SOME APPLICATIONS OF PAPER AND VAPOUR-PHASE CHROMATOGRAPHY TO POLYSACCHARIDES

MODEL STUDIES ON A SYNTHETIC D-MANNAN*

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This paper reports the application of vapour-phase chromatography (v.p.c.) to the analysis of complex mixtures of isomeric methylated sugars and to the isomeric and anomeric glycosides which may arise by application of the SMITH periodate technique. Some preliminary separations were carried out by paper chromatography and the methods developed greatly facilitate the investigation of the structure of polysaccharides.

The substrate in these studies has been a synthetic D-mannan which, from its method of preparation, was expected to have a highly branched random structure and to contain an appreciable proportion of D-mannofuranose residues[1]. Such a polysaccharide would give rise to several glycosyl glycitols and the methylated mannan would give virtually all possible methyl ethers of D-mannose. This mannan was therefore an excellent choice for the model experiments described below.

METHYLATED SUGARS

The separation of methylated sugars as their glycosides has been reviewed by BISHOP[2] but since isomeric glycosides may have similar retention times and each component may give 2 peaks corresponding to the anomeric glycosides the methods so far reported fail with complex mixtures. A method is described here whereby isomeric methylated sugars may be selectively degraded by periodate to compounds with greatly different retention times. In addition monomethyl hexoses, whose glycosides have inconveniently long retention times, may be degraded to products which have lower retention times.

The fully methylated mannan was subjected to hydrolysis and the mixture of sugars was resolved by paper chromatography into the 7 fractions shown in Table I. The separation of the 2,3,4,6- and 2,3,5,6-tetra-O-methyl-D-mannose was readily achieved by v.p.c. of the lactones, each of which was obtained crystalline.

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Fraction 2 was shown by paper chromatography and electrophoresis to contain (a) 2,3,5-, (b) 2,5,6-, and (c) 3,5,6-tri-O-methyl-D-mannoses. By successive reaction with borohydride, periodate and borohydride these isomers were converted into (a) 2,3,5-tri-O-methyl-D-mannitol, (b) 2-O-methylglycerol plus 1,2-di-O-methyl-L-glycerol, and (c) 2,4,5-tri-O-methyl-D-arabinitol. Each of these compounds was

TABLE I

HYDROLYSIS OF METHYLATED MANNAN

Fraction	*Nature*	*Weight (mg)*
1	Tetra-O-methyl	495
2	Tri-O-methylfuranose	70
3	Tri-O-methylpyranose	460
4	Di-O-methylfuranose	55
5	Di-O-methylpyranose	210
6	Mono-O-methyl	60
7	D-Mannose	9
	Recovery 82%	

readily separated by v.p.c. and each was demethylated by boiling with hydrobromic acid[3]. A crystalline derivative of the parent alditol was prepared where possible. This sequence is summarized in Table IIA.

Fraction 3 containing the tri-O-methyl-D-mannopyranose sugars was converted to the methyl glycosides and tritylated, a procedure which has been used successfully for the separation of glucose isomers[4]. The tritylated sugar was identified as 2,3,4-tri-O-methyl-D-mannose by conversion to the crystalline 2,3,4-tri-O-methyl-D-mannonamide. The glycosides which did not react with trityl chloride were hydrolyzed and degraded as for fraction 2 with the results shown in Table IIB. In each case characteristic fragments were produced which were readily separable by v.p.c.

The dimethylmannoses differed sufficiently in mobility on paper chromatography that the furanose isomers were obtained in fraction 4 and the pyranose isomers in fraction 5. The former were directly degraded and analyzed by v.p.c. with the results shown in Table IIC. Fraction 5 contained a larger number of components and they were therefore initially separated as the glycosides by their reaction with trityl chloride. The non-tritylated glycosides were directly oxidized with periodate, reduced with borohydride, hydrolyzed and again treated with borohydride. On this occasion the mixture was demethylated directly and the parent alditols separated by paper chromatography with the results shown in Table IID. The tritylated glycosides were hydrolyzed and the free sugars (2,3-, 2,4- and 3,4-di-O-methyl-D-mannoses) were oxidized with periodate. Paper chromatography showed unchanged material and a fast component corresponding to 3,4-di-O-methyl-D-arabinose. These were separated on paper and the pentose was degraded to threitol thus confirming the presence of 3,4-di-O-methyl-D-mannose in the original mixture. A similar degradation of the slow moving material gave mostly erythritol, from 2,3-di-O-methyl-D-mannose, with traces of D-lyxitol, from 2,4-di-O-methyl-D-mannose.

