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DETERMINATION OF THE DEGREE OF POLYMERISATION OF OLIGO- AND POLYSACCHARIDES BY GAS–LIQUID CHROMATOGRAPHY*

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SUMMARY

A model study has been carried out on the estimation of glycitols and reducing sugars as their trimethylsilyl derivatives in ratios of 1:1 up to 1:150. Application of this method to polysaccharides after reduction and hydrolysis will permit the determination of the degree of polymerisation.

INTRODUCTION

In a previous paper we reported on the feasibility of simultaneously estimating by gas–liquid chromatography (GLC) a mixture of reducing sugars and polyhydric alcohols[1]. At that time we pointed out that these estimations could be extended to amino- and deoxy-sugars and also to the determination of the degree of polymerisation (D.P.) of oligosaccharides. The former is described in another paper[2] and the present communication presents the results obtained in a study of D.P. measurements by GLC.

Oligosaccharides may be obtained by acetolysis or by partial acid or enzymatic hydrolysis of polysaccharides. A knowledge of the D.P. of these fragments and, if possible, the nature of the reducing end group is important in elucidating the structure of the original polysaccharide. For measurement of the D.P. of oligosaccharides various chemical methods have been proposed. These are often based on the liberation of formaldehyde[3] or on a differential colorimetric method involving the original and the reduced sample (*e.g.* ref. 4). Neither method gives any information on the nature of the reducing group and the former requires a knowledge of the manner in which this group is linked, while colorimetric methods are seldom sufficiently precise for D.P. values in excess of ten.

An oligosaccharide contains only one reducing group and thus on reduction only

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one glycitol group. Hydrolysis of such a reduced oligosaccharide yields 1 mole of glycitol and *n* moles of reducing sugar. It thus follows that a knowledge of the ratio 1:*n* provides a measure of D.P. If, in addition, the glycitol is identified this indicates the nature of the reducing group. To test the validity of this approach model systems have been prepared with glycitol: sugar ratios of approximately 1:1, 1:10 and 1:100. These mixtures have been converted to their trimethylsilyl derivatives[5] and their composition determined by GLC using flame ionisation detectors.

The results are presented in Table III and it is clear that even in the range of D.P. 150 acceptable accuracy is obtained. For ratios in this range flame ionisation detectors are mandatory. Flame ionisation in conjunction with the liquid phase chosen also permitted the use of small samples and isothermal column operation thus cleanly separating the solvent peak from the fastest pentose peaks. Molar response factors should be verified regularly and if a change in attenuation is necessary during runs involving small amounts of glycitol, a control experiment should be carried out to determine the *exact* multiple introduced by such a change.

The data given here show that the technique is applicable to those sugars commonly found in polysaccharides with the exception of xylose. On columns of SE-52 and SF-96 the peak for xylitol overlapped with one of the xylose peaks. When the ratio of xylose to xylitol was small reasonable accuracy could be obtained by calculating the quantity of xylose on the basis of one well-separated peak[6] but as the proportion of xylitol decreased the method was too inaccurate.

It is, of course, evident that in all cases rather than the trimethylsilyl compounds other derivatives such as acetates might have been used. This would have had the advantage that the hexitol acetates are separable and crystalline but because of other work in our laboratories we preferred to use the TMS derivatives.

The determination of the D.P. oligosaccharides is thus another application of GLC to the methods of polysaccharide chemistry. No previous systematic study of this aspect of GLC has been published but related estimations in the D.P. range of 1–4 have been made *inter alia* by PERCIVAL[7] (fucose:fucitol), ASPINALL *et al.*[8] (rhamnose:rhamnitol) and BEMILLER AND WING[9] (glucose:4-O-methylglucose).

EXPERIMENTAL

An F & M Model 402 chromatograph fitted with dual flame ionisation detectors was used with dual 8 ft. × 0.25 in. O.D. copper columns packed with Diatoport S 80–100 mesh coated with 10% SF-96. The columns were operated isothermally at 190° and peak areas were measured with a Disc integrator. Under these conditions the trimethylsilyl ether of glucitol had a retention time of 26–28 or 29–32 min on two different columns. Relative retention times are shown in Table I.

