ELSEVIER

Carbohydrate Research 264 (1994) 293–304

CARBOHYDRATE RESEARCH

Analysis of oligosaccharides containing 2-deoxy-α-D-*arabino*-hexosyl residues by the reductive-cleavage method

Petra Mischnick *, Britta Evers, Joachim Thiem

University of Hamburg, Institute of Organic Chemistry, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

Received 15 October 1993; accepted in revised form 17 May 1994

Abstract

A mixture of oligosaccharides containing (1→4)-linked 2-deoxy-α-D-*arabino*-hexosyl (''2-deoxyglucosyl'') and (1→4)-linked α-D-glucosyl residues (**1**) was analyzed by reduction, permethylation (perethylation), degradation to monomers, and GLC–MS. Degradation was performed either by hydrolysis with subsequent reduction, by methanolysis, or by reductive cleavage, always followed by acetylation. Reductive cleavage turned out to be the method of choice for the acid-labile 2-deoxy sugars. The main degradation product formed during acid hydrolysis of 2-deoxy-D-*arabino*-hexosyl residues yielded, after reduction and acetylation, (4*R*,*S*)-6-*O*-acetyl-2,3,5-trideoxyhexono-1,4-lactone (**7**). By methanolysis, in addition to the expected methyl glycosides, methyl 2,3,5-trideoxy-6-*O*-methyl-4-hexulosonate (**12**) is formed as a by-product. For determination of the distribution of chain lengths, the permethylated oligomers were separated by reversed-phase HPLC. For peak assignment, one isolated oligomer was investigated by FABMS and ^{1}H NMR spectroscopy. The average degree of polymerization (dp) calculated from the HPLC chromatogram is in good agreement with the reductive-cleavage results.

Keywords: Reductive cleavage; 2-Deoxy sugar

1. Introduction

The composition and linkage positions of oligo- or poly-saccharides are commonly determined by standard methylation analysis, comprising permethylation, acid hydrolysis, reduction, and acetylation [1]. However, for heteroglycans it may be difficult to find

* Corresponding author.

SSDI 0008-6215(94)00203-R

conditions sufficiently mild for acid-labile components yet strong enough to cleave the most acid-resistant glycosides.

Reductive cleavage as an alternative to standard methylation analysis has been successfully applied in the structural analysis of polysaccharides and derivatives. Especially for samples containing constituents sensitive to acidic conditions, such as pentosyl [2] or ketosyl residues [3], pyruvic acetals [4], and acylated [5–7] or 3,6-anhydro sugars [8], it proved to be superior to standard methylation analysis.

In this paper, the application of standard methylation analysis, methanolysis, and reductive cleavage to a glycan containing 2-deoxy-α-D-*arabino*-hexosyl and α-D-glucopyranosyl residues is reported. Methyl 2-deoxy-*arabino*-hexopyranosides are hydrolyzed ca. 2000 times faster than the corresponding methyl glucosides. The deoxy sugar can be liberated without destruction only under particularly mild conditions which do not cleave glucosides [9]. Levulinic acid was reported as a main degradation product of 2-deoxyribose under acidic conditions [10,11]. From tri-*O*-acetyl-D-glucal, which is a precursor of 2-deoxy-D-*arabino*-hexose, the corresponding methyl δ-methoxylevulinate was formed under reflux with methanolic HCl [12].

Deoxy analogues of carbohydrates are used, for example, in studies of carbohydrate–antibody interactions [13,14] or as enzyme inhibitors. The analysis of 2-deoxy and the even more acid-labile 2,6-dideoxy sugars as constituents of cardiac glycosides [15] or of 3,6-anhydro-2-deoxy sugars, which are present in several products of biological importance [16], is also of interest.

