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Intestinal Glycoproteins of Germfree Rats. IV. Oligosaccharides Obtained by Chemical Degradation of a Water-soluble Glycoprotein Fraction*

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Removal of sialic acid from the glycoprotein and subsequent degradation with sodium hydroxide—sodium borohydride resulted in the formation of a number of low-molecular weight carbohydrate compounds of which three were isolated and characterized: 2-deoxy-2-acetamido-D-galactitol; *O*-α-L-fucopyranosyl (1→2)-*O*-β-D-galactopyranosyl-(1→3)-2-deoxy-2-acetamido-D-galactitol (oligosaccharide I); *O*-β-D-galactopyranosyl-(1→3)-2-deoxy-2-acetamido-D-galactitol (oligosaccharide II). From an acetolysate of the desialized glycoprotein a fourth component was obtained and characterized to be *O*-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-D-glucose (oligosaccharide III).

The mucin layer that covers the epithelial surface of the intestinal mucosa in mammals is known to contain glycoproteins as major constituents.[1-3] Detailed chemical studies of these biopolymers have been impeded by the fact that they are rapidly degraded by the intestinal microflora; however, by the use of germfree animals the intestinal mucin glycoproteins may be obtained essentially in the undegraded state. A water-soluble glycoprotein fraction from the intestinal mucus of germfree rats has been characterized recently [4] and the present communication reports studies on the carbohydrate side chains of this glycoprotein.

RESULTS AND DISCUSSION

It has been well established for glycoproteins of the mucin type that the carbohydrate-peptide linkage involves an α-*O*-glycosidic bond between *N*-acetylgalactosamine and the hydroxyl group of serine or threonine in the polypeptide chain. This linkage is labile to alkali, resulting in β-elimination of the carbohydrate moiety. By performing the alkali treatment in the presence of excess borohydride the alkali catalysed peeling reaction with concomitant degradation of the carbohydrate chains is greatly diminished, as first demonstrated by Carlson [5] who succeeded in isolating from pig submaxillary mucin a number of oligosaccharides, all terminating in *N*-acetylgalactosaminitol. The germfree rat intestinal mucin was subjected to alkali treatment under similar conditions, followed by separation of acidic and neutral oligosaccharides on an AG 1 formate ion exchange column. Further fractionation by gel chromatography and preparative paper chromatography (PC) revealed a large number of compounds present in quantities too small for extensive examination. More than half of the carbohydrate content of the mucin was excluded from a Bio-Gel P-2 column indicating the presence of long-chain oligosaccharides or incomplete release of carbohydrate from the polypeptide chain. The failure to detect *N*-acetylgalactosaminitol in this high-molecular weight fraction would favour the latter possibility. Rechromatography of the front peak from the P-2 column on Bio-Gel P-6 gave no further resolution, the peak being excluded also from this gel; evidently a molecular weight of more than 5000 must be assumed for this fraction. Removal of the sialic acid residues from the glycoprotein and sub-

* Part III, see Ref. 4.

sequent treatment with alkali-borohydride furnished an oligosaccharide mixture of a less complex composition, thus facilitating the isolation of individual sugars. After deionization the degraded material was fractionated on a Bio-Gel P-2 column (Fig. 1). Final separation was achieved by preparative PC. However, only two oligosaccharides (I and II) in addition to *N*-acetylgalactosaminitol were isolated in sufficient yield and purity for structural investigation.

The final peak from the P-2 column (fractions 35 – 39) contained *N*-acetylgalactosaminitol and small amounts of fucose and *N*-acetylglucosamine. The *N*-acetylgalactosaminitol was isolated by chromatography on thick paper; it had the same mobility on PC and the derived trimethylsilyl ether had the same retention time on gas liquid chromatography (GLC) as an authentic sample, while it could be easily separated from *N*-acetylglucosaminitol by PC and GLC. Periodate oxidation of the *N*-acetylgalactosaminitol followed by treatment with sodium borohydride gave 2-*N*-acetylamino-1,3-propanediol, identified by cochromatography (PC and GLC) with a reference sample.

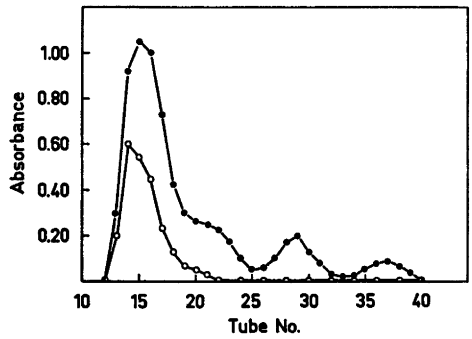

Fig. 1. Chromatography on Bio-Gel P-2 of the products obtained by degradation of desialized glycoprotein with alkali-borohydride. Effluent fractions were analysed for neutral sugar (●) and protein (○). Tubes 26 to 32 and 35 to 39 were pooled, respectively, concentrated and subjected to preparative PC. Experimental details are given in the text.

