ELSEVIER

Carbohydrate Research 278 (1995) 181–194

CARBOHYDRATE RESEARCH

Note

A reinvestigation of the derivatization of monosaccharides as aldononitrile peracetates ☆

Ivo B. Niederer, Giuseppe G.G. Manzardo, Renato Amadò *

Swiss Federal Institute of Technology, Department of Food Science, ETH-Zentrum, CH-8092 Zurich, Switzerland

Received 26 April 1994; accepted in revised form 19 June 1995

Keywords: Monosaccharides; GC; Aldononitrile peracetates; Aldose oxime peracetates; *N*-Hydroxyglycofuranosylamine peracetates

For the chromatographic separation and quantitative analysis of saccharides, derivatization to peracetylated aldononitriles has been widely used (see refs [2,3] and references cited therein). The usual procedure involves base-catalyzed acetylation of the corresponding aldose oximes with acetic anhydride. However, formation of significant proportions of acyclic, as well as cyclic, peracetylated oximes (furanose and pyranose derivatives) has been evidenced [4,5]. Furneaux [5] investigated the derivatization of D-galactose and D-glucose in pyridine. This author isolated furanose derivatives in 18% and 14% and pyranose derivatives in 2% and 8% yield for galactose and glucose, respectively. Hence, the reliability of the method for quantitative GLC analysis has been questioned [5,6].

We have now reinvestigated the aldononitrile peracetate derivatization of 6-deoxy-L-mannose (L-rhamnose), L-arabinose, D-xylose, D-mannose, D-glucose, and D-galactose under various conditions. Based on the results obtained in this work, the scope and limitation of this reaction for the purpose of quantification will be commented on.

The derivatization of a mixture containing the above sugars, using 1-methylimidazole as solvent and catalyst [7,8], showed in the gas chromatogram several peaks besides those due to the expected aldononitrile peracetates (Fig. 1, peaks 8–13). In the gas chromatogram of each individually derivatized sugar, a smaller peak was detected, the retention time of which was 4–5 times longer than that of the aldononitrile peracetate.

☆ Part of the PhD thesis of I. Niederer [1].

* Corresponding author.

SSDI 0008-6215(95)00244-8

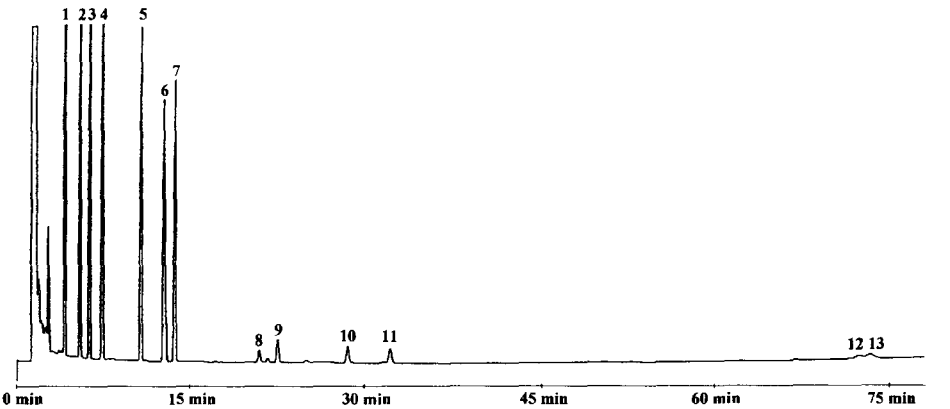


Fig. 1. Gas chromatogram of a mixture of monosaccharides derivatized to aldononitrile peracetates: **1** L-Rha, **2** L-Ara, **3** D-Xyl, **4** Xylitol (internal standard), **5** D-Man, **6** D-Glc, **7** D-Gal, **8–13** by-products.

These peaks correspond to by-products formed during the derivatization. In accordance with the by-products characterized by Furneaux for the derivatization of D-galactose and D-glucose [5], those obtained in this work were also identified (vide infra) as *N*-hydroxyglycofuranosylamine peracetates. Additional peaks in the gas chromatograms were at least 5 times smaller than this *N*-hydroxyglycofuranosylamine peracetate peak and were not identified.

The HPLC analysis of these reaction mixtures resulted in the detection of an additional main peak. Fig. 2 shows a representative HPLC chromatogram of the galactose product mixture. The three fractions identified (vide infra) are D-galactononitrile peracetate (peak 1), D-galactose oxime peracetate (peak 2) and *N*-hydroxy-D-galactofuranosylamine peracetate (peak 3). GLC analysis of the pure compounds showed, in