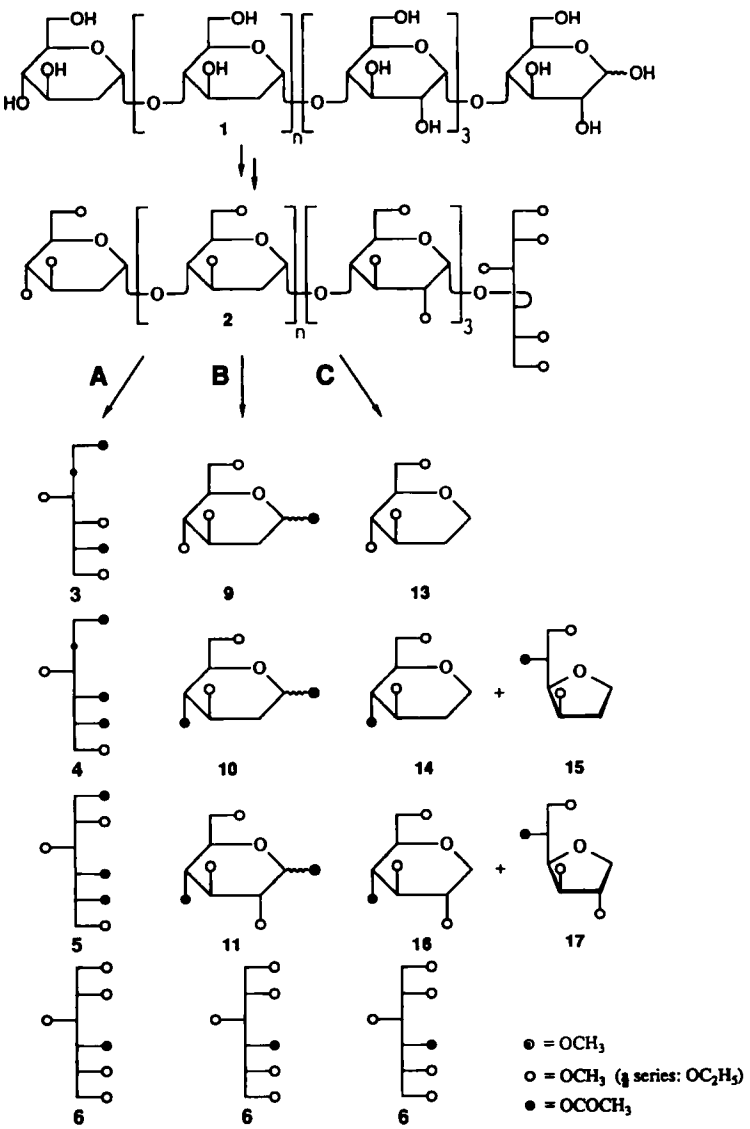
2. Results and discussion

A mixture of oligosaccharides (**1**) containing (1→4)-linked 2-deoxy-D-*arabino*-hexose linked to maltotetraose was prepared from D-glucal and maltotetraose as acceptor by the action of potato phosphorylase [17].The oligomers were insoluble in water, but slightly soluble in dimethyl sulfoxide and in aqueous alkaline solution.

In order to characterize the oligomeric mixture with respect to the linkage positions of the 2-deoxy-D-*arabino*-hexosyl residues and the chain length (average dp), various approaches were applied.

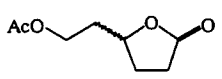
Peralkylation.—The oligomers (**1**) were permethylated with lithium dimsyl and methyl iodide in dimethyl sulfoxide after reduction to give **2**. For comparison a second portion was perethylated to give **2a** (Scheme 1).

Acid hydrolysis of the permethylated oligomers.—Acid hydrolysis of **2** with 0.1–2 M trifluoroacetic acid at 90–120°C led to partial or complete destruction of the 2-deoxy-D-*arabino*-hexose. After reduction and acetylation (Scheme 1, path A), little or no 1,5-di-*O*-acetyl-2-deoxy-D-*arabino*-hexitol (**3**) and the corresponding 1,4,5-tri-*O*-acetyl derivative **4** were observed in addition to the glucitol derivative **5** and **6**. Independent of the type of pre-treatment (none, methylation, or ethylation) prior to hydrolysis, the same products **7** and **8** with a molecular mass of 172 and 258 (determined by CIMS) were always formed. Apparently, degradation of the liberated 2-deoxy-*arabino*-hexoses proceeded even before hydrolysis of these acid-labile glycosidic bonds was complete. As evidence for this, products **7** and **8** were present in considerable amounts while dimers could still be detected by GLC–

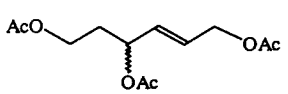


Scheme 1.

MS. Compound **7** could be identified as (4*R*,*S*)-6-*O*-acetyl-2,3,5-trideoxyhexono-1,4-lactone, and **8** as the mixture of stereoisomers of 1,4,6-tri-*O*-acetyl-2,3,5-trideoxyhex-2-enitol. Identification and formation are discussed below.



7



8

Methanolysis.—Methanolysis is known to be a milder cleavage method especially for acid-labile sugars, because the aldehyde functions of liberated sugars are protected by

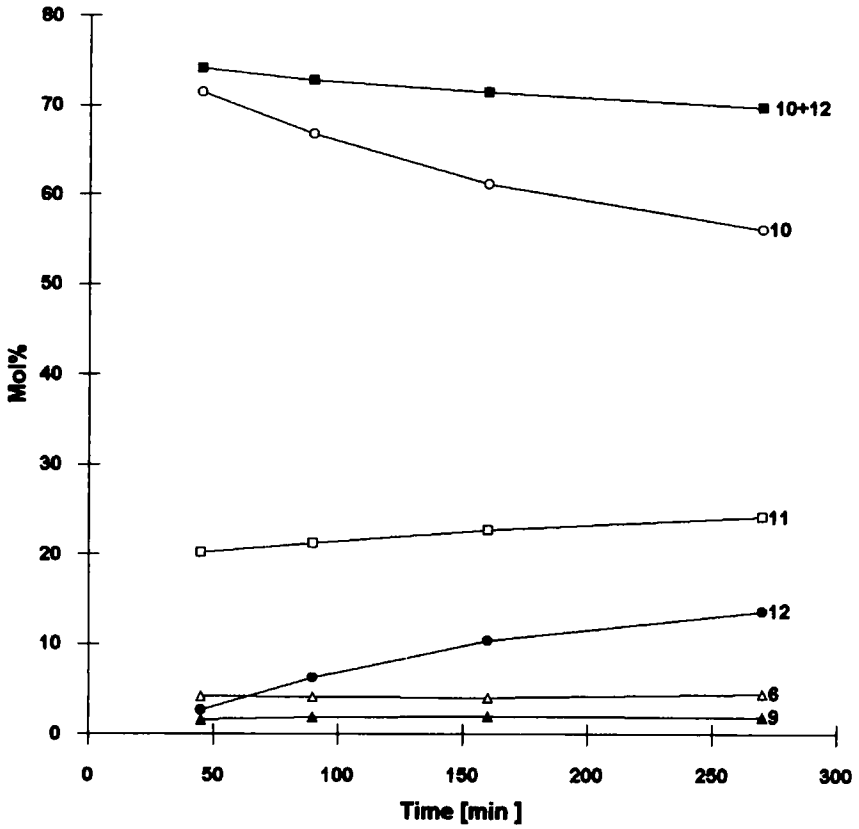
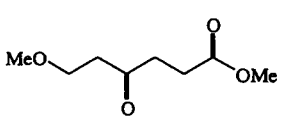


