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BYPRODUCTS IN THE PREPARATION OF PENTA-*O*-ACETYL-D-HEXONONITRILES FROM D-GALACTOSE AND D-GLUCOSE

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ABSTRACT

The procedures commonly employed for preparing per-*O*-acetylated aldononitrile derivatives of D-galactose and D-glucose for use in g.l.c. analysis involve oxime formation, and acetylation. They do not, however, give the nitriles as the sole products, as is generally assumed. Instead, *N*-hydroxy-D-glycosylamine hexaacetates are formed as byproducts, in part by a kinetically controlled ring-closure of the sugar oxime, concomitant with acetylation. From preparative isolation of the products, D-galactose gave the nitrile (67%), and the β-furanose (16%), α-furanose (1%), and β-pyranose (2%) isomers of the cyclic hexaacetate, whereas D-glucose gave the nitrile (63%), and the β-furanose (14%) and β-pyranose (8%) isomers.

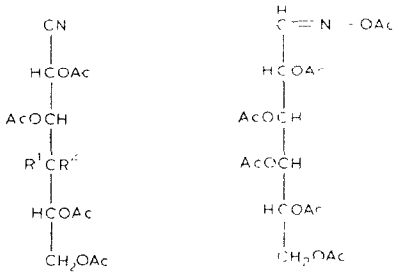
INTRODUCTION

An apparently attractive method for the quantitative analysis of sugars involves their conversion into per-*O*-acetylated aldononitrile (PAAN) derivatives followed by g.l.c. analysis, by which means, the derivatives are readily separable[1–13]. The traditional preparation of PAAN derivatives involves treatment of the sugars in pyridine with hydroxylamine hydrochloride (to form the oxime) and then acetic anhydride (to form the per-*O*-acetylated nitrile), both steps being conducted *in situ* at 60–100°. It has been tacitly, or specifically, assumed by a number of workers, who have published extensively on the advantageous use of PAAN derivatives, that the nitriles are formed in virtually quantitative yield[1–13].

The early work of Deulofeu and Wolfrom and their co-workers[14–17], which examined the acetylation of the *crystalline* oximes of various sugars had indicated, however, that both cyclic and acyclic hexaacetates can be isolated from product mixtures, along with the nitriles. D-Galactose oxime in acetic anhydride–pyridine at 25° yielded a mixture containing D-galactononitrile pentaacetate (**1**), an acyclic oxime hexaacetate (**3**), and a cyclic oxime hexaacetate for which a specific ring-structure was not assigned in the original paper[14], but for which the β-pyranose form (**4**) was indicated in a later review[15]. When strongly heated (*e.g.*, with acetic anhydride–sodium acetate at reflux), acyclic oxime **3** eliminated acetic acid to give

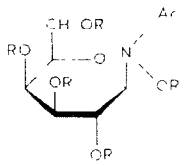
nitrile **1**, but it was stable in acetic anhydride–pyridine for 1 h at 70°. The cyclic oxime derivative could not be converted into **1**.

Treatment of D-glucose oxime with acetic anhydride–pyridine also provided a cyclic oxime hexaacetate (tentatively assigned[17] the β-pyranose form **6**), in addition to the D-gluconononitrile **2**, but D-mannose oxime gave only acyclic products, the nitrile being the exclusive product at reaction temperatures above 30°. By contrast, the oximes of D-arabinose, D-xylose, and L-rhamnose gave the nitriles exclusively at all temperatures[16].

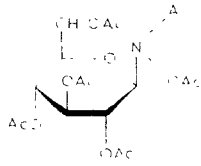


1 R^1 = OAc, R^2 = H
2 R^1 = H, R^2 = OAc

3



4 R = Ac
5 R = H



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Thus, when Lance and Jones[1] described the first application of PAAN derivatives for the g.l.c. analysis of partially methylated xylose derivatives, it may well have been the case that a single derivative was formed in quantitative yield from each pentose. The procedure was then generalized for the analysis of monosaccharides and their partially methylated derivatives by Eastwood and Huff[2] and by Dmitriev and co-workers[3], who apparently were unaware of the early results[14–17], and the erroneous assessment that nitriles are always the sole products of the derivatization procedure appears to stem from their work.

Because of the apparent conflict between the early synthetic studies and the more recent work related to g.l.c. analysis, the PAAN derivatization of D-galactose and D-glucose has now been re-examined, in order to clarify the nature of the reaction mixtures, and to determine the structures of any byproducts.

RESULTS AND DISCUSSION

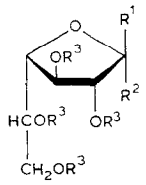
PAAN derivatization of D-*galactose.* — The derivatization of D-galactose was conducted under the various conditions of temperature, time, and concentration that have been recommended as suitable for subsequent g.l.c. analysis of the samples[2,4], including the modification employing 1-methylimidazole as the solvent and catalyst[12]. In each case, t.l.c. and l.c. examination revealed components of mobility lower than that of nitrile **1**. On a capillary, g.l.c. column[9], these components gave broad, poorly shaped peaks at retention times much longer than that of nitrile **1**. On a packed g.l.c.

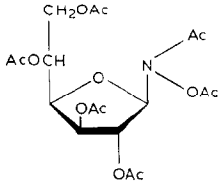
column, they were even more diffuse, leading to base-line disturbances during sequential analyses, but their presence could be confirmed by using an extended temperature-program.