Oligosaccharide I was a trisaccharide consisting of fucose, galactose, and *N*-acetylgalactosaminitol. Weak acid hydrolysis gave fucose and a non-reducing compound with the chromatographic mobility of oligosaccharide II. By subsequent incubation of this compound with β-galactosidase, galactose and *N*-acetylgalactosaminitol were liberated.

Oligosaccharide I was methylated with methyl iodide-methylsulfinyl carbanion in dimethyl sulfoxide [6] according to the modification of Sandford and Conrad. [7] After hydrolysis and conversion into their alditol acetates the methylated sugars were analysed by GLC [8] – mass spectrometry. [9] The three peaks obtained on the gas chromatogram indicated equimolar proportions of the three component sugars. The first and second peak had the retention time of 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylgalactitol, and the respective mass spectra showed the primary fragments characteristic of the per-

Table 1. Methyl ethers from hydrolysates of methylated oligosaccharides I and III, and the major ions (*m/e*) observed in the mass spectra of their alditol acetates.

Oligosaccharide	Methyl ether	r^a	Major ions (m/e)
I	2,3,4-Tri-*O*-methylfucose	0.60	117,[b] 131,[b] 161,[b] 175[b]
	3,4,6-Tri-*O*-methylgalactose	1.96	45,[b] 101, 129, 145, 161,[b] 189,[b] 205, 234
	1,4,5,6-Tetra-*O*-methyl-2-deoxy-2-*N*-methylacetamidogalactitol	3.25	45,[b] 56, 88, 101, 130,[b], 133,[b] 142, 246,[b] 290[b]
III	2,3,4,6-Tetra-*O*-methyl-galactose	1.15	45,[b] 101, 117,[b] 129, 145, 161,[b] 205[b]
	3,6-Di-*O*-methyl-2-deoxy-2-*N*--methylacetamidoglucitol	2.87	45,[b] 74, 116, 128, 142, 158[b] 202,[b] 233[b]

[a] Retention times of the corresponding alditol acetates relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol. [b] Primary fragments.

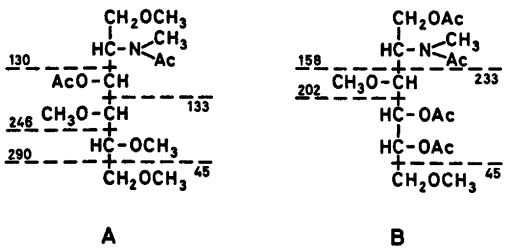

Fig. 2. Primary fragments formed by electron impact on the alditol acetates of the partially methylated amino sugar components of oligosaccharide I (A) and III (B).

acetates of a 2,3,4-tri-*O*-methyl-6-deoxyhexitol and a 3,4,6-tri-*O*-methylhexitol [10] (Table 1). The mass spectrum of the compound in the third peak on the gas chromatogram showed predominant ions at *m/e* 45, 130, 133, 246, and 290 (Table 1). The main diagnostic ion for 2-deoxy-2-*N*-methylacetamidoalditol acetate derivatives is that at *m/e* 158, which is formed by fission between C_2 and C_3 resulting in the ion $[CH_3CO-O-CH_2-CH-N(CH_3)COCH_3]^+$. This ion is absent in the spectrum of the amino sugar derivative from oligosaccharide I. However, methylation of a 2-deoxy-2-acetamidoalditol would introduce a methyl ether group at C_1; thus, in the mass spectrum of such a compound a peak at *m/e* 130 should be expected (Fig. 2 A). The formation of the other primary ions present in the mass spectrum of the amino sugar component is evident from Fig. 2 (A). From the experimental data the following structure is proposed for oligosaccharide I: *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→3)-2-deoxy-2-acetamido-D-galactitol. The α anomeric configuration is assigned to the fucose since this sugar was released from the rat intestinal mucin [4] on incubation with a specific α-(1→2)-L-fucosidase.[11]