Fraction 6 containing the monomethyl ethers was successively oxidized with

TABLE II

DEGRADATION FRAGMENTS OF METHYLATED D-MANNOSES

He flow 95 ml/min. For column size and packing, see p. 84.

1 *Ether*	2 *Product(s)*	3 *Retention time (min)*	4 *Demethylated product*	5 *Derivative of 4 (m.p.)*
A. Fraction 2, tri-O-methyl-D-mannofuranoses[a]				
2,3,5-	2,3,5-tri-O-methyl-D-mannitol	12.3	mannitol	164°
2,5,6-	2-O-methylglycerol	2.5	glycerol	tri-*p*-nitrobenzoate, 189–191°
	+			
	1,2-di-O-methyl-L-glycerol	1.2	glycerol	
3,5,6-	2,4,5-tri-O-methyl-D-arabinitol	7.2	arabinitol	—
B. Fraction 3, tri-O-methyl-D-mannopyranoses[a]				
2,3,6-	mono-O-methyl ethylene glycol	0.8	ethylene glycol	di-*p*-nitrobenzoate, 145–147°
	+			
	2,3-di-O-methyl-erythritol	3.5	erythritol	tetra-*p*-nitrobenzoate, 252–254°
2,4,6-	2,4,6-tri-O-methyl-D-mannitol	12.0	mannitol	164°
3,4,6-	2,3,5-tri-O-methyl-D-arabinitol	6.6	arabinitol	—
C. Fraction 4, di-O-methyl-D-mannofuranoses[b]				
2,5-	2-O-methyl-glycerol	2.5	glycerol	tri-*p*-nitrobenzoate, 189–192°
3,5-	2,5-di-O-methyl-D-arabinitol	10.8	arabinitol	—
5,6-	1,2-di-O-methyl-L-glycerol	1.2	glycerol	tri-*p*-nitrobenzoate, 189–192°
D. Part of fraction 5, di-O-methyl-D-mannopyranoses				
2,6-	1-O-methyl-L-glycerol	not	glycerol	tri-*p*-nitrobenzoate, 189–191°
	+			
	2-O-methyl-glycerol	separated	glycerol	
3,6-	3,6-di-O-methyl-D-mannitol	by	mannitol	163–164°
4,6-	ethylene glycol	v.p.c.	ethylene glycol	di-*p*-nitrobenzoate, 145–146°
	+			
	1,3-di-O-methyl-L-erythritol		erythritol	tetra-*p*-nitrobenzoate, 251–253°
E. Fraction 6, mono-O-methyl-D-mannoses[c]				
2-	glycerol	4.2	glycerol	tri-*p*-nitrobenzoate, 189–191°
	+			
	2-O-methyl-glycerol	2.5	glycerol	
3-	2-O-methyl-D-arabinitol	14.5	arabinitol	—
4-	2-O-methyl-D-erythritol	7.4	erythritol	tetra-*p*-nitrobenzoate, 251–254°
5-	2-O-methyl-L-erythritol	7.4	erythritol	
6-	1-O-methyl-glycerol	1.8	glycerol	tri-*p*-nitrobenzoate, 189–191°

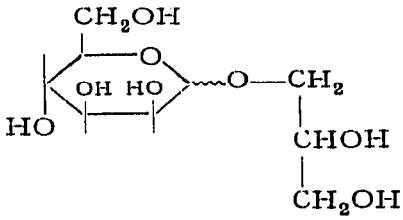
[a] Initial temp. 160°, programmed at 6°/min, 3 min after injection.
[b] Initial temp. 160°, programmed at 8°/min, 3 min after injection.
[c] Initial temp. 160°, programmed at 10°/min, 3 min after injection.

periodate, reduced, hydrolyzed and again reduced. The products were separated by v.p.c. with the results shown in Table IIE.