In an initial series of determinations glycitol to glycoses ratios of about 1:1 and 1:10 were examined and molar response factors (MRF) calculated. Hexose (about 18 mg, accurately weighed) and hexitol (18 or 1.8 mg) or pentose (15 mg) and pentitol (15 or 1.5 mg) were dissolved in pyridine (8 ml) with 2-hydroxypyridine (95 mg) and incubated at 40° overnight. The use of 2-hydroxypyridine to promote mutarotation has been described[2] and although in those cases examined mutarotation was complete in 3 h the overnight incubation was convenient for handling many samples. Hexamethyldisilazane (HMDS, 1 ml) and trimethylchlorosilane (TMCS, 0.5 ml) were

TABLE I

RELATIVE RETENTION TIMES OF TRIMETHYLSILYL DERIVATIVES

Dual 8 ft. × 0.25 in. copper columns packed with 10% SF-96 on 80–100 mesh Diatoport S at 190°.

			Relative retention time
Arabinose 1	*vs.*	Arabinitol	0.61
Arabinose 2		Arabinitol	0.69
Arabinose 3		Arabinitol	0.78
Arabinitol		Glucitol	0.40
Fructose 1		Glucitol	0.57
Fructose 2		Glucitol	0.60
Fucose 1		Fucitol	0.50
Fucose 2		Fucitol	0.59
Fucose 3		Fucitol	0.70
Fucitol		Glucitol	0.53
Galactose 1		Galactitol	0.57
Galactose 2		Galactitol	0.68
Galactose 3		Galactitol	0.80
Mannose 1		Mannitol	0.57
Mannose 2		Mannitol	0.85

added to a 1-ml aliquot of the sample and the mixture shaken for 30 min at room temperature[5]. Samples were injected directly and the response factors given in Table II are mean values from four 2-μl injections. In the case of mixtures containing galactitol, silylation was carried out in dimethylformamide rather than pyridine.

In a second set of experiments known mixtures of glycitol and reducing sugar in the range of 1:1 to 1:150 (accurately weighed) were prepared and analysed using the response factors given in Table II. These results are presented in Table III. Each entry in the table represents the mean of four injections from at least two separately prepared solutions. In the case of arabinitol–arabinose at a ratio of 1:100 the results of three separate experiments are shown.

TABLE II

MOLAR RESPONSE FACTORS OF TMS DERIVATIVES

Silylation in pyridine except dimethylformamide used for galactose–galactitol.

			Molar response factor
Arabinose	*vs.*	Arabinitol	0.831
Arabinose		Glucitol	0.720
Fructose		Glucitol	0.815
Fucose		Fucitol	0.824
Galactose		Galactitol	0.810
Glucose		Glucitol	0.875
Mannose		Mannitol	0.834

TABLE III

MOLAR RATIOS OF SYNTHETIC MIXTURES

Compound	*Standard*	*Molar ratio*	
		Theory	*Found*
Arabinose	Glucitol	1.00:1	1.00:1
Fructose	Glucitol		1.00:1
Fucose	Fucitol		0.992:1
Galactose	Galactitol		1.03:1
Glucose	Glucitol		0.995:1
Mannose	Mannitol		1.00:1
Arabinose	Arabinitol	10.0:1	9.80:1
Fructose	Glucitol		9.60:1
Fucose	Fucitol		9.75:1
Galactose	Galactitol		10.4:1
Mannose	Mannitol		9.30:1
Fucose	Fucitol	50.0:1	50.5:1
Mannose	Mannitol		49.1:1
Arabinose	Arabinitol	100:1	98.2:1
			98.0:1
			100:1
Galactose	Galactitol		95.7:1
Fructose	Glucitol	150:1	156:1
Glucose	Glucitol		150:1

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