The small quantity isolated of oligosaccharide II did not permit methylation analysis. It was composed of galactose and *N*-acetylgalactosaminitol and was indistinguishable from the compound obtained on the removal of fucose from oligosaccharide I. Incubation of oligosaccharide II with a β-galactosidase furnished galactose and *N*-acetylgalactosaminitol. Periodate oxidation of the disaccharide followed by treatment with sodium borohydride and mild acid hydrolysis led to total destruction of the galactose. PC of the resulting product revealed, in addition to glycerol, the presence of a compound which could be detected with periodate-benzidine and also by a hypochlorite-potassium iodide-starch reagent. When oligosaccharide I was subjected to this Smith degradation procedure, again the same compound was detected; furthermore it was also obtained, together with 2-*N*-acetylamino-1,3-propanediol, by incomplete periodate oxidation of *N*-acetylgalactosaminitol and subsequent borohydride reduction. The formation of this compound, possibly *N*-acetylthreosaminitol, from oligosaccharide I and II strongly indicates that galactose is linked to C_3 of the *N*-acetylgalactosaminitol in both oligosaccharides. Accordingly the structure of oligosaccharide II is proposed to be *O*-β-D-galactopyranosyl-(1→3)-2-deoxy-2-acetamido-D-galactitol.

Baig and Aminoff [12] have also reported the isolation of oligosaccharides I and II after alkali-borohydride treatment of a hog submaxillary glycoprotein.

Besides the alkali-borohydride degradation procedure acetolysis was also attempted to obtain oligosaccharides from the germfree rat intestinal glycoprotein. The desialized glycoprotein was acetylated to give a product which was soluble in the acetolysis medium. After acetolysis the products were deacetylated and fractionated on a Bio-Gel P-2 column (Fig. 3.)[13] The front peak contained high-molecular weight

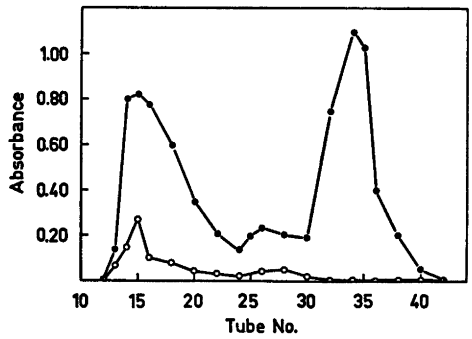

Fig. 3. Chromatography on Bio-Gel P-2 of the products obtained after acetolysis of desialized glycoprotein. The effluent was analysed for neutral sugar (●) and protein (○). Tubes 24 to 30 were pooled, concentrated and subjected to preparative PC. Experimental details are given in the text.

material, the middle one oligosaccharides and glycopeptides, and the last peak mostly monosaccharides. The middle fraction (tubes 24 – 30) was further fractionated by chromatography on thick paper. A large number of compounds was present but only one (oligosaccharide III) was isolated for further examination. It was a disaccharide consisting of galactose and *N*-acetylglucosamine; incubation with β-galactosidase gave the two component sugars. Oligosaccharide III was methylated, hydrolysed, the sugars converted into their alditol acetates and analysed by GLC-mass spectrometry. The first peak on the gas chromatogram had the same retention time as a reference sample of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, and the mass spectrum showed peaks corresponding to the typical fragmentation pattern of a peracetylated 2,3,4,6-tetra-*O*-methylhexitol [10] (Table 1). The mass fragments of the amino sugar derivative (Table 1) are consistent with those obtained by Stellner *et al.*[14] and by Schwarzmann and Jeanloz [15] for 1,4,5-tri-*O*-acetyl-3,6-di-*O*-methyl-2-deoxy-2-*N*-methylacetamidoglucitol; the primary fragments are depicted in Fig. 2 (B). The data presented agree with the following structure for oligosaccharide III: *O*-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-D-glucose.

It is evident from the present work that neither of the two methods employed for degradation appeared ideal for oligosaccharide formation. Both the acidic and the alkaline mode of degrading the glycoprotein resulted in a large number of oligomers of which none could be demonstrated to occur in a comparatively high proportion. This may reflect the presence of microheterogeneity in the polymer as well as a generally intricate molecular structure, both well known features of most glycoproteins. However, the results obtained so far suggest that the molecular constitution of the carbohydrate portion of the germfree rat intestinal mucin does not differ basically from that of other mucin glycoproteins.

EXPERIMENTAL

N-Acetylgalactosaminitol and *N*-acetylglucosaminitol were prepared as described by Crimmin;[16] the two recrystallized products had m.p. 175 – 176 °C and 148 – 150 °C, respectively, and both were chromatographically homogeneous on PC and GLC.