Similar schemes involving a combination of periodate and v.p.c. techniques have also been successfully employed with mixtures of methylated arabinoses[5], galactoses[6] and rhamnoses[7].

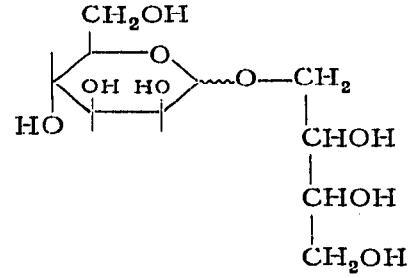
SMITH DEGRADATION

When a polyalcohol, obtained by periodate oxidation and borohydride reduction, is hydrolyzed under mild acid conditions the acetal linkages are cleaved and the glycosidic bonds remain intact. This is known as the SMITH periodate degradation and gives rise to a series of glycosides in which the aglycone is commonly glycerol or a tetritol (ref. 8 and refs. therein). Table III shows the fragments which were separated by paper chromatography when this technique was applied to the synthetic mannan. Compounds I–V shown in Fig. 1 represent typical glycosides which may be obtained, the D-lyxose derivatives arising from cleavage of C-6 from a D-mannofuranose unit by periodate.



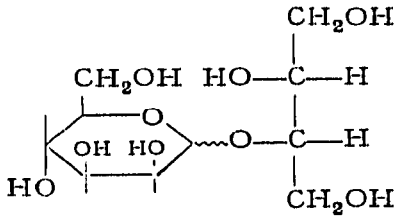
1-O-(D-mannopyranosyl)-glycerol

(I)



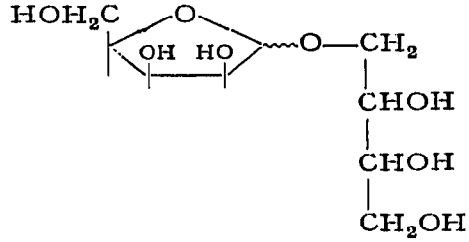
1-O-(D-mannopyranosyl)-erythritol

(II)



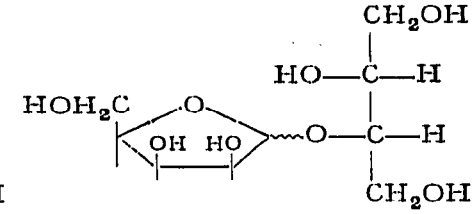
2-O-(D-mannopyranosyl)-D-erythritol

(III)



1-O-(D-lyxofuranosyl)-erythritol

(IV)



2-O-(D-lyxofuranosyl)-D-erythritol

(V)

Fig. 1. Some glycosides obtained by SMITH degradation of a D-mannan.

It has been shown previously[8] that when hexosyl-triitols and pentosyl-tetritols are produced these may be inseparable by paper chromatography since, being isomers, they have similar mobilities. In these cases conversion of the glycosyl-glycitols to their trimethylsilyl ethers[9] has permitted the separation by v.p.c. of both isomers and anomers. The application of the SMITH degradation to the synthetic mannan is of particular interest since it illustrates the separation of certain glycosides by paper chromatography and others by v.p.c.

When fraction 4 (Table III) was rerun on paper it was resolved into 5 fractions 4a–e (Table IV). From the products of hydrolysis and the products of periodate degradation and hydrolysis the structures shown in Table IV were deduced but owing

TABLE III

SMITH DEGRADATION OF MANNAN

Fraction	*Nature*	R_G*	*Weight (mg)*
1	glycerol	3.34	365
2	erythritol	2.42	157
3	D-lyxose	2.24	26
4	see Table IV	1.52	109
5	mannopyranosyl erythritols	1.08	61
6		0.94	122
7	complex	0.64	85
8	complex	0.36	—
9	complex	0.21	—

* Solvent A.

to the small quantities isolated it was not possible to measure optical rotations and thus determine the configuration of the glycosidic linkages.

