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Note

A gas-chromatographic method for identification of the reducing units of disaccharides *via* reduction of the methoximes with borane

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Identification of the reducing unit of di- and oligo-saccharides can be effected by taking advantage of the reactivity of the carbonyl group. Reduction by sodium borohydride or borotritide[1] is a method used frequently. This is followed by methanolysis of glycosidic bonds, which commonly is considered to be superior to hydrolysis; side reactions are diminished, since the liberated monosaccharides are protected as the methyl glycosides. The recoveries of neutral sugars are higher than with hydrolysis[2], although methanolysis can never be carried out under strictly anhydrous conditions[3], and low recoveries of sugar monomers have often been reported[4–6]. Each reducing sugar gives a mixture of methyl glycosides. The non-reducing residues are identified by the proportions of the components and their gas-chromatographic characteristics[7–9].

We now report an alternative method, namely, conversion into the corresponding methoxime (RCH=NOMe), which does not affect glycosidic bonds[10], followed by reduction with borane[11] to the corresponding amine (RCH_2NH_2). A constitutional asymmetry is thereby introduced into the molecule before cleavage into the monosaccharides. Methanolysis of the glycosyl-aminodeoxyalditol then affords a mixture of methyl glycosides and the aminodeoxyalditol. Treatment of the mixture with ethyl chloroformate ($\rightarrow R'CH_2NHCOOEt$) followed by trimethylsilylation gives the Me_3Si derivatives of the methyl glycosides and the 1-deoxy-1-ethoxycarbonylaminoalditol. The two types of products are well separated by g.l.c. and the usefulness of the former

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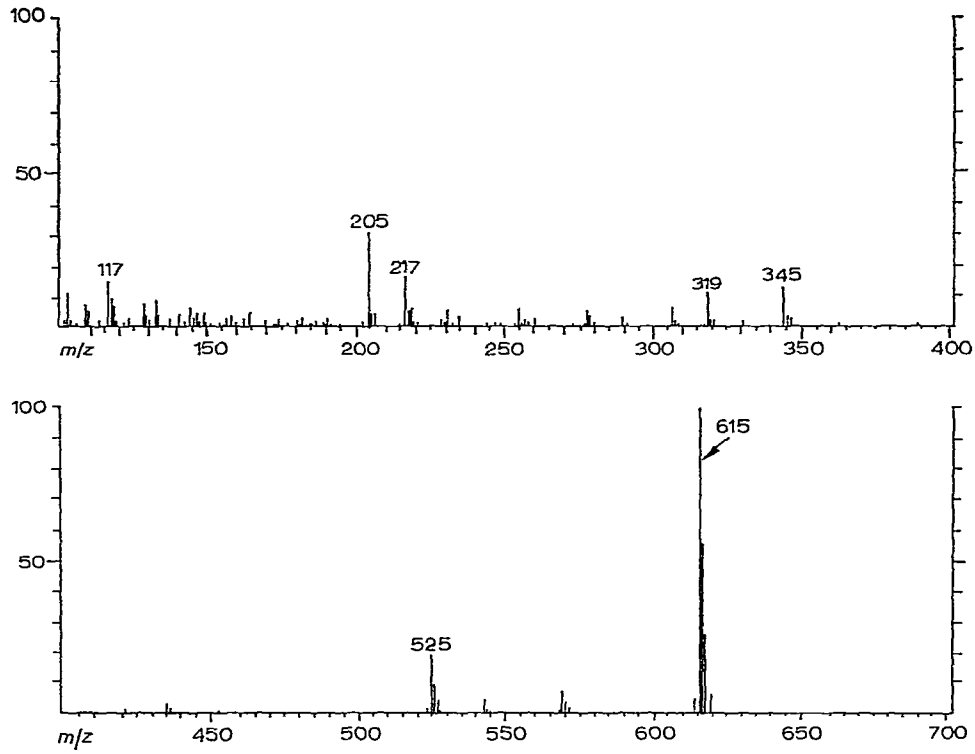

Fig. 1. Chemical-ionisation (isobutane) mass spectrum of trimethylsilylated 1-deoxy-1-ethoxycarbonylaminoglucitol, obtained by reduction of the precursor methoxime with B^2H_3 (see Experimental).

has been demonstrated for analysis of mixtures of monosaccharides[4]. These products were characterised by mass spectrometry; the chemical-ionisation mode gave abundant quasi-molecular ions (Fig. 1).

For disaccharides, the identity of the products obtained after methanolysis of the modified molecule reveals the sequence of the monosaccharides. The derivative of the reducing monosaccharide affords a single peak in g.l.c., which is well separated from those for the methyl glycosides arising from the non-reducing moiety (Fig. 2).