The products from preparative-scale, PAAN derivatization of D-galactose were fractionated by a combination of crystallization and column chromatography. Whereas the major product expected, D-galactononitrile pentaacetate (**1**), was isolated crystalline in 66.5% yield, three *N*-hydroxy-D-galactosylamine hexaacetate isomers were also obtained, in a combined yield of 19.0%. Specifically, the crystalline β-furanose (**7**) and α-furanose (**8**) isomers, and the syrupy β-pyranose (**4**) isomer were respectively isolated in 15.9, 1.2, and 1.9% yield. Evidence for the structures assigned is presented later. The sample containing the β-pyranose isomer **4** was contaminated by 10% each of the other isomers (**7** and **8**), but on *O*-deacetylation and crystallization, it afforded pure *N*-acetyl-*N*-hydroxy-β-D-galactopyranosylamine (**5**). Syrupy *N*-acetyl-*N*-hydroxy-β-D-galactofuranosylamine (**9**) was similarly obtained by *O*-deacetylation of **7**.

On the basis of melting-point and specific-rotation data, the major cyclic isomer characterized here as *N*-hydroxy-β-D-galactofuranosylamine hexaacetate (**7**) corresponds to the cyclic oxime hexaacetate isolated by Deulofeu and co-workers[14,15] from the acetylation of crystalline D-galactose oxime, which was reported[15] to have the β-pyranose structure **4**.

PAAN derivatization of D-*glucose.* — Next, the PAAN derivatization of D-glucose was examined. As expected, D-gluconononitrile pentaacetate (**2**) was the major product, isolated crystalline in 63.0% yield, but again, cyclic oxime derivatives were present in significant proportions. These isomeric *N*-hydroxy-D-glucosylamine hexaacetates had very similar chromatographic mobilities, and they were initially isolated as a syrupy mixture in 28% yield. ^{13}C-N.m.r. examination of this mixture indicated that the β-pyranose isomer **6**, the β-furanose isomer **10**, and two minor isomers,





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7 R^1 = N(Ac)(OAc), R^2 = H, R^3 = Ac

8 R^1 = H, R^2 = N(Ac)(OAc), R^3 = Ac

9 R^1 = N(Ac)(OH), R^2 = R^3 = H

TABLE I

^{1}H-N.M.R. DATA[a] FOR COMPOUNDS **4–10**

Compound	*Solvent*	*Temp* (°C)	*Chemical shift (δ) and multiplicity*							
			H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	*Acetate*
4	C_6D_6	75	← 5.6–5.8m[b] →		5.23dd	5.47dd	3.70o	← 4.0–4.2m →		1.89, 1.85, 1.85, 1.79, 1.79, 1.73
5	D_2O	30	5.43d (0.75H) 5.11d (0.25H)				← 3.4–4.3m →			2.29
6	C_6D_6	75	← 5.0–6.0m[b] →				3.41o[a]	4.25dd	3.97dd	1.82, 1.81, 1.76, 1.74, 1.73, 1.72
	$C_6D_5NO_2$	140	5.91m[c]	← 5.1–5.7m →			4.00o	← 4.2–4.4m →		2.20, 2.15, 2.04, 2.02, 2.00, 1.99
7	$CDCl_3$[d]	30	6.05dt[e]	5.47t[e]	5.17dd	4.31dd	5.27dt	4.32dd	4.18dd	2.28, 2.16, 2.11, 2.10, 2.05
8	C_6D_6	75	6.30d[b]	5.34dd	5.79t[b]	3.90dd	5.50o[b]	4.43dd	4.20dd	2.03, 1.89, 1.86, 1.83, 1.77, 1.72
9	$(CD_3)_2SO$[f]	30	5.66d	← 3.6–4.3m[g] →				← ~3.4m →		2.05
10	C_6D_6	75	6.20d	5.35dd	5.51dd	4.22dd	5.42o[h]	4.68dd	4.11dd	1.94, 1.88(×2), 1.82, 1.79, 1.71

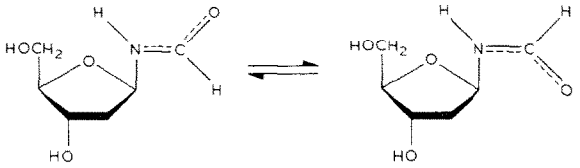
[a]80-MHz. [b]Strongly broadened in 30° spectrum. [c]Virtually coupled doublet-like resonance. [d]270-MHz. [e]Broadened. [f]4,4-Dimethyl-4-silapentane-1-sulfonic acid as the internal standard. [g]Includes 4.23, t, J ~ 7 Hz. [h]Partly merged with adjacent resonances.

probably the α-pyranose and α-furanose compounds, were present in the ratios of 3 : 5 : 1 : 1. The two minor compounds were not further investigated, but small samples of crystalline **6** and syrupy **10** were obtained by repeated chromatographic fractionation. The isomer identified here (see later) as the β-pyranose **6** was found to be identical to the product obtained by low-temperature acetylation of crystalline D-glucose oxime. The β-pyranose structure had been assigned only tentatively to the latter product by Wolfrom and Thompson[17], but this ring form would be anticipated from more recent work that has shown that crystalline D-glucose oxime exists in the β-pyranose form[18].

