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SEPARATION BY GAS–LIQUID CHROMATOGRAPHY OF THE GLYCEROL AND ERYTHRITOL GLYCOSIDES OF D-GLUCOPYRANOSE AND MALTOSE

PERIODATE AND METHYLATION STUDIES ON A SYNTHETIC "POLYMALTOSE"

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SUMMARY

During the investigation of a polysaccharide prepared by polymerising maltose the following compounds have been separated by gas–liquid chromatography on a column of SF 96: (1) 1-O-(α-D-glucopyranosyl)-glycerol, (2) 1-O-(β-D-glucopyranosyl)-glycerol, (3) 2-O-(α-D-glucopyranosyl)-glycerol, (4) 2-O-(β-D-glucopyranosyl)-glycerol, (5) 1-O-(α-D-glucopyranosyl)-erythritol, (6) 1-O-(β-D-glucopyranosyl)-erythritol, (7) 2-O-(α-D-glucopyranosyl)-erythritol, (8) 2-O-(β-D-glucopyranosyl)-erythritol, (9) 1-O-(α-maltosyl)-glycerol, (10) 1-O-(β-maltosyl)-glycerol, (11) 1-O-(α-maltosyl)-erythritol, (12) 1-O-(β-maltosyl)-erythritol, (13) 2-O-(α-maltosyl)-glycerol, (14) 2-O-(β-maltosyl)-glycerol, (15) 2-O-(α-maltosyl)-erythritol, (16) 2-O-(β-maltosyl)-erythritol. Compounds 13–16 were only tentatively identified.

The methylated polysaccharide indicated that some hydrolysis to glucose and recombination occurred during polymerisation. There was a small percentage of glucofuranose units.

Previous papers[1–7] have discussed the nature of various polysaccharides produced by MORA and his colleagues by the polymerisation of monosaccharides[8]. These polymers were characterised by containing a high proportion of furanoside residues and virtually all possible linkages. In such cases the term "structure of the polysaccharide" has little meaning and one of the more fruitful results of these investigations has been the development of methods for dealing with the complex mixtures of degradation products obtained. In addition to polymerising monosaccharides MORA's group had also prepared a polysaccharide by the polymerisation of maltose. We were interested to compare this polymer with that obtained from

glucose and the work reported here is the study of this synthetic "polymaltose". These results are now presented in order to give further examples of the contributions of gas–liquid chromatography to the methods of polysaccharide chemistry and discuss only incidentally the "structure of the polymaltose". Since the principle of the methods has been fully described in earlier papers[1–7] only brief comment is necessary here.

PERIODATE OXIDATION

The "polymaltose" consumed 1.2 moles of periodate per hexose unit, a value which could be ascribed to a highly-branched structure and/or a high incidence of 1–6 linkages. Reduction of the polyaldehyde and total hydrolysis gave glycerol and erythritol (4:1) together with small amounts of glucose and xylose. These same four compounds were formed when the "polymaltose" was subjected to a Smith degradation but in addition several other non-reducing fractions were obtained. The nature of these fractions (5, 6 and 7) is the main concern of this paper.

Fraction 5 contained a small amount of glucose which was removed on an anion exchange resin after oxidation to gluconic acid. The quantity of formaldehyde produced by periodate oxidation of part of the neutral sirup remaining indicated the presence of both 1–1 and 1–2 glucopyranosylglycerols. Reduction of the periodate oxidised material and hydrolysis gave glycerol and ethylene glycol in a molar ratio of 1:1. When the remainder of the neutral material was trimethylsilylated and examined by gas–liquid chromatography four main components were observed (Fig. 1). These were identified by their behaviour with periodate[3] as (5a) 2-O-(α-D-glucopyranosyl)-glycerol, (5b) the β-anomer, (5c) 1-O-(α-D-glucopyranosyl)-glycerol and (5d) the β-anomer.

Fraction 6 was shown by estimation of the formaldehyde produced on periodate oxidation to have a mol.wt. of about 290 consistent with a glucosylerythritol. Subsequent reduction and hydrolysis gave glycol and ethylene glycol in the ratio of 3:2, which indicated a high proportion of 1–1 linkages in the glucosides. The remainder of the fraction was trimethylsilylated and found to give the four components shown in Fig. 2. These were identified as (6a) 2-O-(α-D-glucopyranosyl)-erythritol, (6b) the β-anomer, (6c) 1-O-(α-D-glucopyranosyl)-erythritol and (6d) the β-anomer. The total amount of 6c + 6d was about eight times that of 6a + 6b, indicating the importance of 1–6 linkages in the polymer.