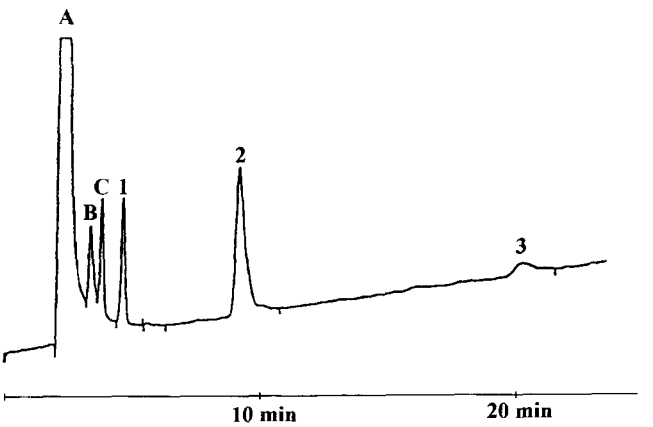
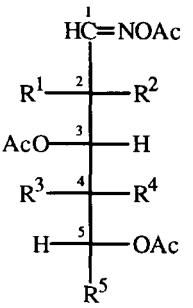
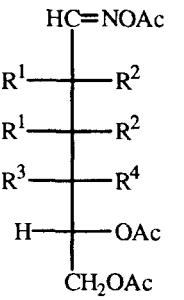


Fig. 2. HPLC of galactose reaction mixture. Peaks: A–C solvent and acetylated hydroxylamine, **1** D-galactononitrile peracetate, **2** D-galactose oxime peracetate **6**, **3** *N*-hydroxy-D-galactofuranosylamine peracetate **12**.

the case of the second fractions, a peak identified as the corresponding aldononitrile peracetates from their co-elution with characterized samples. Consequently, the aldose oxime peracetates are converted to aldononitrile peracetates under gas chromatographic conditions.

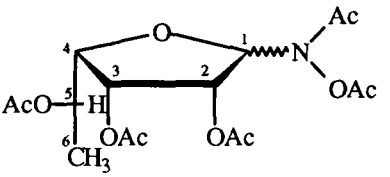




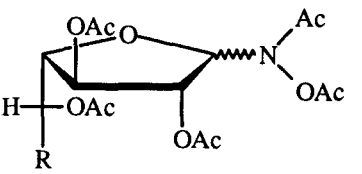
1 $R^1 = R^4 = OAc$, $R^2 = R^3 = H$, $R^5 = CH_3$
2 $R^1 = R^4 = OAc$, $R^2 = R^3 = H$
3 $R^1 = R^4 = H$, $R^2 = R^3 = OAc$
4 $R^1 = R^4 = OAc$, $R^2 = R^3 = H$, $R^5 = CH_2OAc$
5 $R^1 = R^3 = H$, $R^2 = R^4 = OAc$, $R^5 = CH_2OAc$
6 $R^1 = R^4 = H$, $R^2 = R^3 = OAc$, $R^5 = CH_2OAc$

Derivatization on a preparative scale of each individual monosaccharide resulted in reaction mixtures which could be separated by preparative LC yielding, in each case, three fractions. They were identified (in the order of their elution) as the aldononitrile peracetates, the aldose oxime peracetates, namely penta-*O*-acetyl-6-deoxy-L-mannose oxime **1**, penta-*O*-acetyl-L-arabinose oxime **2**, penta-*O*-acetyl-D-xylose oxime **3**, hexa-*O*-acetyl-D-mannose oxime **4**, hexa-*O*-acetyl-D-glucose oxime **5**, hexa-*O*-acetyl-D-galactose oxime **6**, and the furanose derivatives, namely *N*-hydroxy-6-deoxy-L-mannofuranosylamine pentaacetate **7**, *N*-hydroxy-L-arabinofuranosylamine pentaacetate **8**, *N*-hydroxy-D-xylofuranosylamine pentaacetate **9**, *N*-hydroxy-D-mannofuranosylamine hexaacetate **10**, *N*-hydroxy-D-glucofuranosylamine hexaacetate **11**, *N*-hydroxy-D-galactofuranosylamine hexaacetate **12**, respectively.

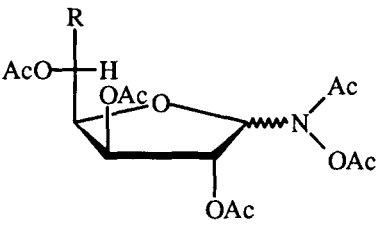
The NMR data of all the aldononitrile peracetates were in agreement with those described by Velasco et al. [9], with the exception of one CH carbon chemical shift of D-mannononitrile peracetate which was found to be 66.8 ppm instead of 63.873 ppm. The ^1H and ^{13}C NMR data for the aldose oxime peracetates **1–6** are given in Tables 1 and 2. The characteristic signals for an oxime group are found at 153 ppm in the ^{13}C NMR (C-1), at 7.5 ppm in the ^1H NMR (doublet, H-1) and at about 1640 cm^{-1} in the IR spectrum (C=N stretching vibration) [10]. Based on the NMR spectra, it was evident that a single product was formed; however, it was not ascertained whether the product was the *syn*- or the *anti*-diastereomer. In the derivatization of the same sugars used in this work, as well as of L-fucose using the method of McGinnis [7], Calvey et al. [6]