2-*N*-Acetylamino-1,3-propanediol was prepared from DL-serinol (2-amino-1,3-propanediol), Sigma, by acetylation with acetic anhydride in pyridine at 20 °C for 15 min. The product after recrystallization from ether, had m.p. 84 – 85 °C. The ion exchange resins AG 1, AG 3, and AG 50 and the Bio-Gel P-2 for gel chromatography are products of Bio-Rad Laboratories, Richmond, California, U.S.A. The mixed bed ion exchange resin Zerolit DM-F was purchased from BDH Chemicals, Poole, England.

Analytical methods. Gas-liquid chromatography (GLC) was performed with a Varian 1400 gas chromatograph on a stainless steel column (200 × 0.3 cm) packed with 3 % SE-52 on Varaport 30 for the separation of pertrimethylsilyl ethers of free sugars, alditols, and methyl glycosides. Partially methylated alditol acetates were separated on a glass column (200 × 0.3 cm) containing 3.5 % OV-225 on Varaport 30 at 185 °C.

For GLC-mass spectrometry of partially methylated alditol acetates the OV-225 column was mounted in a Varian 1400 gas chromatograph combined with a Varian CH 7 low-resolution mass spectrometer. The instrumental details are published elsewhere.[17]

Paper chromatography (PC) was carried out on Whatman No. 1 and, for preparative purposes, on Whatman No. 3 MM filter paper in solvent systems (v/v): A. Ethyl acetate, pyridine, acetic acid, water, 5:5:1:3. B. Butanol, pyridine, water, 6:4:3. C. Ethyl acetate, pyridine, water, 8:2:1. Sugars were located on chromatograms by a periodate-benzidine reagent (*a*)[18] or by a hypochlorite-potassium iodide-starch reagent (*b*)[19] which reacts with CH_3CONH-groups.

Methanolysis was performed by heating the oligosaccharide (0.25 mg) with 1 M hydrogen chloride in methanol (0.5 ml) at 85 °C for 20 h, and then preparing the product for GLC as described by Reinhold.[20] Incubation with β-D-galactosidase (Grade IV, Sigma, purified from *E. coli*) was performed by adding the enzyme (5 units) to the sample (1 mg) dissolved in 0.10 M phosphate buffer pH 7.0 (0.5 ml), and keeping the digest at 35 °C for 16 h under toluene. The mixture was then diluted with water (2 ml), deionized by treatment with Zerolit DM-F resin, and the product concentrated to dryness and examined by PC and by GLC.

The elution of ion exchange or gel chromatography columns was monitored by the phenol-sulfuric acid test [21] and by the method of Lowry *et al.*[22] for neutral sugars and protein, respectively.

Removal of sialic acid from the intestinal glycoprotein. The purified glycoprotein (1.1 g) with a sialic acid content of 13.5 % was dissolved in 0.05 M hydrochloric acid (200 ml) and the solution kept at 80 °C for 80 min. After cooling

and neutralization with sodium hydroxide the mixture was concentrated to 50 ml and dialysed against distilled water (4 × 1 l) during 36 h at room temperature. Deionization of the dialysate followed by concentration and PC revealed trace amounts of fucose as the only neutral sugar. The non-dialysable fraction was freeze-dried to give a product (0.92 g) with a sialic acid content of 1.3 %, as estimated by the method of Jourdian *et al.*[23]

Isolation of oligosaccharides after alkali-borohydride treatment of the desialized glycoprotein. The desialized material (0.9 g) was dissolved in 2 M sodium borohydride (100 ml); then 0.1 M sodium hydroxide (100 ml) was added to give a final concentration of 0.05 M sodium hydroxide-1 M sodium borohydride and the solution was kept at 45 °C for 15 h. After cooling and neutralization with acetic acid to pH 6 the turbid solution was clarified by filtration and passed through a column (3 × 50 cm) of AG 50 × 2 (H^+) resin, 50 – 100 mesh. The column was eluted with water until the eluate gave a negative reaction for carbohydrate. After concentration and removal of boric acid by repeated evaporations with methanol the eluate was still acidic, and was neutralized by treatment with AG 3 × 4 A (OH^-) resin, 100 – 200 mesh. The resulting neutral solution was concentrated to a syrup (0.61 g), which was subjected to fractionation on a column (2.5 × 80 cm) of Bio-Gel P-2, 200 – 400 mesh. The syrup was dissolved in 10 % ethanol in water (4 ml) and a third of the portion was taken out each time for chromatography on the P-2 column, which was eluted with 10 % aqueous ethanol. Fractions of 5 ml were collected and aliquots analysed for carbohydrate and protein. The three runs gave almost identical elution curves (Fig. 1) and appropriate fractions were pooled; yield, tubes 13 to 25: 393 mg; tubes 26 to 32: 42 mg; tubes 35 to 39: 38 mg.