Fig. 1. Composition of the degradation products of permethylated oligosaccharides 2 obtained by methanolysis with 0.5 M MeOH–HCl at 80°C and subsequent acetylation. The graphs are labelled with the compound numbers.

acetalation and enolisation is thus prevented. The expected products of methanolysis of **2** (**6**, **9**, **10**, and **11**) are shown in Scheme 1, path B. All these compounds could be identified by GLC–MS. However, a by-product (**12**) with a molecular mass of 174 (determined by CIMS), derived from 2-deoxy-D-*arabino*-hexose, increased with progressing methanolysis and higher concentration of acid. This product is identical with a by-product obtained from the ethylated sample (**2a**); **12** could be identified as methyl 2,3,5-trideoxy-6-*O*-methyl-4-hexulosonate. Identification and formation are discussed below.



12

Methanolysis with 0.1 M MeOH–HCl at 85°C was incomplete. Only small amounts of **12** were formed under these conditions within 4 h. The composition of the degradation products after methanolysis with 0.5 M MeOH–HCl at 80°C was determined at different times. Peak areas of the gas chromatograms were corrected according to the "effective-carbon-response" (ECR)-concept [18,19]. Results are shown in Fig. 1. The amount of the

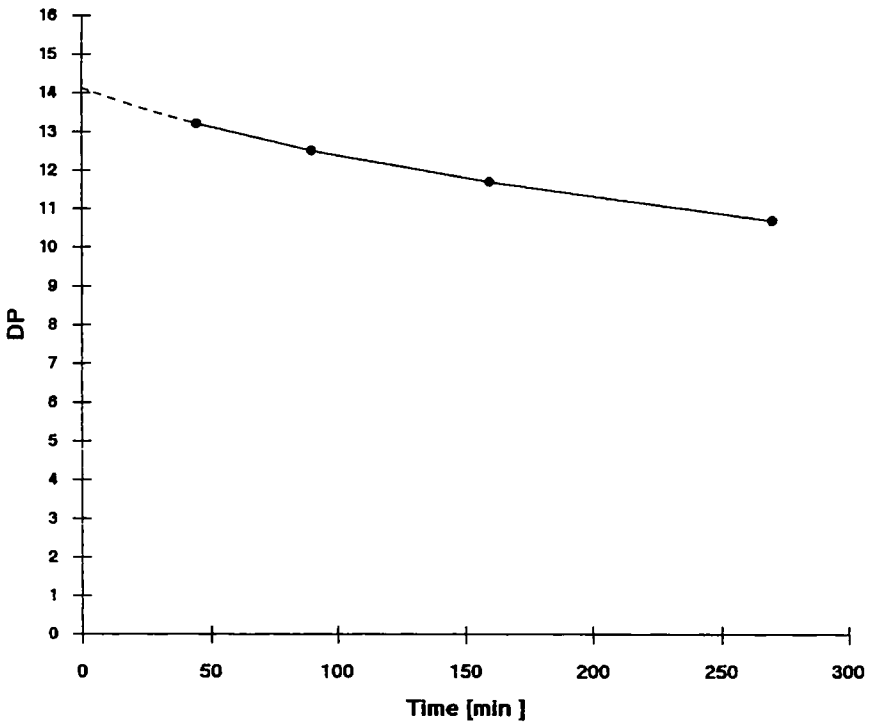


Fig. 2. Average dp value of **2** without the maltotetraose part, calculated from (**10**+**12**):[1/4 (**6**+**11**)]+1 (data from Fig. 1).

terminal deoxy sugar residues **9** was too low, presumably due to losses during evaporation steps. The ratio of the methyl glucosides **11** slowly increased with time, whereas the glucitol **6** remained almost constant. The ratio of **10** decreased with progressing reaction, whereas that of the side-product **12** increased at a comparable rate. Fig. 2 shows the average dp for the 2-deoxy sugar chain calculated from the ratio of the summarized products from the 4-*O*-linked 2-deoxy sugar (**10**+**12**) to one-quarter of the products formed from the maltotetraose part (**6**+**11**). One unit was added for the terminal 2-deoxyglucose. Extrapolated to zero time, a total dp of ca. 14+4 was obtained. Losses of the most volatile component **12** during evaporation of methanol–HCl must also be accounted for.