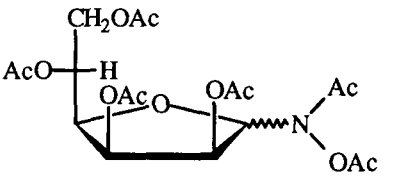
7



8 R = H
12 R = CH_2OAc



9 R = H
11 R = CH_2OAc



10

postulated the formation of aldose oxime peracetates based on the molecular ion in the SFC/MS spectra. The ^{1}H and ^{13}C NMR data for the furanose derivatives **7–12** are given in Tables 3 and 4. The data for the *N*-hydroxy-D-glucofuranosylamine peracetate **11** and the *N*-hydroxy-D-galactofuranosylamine peracetate **12** are in agreement with those obtained by Furneaux [5] for the β anomers. The ring size —furanose or pyranose —was determined from the ^{1}H NMR spectral data. Chemical shifts of protons bound to the secondary carbon bearing acetoxy groups are in the range of 5.0–5.5 ppm while those for the proton bound to the secondary carbon atom next to the ring oxygen is about 1 ppm upfield. In all spectra, the H-4 resonance is at higher field than that for H-2, H-3, or H-5, indicating a furanose derivative [5]. Moreover, in the H–C–long-range correlation spectrum (HMBC) of the furanose derivative of D-glucose, a correlation between a carbonyl carbon of an acetoxy group and H-5 was evident. The configuration at C-1 was not determined. In most of the ^{1}H NMR spectra of these compounds, line broadening of the H-1 signals were observed due to restricted rotation phenomena (*N*-Ac group).

The quantification of the aldose oxime peracetates and the *N*-hydroxyglycofuranosylamine peracetates was done by mass balance. Analytical scale reactions were carried out simultaneously eight times according to McGinnis [7] and the combined reaction mixtures separated by preparative LC. In this way, enough material could be isolated without having to alter the concentration and size of the analytical derivatization reaction. Table 5 shows the isolated yields of the aldononitrile peracetates, aldose oxime

Table 1
^{1}H NMR data ($CDCl_3$) for **1**–**6**

Compound	Chemical shifts (δ)							
	H-1	H-2	H-3	H-4	H-5	CH_3		$OCOCH_3$
1 [a]	7.52–7.62m	5.27–5.33m [c]	5.48–5.50m (2 H) [c]		5.00dq	1.20d		2.03, 2.09, 2.10, 2.11, 2.15
	H-1	H-2	H-3	H-4	H′-5	H″-5		$OCOCH_3$
2 [a]	7.56d	5.79dd	5.49dd	5.24ddd	4.18dd	4.29dd		2.07, 2.08, 2.140, 2.142, 2.153
3 [a]	7.64d	5.69dd	5.51dd	5.33ddd	4.01dd	4.34dd		2.06, 2.11, 2.140, 2.145, 2.16
	H-1	H-2	H-3	H-4	H-5	H′-6	H″-6	$OCOCH_3$
4 [b]	7.56dd	5.43–5.48m (2 H)		5.52dd	5.16ddd	4.10dd	4.22dd	2.06, 2.07, 2.088, 2.099, 2.102, 2.15
5 [a]	7.66d	5.54–5.65m (2 H) [d]		5.43dd [d]	5.11ddd	4.09dd	4.26dd	2.07 (2×), 2.11, 2.13, 2.14, 2.17
6 [b]	7.50d	5.66dd	5.40dd [e]	5.45dd [e]	5.34ddd	3.87dd	4.28dd	2.03, 2.08, 2.11, 2.12, 2.14, 2.15
	Coupling constants (Hz)							
1	$J_{5,6}$ 6.3 J 8.5 and 6.3 (5.00 ppm)							
2	$J_{1,2}$ 5.4 $J_{2,3}$ 2.6 $J_{3,4}$ 9.0 $J_{4,5'}$ 4.2 $J_{4,5''}$ 2.7 $J_{5',5''}$ 12.6							
3	$J_{1,2}$ 5.6 $J_{2,3}$ 5.6 $J_{3,4}$ 4.8 $J_{4,5'}$ 6.1 $J_{4,5''}$ 5.0 $J_{5',5''}$ 11.9							
4	$J_{4,5}$ 9.3 $J_{5,6'}$ 5.0 $J_{5,6''}$ 2.7 $J_{6',6''}$ 12.5 J 9.3 and 1.6 (5.52 ppm), J 6.0 and 1.0 (7.56 ppm)							
5	$J_{5,6'}$ 5.3 $J_{5,6''}$ 3.3 $J_{6',6''}$ 12.4 J 8.2, 5.3 and 3.3 (5.11 ppm), J 7.7 and 3.0 (5.43 ppm), J 5.0 (7.66 ppm)							
6	$J_{1,2}$ 5.3 $J_{2,3}$ 1.8 $J_{3,4}$ 10.0 $J_{4,5}$ 1.8 $J_{5,6'}$ 7.3 $J_{5,6''}$ 5.1 $J_{6',6''}$ 11.7							

[a] At 200 MHz.
[b] At 300 MHz.
[c–e] Assignments are interchangeable.