Fraction 5 crystallized spontaneously and had a negative optical rotation. Hydrolysis gave D-mannose and erythritol (1:1) while periodate degradation gave only glycerol thus indicating 2-O-(β-D-mannopyranosyl)-D-erythritol. Conversion of part of fraction 5 to the trimethylsilyl derivative and analysis by v.p.c. showed the presence of about 15% of the α-anomer, which had the lower retention time[9]. The β-anomer was recovered by alcoholysis and characterized as its *p*-nitrobenzoate.

TABLE IV

PAPER CHROMATOGRAPHIC SEPARATION OF FRACTION 4

EG = ethylene glycol; Ery = erythritol; Gly = glycerol.

Component	*Weight (mg)*	R_G*	*Composition*	*Periodate degradation (Gly:EG)*
			Man:Gly	
(Man$_p$ $\overset{4a}{\overset{1}{\underset{1}{\text{———}}}}$ Gly)	10.4	1.05	1:1	1:1
(Man$_p$ $\overset{4b}{\overset{1}{\underset{1}{\text{———}}}}$ Gly)	8.0	1.18	1:1	1:1
			Lyx:Ery	
(Lyx$_f$ $\overset{4c}{\overset{1}{\underset{1}{\text{———}}}}$ Ery)	12.2	1.31	1:1	1:1
(Lyx$_f$ $\overset{4d}{\overset{1}{\underset{2}{\text{———}}}}$ Ery)	28.2	1.41	1:1	Gly only
(Lyx$_f$ $\overset{4e}{\overset{1}{\underset{1}{\text{———}}}}$ Ery)	10.1	1.50	1:1	1:1

* Solvent A.

Fraction 6 when similarly examined by v.p.c. gave only one main peak which was shown to be due to 1-O-(β-D-mannopyranosyl)-erythritol. Fig. 2 is an actual tracing from fraction 6 and fraction 5 gave a similar pattern except that component (b) was the major one and the others were minor. Since α-anomers have lower retention times than β-anomers[6] it is likely that (a) was 2-O-(α-D-mannopyranosyl)-D-erythritol and that (c) was 1-O-(α-D-mannopyranosyl)-erythritol.

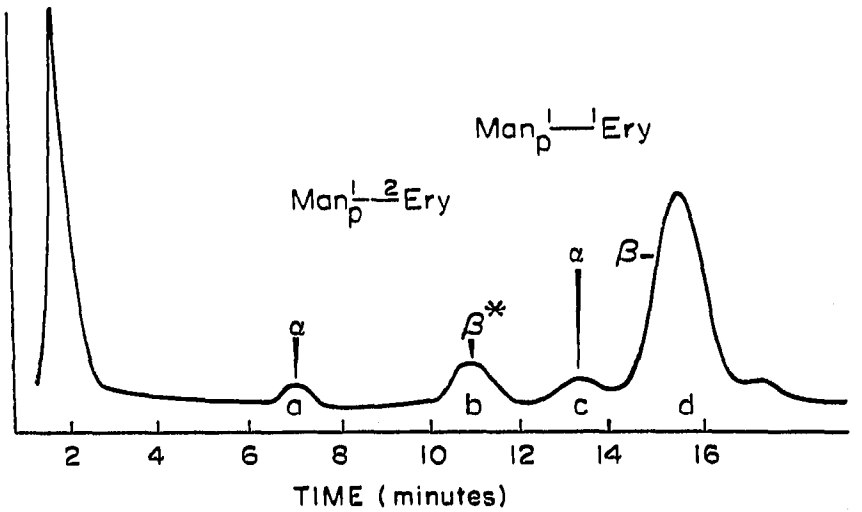


Fig. 2. Vapour-phase chromatography of trimethylsilylated mannosides. Silyl derivatives of mannan fraction 6 (5*). Aerograph A90-P2; 4 ft. column 1/4 in. O.D.; 20% GESF-96 on 60/80 firebrick, 235°; He flow 85 ml/min.

