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Note

A sequencing method for the reductive-amination derivatives of oligo- and poly-saccharides

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The biological significance of natural oligo- and poly-saccharides has stimulated increased interest in developing methods for determining structures of these biomolecules. Recently, methods based on high-pressure liquid chromatographic (hplc) separation of partial hydrolyzates[1] and n.m.r. spectroscopy[2] have been described for sequencing oligo- and poly-saccharides. The former method is relatively complex and the carbohydrate sequence is deduced indirectly from the data obtained; the latter method requires expensive instrumentation that may discourage routine analyses. A recent approach for sequencing oligosaccharides involves reduction of the reducing end of the polymer in order to generate a "marker" for sequencing by partial hydrolysis[3]. We propose a simpler and more-direct method in which the reducing end of a saccharide is initially marked by reductive amination with aniline, and the product is permethylated, partially hydrolyzed, and the partial hydrolyzate separated by hplc. Characterization of these hydrolyzates can then lead to the structure of the original oligomer.

A flow diagram of the method is shown in Fig. 1. The oligosaccharide is reductively aminated[4] with aniline and sodium cyanoborohydride in *N,N*-dimethylformamide. Permethylation (step 2) employs standard conditions[5,6].

The permethylated 1-anilino-1-deoxyalditol is then partially hydrolyzed (usually with 90% formic acid) to yield a series of oligomeric anilino derivatives. Hydrolysis conditions may be optimized by monitoring with hplc. Individual oligomers are then isolated by preparative hplc. The monosaccharide composition of the oligomers is determined by completely hydrolyzing the compound and preparing the alditol acetates, which are identified by g.l.c.–m.s.[4] Linear polysaccharides would ideally give the composition of a succession of compounds of increasing d.p.

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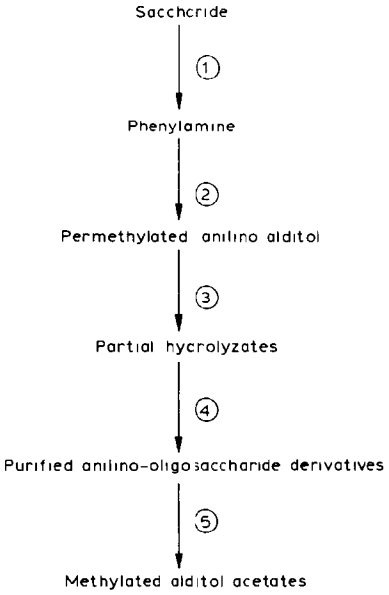
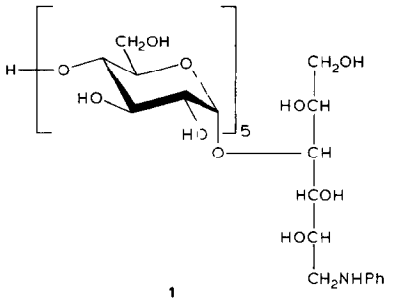


Fig. 1. Flow diagram of sequencing procedure.



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To illustrate the procedure, we have sequenced the homogeneous oligosaccharide, maltohexaose.

A commercial oligosaccharide mixture (d.p. 1–7) prepared by the partial hydrolysis of amylose was converted into *N*-phenyl-[1-deoxy-(4-*O*-α-malto-oligosyl)-D-glucitol-1-yl] amines[1]. The derivatized mixture is identical in composition to the starting material (Fig. 2). The maltohexaose derivative, *N*-phenyl-[(1-deoxy-4-*O*-α-maltopentaosyl)-D-glucitol-1-yl] amine (**1**) was isolated from this sample and permethylated. Partial hydrolysis of **1** by 90% formic acid for 5.5 h at 50° resulted in good yields of the monomer through hexamer (Fig. 3). The individual anilinooligosaccharide derivatives were isolated by preparative hplc and then completely hydrolyzed (0.5M sulfuric acid, 1 h at 100°). After being made neutral with barium carbonate, the hydrolyzates were converted into the alditol acetate derivatives and analyzed[5] by g.l.c.–m.s. The reconstructed total-ion chromatogram of each hydrolyzate (Fig. 4) contained two components, 1,4,5-tri-*O*-acetyl-2,3,6-tri-