Reduction of methoximes with borane affords a stable amine–borane complex from which the free amine can be liberated by heating with M methanolic hydrogen chloride[12], a treatment which simultaneously cleaves the glycosidic bonds.

The rate of methanolysis is strongly influenced by the nature of the glycosidic bond[13], and the order gentiobiose < cellobiose < maltose was observed (Fig. 3). The same sequence has been reported for acid hydrolysis: galactosides are more rapidly cleaved than mannosides[13,14]. The yield of glucose from lactose reached a maximum after 4 h, and then rapidly decreased, possibly due to destruction of galactose residues and formation of reactive decomposition products[4,6,8]. Therefore, the optimum conditions of cleavage for each disaccharide must be established.

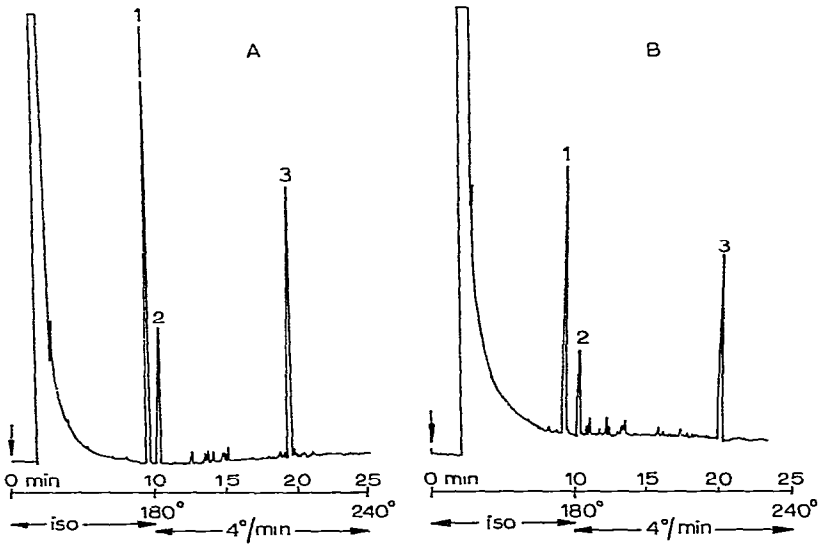

Fig 2. Gas chromatograms of the trimethylsilylated methanolysis products of disaccharide methoximes· A, cellobiose: 1, methyl α-D-glucopyranoside; 2, methyl β-D-glucopyranoside; 3, 1-deoxy-1-ethoxycarbonylamino-D-glucitol; B, lactose: 1, methyl α-D-galactopyranoside; 2, methyl β-D-galactopyranoside; 3, 1-deoxy-1-ethoxycarbonylamino-D-glucitol.

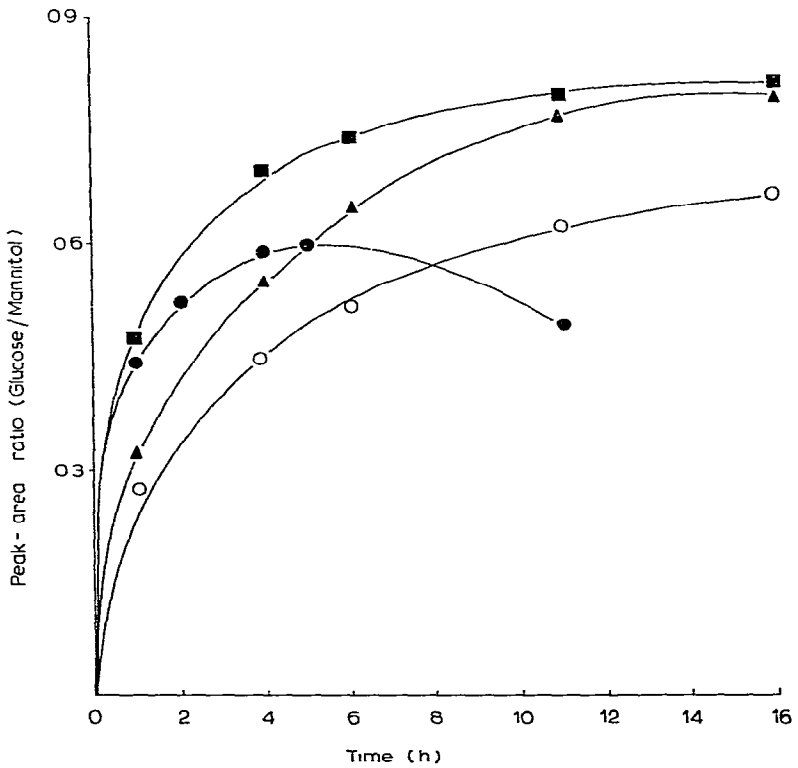

Fig. 3. Methanolysis of the glycosyl-aminodeoxyglucitols with M HCl/MeOH at 80°: liberation of aminodeoxy-D-glucitol relative to the internal standard D-mannitol; ■, maltose; ▲, cellobiose; ○, gentiobiose; ●, lactose.