In order to diminish the losses of the terminal 2-deoxy sugar unit **9**, a perethylated sample **2a** was submitted to methanolysis (0.25 M MeOH–HCl, 90°C, 0.75–4.5 h). As expected the molar ratio of **9a** was much higher than for methylated **9**. The dp value calculated from (**9a**+**10a**+**12**):[1/4 (**11a**+**6a**)] and extrapolated to zero time was 16.5+4 (results not depicted).

Reductive cleavage.—As an alternative to the degradation methods already described, **2** was submitted to reductive cleavage (Scheme 1, path C). To a solution of **2** in dichloromethane was added trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$) or a mixture of trimethylsilyl methanesulfonate (Me_3SiOSO_2Me), trimethylsilyl trifluoroacetate ($Me_3SiOCOCF_3$), or trimethylsilyl trichloroacetate ($Me_3SiOCOCCl_3$) with $BF_3 \cdot OEt_2$ (5:1), together with triethylsilane (Et_3SiH) as hydride donor. After different reaction times at room temperature, aliquots were taken from the mixture and directly acetylated with a

Table 1
Mass-spectral data of the 1,5-anhydro-2-deoxy-*D-arabino*-hexitol derivatives obtained by reductive cleavage of **2** (**13**, **14**, and **18**). Values of *m/z* < 100 are given only with a relative intensity > 10%

Compound	*m/z* (relative intensity, %)
13	CIMS (NH_3): 191 (100), 208 (24) EIMS: 43(26), 45(86), 53(10), 55(11), 57(14), 58(18), 59(21), 71(100), 72(24), 73(15), 75(19), 85(36), 87(18), 88(19), 89(16),101(55), 102(22), 113(24), 145(28), 158(4)
14	CIMS (NH_3): 219 (100), 236(16) EIMS: 41(18), 43(100), 45(28), 57(10), 71(39), 72(10), 87(12), 99(21), 101(6), 113(26), 127(3), 141(75), 142(7), 173(16)
18	CIMS (NH_3): 247(100), 264(24) EIMS: 43(100), 45(10), 57(13), 71(29), 72(62), 101(34), 112(16), 113(3), 115(4), 126(1), 127(1), 131(1), 141(2), 145(2), 154(5), 155(1), 203(2)

mixture of CF_3CO_2H and Ac_2O (1:10) within 15 min [8,20]. After washing, these diluted aliquots were measured by GLC without further evaporation. All products shown in Scheme 1, path C, could be identified by GLC–MS (EIMS and CIMS); **13**, **16**, **17**, and **6** also by comparison with authentic standards. MS data of **13**, **14**, and **18** are shown in Table 1. 2-Deoxy-D-*arabino*-hexose shows a strong tendency for ring contraction. Depending on the catalyst, up to 65% of 5-*O*-acetyl-1,4-anhydro-2-deoxy-3,6-di-*O*-methyl-D-*arabino*-hexitol (**15**) was formed in addition to the expected 1,5-anhydro isomer **14**. Fig. 3 shows a gas chromatogram; **14** and **15** (peak 2) coelute on this column (CP Sil-5 CB), but can be separated on a column with CP Sil-19 CB as the stationary phase.

Trimethylsilyl trifluoromethanesulfonate was the strongest Lewis acid used. In a single experiment, a considerable amount of demethylation products of the 2-deoxy sugar was obtained.

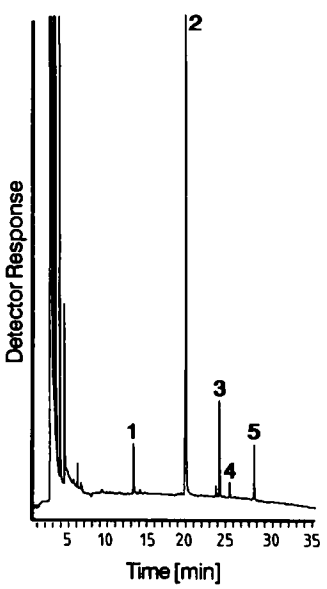


Fig. 3. Gas chromatogram of the degradation products of **2** obtained by reductive cleavage accomplished with Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ and subsequent in situ acetylation: 1, **13**; 2, **15** + **14**; 3, **16**; 4, **17**; 5, **6**.