Temperature-dependent, n.m.r. spectra. — A characteristic feature of these cyclic oxime derivatives is the temperature-dependence of both their ^{1}H- and ^{13}C-n.m.r. spectra (see Tables I and III). Resonances, particularly those of H-1 and C-1, were in many instances broadened in spectra recorded at 30°, but were considerably sharpened at higher temperatures. In addition, the relatively sharp, H-1 and H-2 signals in the spectrum ($CDCl_3$, 30°) of the β-D-galactofuranose derivative **7**, recorded at field strengths of 60 and 80 MHz, were severely broadened at 270 MHz.

The dependence of these spectra on the temperature and field strength is consistent with the presence in solution of interconverting, geometric isomers associated with restricted rotation within the amide (*i.e.*, *N*-acetyl) functionality of these *N*-acetyl-*N*-hydroxy-glycosylamine derivatives. The lowest temperature (30°) used in these studies was above the coalescence temperature for all of the compounds reported, with the exception of *N*-acetyl-*N*-hydroxy-β-D-galactopyranosylamine (**5**). This compound displayed clearly different H-1 and C-1 resonances for the two isomeric forms at 30°, these resonances being severely broadened at 65°.

In agreement with such an explanation, the ^{1}H- and ^{13}C-n.m.r. spectra of the related *N*-formyl-(2-deoxy-β-D-*erythro*-pentofuranosylamine) (**11**) have been reported to display temperature-dependence (with a coalescence temperature of 96° for the proton spectrum of **11** in D_2O), due to *cis-trans* amide isomerism in the preponderant, *anti* orientation shown[19].



For the hydroxylamine derivatives reported here, an alternative explanation that would also account for the observed n.m.r. effects, could be that the nitrogen atom is non-planar and, hence, chiral, and that the resulting diastereoisomers interconvert by inversion at the nitrogen atom. The validity of this postulate was not determined, except to note that the barrier to inversion at nitrogen is generally in-

TABLE II

^{1}H-N.M.R. COUPLING-CONSTANTS[a] FOR COMPOUNDS **4**–**10**

Compound	*Solvent*	*Temp.* (°)	*Coupling constants (Hz)*						
			$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
4	C_6D_6	75	- -	10.0	3.4	1.3	5.9	7.2	-
5	D_2O	30	8.6 8.4						
6	C_6D_6	75	-		-	9.2	4.6	2.5	12.6
	$C_6D_5NO_2$	140	9.3[b]	-					
7	$CDCl_3$[c]	30	4.2	4.0	5.4	4.0	4.5	7.0	11.9
8	C_6D_6	75	6.8	7.7	7.7	5.6	4.2	6.5	12.0
9	$(CD_3)_2SO$	30	6.4						
10	C_6D_6	75	3.2	1.4	4.5	8.5	2.7	6.0	- 12.2

[a]80-MHz. [b]Separation of strong, outer lines in multiplet. [c]270-MHz.

creased by an oxygen substituent[20], and that substantial barriers, of ~ 50 kJ.mol^{-1} (~ 12 kcal.mol^{-1}), have been reported for trialkyl-substituted hydroxylamines[21].

Assignment of ring size and anomeric configuration. – The ring sizes of the five acetylated, cyclic oximes encountered in this study were determined from ^{1}H-n.m.r. data (see Tables I and II), and their anomeric configurations were elucidated from optical-rotation and ^{13}C-n.m.r.-spectral data (see Table III).

The ^{1}H-n.m.r. spectra of compounds **7**, **8**, and **10** were completely assigned by first-order analysis. Confirmation that correct assignments had been made came from decoupling (for **7**), and, for **8** and **10**, simulation experiments. These three products were identified as furanosyl derivatives, as, in each spectrum, the H-4 resonance was at higher field than those of H-2, -3, and -5.

The ^{1}H-n.m.r. spectra of compounds **4** and **6** were only partially resolved. In both, however, the ring-proton resonance at highest field was readily assigned to H-5 from its coupling to three adjacent protons, indicating that compounds **4** and **6** have the pyranoid structure

The position of the C-1 resonance was not a useful criterion of ring size in these derivatives; *e.g.*, the C-1 resonances for the galactopyranosyl (**4**) and galactofuranosyl (**8**) derivatives have similar chemical shifts. The remaining ring-carbon resonances were, however, of value in confirming the assignments made. Although specific assignments for C-2, -3, and -4 were not attempted, the spectral region containing these signals in the case of the galactofuranosyl derivatives **7** and **8** extended to substantially lower field than that containing the same resonances in the spectrum of the galactopyranosyl derivative **4**, although there was some overlap between these regions. The same comparison holds for the glucofuranosyl (**10**) *versus* the glucopyranosyl derivative **6**. These observations are consistent with the generalization that the ring-carbon resonances of underivatized furanose compounds are located