Fraction 7 was considerably more complex and gave the gas phase separation shown in Fig. 3. The two principal peaks 7d and 7f were identified as 1-O-(α-maltosyl)-glycerol and the β-anomer respectively. Component 7j was identified as 1-O-(α-maltosyl)-erythritol from which it may be deduced that 7k is the β-anomer and that components 7h and 7i are respectively the α and β anomers of 2-O-(maltosyl)-erythritol. Higher molecular weight fractions separated on paper were not sufficiently volatile to be resolved by gas–liquid chromatography.

METHYLATION

When a polysaccharide contains a variety of different linkages the methylation method of structure elucidation gives several isomeric compounds which may be

difficult to separate by standard chromatographic procedures especially when both furanose and pyranose forms are involved. One approach is to separate the isomeric compounds by cellulose chromatography and then by sequential reduction, periodate oxidation and reduction to degrade the isomers into compounds of varying complexity which are readily separable by gas–liquid chromatography. This approach which has been used previously[4,5] was most successful in the case of methylated rhamnoses[5] and a further example is given in this paper. Ideally the separated fragments should be characterised directly but where insufficient material is collected or standards are not available demethylation to the parent polyhydric alcohol is satisfactory since, in most cases, each member of an isomeric group gives a unique polyhydric alcohol. For complex mixtures it may be useful to react the mixture with trityl chloride thus dividing the components into two groups, a water insoluble group of trityl derivatives and a water soluble group which is not tritylated. The compounds in the latter group must all carry a methyl substituent at C-6. This method used previously in connection with a synthetic glucan[2] has also been applied in the present study. The results of these various separation procedures are given in the experimental section.

The most interesting point to emerge from the present study is the ability of a suitable gas phase system to separate mixtures of glycosylalditols which appear homogeneous by paper chromatography. It should be emphasized that this technique will resolve both isomers and anomers and therefore gives useful information on the structure of the polysaccharide. Thus the isolation of both 1-O-(α-D-glucopyranosyl)-glycerol and the β-anomer is evidence for the existence in the polymer of 1–6 linkages in both the α and β configurations and implies that the glucopyranose unit was itself substituted in such a way as to render it immune to periodate attack. Admittedly such a result is not surprising in view of the manner in which the polysaccharide was synthesized. It is hoped that these methods and separations will be useful for studying natural products where the word structure is more meaningful. In addition to their application in studying fragments obtained by Smith degradations[9] of polysaccharides or lead tetraacetate oxidation products of oligosaccharides[10] they may also be of use in examining bacterial lipids[11].

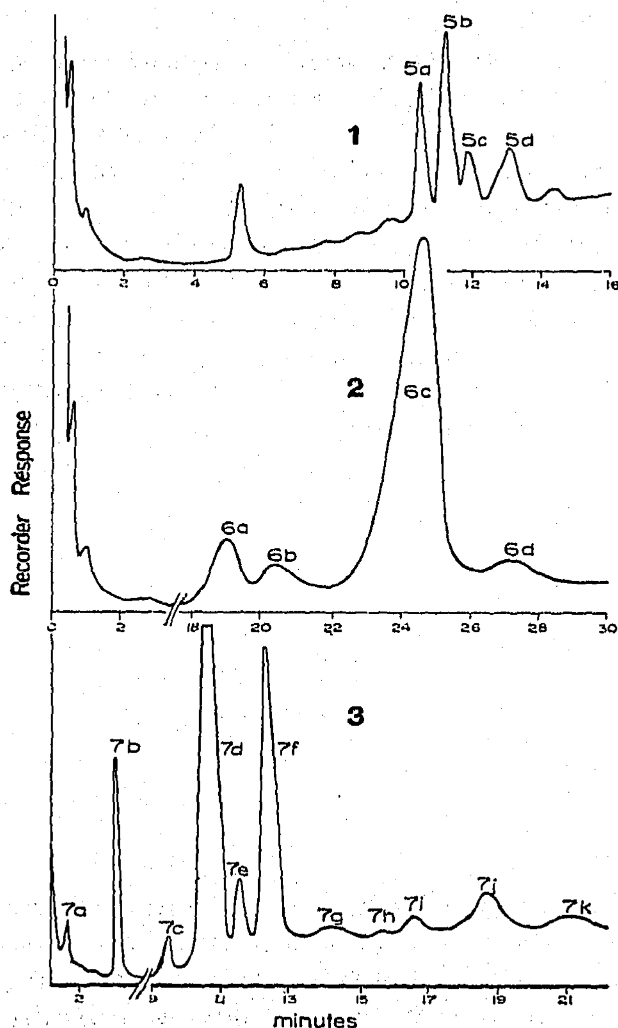
As far as the methylation study is concerned these results suggest that during polymerisation part of the maltose is hydrolysed to glucose which then recombines, partly in the furanose form, and that all possible linkages are formed to some extent. This behaviour may be compared with that of maltose in solution[12,13].