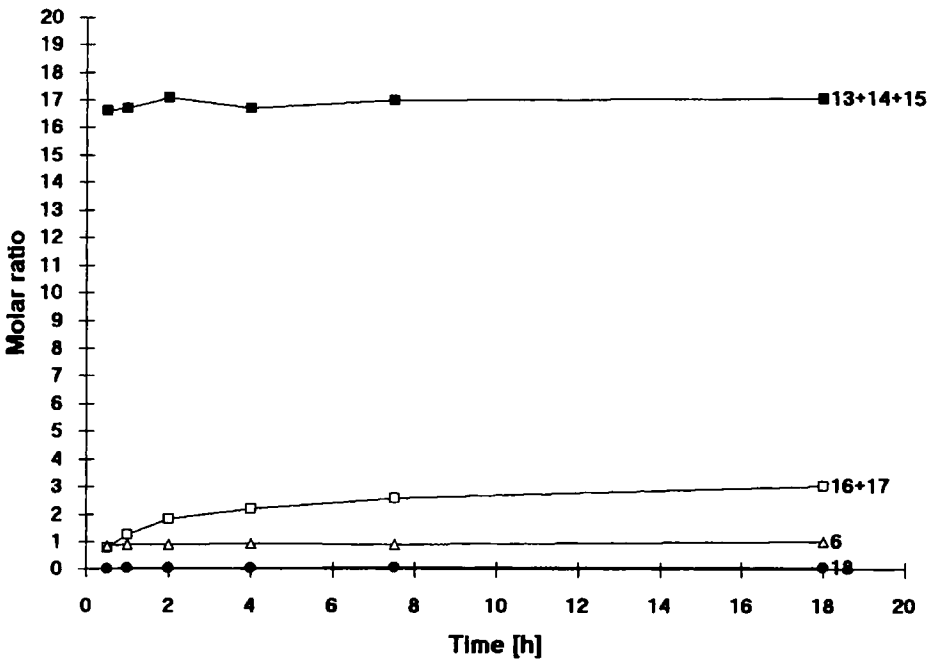
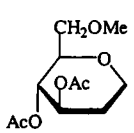


Fig. 4. Composition of the degradation products of **2** obtained by reductive cleavage and subsequent in situ acetylation [0.025 mmol/mL with respect to the glycosidic bonds, 4.5 equiv of Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ (5:1)]. The graphs are labelled with the compound numbers.

With a mixture of Me_3SiOSO_2Me and $BF_3 \cdot OEt_2$ as Lewis acid, **13** and **14**+**15** were obtained in a molar ratio of ca. 1:15. With increasing reaction time, a by-product (**18**) appeared, which could be identified as 3,4-di-*O*-acetyl-1,5-anhydro-2-deoxy-6-*O*-methyl-D-*arabino*-hexitol by comparison with the well known fragmentation pattern of 3,4-di-*O*-acetyl-1,5-anhydro-2,6-di-*O*-methyl-D-glucitol [21]. The ratio of the glucitol derivative **6** decreased with proceeding reaction time as reported earlier by Bennek et al. [22]. However, under appropriately mild conditions, up to 90% of the expected amount of **6** could be obtained. (Permethylated maltitol gave 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol and **6** in a ratio of 1:0.97 within 1 h.) The changes of the composition with time are shown in Fig. 4. The glycosidic bonds of the deoxy sugar are cleaved very quickly with Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ as Lewis acid, whereas degradation of the maltotetraose part is complete within 1 to 18 h, depending on the exact conditions applied. An optimal concentration of 0.025 mmol/mL with respect to the glycosidic bonds of **2** was found to avoid demethylation. The average dp calculated from the summarized amounts of 1,4(5)-anhydro-2-deoxy-D-*arabino*-hexitols (**13**, **14**, **15**, and **18**) to one-third of the anhydroglucitols (**16** and **17**) was 16.6 + 4. Isolated oligomers of a single dp were also submitted to reductive cleavage (see below).



18

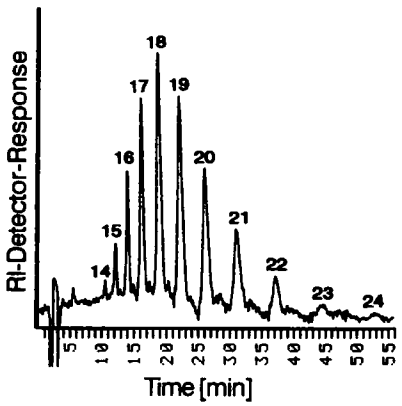


Fig. 5. HPLC chromatogram of **2**. For conditions, see Experimental section. Peaks are assigned with the total dp.

With weaker Lewis acid mixtures $Me_3SiOCOCF_3$–$BF_3 \cdot OEt_2$ and $Me_3SiOCOCCl_3$–$BF_3 \cdot OEt_2$, the glucosidic bonds are no longer quantitatively cleaved. However, the conditions are more appropriate for the less stable glycosidic linkages of the 2-deoxyhexosyl residues. $Me_3SiOCOCCl_3$–$BF_3 \cdot OEt_2$ can even be used to cleave these linkages nearly selectively as described for 5-*O*-linked galactofuranosyl residues [23]. Formation of the demethylation product **18** can be totally suppressed by these milder reductive cleavage systems, thus avoiding erroneous interpretation of linkage positions in an unknown sample. The average dp was calculated from **13** to (**14**+**15**) and was again 16.6 without the maltotetraose.

Reductive cleavage of the ethylated sample **2a** gave **13a**, **14a**, **15a**, **16a**, **17a**, and **6a**. The isomers **14a** and **15a** could be separated by means of GLC with CP-Sil 5 CB as the stationary phase. The ratio of terminal to 4-*O*-linked 2-deoxy-*arabino*-hexosyl residues was 1:15 under appropriate conditions (results not shown).