Table 2
^{13}C NMR data ($CDCl_3$) for **1–6**

Compound	Carbon				
	C-1	C-2–C-5	C-6	$(OCO)CH_3$	C=O
1 [a]	153.7	66.8, 67.6, 68.1, 70.6	16.6	19.2, 20.6 (2×), 21.0	167.8, 169.0, 169.8, 170.0, 170.2
	C-1	C-2–C-4	C-5	$(OCO)CH_3$	C=O
2 [a]	153.1	67.9, 68.0, 69.2	61.5	19.4, 20.5, 20.67, 20.71, 20.79	167.9, 169.5, 169.7 (2×), 170.6
3 [a]	152.5	68.7, 68.8, 69.6	61.4	19.3, 20.52, 20.56, 20.62, 20.69	167.8, 169.3, 169.6, 169.7, 170.4
	C-1	C-2–C-5	C-6	$(OCO)CH_3$	C=O
4 [b]	153.7	66.8, 67.5, 67.8, 68.1	61.8	19.3, 20.6 (3×), 20.8 (2×)	167.8, 169.0, 169.6, 169.8, 170.4, 170.6
5 [a]	152.2	68.3, 68.4, 68.9, 69.1	61.5	19.4, 20.5 (2×), 20.7 (2×), 20.8	167.8, 169.3, 169.6, 169.7, 169.8, 170.5
6 [b]	153.0	67.4, 67.6, 68.5, —	62.0	19.3, 20.5 (3×), 20.6 (2×)	167.8, 169.6 (2×), 169.8, 170.2, 170.4

[a] At 50 MHz.
[b] At 75 MHz.
—means weak or not visible due to peak overlapping.

Table 3
^{1}H NMR data ($CDCl_3$) for **7–12**

Compound	Chemical shifts (δ)								
	H-1	H-2	H 3	H-4	H-5	CH_3		$OCOCH_3$	$NCOCH_3$
7 [a]	5.99bd	5.51dd	5.56dd	4.18dd	5.06dd	1.29d		1.95, 2.01, 2.08 (2×)	2.23
	H-1	H-2	H-3	H-4	H′-5	H″-5		$OCOCH_3$	$NCOCH_3$
8 [a]	5.94d	5.42dd	5.12dd	4.27ddd	4.21dd	4.13dd		2.01 (3×), 2.02	2.18
9 [a]	6.00bd	5.32dd	5.36dd	4.37dt	4.20d (2 H)		2.05, 2.085, 2.090 (2×)	2.24	
	H-1	H-2	H-3	H-4	H-5	H′-6	H″-6	$OCOCH_3$	$NCOCH_3$
10 [b,c]	6.0–6.1bs	5.5–5.6m (2 H)		4.46dd	5.22–5.27ddd	4.08dd	4.54dd	1.99, 2.04, 2.05, 2.073, 2.077, 2.11	2.27
11 [a,d]	6.02–6.08bs	5.14–5.18m	5.38dd	4.23dd	5.21ddd	4.05dd	4.56dd	1.98, 2.04, 2.07 (2×), 2.10	2.26
12 [b]	6.0–6.1bs	5.4–5.5m	5.17dd	4.32dd	5.27ddd	4.18dd	4.33dd	2.05, 2.09, 2.11 (2×), 2.16	2.28
	Coupling constants (Hz)								
7	$J_{1,2}$ 7.1	$J_{2,3}$ 4.5	$J_{3,4}$ 3.1	$J_{4,5}$ 8.9	$J_{5,6}$ 6.3				
8	$J_{1,2}$ 4.0	$J_{2,3}$ 4.0	$J_{3,4}$ 5.5	$J_{4,5'}$ 3.9	$J_{4,5''}$ 5.4	$J_{5',5''}$ 12.0			
9	$J_{1,2}$ 4.3	$J_{2,3}$ 3.1	$J_{3,4}$ 5.5	$J_{4,5'}$ 5.6	$J_{4,5''}$ 5.6				
10	$J_{4,5}$ 9.4	$J_{5,6'}$ 5.2	$J_{5,6''}$ 2.4	$J_{6',6''}$ 12.4	J 9.4 and 2.8 (4.46 ppm)				
11	$J_{3,4}$ 4.4	$J_{4,5}$ 8.6	$J_{5,6}$ 5.6	$J_{5,6''}$ 2.6	$J_{6',6''}$ 12.3	J 4.4 and 1.1 (5.38 ppm)			
12	$J_{2,3}$ 4.0	$J_{3,4}$ 5.4	$J_{4,5}$ 4.4	$J_{5,6'}$ 7.0	$J_{5,6''}$ 4.4	$J_{6',6''}$ 11.9			

[a] At 500 MHz.
[b] At 300 MHz.
[c] One $OCOCH_3$ signal due to EtO*Ac*.
[d] Assignments assisted by an HMBC experiment.

Table 4
^{13}C NMR data for **7–12**

Compound	Carbon						
	C-1	C-2–C-5	C-6	$(NCO)CH_3$	$(OCO)CH_3$	NCO	C=O
7 [a,c]	86.9br	66.9, 70.2, 70.7, 81.3	17.4	18.2	20.3, 20.5, 21.0, 21.1	168.1	169.6, 169.7, —, —
	C-1	C-2–C-4	C-5	$(NCO)CH_3$	$(OCO)CH_3$	NCO	C=O
8 [a,c]	89.5br	76.8, 77.6, 81.1	63.3	18.5	20.7, 20.8, 20.9, —	168.0	169.8, 169.9, 170.6, —
9 [a,c]	88.0br	74.8, 76.2, —	61.5	18.3	20.68, 20.76, 20.80, —	168.1	169.4, 169.5, 170.5, —
	C-1	C-2–C-5	C-6	$(NCO)CH_3$	$(OCO)CH_3$	NCO	C=O
10 [b,c]	88.0br	67.8, 70.2, 70.7, 77.0	62.6	18.2	20.3, 20.5, 20.67, 20.73, 21.1	—	169.5, 170.6, —, —, —
11 [a,c]	—	67.4, 74.0, 77.0, —	62.8	18.3	20.6 (2×), 20.8 (3×)	—	169.0, 169.3, 169.8, 170.6, —
11 [a,d]	89.6br	67.9, 74.3, 77.7, 78.8	63.3	17.8	19.9, 20.28, 20.32, 20.46, 20.51	168.2	169.0, 169.1, 169.6, 170.0, —
12 [b,c]	89.7br	69.8, 76.5, 81.2, —	62.4	18.4	20.7, 20.9, —, —, —	167.9	169.8 (2×), 170.0, 170.5, —

