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Note

Determination of the degree of polymerisation of oligo- and polysaccharides by gas–liquid chromatography

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The degree of polymerisation of oligo- and polysaccharides can be determined in various ways. Physical methods have involved the use of vapour-phase osmometry[1] and isothermal distillation[2] techniques while chemical methods are usually based on the estimation of the proportion of terminal reducing groups to interior chain groups. One group of chemical methods is based on reduction of the reducing end group to the corresponding alditol and then determination of the ratio of alditol to reducing sugar after acid hydrolysis of the polymer. This can be achieved by determining the amount of formaldehyde released by periodate oxidation of the terminal alditol group[3] or by enzymic methods if the alditol is glucitol[4] or xylitol[5]. Dutton *et al.*[6] have demonstrated the feasibility of separating glycitols and reducing sugars as trimethylsilyl derivatives by gas–liquid chromatography (GLC) and Dutton *et al.*[7] have also shown the possibility of extending the method to determine the degree of polymerisation of oligo- and polysaccharides. Disadvantages of this method are that xylans cannot be analysed since xylitol and one of the xylose peaks overlap on all columns tested and that, since derivatives of reducing sugars are used, multiple peaks are obtained from the different anomeric forms making the chromatograms difficult to interpret especially when heteropolymers are being analysed.

In the proposed method, the reducing end group is also converted to the alditol and the polymer is hydrolysed. The reducing sugars released are converted to the oxime derivatives before simultaneously dehydrating and acetylating to the acetylated aldononitriles. At the same time, the alditol is also acetylated. The ratio of acetylated aldononitrile to acetylated alditol gives the degree of polymerisation of the polymer.

EXPERIMENTAL AND RESULTS

A Pye Model 104 gas chromatograph fitted with dual flame ionisation detectors and a Hewlett-Packard Model 3373B integrator were used throughout. Glass columns were used with the following packing materials, column sizes and operational temperatures: (A) 3% ECNSS-M on 100–120 mesh Gas-Chrom Q (1.5 m) at 185°; (B) 5% OV-225 on 100–120 mesh Chromosorb W AW DMCS (2 m) at 210°; (C) 3% OV-17 on 100–120 mesh Supasorb (AW DMCS) (1.5 m) at 210°.

The retention times of the acetylated alditols and aldononitriles, relative to the internal standard of myoinositol hexaacetate are given in Table I.

TABLE I

RELATIVE RETENTION TIMES OF ACETYLATED DERIVATIVES (MYOINOSITOL HEXAACETATE = 1.00)

Acetylated derivative of	*Relative retention times*		
	Column A	*Column B*	*Column C*
Rhamnitol	0.136	0.269	0.271
Fucitol	0.153	0.285	0.282
Arabinitol (≡ Lyxitol)	0.229	0.348	0.339
Xylitol	0.316	0.416	0.414
Ribitol	0.204	0.315	0.320
Mannitol	0.580	0.891	0.836
Galactitol	0.699	1.000	0.906
Glucitol	0.770	1.000	1.000
Rhamnononitrile	0.110	0.176	0.186
Fuconononitrile	0.154	0.194	0.235
Arabinononitrile	0.229	0.236	0.276
Lyxononitrile	0.190	0.219	0.255
Xylononitrile	0.279	0.259	0.313
Ribononitrile	0.168	0.201	0.232
Mannononitrile	0.486	0.542	0.606
Galactononitrile	0.699	0.730	0.782
Gluconononitrile	0.649	0.669	0.723

Synthetic mixtures of pure aldoses and alditols were prepared in various ratios from 1:1 to 500:1. The aldose was converted to the oxime by heating at 100° for 15 min with 5% hydroxylammonium chloride in pyridine (0.2 ml/mg of aldose). After cooling, acetic anhydride (0.2 ml/mg of aldose) was added and heating was continued for a further 1 h to dehydrate the oxime to the nitrile and to acetylate the free hydroxyl groups on the nitrile and alditol. When the ratio of aldose to alditol was high (> 50:1) it proved necessary to stir the mixture continuously when the oxime was being prepared since the acetylated derivatives of the parent aldoses also have similar retention times and could cause extraneous peaks on the chromatograms. The molar ratios found are shown in Table II. The values have been corrected by using molar response factors.

