β-D-glucopyranosylamine (0.5 g, 2.8 mmol) in water (2 mL) was treated with "crude" **7** (0.26 g) and M HCl (3 mL), as described for the preparation of **14**. Preparative t.l.c. (solvent *D*) of the syrup obtained after freeze-drying afforded **18** (50 mg, 5%), isolated as a yellow syrup, R_F 0.54 (solvent *D*), $[\alpha]_D^{15}$ +26° (*c* 0.5, water); λ_{max}^{MeOH} 233, 259, and 372 nm (ε 18 216, 9725, and 7106); λ_{max}^{Ex} 382 nm, λ_{max}^{Em} 446 nm (RMI, 0.70); υ_{max}^{KBr} 3431 (OH), 1667 (C=O), and 1580 cm^{-1} (C=C). The n.m.r. data are given in Table III.

Anal. Calc. for $C_{17}H_{25}NO_9$: C, 52.70; H, 6.50; N, 3.61. Found: C, 52.95; H, 6.68; N, 3.70.

Formation of 3-pyrrolecarbaldehyde (**19**), *5-(α,β-D-erythrofuranosyl)-3-pyrrolecarbaldehyde* (**20αβ**), *and 5-(D-arabino-tetritol-1-yl)-3-pyrrolecarbaldehyde* (**21**) *from* (**12**). — (*a*) A solution of 2-amino-2-deoxy-D-glucose hydrochloride (1.0 g, 4.4 mmol) and **7** (0.45 g, 4.0 mmol) in water (3 mL) was stored at room temperature for 24 h. The λ_{max} at 279 nm and t.l.c. then indicated that the concentration of **12** had reached a maximum. The pH was adjusted to 9 with saturated aq. sodium carbonate and the solution was heated at 90° for 1 h. T.l.c. (solvent *F*) then indicated the complete transformation of **12**, and the formation of the pyrroles **19** (R_F 0.83), **20αβ** (R_F 0.5), and **21** (R_F 0.36, solvent *G*). The mixture was concentrated. Elution of the residue from silica gel with ether gave **19** (60 mg, 14%), isolated as a yellow syrup that turned red on standing; λ_{max}^{MeOH} 245, 271 (sh) nm (ε 9775 and 6030). N.m.r. data (D_2O): ^1H, δ 9.49 (d, 1 H, $J_{CHO,5}$ 0.8 Hz, CHO), 7.64 (t, 1 H, $J_{2,4} = J_{2,5}$ = 1.8 Hz, H-2), 6.90 (ddd, 1 H, H-5), and 6.57 (dd, 1 H, $J_{4,5}$ 3.1 Hz, H-4); ^{13}C, δ 190.3 (CHO), 132.5 (C-2), 125.9 (C-3), 122.9 (C-5), and 106.9 (C-4). Mass spectra: *m/z* 95 [100%, $(M+1)^+$], 94 (83), 60 (11); *m/z* 95.0345 (calc. for C_5H_5NO: 95.1030).

Treatment[11] of **19** (20 mg) with silver oxide gave 3-pyrrolecarboxylic acid (**22**, 4 mg), m.p. 140–143°; lit.[11] m.p. 144°.

Elution with ether–ethanol (6:1) gave **20**$\alpha\beta$, isolated as a syrup (32 mg, 3%), $[\alpha]_D^{15}$ –20° (*c* 0.9, water); λ_{max}^{MeOH} 245 and 271 nm (ε 6571 and 4823). N.m.r. data D_2O): ^1H, α anomer, δ 9.47 (s, 1 H, CHO), 7.67 (d, 1 H, $J_{2,4}$ 1.8 Hz, H-2), 6.59 (d, 1 H, H-4), 5.03 (d, 1 H, $J_{1',2'}$ 4.9 Hz, H-1'), 4.4–4.5 (m, 2 H, H-2',3'), 3.96 (dd, 1 H, $J_{3',4'}$ 6.0, $J_{4',4''}$ 9.3 Hz, H-4'), and 3.82 (dd, 1 H, $J_{3',4''}$ 3.0 Hz, H-4''); β anomer, δ 9.49 (s, 1 H, CHO), 7.69 (d, 1 H, $J_{2,4}$ 1.7 Hz, H-2), 6.65 (d, 1 H, H-4), 4.73 (d, 1 H, H-1'), 4.34 (m, 1 H, H-3'), 4.25 (dd, 1 H, $J_{2',3'}$ 4.6 Hz, H-2'), 4.23 (dd, 1 H, $J_{3',4'}$ 4.1, $J_{4',4''}$ 10.4 Hz, H-4'), and 3.84 (dd, 1 H, $J_{3',4''}$ 1.8 Hz, H-4''); ^{13}C, α anomer, δ 190.2 (CHO), 133.4 (C-2), 133.2 (C-5), 126.0 (C-3), 107.2 (C-4), 77.1, 73.2, 72.1, and 71.5 (C-1',2',3',4'); β anomer, δ 190.2 (CHO), 133.4 (C-2), 133.2 (C-5), 126.0 (C-3), 106.3 (C-4), 76.3, 73.6, and 71.6 (C-1',2',3',4'); $\alpha\beta$-ratio ~1:3.

Anal. Calc. for $C_9H_{11}NO_4$: C, 54.81; H, 5.62; N, 7.10. Found: C, 55.12; H, 5.88; N, 7.31.

Elution with methanol gave **21** (400 mg), preparative t.l.c. (solvent *G*, 2 irrigations) of which afforded **21** (80 mg, 8%), m.p. 144–146°. Recrystallisation from ethanol gave the analytical sample, m.p. 149–150°, $[\alpha]_D^{15}$ –28° (*c* 0.4, water); λ_{max}^{MeOH} 247 and 271 (sh) nm (ε 5093 and 2369); υ_{max}^{KBr} 3500–3200 (OH, NH), 1647 (C=O), and 1524 cm^{-1} (C=C, pyrrole). N.m.r. data (D_2O): ^1H, δ 3.44–3.79 (m, 4 H, H-2',3',4',4'), 4.94 (d, 1 H, $J_{1',2'}$ 2.5 Hz, H-1'), 6.54 (d, 1 H, $J_{2,4}$ 1.8 Hz, H-4), 7.65 (d, 1 H, H-2), and 9.48 (s, 1 H, CHO); ^{13}C, δ 190.3 (CHO), 136.5 (C-5), 132.7 (C-2), 125.9 (C-3), 104.6 (C-4), 74.5, 72.0, and 67.2 (C-1',2',3'), and 63.5 (C-1').

Anal. Calc. for $C_9H_{13}NO_5$: C, 50.23; H, 6.08; N, 6.50. Found: C, 49.97; H, 6.05; N, 6.20.

(*b*) A solution of **12** (28 mg, 0.8 mmol) and Na_2CO_3 (14 mg) in D_2O (0.5 mL, pH 9) was heated at 90°. Monitoring (t.l.c., ^1H-n.m.r. spectroscopy) of the reaction indicated that the transformation of **12** was complete in ~1 h, affording **19**, **20**$\alpha\beta$, and **21** in the ratios 3:1:2.

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