[a] At 50 MHz.
[b] At 75 MHz.
[c] In $CDCl_3$.
[d] In C_6D_6.
—means weak or not visible due to peak overlapping.

Table 5
Yields of products isolated by preparative LC from the derivatization of aldoses

Aldose	Compound (%)			
	Aldononitrile peracetate	Aldose oxime peracetate	Furanose derivative	Total yield
L-Rha	25	56	5	86
L-Ara	35	49	9	93
D-Xyl	29	54	8	91
D-Man	31	58	5	94
D-Glc	39	40	7	86
D-Gal	27	46	9	82

peracetates and the *N*-hydroxyglycofuranosylamine peracetates. In this derivatization using 1-methylimidazole, the aldose oxime peracetate and not the aldononitrile peracetate is the major product, this being the case for all monosaccharides studied. The furanose derivatives were obtained in 5–9% yield.

The examination of the reproducibility for the *N*-hydroxyglycofuranosylamine peracetate formation was carried out with an example from the groups of the 6-deoxyhexoses, pentoses and hexoses. 6-Deoxy-L-mannose, L-arabinose, and D-glucose (2–10 mg) were derivatized six times and the ratio of their furanose derivative to peracetylated nitrile determined by GLC. The percent standard deviations relative to the mean values of these ratios for each set of 6 independent reactions were small and calculated to be $< 7\%$, $< 2\%$ and $< 3\%$, respectively. By way of comparison, the reproducibility of the auto-sampler injection was investigated for one sample, which was injected eight times. A standard deviation of less than 1% relative to the mean value of the ratio of furanose derivative to aldononitrile peracetate was obtained. It may be concluded that the ratio of the furanose derivative to aldononitrile peracetate is constant regardless of the sugar concentration used in the derivatization reaction.

In order to clarify the reproducibility and completeness of the conversion of D-galactose oxime peracetate into D-galactononitrile peracetate, solutions of known molarity of these compounds were analysed by GLC at different injector temperatures using galactitol hexaacetate as internal standard. The extent of conversion increased with increasing temperature of the injector block: at an injector temperature of 220°C it was found to be 84.8% ($s = 0.2$, $n = 4$), at 240°C 90.7% ($s = 0.8$, $n = 4$) and at 300°C 92.4% ($s = 0.3$, $n = 4$), the gas chromatographic peaks being sharp and symmetrical. Hence, the formation of the aldose oxime peracetate is not a major handicap for the GLC analysis, since the elimination of acetic acid is reproducible and nearly complete at a high enough temperature of the injector block.

Taking D-galactose as an example, the influence of the acetylation temperature on the ratio of D-galactononitrile peracetate to D-galactose oxime peracetate **6** (relative HPLC peak area) has been determined for 1-methylimidazole as acetylation catalyst, carrying out the derivatization procedure in the presence of water. Whereas on acetylation at room temperature [7] this ratio amounts to 1:3, acetylation at 100°C changes it to 13:1. Anhydrous derivatization conditions suppress the formation of the D-galactononitrile

Table 6
Composition of reaction mixtures (relative peak area percents) of D-galactose after derivatization by various methods

Method/Acetylation temperature	Compound (%)		
	D-Galactononitrile peracetate [a]	**6** [a]	**12** [b]
1-Methylimidazole [c] [7]/RT	24	68	8
1-Dimethylamino-2-propanol [11]/70°C	55	32	13
4-(Dimethylamino)pyridine [12]/70°C	20	71	9
4-(Dimethylamino)pyridine [c] [12]/70°C	47	46	7
Pyridine [5]/70°C	30	54	16

[a] Obtained by manual peak area integration of HPLC chromatograms.
[b] Obtained by digital peak area integration of GC chromatograms.
[c] In presence of water.

peracetate, the ratio being now 1:14 when acetylation is performed at room temperature. The results with other derivatization procedures, as compared with the derivatization with 1-methylimidazole according to McGinnis [7] on the product mixture, are shown in Table 6.

Acetylation catalyst, acetylation temperature and the presence of water had a definite impact on the D-galactononitrile peracetate/D-galactose oxime peracetate ratio. No significant differences in the relative proportion of peracetylated *N*-hydroxy-D-galactofuranosylamine **12** were observed. In the derivatization reactions using 1-dimethylamino-2-propanol or pyridine, the portion of the *N*-hydroxy-D-galactofuranosylamine peracetate **12** is about 50% higher than that obtained using 1-methylimidazole. All alternative derivatization methods require pyridine and take longer time as compared to the derivatization method of McGinnis. Therefore, this derivatization method is to be preferred.

