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Note

Analysis of the products of Smith degradation of polysaccharides by g.l.c. of the acetylated, derived aldononitriles and alditols

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The sequential subjection of polysaccharides to periodate oxidation, reduction with borohydride, and acid hydrolysis (Smith degradation) gives a variety of products, the analysis of which gives information on the structure of the polysaccharides. The analysis of these products has been carried out by using the *O*-trimethylsilyl derivatives of the free sugars[1] or the methyl glycosides[2]. These methods give anomeric mixtures for each sugar and do not give good results with the free aldehydes produced in Smith degradation. This problem was partially overcome by the use of the *O*-trimethylsilyl derivatives of the oximes of the aldehydes[3,4]. The acetylated aldononitriles have been used recently[5] as derivatives of aldoses amenable to g.l.c., and are obtained by condensation of the free sugar with hydroxylamine and subsequent heating of the oxime with acetic anhydride. Use of these derivatives for g.l.c. should give single peaks for the free sugars, alditols, and aldehydes formed by Smith degradation.

This paper reports a simple method of preparing these derivatives and their use in quantitative g.l.c. of the typical Smith-degradation products of glucans. By using this technique, the chain lengths of various glucans were estimated.

EXPERIMENTAL

Amylopectin and glycogen samples were kindly supplied by Dr. R. J. Sturgeon (Heriot-Watt University), and all other materials were obtained from commercial sources.

G.l.c. — A Pye series 104 chromatograph with a flame-ionisation detector was used. The carrier nitrogen flow was 40 ml/min and the peak areas were measured with a Kent chromalog integrator (series 2) and by triangulation. Derivatives were separated on glass columns (5 ft) containing 10% of poly(neopentylglycol succinate) on Gas-Chrom Q (100–120 mesh), employing an initial temperature of 160° for 10 min and then a linear temperature-gradient of 160–235° at 4°/min.

All solids were dried to constant weight *in vacuo* over phosphorus pentaoxide. Ampoules containing the various standards (100 μmoles) were derivatised by the procedure described below. A mixture of these derivatives was prepared, and a series of dilutions in pyridine was used for the determination of response factors. The ratio of the total peak-area of each standard to the peak area of the internal standards, triethylene glycol and inositol, was taken as the relative, molar response-factor. The values obtained were constant over a ten-fold range of dilution and are shown in Table I.

Smith degradation of polysaccharides. — The polysaccharide (20 mg) was oxidised with an eight-fold excess of 50mM sodium metaperiodate (20 ml) for 5 days at 4° in the dark. The polyaldehyde was then reduced with sodium borohydride (100 mg) for 16 h at 4°. Excess of sodium borohydride was decomposed by the addition of 0.1M hydrochloric acid and the polyol desalted by dialysis against several changes of distilled water. The non-diffusible material was freeze dried.

A sample of the modified polysaccharide (5 mg) was hydrolysed with M sulphuric acid (1 ml) at 100° in a sealed tube for 3 h. If required, an internal standard, triethylene glycol or inositol was added at this stage. The hydrolysate was titrated (Congo Red) at 60–70° with saturated, aqueous barium hydroxide. The precipitated barium sulphate was removed by centrifugation, and the supernatant fraction was evaporated to dryness *in vacuo* at 37° and finally dried in a vacuum desiccator over phosphorus pentaoxide.

Preparation of derivatives for g.l.c. — The dried sample from the Smith degradation was dissolved in pyridine (0.4 ml) containing hydroxylamine hydrochloride (20 mg), and an aliquot (0.2 ml) was heated at 90° in a sealed tube for 1 h. After cooling, acetic anhydride (0.2 ml) was added and the sample again heated for 1 h. The derivatives were examined directly by g.l.c.

Estimation of chain lengths of polysaccharides. — The peak area for each Smith-degradation product was multiplied by the relative, molar response-factor, and the molar ratios of erythritol–glycerol and glucose–glycerol were calculated. For glycogen and amylopectin, the chain length = the molar ratio of erythritol–glycerol + 1, or = (moles of erythritol + moles of glycerol)/moles of glycerol. Similarly, for laminarin, the chain length = the molar ratio of glucose–glycerol + 1.

The values thus obtained for the chain lengths of glycogen, amylopectin, and laminarin samples are shown in Table II.

RESULTS AND DISCUSSION

The separation achieved by this method is shown in Fig. 1, and the relative retention times and the response factors are recorded in Table I. Glycolaldehyde can be separated before the acetic anhydride peak by starting the temperature programme at a lower temperature.