HPLC separation of the permethylated glycan oligomers.—In order to get further information about the exact composition of the oligomeric mixture, especially about the distribution of the dp values, **2** was separated by reversed-phase HPLC. In Fig. 5 the distribution of ca. 11 oligomers can be recognized. An isolated single oligomer was analyzed by positive ion FABMS showing a pseudomolecular ion at m/z 3515 ($[M+Na]^+$), which corresponds to a dp of 15+4 (M of $C_{159}H_{284}O_{81}$ = 3492). After assigning the dp values of the chromatographic fractions, the total composition and the average dp could be calculated by accounting for the slightly different response of the RI-detector for the homologous oligosaccharides (response proportional to the molecular mass). The average dp calculated on this basis is 15.4+4. Due to the exponential increase of the retention times (isocratic conditions), small amounts of higher homologues presumably escaped detection, so that the real dp may be slightly higher. Two isolated oligomers (dp = 15+4 and 16+4) were submitted to reductive cleavage with Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ and chain lengths of 16.0 and 16.8 units, respectively, for the deoxy sugar part were determined.

NMR spectroscopy.—The ^1H NMR spectrum of **2** and of an isolated oligomer (dp = 16+4 as determined by FABMS) were nearly identical. In the anomeric proton area, a large signal (δ 5.45), a smaller one (δ 5.40), and three small doublets (δ = 5.18, 5.55, and 5.63, J 3.57 Hz) were observed, apparently caused by the three differently linked α-D-

glucosyl residues. The integration of the broad signal belonging to H-1 of the interglycosidically linked 2-deoxy-α-D-*arabino*-hexosyl residues and of the H-1 signal of a single deoxy sugar residue, presumably that one which is 4-*O*-linked to glucose, led to a dp of 17.7 + 4 (for the isolated oligomer with dp 16 + 4).

Identification of the degradation products **7**, **8**, *and* **12** *of 2-deoxy*-D-arabino-*hexose.*— Compounds **7** and **8** were also formed when free 2-deoxy-D-*arabino*-hexose was heated in aqueous acid.

The ^{13}C and ^{1}H NMR spectra (for data, see Experimental section) suggested a γ-lactone structure for **7**. This was also confirmed by mass spectra of different derivatives. The trimethylsilyl ether showed a molecular mass of 202, indicating one $OSiMe_3$ group by comparison with the acetyl derivative ($\Delta M = 30$). Under alkaline methylation conditions the main product showed the mass 190. The fragmentation pattern of the EI-mass spectrum could be interpreted and assigned to methyl 4,6-di-*O*-methoxyhexanoate. The enantiomers of **7** could be separated by GLC using a chiral stationary phase [24]. Compound **8** took up two deuterium atoms by reduction with $NaBD_4$ and was found to contain three acetyl groups.

The precursor of **7** formed by acid hydrolysis is assumed to be the corresponding 4-oxo compound, namely, 2,3,5-trideoxy-4-hexulosonic acid. Alkaline methylation resulted in various *C*-methylated products. This is confirmed by the by-product **12** formed during methanolysis and identified as the corresponding 6-*O*-methylated methyl ester by its mass spectrum, and is in agreement with the formation of this compound (**12**) from tri-*O*-acetyl-D-glucal [12]. The formation can be interpreted to be analogous to the formation of levulinic acid from 2-deoxyribose or furfuryl alcohol, including several dehydration and hydration steps and rearrangements [10,11]. By this way a β-acylacrolein is formed giving 1,4,6-tri-*O*-acetyl-2,3,5-trideoxyhex-2-enitol after reduction and acetylation. The mass spectrum of **8** is in agreement with this structure. To our knowledge the formation of **7** and **8** from 2-deoxyhexoses has not been reported previously.

3. Conclusion

The composition of a mixture of oligosaccharides containing acid-labile 2-deoxyhexosyl and much more resistant glucosyl residues could be completely characterized by reductive cleavage, HPLC, FABMS, and ^{1}H NMR spectroscopy. Reductive cleavage allowed the quantitative determination of the 2-deoxy sugar residues without side-reactions, whereas 2,3,5-trideoxy-4-hexulosonic acid was formed when acidic conditions were used.

4. Experimental

General methods.—The mixture of oligomeric glycans containing 2-deoxy-D-*arabino*-hexosyl residues and maltotetraose was prepared as described [17]. Acetic anhydride, MeCN (HPLC grade), $CHCl_3$, CH_2Cl_2, Me_2SO, EtI, MeI, 1-methylimidazole, $NaBH_4$, $NaBD_4$, triethylsilane, and CF_3CO_2H (all analytical grade) were purchased from Merck (Darmstadt, Germany); $Me_3SiOSO_2CF_3$, Me_3SiOSO_2Me, $Me_3SiOCOCF_3$, and

$Me_3SiOCOCCl_3$ were from Fluka (Neu-Ulm, Germany); BF_3 etherate, MeLi, and pyridine were from Aldrich (Steinheim, Germany).

Reduction of the oligomers.—Mixture **1** (9.5 mg) was suspended in 1 mL of a 0.5 M $NaBH_4$ solution in 1 M NH_3 in a V-vial and stirred at 60°C for 2 h and a further 20 h at 40°C. After destruction of the excess of $NaBH_4$ with AcOH, borate was removed by repeated evaporation with 15% AcOH in MeOH. The residue was suspended and desalted by dialysis against H_2O (Spectra/PorCE membrane, MWCO 1000). After lyophilization a white powder was obtained (9.7 mg).