A disadvantage of the derivatization of monosaccharides as aldononitrile peracetates may be the long retention times of the *N*-hydroxyglycofuranosylamine peracetate by-products. Nevertheless, the analysis time can be halved by appropriate choice of the repeat injection times, avoiding interferences of the aldononitrile peracetate peaks by the elution of by-products of a precedent analysis.

1. Experimental

General.—With the exception of galactitol hexaacetate from Supelco, all other chemicals were purchased from Fluka Chemicals and were of the highest available grade. Derivatization reactions were carried out in 10 mL Reacti-Vials (Pierce Nr. 13225) with teflon seals (Pierce Nr. 12422) and magnetic stirrer (Pierce Nr. 16000) in a heating module (Pierce Reacti-Therm Module Nr. 18971). Melting points were determined in open melting tubes using a Büchi SMP-20 apparatus equipped with a thermometer (not calibrated) and are not corrected. Elementary analyses of the crys-

talline compounds were carried out by the service department of Organic Chemistry Laboratories of Swiss Federal Institute of Technology, Zurich.

Chromatography.—HPLC was performed with a Hewlett–Packard apparatus (1090 liquid chromatograph, 1037 A refractometer) and a Brownlee Spheri-10 silica column (250 mm × 4.6 mm) with 1:2 EtOAc–petroleum ether (40–60°C) at 1.5 mL/min and a column temperature of 35°C. For the evaluation of the chromatograms, the peak areas were measured manually with a magnifying lens with scale (0.1 mm intervals) by multiplying the peak heights with the peak widths at one half peak height. Preparative low pressure liquid chromatography was performed with a Waters apparatus (Model 6000A pump, R401 refractometer) and a Lichroprep column Si 60 from Merck (No. 10401, 310 mm × 25 mm) with 2:1 EtOAc–petroleum ether (40–60°C) at 9 mL/min. GLC was conducted in a Hewlett–Packard instrument (5890 gas chromatograph, 3396A integrator, 7673A automatic sampler) with flame-ionization detector on a J&W Scientific fused silica column (DB-225, 30 m × 0.32 mm, 0.25 μm film thickness). Helium was used as carrier gas at a column flow rate of 10 mL/min and a split ratio of 10:1. The column temperature was 215°C isothermal, the injector and detector temperatures were 240 and 260°C, respectively.

Spectroscopy.—The ^{1}H and ^{13}C NMR spectra were recorded at 300 K on a Bruker AC-200 or AM-300 or at 333 K on a AMX-500 spectrometer. $CDCl_3$ was used as solvent. Chemical shifts are given in ppm downfield from internal Me_4Si. The number of protons attached to a carbon atom was determined from DEPT spectra. The IR spectra were run on a Perkin–Elmer 983 G. Solids were measured as KBr pellets, oils in $CHCl_3$. The FABMS spectra were measured in a ZAB-VSEQ instrument, 3-nitrobenzyl alcohol being used as matrix.

Procedures.—(a) Derivatization on analytical scale [7]. Aqueous solutions of monosaccharides (2–10 mg in 0.2 mL) were prepared, a hydroxylamine hydrochloride solution in 1-methylimidazole (2.5% (w/v), 0.4 mL) was added and the mixture stirred at 80°C for 10 min in a heating module. After cooling to room temperature, Ac_2O (1 mL) was added and stirred for 5 min. Then CH_2Cl_2 (1.5 mL) and water (1 mL) were added and the mixture shaken vigorously on a vortex mixer. After removal of the aqueous layer with a Pasteur pipette, the organic phase was washed twice with water (1 mL), dried (Na_2SO_4) and analyzed by GLC and HPLC, respectively.

(b) Derivatization on preparative scale. To a stirred solution of monosaccharide (1.25 g) in water (25 mL) was added a solution of hydroxylamine hydrochloride (1.25 g) in 1-methylimidazole (50 mL) and the mixture heated to 80°C for 10–15 min. After cooling in an ice bath, Ac_2O (125 mL) was added slowly in order to maintain the temperature between 85 and 95°C. Upon cooling, CH_2Cl_2 (125 mL) was added and the organic phase washed three times with water (125 mL), dried (Na_2SO_4) and concentrated in a rotary evaporator.

(c) Isolation of the products. The residue from the reaction mixtures obtained on preparative scale (section b) was subjected to preparative LC to give three fractions: (1) aldononitrile peracetate; (2) aldose oxime peracetate; and (3) *N*-hydroxyglycofuranosylamine peracetate in order of their elution sequence. In order to separate the product mixture from the derivatization of 6-deoxy-L-mannose, the ratio of EtOAc–petroleum ether was changed to 1:1. The combined fractions were concentrated on a rotary

evaporator and dried to constant weight in a Kugelrohr oven (Büchi GKR-50) at 50°C/8 mbar. The acetylated hydroxylamine, which was co-eluted with the aldononitrile peracetate fraction, could be removed during the oven drying process. The GLC purity of aldononitrile peracetate fractions was 97–100%; that of the *N*-hydroxyglycofuranosylamine peracetates was 83–98%. HPLC analysis showed aldose oxime peracetate fractions to be pure. A Kugelrohr distillation of the oily furanose derivatives could not be done. Due to thermal instability of the aldose oxime peracetates, the Kugelrohr oven temperature was not increased above 50°C during drying. In consequence, traces of solvents could be detected in the NMR spectra of these compounds.