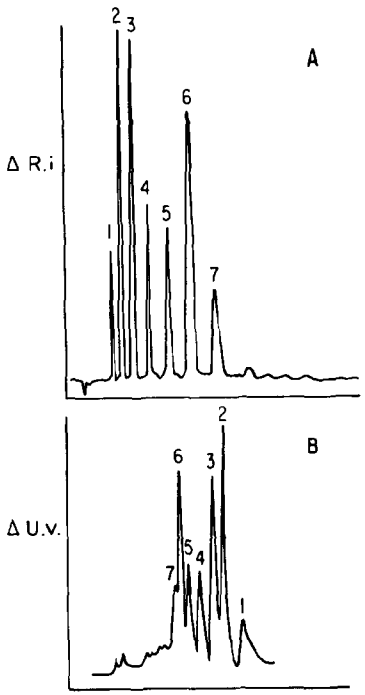

Fig. 2. (*a*) Liquid chromatogram of malto-oligosaccharide hydrolyzate. Bondapak C-18 (10 μ) column, 3:17 acetonitrile–water, 1 mL/min, r.i. detection. (*b*) Liquid chromatogram of anilino derivative from the malto-oligosaccharide hydrolyzate Bondapak C-18 (10 μ), 1:9 acetonitrile–water, 1 mL/min u.v. detection (242 nm). Numbers above peaks refer to the degree of polymerization.

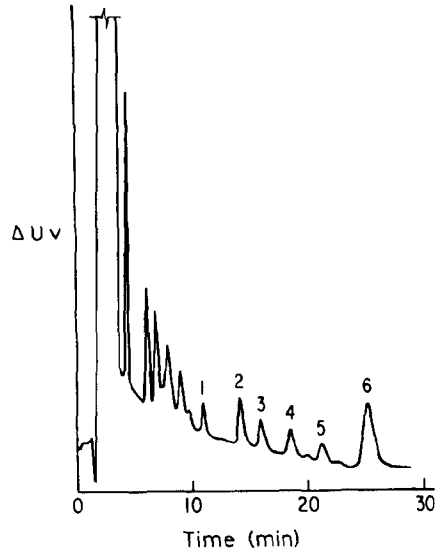

Fig. 3. Liquid chromatogram of partial hydrolyzate of **1**. Bondapak C-18 (10 μ) 11:9 acetonitrile–water, 2 mL/min, u.v. detection (245 nm).

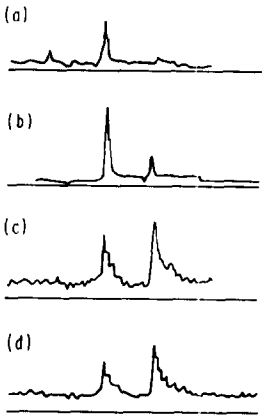

Fig. 4. Reconstructed total-ion chromatogram of derivatized hydrolyzates of (*a*) hexa-, (*b*) penta-, (*c*) tri-, and (*d*) di-mer fragments from permethylated **1**. G.l.c. conditions: 12M OV-1 fused silica column, He flow, 1 mL/min; oven temp. 150–225° at 8°/min.

O-methylglucitol and an unknown product, presumably 4,5-di-*O*-acetyl-1-deoxy-2,3,5,6-tetra-*O*-methyl-1-(*N*-methyl)anilino-D-glucitol. The mass spectrum (*m/z* 341 [4 − 28], 189. 120 [B]) was identical to the aniline-containing product resulting from the hydrolysis of the analogous maltose derivative, the structure of which was confirmed by n.m.r. spectroscopy (details to be given in a full paper). The base peak in the mass spectrum arises from the expected cleavage β to the amino group. The maltohexaose hydrolyzate (A) also contained a minor component identified as a 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol. The structure of the starting compound as a homogenous α-(1→4)-linked D-glucose oligomer is obvious from the results.

Alternatively, **1** was partially hydrolyzed in 0.5M sulfuric acid (90 min, 100°) and, after being made neutral with barium carbonate, the solution was chromatographed on a column of Dowex-50 (H^+ form). The neutral sugars were eluted with water and the desired amine derivatives eluted with 10% ammonium hydroxide. After evaporation, the amine derivatives were permethylated and the oligomers therein isolated by preparative hplc. The purified oligomers were hydrolyzed and derivatized as before, to give the alditol acetates. In contrast to the first procedure, a terminal glucose residue (that is, unsubstituted) is then observed for each hydrolysis product in the g.l.c.–m.s. analysis.

This method has the following advantages: (*a*) the terminal reducing-end of the oligosaccharide is marked with a u.v.-absorbing chromophore that allows greater sensitivity and separation for hplc analysis; (*b*) a simplified chromatogram is provided by hplc detection of only fragments that contain an anilino moiety, and, for linear polymers, a sequence of oligomers in order of increasing d.p. is observed; and (*c*) purification of the desired alkylanilino derivatives at any step of the procedure may be accomplished by ion-exchange chromatography.

With the introduction of a few additional steps, the procedure may be

applied to more complicated, branched polysaccharides. Details of these modifications will be discussed later.

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