Hydrolysis of fraction 7 gave mannose, lyxose, erythritol and glycerol and v.p.c. gave several overlapping peaks which were shown to be due to disaccharide glycosides. It was evident that fractions 8 and 9 were of even greater complexity.

CONCLUSIONS

The results given in Table II demonstrate that the selective degradation of a mixture of isomeric methylated alditols and the separation of the fragments by v.p.c. is a facile method for the analysis of such mixtures. Given the necessary reference compounds it would also be possible to characterize directly the fragments obtained by v.p.c. without the intermediate demethylation step. It should be noted that each member of an isomeric group of ethers gives rise to a unique degradation product.

Fig. 2 clearly shows the application of v.p.c. to the separation of isomeric and anomeric glycosides which were inseparable on paper. Although disaccharide glycosides have been separated by v.p.c. in the case of a 6-deoxy-hexose[7] the situation is complicated with a hexosan by the possible formation of pentose and hexose glycosides. At the present time more fragments are in general produced by a SMITH degradation than can be separated by existing techniques. Thin-layer chromatography has had considerable success in separating a series of oligomers (*e.g.* ref. 10) and it may be that this technique will also be useful for resolving the glycosides obtained by this degradative procedure. Such a technique is urgently required if the maximum amount of information is to be extracted from this method of polysaccharide analysis.

With respect to the mannan the methylation results show that the polymer was highly branched and that approximately 20% of the terminal units were furanose. The presence of D-mannofuranose units was also demonstrated by the isolation of lyxose and glycosides of lyxose. Both the methylation data and the SMITH degradation show that the principal linkages were (1 → 4) and (1 → 6).

The synthetic mannan has a structure very much more complicated than the majority of those mannans occurring in nature but because of the wide variety of polysaccharides in which mannose is found these results are presented here in the hope that they will be of general application to a study of natural mannans in particular and by suitable modification to polysaccharides in general.

EXPERIMENTAL

Paper chromatographic separations were carried out on Whatman 1 or 3MM paper using solvent A (ethyl acetate–pyridine–water, 8:3:2), solvent B (ethyl acetate–acetic acid–water, 8:2:2) or solvent C (butanone–water azeotrope). Compounds were detected either with *p*-anisidine trichloracetate[3] or ammoniacal silver nitrate[11]. Reducing sugars were estimated by the phenol–sulphuric acid method[12] and alditols by chromotropic acid after oxidation with periodate[13]. Periodate uptake was measured by the arsenite method[14].

Vapour-phase chromatography was carried out using an Aerograph A90-P2* instrument. For the separation of the methylated glycitols the column was 4 ft. × 0.25 in. O.D. copper packed with 12% Versamid on Diatoport (F and M Scientific Corporation Inc.). For the separation of trimethylsilyl derivatives the column was 4 ft. × 0.25 in. O.D. copper packed with 20% GESF-96 (Wilkens Instrument and Research Inc.).

All evaporations were carried out *in vacuo* at a bath temperature of less than 40°. Optical rotations are equilibrium values measured for the Na_D line and at 22° ± 2° and melting points are uncorrected.

Electrophoresis was carried out on Whatman No. 1 paper in 0.05 *M* borate at 950–1050 V, 25–30 mA.

Analysis of mannan

The mannan was used as received from Dr. MORA and was part of the same batch on which some measurements have been reported[1]. Periodate oxidation of the mannan gave 0.05 mg formaldehyde per 100 mg of polysaccharide. When the mannan was reduced with borohydride before oxidation the figure was 0.54 mg/100 mg. On the assumption that each mannitol end group gave rise to 2 moles of formaldehyde the mannan[13] had an approximate D.P. of 70.