The accuracy of the method was demonstrated by analysing a number of oligo-

TABLE II

RATIO OF ALDOSE TO ALDITOL IN SYNTHETIC MIXTURES

Calculated ratio	*Glucose to sorbitol ratio*	*Xylose to xylitol ratio*
1:1	0.95:1	1.0:1
2:1	1.95:1	2.0:1
5:1	4.9 :1	5.3:1
10:1	9.9 :1	10.7:1
50:1	51.6 :1	49.7:1
100:1	103.1 :1	98.9:1
250:1	241 :1	238 :1
500:1	450 :1	460 :1

and polysaccharides of known structure to the following procedure. The carbohydrate was reduced with sodium borohydride (10 moles/mole of reducing sugar) for 1 h in experiments with oligosaccharides and overnight in experiments with polysaccharides. The excess borohydride was oxidised to borate ions and sodium ions were removed by adding Amberlite IR-120(H^+) resin. The solution was pipetted off, concentrated to dryness and the borate ions were removed as the volatile methyl borate by repeated evaporation with 5% acetic acid in methanol (five times). The residue was hydrolysed with 2 *N* trifluoroacetic acid at 100° for 16 h, evaporated to dryness and derivatised as above. The results are shown in Table III.

TABLE III

RATIOS OF ALDOSE TO ALDITOL FOUND AFTER REDUCTION AND ACID HYDROLYSIS OF PURE OLIGOSACCHARIDES

Oligo- or polysaccharide	*Aldose to alditol ratio*
Maltose	1.00:1
Maltotriose	1.83:1
Maltopentaose	3.86:1
Maltohexaose	5.01:1
Maltononaose	7.73:1
Cellobiose	1.00:1
Xylobiose	0.99:1
Lactose	0.95:1
Panose	1.00:1
Gentiobiose	0.96:1
Arabinoxylan from grass	145 :1
Galactoarabinoxylan from grass	90 :1

DISCUSSION

The quantitative determination of aldoses by GLC after conversion to their alditol acetates is now a well established procedure in many laboratories (*e.g.* refs. 8–10) whilst the estimation of aldoses as acetylated aldononitriles, although shown to be possible[11–13], has not received the same interest. Their potential in carbohydrate chemistry is good since they are more easily prepared than the alditol acetates and have lower retention times. Derivatisation is virtually 100% and the molar response factors are comparable. The identity of the parent aldose is determined with greater assurance since each aldose gives only one aldononitrile whilst for example D- and L-galactose both give an optically inactive galactitol and arabinose and lyxose give the same alditol.

It has been shown (Table I) that the acetylated alditol and aldononitrile from the same aldose can be separated on a single column whilst, using a combination of columns, all heterogeneous trisaccharides containing any of the five sugars arabinose, xylose, mannose, galactose or glucose can be identified. The three column packings in Table I were the ones which gave the best resolution of all the derivatives examined. Other column packings were examined and may be superior for certain specific separations, *e.g.* 5% Silar 10C for the separation of all aldononitrile acetates and OV-1 for the separation of rhamnitol and fucitol. Using the molar response factors it has been shown that mixtures of acetylated xylitol and xylononitrile and sorbitol and

gluconononitrile in ratios of from 1:1 up to 1:100 can be accurately measured. It is possible to measure mixtures of 1:250 and even 1:500 but the error becomes greater at these ratios.

When the method was tested on a number of oligo- and polysaccharides of known composition (Table III), the calculated values were very close to the theoretical ones. The slightly low value for maltotriose was due to a trace of maltose in the sample. Hence the method can be used to determine the degree of polymerisation of both oligo- and polysaccharides and has the added advantage that the monosaccharide at the reducing end of the molecule is identified.

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