Peralkylation [25].—The reduced oligomeric mixture of **1** (9.7 mg) was dissolved in Me_2SO (1 mL) under N_2. Lithium dimsyl (5 equiv per OH group), prepared from a 5% solution of MeLi in ether and the same volume of Me_2SO, was added and, after 2 h, MeI (6 equiv) was added under cooling. The product was isolated by extraction with $CHCl_3$ and washing with water. A colourless film of **2** (9.2 mg) was obtained. Perethylation was performed in a similar way with EtI.

Acid hydrolysis.—Acid hydrolysis was performed with 0.1–2.0 M CF_3CO_2H at 90 to 120°C for 0.5 to 1.5 h. Reduction (with $NaBD_4$) and acetylation were carried out as described [26].

Methanolysis—To 0.1–0.5 mg of **2** (**2a**) in a V-Vial was added 0.1 (0.5; 0.25) M MeOH–HCl and the mixture was stirred in a heating block at 80 (85; 90)°C for 0.5–4.5 h. After evaporation to dryness, the residue was acetylated with 1-methylimidazole–Ac_2O in CH_2Cl_2 at room temperature.

Reductive cleavage.—To 1 mg of **2** (**2a**) in a silylated screw-cap vial was added CH_2Cl_2 (200 μL) containing 4.5 (9.0) equiv/glycosidic bond of Et_3SiH and Me_3SiOSO_2Me ($Me_3SiOCOCF_3$, $Me_3SiOCOCCl_3$) and 0.9 (1.8) equiv of $BF_3 \cdot OEt_2$ (freshly prepared premix), and the mixture was kept at room temperature. After different times (0.5, 1, 2, 4, 7.5, and 18 h), aliquots of 10 μL (corresponding to 50 mg of the sample) were taken, diluted with CH_2Cl_2 (30 μL), and acetylated with CF_3CO_2H–Ac_2O (4 μL, 1:10) for 15 min at 50°C in a screw-cap vial with a silylated 250-μL inlet (Amchro). After 5 min at room temperature the solutions were washed with aq $NaHCO_3$ (2 times), and the organic phase was separated, dried with a small piece of $CaCl_2$, diluted to 500 μL, and analyzed by GLC.

GLC.—GLC was carried out on a Carlo Erba Fractovap 4160 gas chromatograph, equipped with an on-column injection system, a CP-Sil 8 CB capillary column (25 m × 0.25 mm) and a 2-m retention gap, a flame-ionization detector, and an HP integrator 3390A; and on a Siemens Sichromat 2 instrument equipped with a CP-Sil 5 CB column (25 m × 0.25 mm) and a Merck Hitachi D-2500 Chromatointegrator. Hydrogen was used as carrier gas. Response factors (for multiplication of the peak areas) were calculated according to the ECR-concept [18,19]: **3** (0.779), **4**, **5**, and **8** (0.731), **6** (0.845), **7**, **9**, and **13** (1.090), **10**, **11**, **14**, **15**, **16**, and **17** (1.000), **12** (1.112), and **18** (0.924).

GLC–MS.—Mass spectra were obtained with a VG Analytical VG/70-250S instrument. For CIMS, ammonia was used as reactant gas.

HPLC.—HPLC separation was performed on a Nucleosil 100-C18 column (Knauer, Bad Homburg, Germany, 250 × 4 mm, 5 μm) with a Merck Hitachi L-6200 pump, a Merck differential refractometer RI-71, and a Merck Hitachi D-2500 Chromatointegrator. 7:3

Acetonitrile–water was used as the eluent at a flow rate of 1.0 mL/min, with a column temperature of 55°C.

FABMS.—FAB mass spectra were recorded on a VG Analytical VG-ZAB2-SE-FPD instrument with a cesium gun, from a solution of an HPLC-fraction of **2** in $CHCl_3$ (20 μg/μL) with *m*-nitrobenzyl alcohol as matrix.

NMR spectroscopy.—^{1}H NMR spectra (400 MHz) were obtained for $CDCl_3$ solutions by using a Bruker WM400 instrument. Chemical shifts were expressed as δ relative to tetramethylsilane as internal standard. ^{13}C NMR spectra were recorded with the same instrument at 100 MHz.

*(4*R,S*)-6-*O*-Acetyl-2,3,5-trideoxyhexono-1,4-lactone* (**7**).—Compound *7* was isolated by silica gel liquid chromatography. The racemic mixture could be separated by enantioselective GLC [24] (Lipodex E, 25 m, H_2, 150°C) with an α-value of 1.088. CIMS (NH_3): *m/z* (% rel.int.) 173*(81), 190*(100); EIMS: *m/z* (% rel.int.) 43(95), 56(14), 57*(15), 67*(11), 68*(9), 84*(9), 85*(100), 100*(12), 112(16), 112*(9) 129*(2), 130*(2), 142*(1), 144*(1), 173*(1); *m/z**: fragments are shifted ($\Delta = +1$) when reduction is carried out with $NaBD_4$. ^{1}H NMR: δ 1.99, 2.05 (m, 2 H, H-5a,b), 1.91, (m, 1 H, H-3a), 2.10 (s, 3 H, Ac), 2.38 (m, 1 H, H-3b), 2.55 (dd, 2 H, H_2-2), 4.20, 4.25 (m, 2 H, H-6a,b), 4.61 (m, 1 H, H-4). ^{13}C NMR: δ (ppm) 20.27 (*Me*CO), 27.44, 28.03 (C-3, C-5), 34.10 (C-2), 60.01 (C-6), 76.90 (C-4), 170.22 (Me*C*O), 176.00 (C-1).