Acid hydrolysis of the mannan gave an almost quantitative yield of D-mannose characterized as its phenylhydrazone m.p. and mixed m.p. 198–200°, [α] 33° (*c* 1.8, in pyridine). Partial hydrolysis with 0.1 *N* hydrochloric acid at 50° gave 9% mannose after 1 h and 13% after 8 h with lesser amounts of oligosaccharides and about 55% of the polymer was unhydrolyzed.

Methylation of mannan

The mannan (3 g) was methylated twice by HAWORTH's method[15] and 3 times

by KUHN's method[16] but it was not until after 4 treatments with PURDIE's reagents[17] that a fully methylated product was obtained. The product was purified by extraction with 95 % petroleum ether and 5 % chloroform and the methylated mannan (2.9 g, 45.3 % methoxyl, no absorption in the infrared) was obtained as a friable glass. The methylated polysaccharide (2.4 g) was dissolved in 72 % sulphuric acid and the solution was diluted and boiled. The mixture of methylated sugars was resolved on Whatman 3MM paper using solvent C to give the fractions shown in Table I.

Fraction 1 was oxidized with bromine and analysis of the lactones by v.p.c. gave, in order, 2,3,5,6-tetra-O-methyl-D-mannonolactone which crystallized in the collecting tube m.p. 106–108° (lit.[18,19] 107–108°) and 2,3,4,6-tetra-O-methyl-D-mannonolactone which crystallized on cooling the collection tube m.p. 24–26°, [α] 150 → 68° (*c* 1.0, in water) (lit.[20,21] m.p. 24–25°, [α] 150 → 67° (in water)).

Fraction 2 on paper electrophoresis showed the presence of 3,5,6-tri-O-methyl-D-mannose (M_G 0.3) and non-migrating components. The mixture was reduced ($NaBH_4$, 100 mg, 24 h), acidified (HOAc) and oxidized with periodic acid (0.01 *N*, 5°, 6 h). Periodate and iodate ions were removed ($BaCO_3$) and the mixture again reduced ($NaBH_4$, 100 mg, 24 h). The solution was passed through a column of Amberlite IR-120H^+, evaporated to dryness and distilled several times with methanol. The products were separated by v.p.c. and the individual components demethylated by boiling for 35 min with 45 % hydrobromic acid[3]. The D-mannitol crystallized and the glycerol was characterized as its tri-*p*-nitrobenzoate as shown in Table IIA.

Fraction 3 was converted to the methyl glycosides (3 % MeOH–HCl, reflux 10 h), the sirup was dissolved in pyridine (40 ml) and reacted at room temperature with trityl chloride (200 mg, 48 h). The reaction mixture was poured into water when the tritylated glycoside and tritanol precipitated. The precipitate was washed with water and the free sugar regenerated by hydrolysis. The sirup (65 mg) was essentially pure and was identified by the formation of 2,3,4-tri-O-methyl-D-mannonamide m.p. 140–142° (lit.[22] m.p. 143°).

The mother liquor and washings were evaporated to dryness and those glycosides which did not tritylate were hydrolyzed. The mixture of sugars (370 mg) was reduced ($NaBH_4$), oxidized (HIO_4) and reduced ($NaBH_4$) as described above and the produce examined by v.p.c. with the results shown in Table IIB.

Fraction 4 was subjected to the reduction, oxidation, reduction sequence and analysis of the products by v.p.c. is shown in Table IIC.