TABLE III

^{13}C-N.M.R.-SPECTRAL DATA[a] FOR COMPOUNDS **1** AND **4–10**

Com-	*Solvent*	*Temp. (°C)*	*Signal of atom*						
			C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*Acetate*[a]
1	$CDCl_3$	30	114.1	67.4[b]	67.2[b]	[b]	61.6	59.5	20.1, 20.4, 20.6; 168.8, 168.9, 169.5, 170.1, 170.3
4	$CDCl_3$	30	82.3[c]	72.9[d]	71.8[d]	67.2[d]	64.6[d]	61.1	
	C_6D_6	75	84.2	73.3[d]	72.4[d]	67.9[d]	65.4[d]	61.3	17.6 (*N*Ac); 19.9, 20.0(×2), 20.3, 20.4
5	D_2O	30	83.9 (0.75*C*) 88.6 (0.25*C*)	77.8[d]	74.2[d]	69.5[d]	67.2 (0.75*C*)[d] 66.8 (0.25*C*)	61.7	
		75	84.3[c]	78.1[d]	74.5[d]	69.8[d]	67.3[c,d]	62.1	21.0
6	C_6D_6	30	82.5[c]	67.3[d]	74.2	68.0[d]	74.2	63.2	17.7 (*N*Ac); 19.9, 20.2, 20.4; 168.2, 169.0, 169.5, 169.9
	$C_6D_5NO_2$[e]	140	84.9	70.1[d]	75.4[f]	68.9[d]	75.1[f]	63.2	18.0 (*N*Ac); 20.7, 20.4(×4)
7	$CDCl_3$	30	90.0	81.5[d]	77.4[d]	76.7[d]	69.9	62.4	18.3 & 167.8 (*N*Ac); 20.6, 20.7; 169.5, 169.6, 169.9, 170.3
8	$CDCl_3$	30	83.6[c]	76.4[d]	74.2[d]	72.3[c,d]	69.5	62.2	18.8 (*N*Ac);
	C_6D_6	75	84.1	76.8[d]	74.7[d]	73.1[d]	70.2	62.6	18.3 & 167.5 (*N*Ac); 19.9, 20.0(×2), 20.2, 20.4
9	D_2O	30	87.1[c]	82.0[d]	76.3[d]	75.2[d]	70.9	63.3	20.9
	$(CD_3)_2SO$	30	86.3[c]	81.0[d]	75.3[d]	74.6[d]	70.0	62.5	20.9 & 172.0[c] (*N*Ac)
10	C_6D_6	30	89.8[c]	78.7[d]	77.7[d]	74.3[d]	67.9	63.2	17.7 (*N*Ac); 19.9, 20.2, 20.4; 168.2, 169.0, 169.5, 169.9
		75	90.1	78.9[d]	78.0[d]	74.8[d]	68.4	63.4	17.9 (*N*Ac); 20.0, 20.3, 20.5; 168.3, 169.1(×2), 169.6 170.1, 170.3

[a]Recorded at 20 MHz. [b]Only two signals observed for C-2, -3, and -4. [c]Broadened. [d,f]Assignments interchangeable within a row. [e]Referenced to central peak in the *meta*-^{13}C triplet of nitrobenzene as 129.5 p.p.m. Carbonyl signals were not always sufficiently intense to be detected.

substantially downfield of those in the spectra of configurationally related pyranoses[22], bearing in mind that *O*-acetylation has a relatively small effect on carbon chemical-shift[22].

It was concluded that both the D-galactopyranosyl and D-glucopyranosyl derivatives **4** and **6** have the β configuration. ^{1}H-N.m.r. spectroscopy of the *galacto* hexaacetate **4** was not helpful, as its H-1 resonance was merged with other signals, but compound **5**, produced by *O*-deacetylation of **4**, displayed H-1 resonances for two isomeric forms both of which had a large $J_{1,2}$ value (8.4 and 8.6 Hz). This confirmed the β configuration of **5** and, hence, of **4**. That the *gluco* isomer **6** also had the β configuration may be inferred from the fact that the crystalline form of D-glucose oxime exists in the β-pyranoid form[18], and that acetylation of this form provided **6** as the sole product. In addition, in the ^{1}H-n.m.r. spectrum of **6**, H-1 appears as a doublet-like resonance at lowest field (5.71 p.p.m.). The complexity of this resonance is probably due to virtual coupling. The strong, outer lines of this multiplet are separated by 9.3 Hz, which is a good indication that $J_{1,2}$ is large, and that **6** has the β configuration.

The galactofuranosyl pair **7** and **8** are considered to be the β and α anomer, respectively, as their optical-rotation and ^{13}C-n.m.r.-spectral data independently lead to the same conclusion. Certain reservations as to the interpretation of these data are, however, discussed later. Hudson's rules of isorotation indicate that the more-levorotatory isomer **7** (−27.9°) has the β, whereas the dextrorotatory isomer **8** (+75.4°) has the α, configuration. These rules, have, however, proved unreliable in predicting anomeric configuration in certain related instances [*e.g.*, pyrimidine nucleosides[23] and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-D-glucopyranoses[24]], where it has been postulated that restricted rotation, or inversion at the nitrogen atom, leads to chirality that dominates the rotation observed. With this reservation in mind, independent confirmation of the anomeric configuration of the aforementioned pair was sought from ^{13}C-n.m.r.-spectral data. The C-1 and C-2 resonances of furanosyl derivatives are mutually deshielded in compounds bearing 1,2-*cis*- compared to 1,2-*trans*-related substituents[25,26]. Assuming that this correlation is sufficiently insensitive to conformational variation within the furanose ring (which has not yet been established), the D-galactofuranosyl pair **7** and **8** should have the β and α configuration, respectively, as the C-1 and C-2 resonances of **7** are downfield of the corresponding resonances for **8**, this being evident despite the fact that the C-2 resonances for these compounds were not unambiguously assigned.