EXPERIMENTAL

General methods have been described previously[1–7] and the "polymaltose" was used as received[8].

Graded hydrolysis

The polysaccharide (50 mg) was dissolved in 0.1 *N* HCl (0.5 ml) and maintained at 50°. Periodically samples were withdrawn, chromatographed on Whatman No. 1 paper in EtOAc–HOAc–H_2O (4:1:1) and estimated by the phenol–sulphuric acid method. After 1 h 3.7% glucose was formed and after 8 h 5.2% glucose, 3.7% maltose, 1.7% maltotriose and 0.9% maltotetraose were obtained.



Figs. 1–3. Gas–liquid chromatography of trimethylsilyl derivatives. Aerograph A90-P2, thermal conductivity detector, 4 ft. × 0.25 in. stainless steel column, 20% GESF 96 on 60/80 mesh firebrick. Fractions 5 and 6, column temperature 190° to 250° at 6°/min, helium flow 120 ml/min (Figs. 1 and 2). Fraction 7, column temperature 210° to 265° at 10°/min, helium flow 165 ml/min (Fig. 3).

Fraction 7 (60 mg) was shown to yield glycerol, erythritol and glucose when a portion was hydrolysed. Periodate degradation of a portion (20 mg) yielded ethylene glycol, glycerol and erythritol. The remainder of the fraction was trimethylsilylated to yield the separation shown in Fig. 3. Owing to the small amount of some of the components collected only 7d, 7f and 7j were studied in detail by periodate degradation. Component 7d was shown to be 1-O-(α-maltosyl)-glycerol with 7f being the β-anomer. Component 7j was identified as 1-O-(α-maltosyl)-erythritol, from which it may be deduced that 7k is the β-anomer. Assignment of the other components is more speculative but it is highly probable that 7c and 7e are respectively the α and β-anomers of 2-O-(maltosyl)-glycerol and two of the three peaks 7g, 7h and 7i probably represent the anomers of 2-O-(maltosyl)-erythritol.

A small amount of slower moving material on paper had a sugar:alcohol ratio of about 3:1 but was too complex to investigate in detail and the silyl derivatives were not sufficiently volatile to permit a separation.

Methylation

The polysaccharide (600 mg) was subjected to one Haworth methylation, two treatments using Kuhn's method and finally several Purdie methylations to give in 80% yield a product which showed no hydroxyl absorption in the infrared. The methylated polysaccharide was dissolved in 72% H_2SO_4 at 0° and the solution allowed to come to room temperature over 1 h. The solution was diluted to an acid concentration of 2 *N*, refluxed for 8 h, neutralised ($BaCO_3$) and finally evaporated to a sirup (450 mg). A portion was resolved on paper using butanone–water azeotrope to give the fractions shown in Table I and the phenolsulphuric acid method was used for the estimation. The bulk of the sirup was separated on a cellulose column into tetra-, tri-, di- and monomethyl fractions. These fractions were examined briefly as described below in order to determine the principal components of each.

TABLE I

QUANTITATIVE ANALYSIS OF METHYLATED SUGARS

O-Methyl derivative of D-*glucose*	*Mole per cent*
Tetra, furanose + pyranose	37.2
Tri, furanose	4.5
Tri, pyranose	30.9
Di, furanose	3.2
Di, pyranose	18.4
Mono	5.6

Tetramethylglucoses. The sirup (140 mg) was oxidised with bromine and shown to contain 6% 2,3,5,6-tetra-O-methyl-δ-D-gluconolactone (retention time 1.5 min) and 94% 2,3,4,6-tetra-O-methyl-γ-D-gluconolactone (retention time 2.1 min; 4 ft. × 0.25 in. column of 12% Versamide on Chromosorb W, helium flow 90 ml/min, 165°).