Since mannitol, the internal standard, decomposes during prolonged methanolysis[8], it was added after the methanolysis stage.

For reduction of methoximes to amines, borane in tetrahydrofuran is superior to lithium aluminium hydride[12]. Borane reduction of methoximes is rapid, the yields of amines are high[11], and excess of borane is easily decomposed with methanol[7]. In contrast, lithium aluminium hydride requires long reaction times and generates a voluminous precipitate of highly adsorptive aluminium hydroxide. This seriously affects the recovery of the reduction products[15], especially with small quantities.

The method reported here can be used with quantities in the picomole range. The chromatograms shown in Fig. 2 were obtained by derivatisation of 200 pmol and correspond to an amount of injected products from ~ 1.6 pmol of disaccharide. The peaks at the beginning of the chromatograms are due to the methyl glycosides, well separated from those for the aminodeoxyalditols derived from the reducing monosaccharide moiety of the carbohydrate.

The proposed method offers a simple and sensitive method for determining the identity of reducing-terminal sugars and is also compatible with methylation analysis. Also, the high yields of the derivatisation steps coupled with the high sensitivity of glass-capillary g.l.c. render the method useful for the structural analysis of carbohydrates available in only minute quantities.

EXPERIMENTAL

Pyridine was dried over potassium hydroxide for 48 h and distilled. Gas–liquid chromatography (g.l.c.) was performed on a DANI instrument, model 6800, equipped with a split injector, a flame-ionisation detector, and an OV-101 capillary column (25 m × 0.25 mm); the temperature programme was as indicated in the chromatograms. The carrier gas was hydrogen: inlet pressure, 0.4 kg/cm^2; injector temperature, 250°; detector temperature, 275°. For g.l.c –m.s., the gas chromatographic conditions were as noted above, but helium was used as carrier gas at an inlet pressure of 1 5 kg/cm^2. Chemical-ionisation mass spectra were obtained with a Finnigan 4021 instrument with INCOS data system. Isobutane was used as reagent gas, the interface temperature was 250°, and the ion-source temperature was 225°.

Preparation and degradation of aminodeoxy disaccharides. — Samples (200 μl) of standard solutions of disaccharides (1–2 mg) in water (5 ml) were transferred into 1-ml screw-cap vials with PTFE-lined rubber septa, the water was evaporated *in vacuo*, and the residue was dried for 24 h over phosphorus pentaoxide *in vacuo*. A solution (50 μl) of methoxyammonium chloride in pyridine (25 mg/ml) was added, the mixture was heated to 80° for 2 h and then concentrated in a stream of nitrogen, and 100 μl of M borane in tetrahydrofuran was added. When evolution of hydrogen had subsided, the solution was stirred vigorously and heated to 80° for 2 h, and then cooled to 0°, and excess of borane was decomposed with methanol (200 μl). The solvent was evaporated in a stream of nitrogen, and the residue was treated with M methanolic hydrogen chloride (100 μl) at 80° for 30 min. After evaporation of the

solvent, this procedure was repeated in order to decompose the amine–borane complex and complete the formation of methyl glycosides.

The products of methanolysis were dissolved in saturated, aqueous potassium carbonate (50 μl), and ethyl chloroformate (25 μl) was added. The mixture was agitated vigorously for 1 min and left at room temperature[4]. After 1 h, an aqueous solution (200 μl) of D-mannitol (1 mg/ml) was added as the internal standard, the water was evaporated under a stream of nitrogen, and the residue was dried *in vacuo* over phosphorus pentaoxide for 24 h. Pyridine (40 μl), *N*-trimethylsilylimidazole (10 μl), and chlorotrimethylsilane (10 μl) were added to the dried residue, and the mixture was heated to 50° for 30 min. After centrifugation, aliquots (0.1–0.5 μl) of the supernatant solution were used for g.l.c. Peak areas were determined with a Spectra Physics SP 4100 computing-integrator. Response factors were calculated relative to D-mannitol.

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