The D-glucofuranosyl derivative **10** was tentatively assigned the β configuration from the similarity of its ^{13}C-n.m.r. spectrum, in particular the position of the C-1 signal (89.8 p.p.m.), to that of the β-D-*galacto* isomer **7** (C-1 signal at 90.0 p.p.m.), and from its relatively low optical rotation (+6.2°).

Conformations of the furanose hexaacetates. The anomeric configuration of galacto- and gluco-furanosyl derivatives is frequently determined from ^{1}H-n.m.r. data, in particular from the value of $J_{1,2}$. When $J_{1,2}$ is in the region of 0–1 Hz, the 1,2-*trans* (*i.e.*, β) configuration of substituents may be inferred, but this only occurs

when these substituents adopt quasiaxial orientations[27]. The significant $J_{1,2}$ values exhibited by **7**, **8**, and **10** (4.2, 6.8, and 3.2 Hz, respectively) can be understood by analysis of the conformations of these compounds.

Shugar and co-workers[28,29] studied in detail the relationships between conformational equilibria and ring-proton coupling-constants for α- and β-L-arabinofuranosylnucleosides*, compounds that have the same group configuration of the furanose ring as β- and α-D-galactofuranosyl derivatives, respectively. The α-L-arabinofuranosyl derivatives were shown to exist in a two-state, conformational equilibrium, denoted N (north) ⇌ S (south), each state corresponding to a narrow range of the pseudorotational cycle near ^3E and ^2E, respectively[28]. From the linear relationship derived between coupling constants and population of the N and S states, it was concluded that the β-D-galactofuranosyl derivative **7** has a 50–55% population of the S state.

β-L-Arabinofuranosylnucleosides* also exist in an N ⇌ S conformational equilibrium, but here, the N type of state incorporates a broader range of available conformations[29]. The large coupling constants for the α-D-galactofuranosyl derivative **8** indicate, however, a high population (~80%) of the S type of state, centered on the E_3 conformation.

In the case of β-D-xylofuranosylnucleosides, which are configurationally related to the β-D-glucofuranosyl derivative **10**, values of $J_{3,4}$ in the range of 3.4–.4 Hz are obtained only for the $^3T_4$ conformation, an N type[30]. Because, for **10**, $J_{3,4}$ = 4.5 Hz, it evidently adopts this N type of conformation. In all of these conformations, the bulky C-1 substituent avoids a quasiaxial orientation, and hence, $J_{1,2}$ is non-zero.

Mass-spectral data. — In the early stages of this work, when only the poorly resolved, room-temperature, n.m.r. spectra were at hand, mass-spectral data were of great help in ascertaining that the cyclic hexaacetate derivatives were isomeric. The molecular ion at *m/z* 447 was weak, but each compound had relatively intense peaks at *m/z* 405, 345, and 331, due to loss of CH_2CO, Ac_2O, and ·N(Ac)OAc fragments, respectively, from M^+, and, in most other respects, their spectra were very similar. There were no significant ions that could be assigned to side-chain cleavage, such as $[M - CHOAc \cdot CH_2OAc]^+$, a fragmentation that has proved useful for distinguishing furanosyl and pyranosyl derivatives of acetylated sugars in other instances[31]. Evidently, the fragmentation pattern is dominated here by cleavage at the glycosyl moiety.

Formation of cyclic product. — In an attempt to improve product selectivity, the acetylation step in the PAAN derivatization of D-galactose was examined. Acetyl bromide–trifluoroacetic acid, which selectively acetylates hydroxyl groups in the presence of primary and secondary amino groups[32], could have enhanced the forma-

*Much of the data reported by Shugar *et al.*[28–30] relates to nucleosides in the D series. Accordingly, it should be noted that a change in the configuration of a sugar from D to L results in an N to S type of change in conformational nomenclature. Obviously, a β-D-arabinofuranosylnucleoside in the ^3E (S type) conformation is the enantiomer of a β-L-arabinofuranosylnucleoside in the E_3 (N type) conformation.

TABLE IV

^{13}C-N.M.R. DATA FOR SUGAR OXIMES (**12**–**14**)

Compound	*Solvent*	*Signal of atom*					
		C-1	*C-2*[a]	*C-3*[a]	*C-4*[a]	*C-5*[a]	*C-6*
12	D_2O	153.6	72.0	70.0	69.3	70.8	63.0
	C_5D_5N	152.6	74.2	71.5	70.3	71.8	65.0
13	D_2O	154.5	71.2	70.9	70.1	[b]	65.4
	C_5D_5N	154.8	73.1	71.6	[b]	71.9	66.0
14	D_2O	92.3	[b]	74.3	67.6	77.1	61.9

[a]Not specifically assigned [b]Signal coincident with another in the spectrum of the sample mixture.

tion of nitrile **1**. Acetic anhydride in methanol (followed by peracetylation for product isolation), which is used for selective *N*-acetylation, could have enhanced the formation of the cyclic byproducts, *i.e.*, **4**, **7**, and **8**. Both reagents, however, led to unsatisfactory product-mixtures, as judged by t.l.c.