*1,4,6-Tri-*O*-acetyl-2,3,5-trideoxyhex-2-enitol* (**8**).—CIMS (NH_3): *m/z* (% rel.int.) 199(80), 259(2), 276(39) (after reduction with $NaBD_4$: M = 260). EIMS: *m/z* (% rel.int.) 43(100), 67(16), 68(27), 78(25), 96(52), 103(7), 115(6), 127(2), 138(3), 156(6), 198(1).

*Methyl 2,3,5-trideoxy-6-*O*-methyl-4-hexulosonate* (**12**).—CIMS (NH_3): *m/z* (% rel.int.)143(100), 160(9), 175(10), 192 (3). EIMS: *m/z* (% rel.int.) 45(100), 55(58), 59(21), 87(35), 100(5), 111(19), 114(17), 115(32), 127(2), 142(11), 143(4), 159(1).

Acknowledgements

We are grateful to Dr. H.J. Räder, MPI Mainz, Germany, for recording the FAB-mass spectra, and the Fonds der Chemischen Industrie, the Deutsche Forschungsgemeinschaft, and the Bundesministerium für Forschung und Technologie for financial support.

References

[1] H. Björndal, C.G. Hellerquist, B. Lindberg, and S. Svensson, *Angew. Chem.*, 82 (1970) 643–652; *Angew. Chem., Int. Ed Engl.*, 9 (1970) 610–619.
[2] H. Heims, H. Steinhart, and P. Mischnick, *Carbohydr. Res.*, 191 (1989) 343–350.
[3] D. Rolf and G.R. Gray, *Carbohydr. Res.*, 131 (1984) 17–28.
[4] S.G. Zeller and G.R. Gray, *Carbohydr. Res.*, 198 (1990) 285–303.
[5] P. Mischnick, *Minutes, Proc. Int. Symp. Cyclodextrins, 5th*, Editions de Santé, Paris, 1990, pp 90–94.
[6] J.S. Sherman and G.R. Gray, *Carbohydr. Res.*, 231 (1993) 221–235.
[7] J.D. Stankowski, B.E. Mueller, and S.G. Zeller, *Carbohydr. Res.*, 241 (1993) 321–326.

[8] K. Kiwitt-Haschemie, H. Heims, H. Steinhart, and P. Mischnick, *Carbohydr. Res.*, 248 (1993) 267–275.
[9] W.G. Overend, M. Stacey, and J. Stanek, *J. Chem. Soc.*, (1949) 2841–2845 and 2846–2849.
[10] J.K. Seydel, E.R. Garrett, W. Diller, and K.-J. Schaper, *J. Pharm. Sci.*, 56 (1967) 858–862.
[11] L. Birkofer and F. Beckmann, *Justus Liebigs Ann. Chem.*, 620 (1959) 21–31.
[12] M. Bergmann and H. Machemer, *Ber.*, 66 (1933) 1063–1065.
[13] J. Thiem and W. Klaffke, *Top. Curr. Chem.*, 154 (1990) 285–332.
[14] E. Petráková, P. Kováč, and C.P.J. Glaudemans, *Carbohydr. Res.*, 233 (1992) 101–112.
[15] T. Reichstein and E. Weiss, *Adv. Carbohydr. Chem.*, 17 (1962) 65–120.
[16] A. Serrano and E. Roman, *J. Carbohydr. Chem.*, 12 (1993) 237–246.
[17] B. Evers, P. Mischnick, and J. Thiem, *Carbohydr. Res.*, 262 (1994) 335–341.
[18] D.P. Sweet, R.H. Shapiro, and P. Albersheim, *Carbohydr. Res.*, 40 (1975) 217–225.
[19] A.D. Jorgensen, K.C. Picel, and V.C. Stamoudis, *Anal. Chem.*, 62 (1990) 683–689.
[20] P. Mischnick, M. Hagen, and B. Grab, *Abstracts of Papers EUROCARB VI*, Edinburgh, 1991, A29.
[21] P. Mischnick-Lübbecke, Thesis, University of Hamburg, 1987; P. Mischnick-Lübbecke and W.A. König, *Carbohydr. Res.*, 185 (1989) 113–118.
[22] J.A. Bennek, M.J. Rice, and G.R. Gray, *Carbohydr. Res.*, 157 (1986) 125–137.
[23] P. Mischnick and G.A. De Ruiter, *Carbohydr. Polym.*, 23 (1994) 5–12.
[24] W.A. König, R. Krebber, and P. Mischnick, *J. High Res. Chromatogr. Chromatogr. Commun.*, 12 (1989) 732–738.
[25] A.J. D'Ambra, M.J. Rice, S.G. Zeller, P.R. Gruber, and G.R. Gray, *Carbohydr. Res.*, 177 (1988) 111–116.
[26] P. Mischnick, *Carbohydr. Res.*, 192 (1989) 233–241.