Trimethylglucoses. An attempt to resolve a portion (20 mg) of this fraction as the lactones on SE 30 indicated at least eight compounds but did not give a satisfactory separation. The remainder (110 mg) was separated on paper into a trimethylfuranose portion (10 mg) and a trimethylpyranose portion (80 mg), the area of overlap being discarded. The furanose portion was sequentially reduced, oxidised and reduced and a part examined by GLC (5 ft. × 0.125 in. column of 5% SE 30 on firebrick, nitrogen flow 15 ml/min, 145°, hydrogen flame detector). Three main products were observed, 1,2-di-O-methylglycerol (0.6 min), 2-O-methylglycerol (0.9 min), both formed from 2,5,6-tri-O-methylglucofuranose, and 2,4,5-tri-O-methylarabinitol (10 min, from the 3,5,6-isomer). The remainder of the reduced furanose portion was demethylated and shown to give glycerol and arabinitol. The pyranose portion was treated similarly and demethylation gave xylitol, arabinitol, glucitol, threitol and ethylene glycol in the ratio (chromotropic acid) of 1:1:1:8:8. This shows that 2,3,6-tri-O-methyl-D-glucose is the principal member of this group and confirms that the 1–4 linkage in maltose is largely preserved in the polysaccharide.

Dimethylglucoses. Electrophoresis in 0.07 *M* borate and estimation by phenolsulphuric acid showed 2,3-di-O-methylglucose (and 2,6-) 78% with about 5% of each of 2,4-, 3,4-, 3,6-, and 4,6-isomers. The bulk of the material (91 mg) was refluxed with

1% methanolic hydrogen chloride and divided into a tritylated water insoluble fraction and water soluble fraction. The latter was sequentially oxidised with periodate, reduced with borohydride, hydrolysed and again reduced. The sirup thus obtained was demethylated and shown to contain glucitol (from 3,6-), glycerol (from 2,6-) and erythritol and ethylene glycol (from 4,6-) in a molar ratio of 2:2:1:1. The water insoluble tritylated fraction was refluxed with methanolic hydrogen chloride, concentrated and water added to precipitate tritanol. The filtrate was made 1 *N* with sulphuric acid, refluxed 10 h, neutralised and concentrated. Electrophoresis showed 2,3-, 2,4- and 3,4-di-O-methylglucoses, the first comprising about 85% of the three. This was confirmed by the usual reduction and demethylation sequence to give threitol and xylitol (from 2,4-) in the ratio of 9:1. It may be noted that in this instance 2,3-di-O-methyl-D-glucose gives 2,3-di-O-methyl-D-threitol while the 3,4-isomer gives the L-enantiomorph.

Monomethylglucoses. The 2-, 3-, 4- and 6-O-methylglucoses were identified by electrophoresis.

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REFERENCES

1 G. G. S. Dutton and A. M. Unrau, *Can. J. Chem.*, 40 (1962) 1479, 2101, 2105.
2 G. G. S. Dutton and A. M. Unrau, *Can. J. Chem.*, 41 (1963) 2439.
3 G. G. S. Dutton and A. M. Unrau, *Can. J. Chem.*, 42 (1964) 2048.
4 G. G. S. Dutton and A. M. Unrau, *Can. J. Chem.*, 43 (1965) 924.
5 G. G. S. Dutton and A. M. Unrau, *Can. J. Chem.*, 43 (1965) 1738.
6 G. G. S. Dutton and A. M. Unrau, *Carbohydrate Res.*, 1 (1965) 116.
7 G. G. S. Dutton and A. M. Unrau, *J. Chromatog.*, 20 (1965) 78.
8 P. T. Mora, J. W. Wood and V. W. McFarland, *J. Am. Chem. Soc.*, 82 (1960) 3418.
9 I. J. Goldstein, G. W. Hay, B. A. Lewis and F. Smith, in R. L. Whistler (Editor), *Methods in Carbohydrate Chemistry*, Academic Press, New York, 1965, p. 361.
10 A. S. Perlin, *Advan. Carbohydrate Chem.*, 14 (1959) 9.
11 N. Shaw and J. Baddiley, *Nature*, 217 (1968) 142.
12 D. J. Manners, G. A. Mercer and J. J. M. Rowe, *J. Chem. Soc.*, (1965) 2150.
13 M. L. Wolfrom, A. Thompson and R. H. Moore, *Cereal Chem.*, 40 (1963) 182.
14 C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Am. Chem. Soc.*, 85 (1963) 2497.