The nature of D-galactose oxime in solution was examined by ^{13}C- (see Table IV) and ^{1}H-n.m.r. spectroscopy in order to determine whether, in the PAAN derivatization procedure, the acyclic ⇌ cyclic oxime equilibrium position is merely "frozen" by acetylation, or whether further ring-closure occurs, concomitant with acetylation. Spectral assignments for the acyclic oximes were based on the generalization that the H-1 signal for the *syn*-oxime is always at lower field than that for its *anti* isomer[18]. Because of the greater solubility of the oxime in D_2O than in pyridine-d_5, initial studies were conducted for solutions in D_2O. The *syn*-oxime **12** appears to be the preponderant form in the crystalline state, as it was the major component in D_2O solution after only a few minutes, the ratio of *syn*-**12**:*anti*-**13** then being 7:2. After equilibrium had been established, this ratio decreased to 3:2. The only cyclic, *N*-hydroxyglycosylamine isomer then detected by ^{13}C-n.m.r. spectroscopy was present as $\leq 3\%$ of the total mixture, and it appeared to be the β-pyranose isomer **14**, as judged from the ^{13}C-n.m.r. data, although only five of its six carbon atoms were distinguishable in the spectrum of the mixture.

Mutarotation of D-galactose oxime in pyridine was slower than in water, but the equilibrium mixture appeared to be very similar. In particular, the cyclic forms were no more significant than in D_2O solution. The absence of detectable proportions of *N*-hydroxy-furanosylamine forms in solutions of D-galactose oxime implies that **7** and **8** probably result from a kinetically controlled reaction involving acetylation of the nitrogen atom of the acyclic oxime, with concomitant ring-closure, which would be expected to favor the formation of a furanosyl over a pyranosyl derivative.

D-Glucose oxime in aqueous solution has been shown[18] to be a mixture of the

β-pyranose (23%), α-pyranose (7%), *anti* (13.5%), and *syn* (56.5%) forms. Again, the formation of a substantial proportion of the furanosyl hexaacetate **10** is consistent with kinetically controlled ring-closure of the acyclic oxime on acetylation.

CONCLUSIONS

The implications of this work for the g.l.c. analysis of sugars as their PAAN derivatives must be considered. Some sugars do not give a single nitrile derivative in quantitative yield, as demonstrated here with D-galactose and D-glucose. The relatively long, g.l.c. retention-times of the unavoidable byproducts necessitate long analysis times using the capillary column, and lead to baseline disturbances during sequential analyses on packed columns. For quantitative, g.l.c. analysis, the use of a derivatization procedure that provides a kinetically controlled mixture of products, only one of which is detected, is clearly undesirable. All of these factors significantly detract from the reliability of the PAAN derivatization procedure in the quantitative, g.l.c. analysis of carbohydrates.

EXPERIMENTAL

General. — D-(+)-Galactose, purified grade, was obtained from Sigma Chem. Co., and contained a negligible proportion of D-glucose, as shown by per(trimethylsilylation)–g.l.c. analysis. Optical rotations were determined with a Perkin–Elmer Model 241 polarimeter, for solutions in chloroform (*c* 1.0–3.0, unless otherwise noted). Analytical l.c. was performed with a Waters apparatus (M6000A pump, R401 differential refractometer) and a Brownlee silica-gel column (SI-100, 25 cm × 4.6 mm i.d.), and preparative l.c., in a Waters PrepLC/System 500, using a single silica-gel cartridge. For t.l.c., Merck silica gel $60F_{254}$ aluminum-backed plates and 95:5:3 ethanol–sulfuric acid–anisaldehyde spray reagent were employed. G.l.c. was conducted in a Pye GC-D instrument on both a SCOT capillary column (SGE, 25 m; split ratio 10:1; N_2 flow 2 mL.min^{-1}) coated with SE-30, and a glass column (2 m × 2 mm i.d.; N_2 flow 25 mL.min^{-1}) packed with 3% of poly(neopentyl glycol succinate) on Gas Chrom Q (100–120 mesh). N.m.r. spectra (^1H and ^{13}C) were recorded with a Varian FT-80A spectrometer, with internal (or external, for D_2O solutions) tetramethylsilane as the reference. Mass spectra were recorded with AEI-MS-30 and MS-902 spectrometers.

Chromatographic analysis of the PAAN derivative of D-*galactose.* — The mixture of products isolated following treatment[3] of D-galactose in pyridine with hydroxylamine hydrochloride, and then acetic anhydride at 70°, was shown to contain the following components, (*a*) *by t.l.c.* with 2:1 ethyl acetate–light petroleum ether: R_F (compound), 0.67 (**1**), 0.65 (Ac_2NOAc), 0.42 and 0.39 (minor, unidentified), 0.28 (**8** + **4**), and 0.23 (**7**); (*b*) *by analytical l.c.* with 1:1 ethyl acetate–light petroleum ether at 1.5 mL.min^{-1}: R_T (min; compound), 2.1 (Ac_2NOAc), 3.5 (**1**), 4.4 and 4.9 (minor, unidentified), 8.7 and 9.0 (merged peaks, **4** + **8**), and 10.7 (**7**), and the

relative peak-area ratio of **7**: (**4** + **8**) was 3.1; (*c*) *by capillary g.l.c.* (175° to 250° at 8°/min, then isothermal at 250°): R_T (min; compound), 31.5 (**1**), and 55.1, 60.3, and 61.3 (**4**, **7**, and **8**); and (*d*) *by packed-column g.l.c.* (230° for 10 min, then to 250° at 4°/min): R_T (min; compound), 6.1 (**1**), and 18.5 and 20.6 (**4**, **7**, and **8**).