The Smith degradation of (1→4)-linked glucans with (1→6) branch-points gives glycerol from the terminal non-reducing glucose residue and erythritol from all other glucose residues in the chain. Thus, the ratio of glycerol to erythritol produced is a measure of the chain length of the polymer. For a (1→3)-linked glucan, the ratio of glycerol formed to undegraded glucose on Smith degradation would also give a

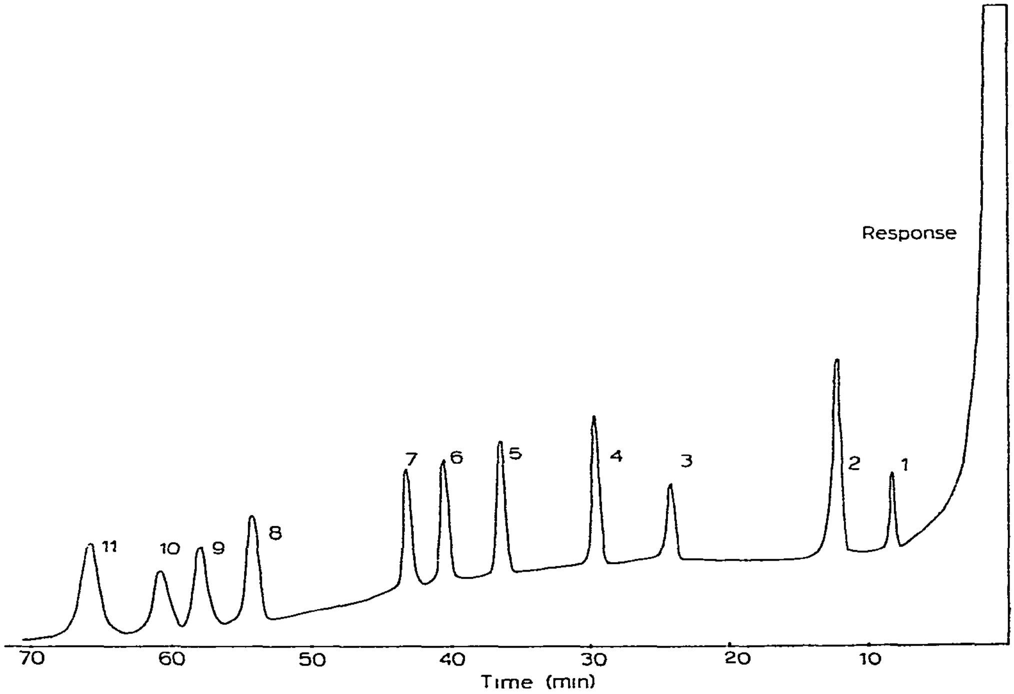

Fig 1 G l c separation of a mixture of acetylated aldononitriles and acetylated alditols, determined on 1 μl of a mixture containing 10 nmoles of each derivative, at attenuation 2 × 10^3 1, glyceraldehyde, 2, glycerol, 3, triethylene glycol, 4, erythritol, 5, L-rhamnose, 6, D-ribose, 7, D-arabinose, 8, D-mannose, 9, D-glucose, 10, D-galactose, 11, inositol

TABLE I

G L C PROPERTIES OF DERIVATIVES OF POLYALCOHOLS AND MONOSACCHARIDES

Compound	T_G[a]	*Molar response factors*[b]	
		A	B
Glyceraldehyde	0 17	1 43	1 90
Glycerol	0 26	0 41	0 57
Triethylene glycol	0 47	1 0	1 25
Erythritol	0 55	0 47	0 61
Rhamnose	0 65	0 48	0 62
Ribose	0 69	0 51	0 64
Arabinose	0 73	0 59	0 74
Mannose	0 93	0 59	0 76
Glucose	1 0	0 61	0 79
Galactose	1 06	0 8	1 0
Inositol	1 14	0 8	1 0

[a] Retention times relative to that for D-glucose (58 min) [b] Relative to those for triethylene glycol (*A*) and inositol (*B*)

measure of the chain length Using the method described in this paper, we have determined the chain lengths of samples of glycogen, amylopectin, and laminarin

TABLE II

CHAIN LENGTH OF POLYSACCHARIDES

Polysaccharide	*Present method*[c]	*Enzymic method*[6]
Glycogen		
Rabbit liver[a]	13 8 ±0 8	14
Ascaris lumbricoides[a]	12 1 ±0 5	12
Amylopectin		
Potato red star[a]	25 ±0 9	23
Waxy maize[a]	26 ±0 8	—
Laminarin		
(ex *L hyperborea*) 1[b]	21 6 ±0 5	—
2[a]	26 7 ±0 5	25

[a] Kindly supplied by Dr R J Sturgeon [b] Obtained from Koch-Light [c] Each determination was carried out twice on four separate Smith degradations

The results shown in Table II are in good agreement with those of Noble and Sturgeon[6] who used an enzymic method to determine the glycerol formed on Smith degradation of these polymers The method gives reproducible results and, since the determinations of glycerol, erythritol, and glucose are carried out in the same run, accurate results for chain-length determination are possible

It should also be possible to determine the glyceraldehyde formed from (1→2)-linked glucans, so that determination of the chain lengths of these types of polymers could be made

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