The low-molecular weight fraction (38 mg) on GLC and PC proved to contain a compound with the chromatographic mobility of *N*-acetylgalactosaminitol together with minor proportions of fucose and *N*-acetylglucosamine. By preparative PC in solvent system C and B, successively, the substance was isolated in a pure state, yield 10 mg. It cochromatographed with authentic *N*-acetylgalactosaminitol as one compound on GLC and PC, but was clearly separated from *N*-acetylglucosaminitol. The compound (2 mg, *ca.* 10 μmol) was dissolved in an unbuffered aqueous solution (2.5 ml) of 0.02 M sodium metaperiodate (50 μmol) and left in the dark at 20 °C for 2 h. After addition of ethylene glycol (1 drop) and sodium borohydride (5 mg) the solution was left overnight at 5 °C. Then excess acetic acid was added and boric acid was removed by codistillation with methanol; the resulting product was deionized with mixed bed resin prior to analysis by GLC and PC.

The trimethylsilyl derivative of the compound when cochromatographed with the di-trimethylsilyl ether of 2-*N*-acetylamino-1,3-propanediol gave one peak on the gas chromatogram. To separate this peak from that of the solvent a temperature programme was used, starting at 70 °C with an increase of 8 °C per min. Under these conditions the di-trimethylsilyl ether of ethylene glycol, also present in the sample, was eluted in the solvent peak. On PC in solvent systems A and B the isolated compound had the same mobility as 2-*N*-acetylamino-1,3-propanediol.

The middle fraction from the P-2 column (42 mg) was further fractionated by preparative PC in solvent system B. Several compounds were revealed by the periodate-benzidine reagent, of which two, oligosaccharides I (11 mg) and II (4 mg) were obtained as chromatographically homogeneous products.

Oligosaccharide I had R_{Lactose} 1.25 and 1.34 in solvent system A and B, respectively. Methanolysis and subsequent GLC showed the presence of fucose, galactose, and *N*-acetylgalactosaminitol. When oligosaccharide I (2 mg) was hydrolysed with 0.1 M hydrochloric acid (0.5 ml) at 100 °C for 1 h fucose was detected in addition to a non-reducing compound with the chromatographic mobility of oligosaccharide II in solvent systems A and B. The deionized hydrolysate was evaporated to dryness, dissolved in 0.1 M phosphate buffer pH 7.0 and incubated with β-galactosidase. Examination of the product by PC and GLC showed the presence of fucose, galactose, and *N*-acetylgalactosaminitol. After methanolysis and trimethylsilylation *N*-acetylgalactosaminitol gave one, and sometimes two, minor peaks on the gas chromatogram besides the main peak obtained as the single one by direct trimethylsilylation of the alditol. The origin of these smaller peaks is unknown.

Methylation.[6] Oligosaccharide I (6 mg) in a 10 ml serum bottle sealed with a rubber cap was dissolved in dry dimethyl sulfoxide (1 ml). Methylsulfinyl sodium in dimethyl sulfoxide (1 ml, 2 M)[7] was added and the reaction mixture stirred for 1 h at room temperature. Methyl iodide (0.5 ml) was added slowly through a syringe under external cooling and the stirring was continued for 1 h. Water (5 ml) was added and excess methyl iodide was evaporated by bubbling nitrogen gas through the solution. After a further addition of water (50 ml) the methylated sugar was extracted into chloroform by shaking with 2 × 15 ml in a separatory funnel. The combined chloroform extracts (30 ml) were then shaken with water (6 × 100 ml) to remove dimethyl sulfoxide, and the organic phase was dried with anhydrous sodium sulfate and concentrated to dryness. The methylated product was hydrolysed with 90 % formic acid (1 ml) at 100 °C for 3 h. After dilution of the mixture with 4 volumes of water the heating was continued for another 2 h followed by evaporation

of the formic acid. The residue was dissolved in water (2 ml), sodium borohydride (5 mg) was added and the solution left overnight at 5 °C. After acidification with acetic acid and concentration, boric acid was removed by distillation with methanol, the product dissolved in a 1:1 (v/v) mixture of acetic anhydride and pyridine (2 ml) and heated at 100 °C for 15 min. After concentration to dryness the residue was dissolved in chloroform and subjected to GLC-mass spectrometry; the results are given in Table 1.