Preparation of the P-1,1N derivative of D-*galactose.* D-Galactose (20 g) and hydroxylamine hydrochloride (12.5 g, 1.6 mol. equiv.) were stirred and heated in pyridine (200 mL) for 20 min at 70° during which time dissolution occurred. The mixture was then removed from the hot bath, and acetic anhydride (160 mL) was gradually added, so that the temperature did not rise above 100°. The resulting mixture was heated for a further 30 min at 70°, after which, most of the solvent was evaporated *in vacuo*. The residue was partitioned between chloroform and water, and the organic phase was washed with aqueous sodium hydrogencarbonate, evaporated to a crystalline mass, and several portions of toluene were added and evaporated, to remove residual pyridine. Crystallization of the product from ethanol yielded D-galactononitrile pentaacetate (**1**; 26.6 g, 62.6%). Recrystallized from ethanol, it had m.p. 138–139°, $[\alpha]_D$ +43.2°; lit.[14] m.p. 138–139°, $[\alpha]_D$ +43.2°, and its ^{1}H-n.m.r. spectrum was identical to that reported[33,34] for **1**.

The mother liquors were evaporated to a syrup from which hydroxylamine triacetate, identified by its ^{1}H-n.m.r. spectrum ($CDCl_3$): δ 2.27 (s, OAc) and 2.31 (s, 2 NAc), was removed as a mobile liquid by distillation at 0.1 torr/80° (bath temp.). The remaining syrup yielded *N*-hydroxy-β-D-galactofuranosylamine hexaacetate (**7**) as crystals (6.28 g) from ethanol (with the aid of a nucleating crystal obtained by preparative-l.c. fractionation of a similar reaction product, and storage of syrupy **7** for several months, during which it spontaneously crystallized). Recrystallized from ethyl acetate–light petroleum ether, it had m.p. 108–110°, $[\alpha]_D$ 27.9°. The cyclic oxime hexaacetate prepared by Deulofeu *et al.*[14] by acetylation of D-galactose oxime had m.p. 106°, $[\alpha]_D$ 27.4°.

Anal. Calc. for $C_{18}H_{25}NO_{12}$: C, 48.3; H, 5.6; N, 3.1. Found: C, 48.5; H, 5.9; N, 3.2.

A second crop of crystalline material (2.6 g) proved to be a 2:1 mixture (^{1}H-n.m.r.) of nitrile **1** and the β-furanosyl derivative **7**, raising the total yield of nitrile **1** to 66.5%. The remaining liquors were fractionated on a column of silica gel eluted with 1:2 to 1:1 ethyl acetate–light petroleum ether, the eluates being pooled into a more- and a less-mobile fraction.

From an ethanol solution of the more-mobile fraction, *N*-hydroxy-α-D-galactofuranosylamine hexaacetate (**8**) was obtained as fine needles (0.47 g) (with the aid of a nucleating crystal obtained on prolonged storage of another fraction from the preparative-l.c. experiment already mentioned). Recrystallized from ethanol, it had m.p. 128–130°, $[\alpha]_D$ +75.4°, m/z 447.139 (M^+: calc. for $C_{18}H_{25}NO_{12}$, 447.138).

Anal. Calc. for $C_{18}H_{25}NO_{12}$: C, 48.3; H, 5.6; N, 3.1. Found: C, 48.2; H, 6.0; N, 3.1.

The syrup residue (1.11 g), $[\alpha]_D$ +30.5°, from the latter crystallization was shown by ^{13}C-n.m.r. spectroscopy to consist of *N*-hydroxy-β-D-galactopyranosyl-

amine hexaacetate (**4**, 1.9%) and its α- and β-furanosyl isomers **8** and **7**, in the ratio of 8:1:1.

The second, less-mobile, chromatographic fraction yielded a further crop of the β-furanosyl derivative **7** (0.74 g; total yield, 15.9%) and of the α-furanosyl derivative **8** (0.14 g; total yield, 1.2%) on fractional recrystallization from ethyl acetate–light petroleum ether, and then ethanol.

N-*Acetyl*-N-*hydroxy*-β-D-*galactopyranosylamine* (**5**). — A portion (0.56 g) of the syrupy mixture from the preceding experiment (which was an 8:1:1 mixture of the isomeric hexaacetates **4**, **7**, and **8**) was *O*-deacetylated with sodium methoxide in methanol, and the base was then neutralized with an ion-exchange resin (H^+). The product yielded crystalline **5** (0.16 g, 52%) from ethanol–ethyl acetate. Recrystallized from aqueous ethanol, it largely melted at 200–202°, with decomposition and gas evolution, although portions of the sample decomposed as low as 191°, and it had $[\alpha]_D$ +37.6° (*c* 2.4, water).