(d) Characterization of aldose oxime peracetates. All aldose oxime peracetates were obtained as white solids with the exception of penta-*O*-acetyl-D-xylose oxime **3** which was obtained as an oil. The FABMS spectra of all compounds contained the quasi molecular ion peak $[M + H]^+$. The ^1H and ^{13}C NMR data are given in Tables 1 and 2, respectively.

Penta-*O*-acetyl-6-deoxy-L-mannose oxime **1**: mp 43–44.5°C; IR: 1750, 1635 (C=N), 1370, 1225, 1060, 1035, 920 cm^{-1}. Anal. Calcd for $C_{16}H_{23}NO_{10}$: C, 49.36; H, 5.95; N, 3.60. Found: C, 49.27, H, 6.07; N, 3.60.

Penta-*O*-acetyl-L-arabinose oxime **2**: mp 61.5–63.5°C; IR: 1745, 1645 (C=N), 1375, 1225, 1075, 1050, 1010, 975 cm^{-1}. Anal. Calcd for $C_{15}H_{21}NO_{10}$: C, 48.00; H, 5.64; N, 3.73. Found: C, 48.12, H, 5.61; N, 3.78.

Penta-*O*-acetyl-D-xylose oxime **3**: IR: 1755, 1640 (C=N), 1370, 1050 cm^{-1}.

Hexa-*O*-acetyl-D-mannose oxime **4**: mp 76.5–79°C ([13]: 94°C, [14]: 91–92°C); IR: 1780, 1750, 1645 (C=N), 1370, 1220, 1045, 910 cm^{-1}.

Hexa-*O*-acetyl-D-glucose oxime **5**: mp 72.5–74°C ([15]: 79°C); IR: 1750, 1640 (C=N), 1370, 1220, 1085, 1060, 1025, 965 cm^{-1}.

Hexa-*O*-acetyl-D-galactose oxime **6**: mp 143–143.5°C ([16]: 145.5–146.5°C); IR: 1775, 1755, 1640 (C=N), 1375, 1210, 1080, 1045, 1020, 960, 930 cm^{-1}.

(e) Characterization of N-*hydroxyglycofuranosylamine peracetates.* All furanose derivatives were obtained as oils with the exception of *N*-hydroxy-D-galactofuranosylamine hexaacetate **12**, which was obtained as a white solid. The FABMS spectra of all compounds contained the quasi molecular ion peak $[M + H]^+$. The ^1H and ^{13}C NMR data are given in Tables 3 and 4, respectively.

N-Hydroxy-6-deoxy-L-mannofuranosylamine pentaacetate **7**: IR: 1800, 1755, 1375, 1250, 1175, 1060, 1045 cm^{-1}.

N-Hydroxy-L-arabinofuranosylamine pentaacetate **8**: IR: 1800, 1750, 1705, 1370, 1230, 1175, 1050 cm^{-1}.

N-Hydroxy-D-xylofuranosylamine pentaacetate **9**: IR: 1805, 1750, 1710, 1370, 1230, 1165, 1050 cm^{-1}.

N-Hydroxy-D-mannofuranosylamine hexaacetate **10**: IR: 1800, 1750, 1715, 1375, 1235, 1045 cm^{-1}.

N-Hydroxy-D-glucofuranosylamine hexaacetate **11**: IR: 1805, 1750, 1705, 1370, 1230, 1075, 1045 cm^{-1}.

N-Hydroxy-D-galactofuranosylamine hexaacetate **12**: mp 102–103°C ([5]: 108–110°C β anomer, 128–130°C α anomer); IR: 1800, 1745, 1690, 1370, 1220, 1080, 1045 cm^{-1}.

(f) Derivatizations on analytical scale for mass balance. Derivatizations were carried out as described in section (a) with eight simultaneous reactions. After derivatization, the aqueous layer of each reaction mixture was removed with a Pasteur pipette and combined in a beaker. The combined organic phase was washed with water (8 mL). The combined aqueous phase was extracted with CH_2Cl_2 (5 mL). The organic phases were then combined, dried (Na_2SO_4), concentrated to 2 mL on a rotary evaporator and subjected to Lobar chromatography (see section c). From the yields of the isolated aldononitrile peracetate, aldose oxime peracetate and furanose derivative fractions, the compositional percentages were calculated.

(g) Derivatization of galactose under anhydrous conditions. D-Galactose (approx. 10 mg) was weighed in a Reacti-Vial, dissolved in a hydroxylamine hydrochloride solution in 1-methylimidazole (2.5% (w/v), 0.4 mL) and the derivatization carried out as described in section (a). In contrast to the acetylation in the presence of water, the addition of Ac_2O under non-aqueous conditions caused a dark-brown colouring of the reaction mixture. The work up was similar to that done for the aqueous solutions, except that the organic layer was washed three times instead of twice with water.