Oligosaccharide II had $R_{Lactose}$ 1.65 and 1.80 in solvent system A and B, respectively. Methanolysis followed by GLC showed the presence of galactose and *N*-acetylgalactosaminitol. When the oligosaccharide (1 mg) was incubated with β-galactosidase, the resulting material after deionization was shown by PC and GLC to contain galactose and *N*-acetylgalactosaminitol as the only free sugars. Oligosaccharide II (2.2 mg, *ca.* 6 μmol) was dissolved in 0.02 M sodium metaperiodate (2.5 ml, 50 μmol) and kept at 20 °C in the dark for 2 h. After addition of ethylene glycol (1 drop) and sodium borohydride (5 mg) the solution was left overnight at 5 °C and then neutralized and freed of boric acid the usual way. The product was dissolved in 0.05 N sulfuric acid and kept at 80 °C for 70 min, followed by neutralization, deionization, and concentration to dryness. PC of the resulting material established the absence of galactose and the presence of glycerol and another non-reducing compound which could be detected with both reagents *a* and *b*. This latter compound had a $R_{N\text{-acetylglucosamine}}$ of 1.18 and 1.37 in solvent system A and B, respectively, while the corresponding values for 2-*N*-acetylamino-1,3-propanediol were 1.47 and 1.80.

When oligosaccharide I (1 mg, *ca.* 2 μmol) was subjected to the same procedure (oxidation with 50 μmol) of periodate, followed by reduction and acid hydrolysis) a non-reducing compound with the same chromatographic mobility on paper as the one obtained from oligosaccharide II was detected with reagent *a;* however, no spot was revealed with the less sensitive reagent *b*. By oxidation of *N*-acetylgalactosaminitol (2 mg, *ca.* 10 μmol) in 0.02 M periodate (1 ml, 20 μmol) at 20 °C in the dark for 2 h and subsequent treatment with borohydride the same non-reducing compound was detected together with a small proportion of 2-acetylamino-1,3-propanediol.

Isolation of oligosaccharide III after acetolysis of the desialized glycoprotein. The desialized glycoprotein (0.89 g) was dissolved in formamide (20 ml) by stirring 1 h at room temperature. A 2:1 (v/v) mixture of acetic anhydride and pyridine (66 ml) was added and the stirring continued for another 48 h. The brown liquid was diluted with water and dialysed against distilled water for 3 days. The acetylated polymer which precipitated gradually, was filtered off and dried, yield 0.85 g. The acetylated product (0.85 g) was stirred with a 10:10:1 (v/v) mixture of acetic anhydride, acetic acid, and sulfuric acid (120 ml) at 40 °C for 5 h.[18] After cooling pyridine (30 ml) was added to terminate the reaction and the mixture was poured into ice water and left overnight at 5 °C. The acetolysis products were recovered by extraction with chloroform (3 × 200 ml) and the combined chloroform phases were extracted with water to remove acetic acid. After drying with anhydrous sodium sulfate the chloroform was evaporated off and the residual syrup dissolved in methanol (60 ml). A 0.5 % solution of sodium methoxide in methanol (20 ml) was added and the deacylation was allowed to proceed for 30 min at room temperature. The reaction was terminated by the addition of ethyl acetate (15 ml) followed by concentration to *ca.* 20 ml, dilution with water and treatment with Dowex 50 (H^+). The resulting solution was concentrated to a syrup (0.57 g) which was dissolved in 10 % ethanol in water (4 ml). This solution was divided into three parts, each being successively subjected to gel chromatography on a Bio-Gel P-2 column; fractions of 5 ml were collected. The fractions (tubes 24 to 30) corresponding to the middle peak of the elution curve (Fig. 3) were pooled and concentrated to a syrup (59 mg) which was further fractionated by preparative paper chromatography. One sugar, oligosaccharide III, (8 mg) was isolated in the pure state; it has $R_{Lactose}$ 1.25 and 1.45 in solvent system A and B, respectively. GLC after methanolysis showed the presence of galactose and *N*-acetylglucosamine, and incubation of the oligosaccharide (1 mg) with β-galactosidase gave rise to the same sugars, as demonstrated by GLC and PC. Oligosaccharide III (5 mg) was methylated, hydrolysed and the products converted into alditol acetates as described for oligosaccharide I, followed by examination by GLC-mass spectrometry; the results are summarized in Table 1.

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