Anal. Calc. for $C_8H_{15}NO_7$: C, 40.5; H, 6.4; N, 5.9. Found: C, 40.2; H, 6.6; N, 5.8.

N-*Acetyl*-N-*hydroxy*-β-D-*galactofuranosylamine* (**9**). — The hexaacetate **7** was *O*-deacetylated with sodium methoxide in methanol, to give a single component (t.l.c.: R_F 0.40 in 15:2:2:1 acetone–chloroform–methanol–water). The base was neutralized with an ion-exchange resin (H^+), and the solution evaporated to a colorless syrup, characterized by its ^{1}H-n.m.r.- (Tables I and II) and ^{13}C-n.m.r.-spectral (Table III) data.

Preparation of the PAAN derivative of D-*glucose.* — D-Glucose (5.0 g) and hydroxylamine hydrochloride (3.15 g, 1.6 mol. equiv.) were stirred and heated in pyridine (55 mL) for 15 min at 70°, during which time, dissolution occurred. The mixture was removed from the hot bath, acetic anhydride (45 mL) was gradually added, so that the temperature remained at 70–80°, and then the solution was kept for a further 30 min at 70°, cooled, evaporated *in vacuo*, and the residue partitioned between chloroform and dilute hydrochloric acid. The organic phase was washed with aqueous sodium hydrogencarbonate solution, and evaporated to a syrup that crystallized from ethanol to yield D-gluconononitrile pentaacetate (**2**, 5.55 g). Recrystallized from ethanol, it had m.p. 83–86°, $[\alpha]_D$ +47.7°; lit.[17] m.p. 83–84°, $[\alpha]_D$ +48°. The mother liquors were fractionated by flash chromatography on silica gel eluted with 1:1 ethyl acetate–light petroleum ether, to yield a further crop of **2** (1.12 g; total yield, 62.0%) and a less-mobile, syrupy mixture of *N*-hydroxy-β-D-glucosylamine hexaacetates (3.52 g, 28.3%), shown by ^{13}C-n.m.r. spectroscopy (with n.O.e. suppression) to be a 3:5:1:1 mixture of the β-pyranose isomer **6**, the β-furanose isomer **10**, and two minor isomers. Although these compounds were of very similar chromatographic mobility, pure samples of the major two were obtained by repeated chromatography. The more-mobile, β-pyranose isomer **6** crystallized from aqueous ethanol as fine needles, m.p. 113–116°, $[\alpha]_D$ +7.3°; lit.[17] m.p. 113–115°, $[\alpha]_D$ +7.3°.

The less-mobile, furanose isomer **10** was obtained as a colorless syrup, $[\alpha]_D$ +6.2°, *m/z* 447.139 (M^+; calc. for $C_{18}H_{25}NO_{12}$: 447.138).

Anal. Calc. for $C_{18}H_{25}NO_{12}$: C, 48.3; H, 5.6; N, 3.1. Found: C, 48.6; H, 5.9; N, 3.0.

Low-temperature acetylation of D-*glucose oxime.* – This acetylation was conducted in acetic anhydride–pyridine for 24 h at 0°, and then for 24 h at room temperature, as described by Behrend[35]. Thus, crystalline D-glucose oxime (1.1 g) yielded *N*-hydroxy-β-D-glucopyranosylamine hexaacetate (**6**) as white needles (2.04 g, 81%) from ethanol–water; m.p. 115–119°, $[\alpha]_D$ +7.3°, identical (by t.l.c. and ^{1}H-n.m.r. spectrum) to the material from the preceding experiment.

D-*Galactose oxime.* – To a solution of hydroxylamine hydrochloride (11.65 g) in dry methanol (75 mL) was added a solution of sodium metal (3.6 g) in dry methanol (60 mL). The resulting suspension was filtered from precipitated salt directly into a flask containing D-galactose (15 g, anhydrous), and the whole was heated under reflux for 0.5 h. The sugar soon dissolved, and towards the end, the oxime began to crystallize. The suspension was kept for 1 day at room temperature, and then the D-galactose oxime was isolated by filtration, and recrystallized from ethanol–water, to give white crystals (10.2 g, 63%), m.p. 175–178° (dec.), $[\alpha]_D^{20}$ (*c* 1.0, water): +45.7° (3 min) → +35.3° (85 min) → +15.4° (17 h); (*c* 1.1, pyridine): +52.6° (10 min) → +52.4° (65 min) → +53.2° (20 h); lit.[36] m.p. 176–178°, $[\alpha]_D^{25}$ +84° (extrapolated, initial value) → +14.5° (final) (*c* 1.01, water); ^{1}H-n.m.r. data (D_2O): δ 7.60 (d, $J_{1,2}$ 5.8 Hz, H-1; **12**), 6.97 (d, $J_{1,2}$ 5.6 Hz, H-1; **13**), 5.13 (dd, $J_{2,3}$ 1.9 Hz, H-2; **13**), 4.54 (dd, $J_{2,3}$ 1.8 Hz; **12**), and 4.1–3.4 (m, remaining protons); (C_5D_5N): δ 12.80 (br s, N-O*H*), 8.44 (d, $J_{1,2}$ 6.9 Hz, H-1; **12**), and 7.69 (d, $J_{1,2}$ 5.7 Hz, H-1; **13**, partially merged with solvent resonances).

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