(h) Derivatization of galactose at different acetylation temperatures. A solution of D-galactose (approx. 10 mg) in water (0.2 mL) was mixed with a hydroxylamine hydrochloride solution in 1-methylimidazole (2.5% (w/v), 0.4 mL) and the mixture stirred at 80°C in a heating module for 10 min. The acetylation reactions were carried out by dropwise addition of Ac_2O (1 mL) at 0°C (ice bath) and 100°C respectively, with further stirring for 6 min. The work up for all these reactions proceeded as described in section (a). The reaction mixtures were analyzed by HPLC.

(i) Reproducibility and extent of conversion of galactose oxime peracetate into galactononitrile peracetate. D-Galactononitrile peracetate and D-galactose oxime peracetate **6** (20 mg each) were weighed in separate 10 mL volumetric flasks. D-Galactitol hexaacetate (approx. 10 mg, internal standard) was dissolved in CH_2Cl_2 (25 mL) and this solution was used to fill up the two 10 mL volumetric flasks up to the mark. By means of an automatic sampler, the solutions were injected 4–5 times each into the gas chromatograph at injector temperatures of 220°C, 240°C and 300°C and the peaks were quantified by means of an integrator. The extents of conversion D-galactose oxime peracetate/D-galactononitrile peracetate were determined according to the formula below. The factor 1.155 corresponds to the ratio of the molar mass of D-galactose oxime peracetate/D-galactononitrile peracetate.

$$\text{Extent of conversion} = \frac{A_{IS,N} \times A_O}{A_{IS,O} \times A_N} \times 1.155 \times 100\,[\%]$$

$A_{IS,O}$, A_O, areas of the D-galactitol hexaacetate (internal standard) and the D-galactononitrile peracetate peaks, respectively, of the galactose oxime peracetate injection; $A_{IS,N}$, A_N, areas of the D-galactitol hexaacetate (internal standard) and the D-galactononitrile peracetate peaks, respectively, of the galactononitrile peracetate injection.

(j) Derivatization of galactose by various methods. The reaction mixtures were analyzed by GLC and HPLC. The peaks were identified from their co-elution with characterized samples of D-galactononitrile peracetate, D-galactose oxime peracetate and *N*-hydroxy-D-galactofuranosylamine peracetate. (i) *Using pyridine as both solvent and*

catalyst; the derivatization was carried out using the method described by Furneaux [5]. The reaction scale was reduced to 1/10 of the reported procedure and $CHCl_3$ was replaced by CH_2Cl_2. (ii) *Using 1-dimethylamino-2-propanol*; the derivatization method of Mawhinney et al. [11] was employed, 10 mg of D-galactose being used and the reaction scale doubled. Because Pierce Reacti Vials have thick glass walls (slower heat transmission), the mixture was stirred at 70°C for 10 instead of 5 min. Also here the extraction solvent was replaced by CH_2Cl_2. (iii) *Using 4-(dimethylamino)pyridine*; according to the procedure of Guerrant and Moss [12], 10 mg of D-galactose was derivatized. In the same way, 10 mg of D-galactose in water (0.15 mL) was subjected to derivatization. The oxime formation and the acetylation were carried out using a stirrer so that the ultrasonic treatment step could be left out. 1,2-Dichloroethane was replaced by CH_2Cl_2.

References

[1] I.B. Niederer, Diss. ETH Nr. 10070, 1993.
[2] F.R. Seymour, *Methods Carbohydr. Chem.*, 9 (1993) 59–85.
[3] G.D. McGinnis and C.J. Biermann, in C.J. Biermann and G.D. McGinnis (Eds.), *Analysis of Carbohydrates by GLC and MS*, CRC Press, Boca Raton, FL, 1989, pp 119–125.
[4] V. Deulofeu, *Adv. Carbohydr. Chem.*, 4 (1949) 119–151.
[5] R.H. Furneaux, *Carbohydr. Res.*, 113 (1983) 241–255.
[6] E.M. Calvey, L.T. Taylor, and J.K. Palmer, *J. High Resolut. Chromatogr., Chromatogr. Commun.*, 11 (1988) 739–741.
[7] G.D. McGinnis, *Carbohydr. Res.*, 108 (1982) 284–292.
[8] C.C. Chen and G.D. McGinnis, *Carbohydr. Res.*, 90 (1981) 127–130.
[9] D. Velasco, J. Castells, and F. López-Calahorra, *J. Org. Chem.*, 55 (1990) 3526–3530.
[10] H. Günzler and H. Böck, *IR-Spektroskopie*, Verlag Chemie, Weinheim, 1983, p 238.
[11] T.P. Mawhinney, M.S. Feather, G.J. Barbero, and J.R. Martinez, *Anal. Biochem.*, 101 (1980) 112–117.
[12] G.O. Guerrant and C.W. Moss, *Anal. Chem.*, 56 (1984) 633–638.
[13] V. Deulofeu, P. Cattaneo, and G. Mendivelzua, *J. Chem. Soc.*, (1934) 147–148.
[14] M.L. Wolfrom and L.W. Georges, *J. Am. Chem. Soc.*, 58 (1936) 1781–1782.
[15] M.L. Wolfrom and A. Thomson, *J. Am. Chem. Soc.*, 53 (1931) 622–632.
[16] V. Deulofeu, M.L. Wolfrom, P. Cattaneo, C.C. Christman, and L.W. Georges, *J. Am. Chem. Soc.*, 55 (1933) 3488–3493.