Fraction 5 consisted of at least 6 components and it was therefore separated into 2 portions by tritylation as described for fraction 3. Those glycosides (85 mg) incapable of forming a trityl derivative were hydrolyzed, degraded and examined by v.p.c. with the results shown in Table IID. The tritylated glycosides were hydrolyzed and the free sugars (120 mg) oxidized (HIO_4, 0.01 *M*, 5°, 12 h). Addition of barium carbonate removed iodate and periodate ions and paper chromatographic examination of the resulting sirup showed a new sugar (R_F 0.24, solvent C) and unchanged di-O-methyl-D-mannoses. The mixture was resolved (solvent C) and the new sugar (22 mg), believed to be 3,4-di-O-methyl-D-arabinose was reduced, oxidized and reduced as above and the product demethylated to yield threitol [α] 4.3° (*c* 1.5, in water), tetra-*p*-nitrobenzoate m.p. and mixed m.p. 219–221°, thus confirming this assignment. The dimethyl hexose portion was treated similarly and was shown to yield mainly erythri-

tol (65 mg), tetra-*p*-nitrobenzoate m.p. and mixed m.p. 252–253° and a small amount of D-lyxitol (7 mg).

Fraction 6 was oxidized (HIO_4, 0.01 *M*, 15 ml, 6 h), neutralized ($BaCO_3$) and reduced ($NaBH_4$, 100 mg, 48 h). The mixture was hydrolyzed and again reduced ($NaBH_4$, 100 mg, 48 h). After deionization and removal of borate the product was analyzed by v.p.c. with the results shown in Table IIE.

Smith periodate degradation

The mannan (3.0 g) was dissolved in water (55 ml) and periodic acid added (0.5 *M*, 45 ml). The consumption of periodate reached a constant value of 1.1 moles of periodate per hexose unit after 72 h. The solution was neutralized ($BaCO_3$), sodium borohydride (2.5 g) was added and the reduction was allowed to proceed for 48 h. The solution was passed through Amberlite IR-120H+, evaporated and distilled with methanol to remove borate. The dry residue was dissolved in acid (0.1 *N* HCl, 40 ml) and left at room temperature for 7 h. The solution was deionized and evaporated to a sirup which on examination by paper chromatography showed at least 9 components, 2 of which were reducing (Table III).

Fraction 1 was identified as glycerol (tri-*p*-nitrobenzoate m.p. and mixed m.p. 188–190°); fraction 2 crystallized spontaneously to give erythritol m.p. and mixed m.p. 118–120° (tetra-*p*-nitrobenzoate m.p. and mixed m.p. 250–252°); fraction 3 crystallized on seeding with D-lyxose and after washing with ethanol had m.p. and mixed m.p. 106–108°, D-lyxose 2,4-dinitrophenylhydrazone m.p. and mixed m.p. 169–171°, [α] 30.5° (*c* 1.2, in pyridine) (lit.[23] m.p. 169–170°, [α] 27.6°).

Fraction 4 (109 mg) had an R_F similar to mannose and showed reducing properties. A portion (35 mg) was converted to D-mannose phenylhydrazone m.p. and mixed m.p. 198–200°, [α] 33° (*c* 1.2, in pyridine). The remaining material was dissolved in water (15 ml) containing lead carbonate and bromine (2 ml). After 24 h the solution was aerated and deionized and the neutral effluent evaporated to a sirup (60 mg) which was separated on Whatman 1 paper using solvent A. Part of each component was hydrolyzed and part was oxidized (HIO_4), reduced ($NaBH_4$) and hydrolyzed with the results shown in Table IV.

Fraction 5 crystallized spontaneously and had m.p. 109–112°, [α] —31° (*c* 0.9, in water). Hydrolysis of a small portion (8 mg) gave mannose and erythritol (1:1) while periodate oxidation of a second portion (10 mg) gave formaldehyde (1.04 mg) indicating a mol.wt. of about 280 (theory 284). Reduction ($NaBH_4$) and hydrolysis of the oxidized material gave only glycerol. The remainder of the fraction was reacted with trimethylchlorosilane and hexamethyldisilizane and analyzed by v.p.c.[9]. A tracing similar to that shown in Fig. 2 was obtained except that (b) was the major component. Material corresponding to peaks (a) and (b) was desilylated by boiling in 90% methanol for 12 h. Component (a) (7 mg) had [α] 35° (*c* 1, in water) and was thus 2-O-(α-D-mannopyranosyl)-D-erythritol while component (b) (38 mg) which had [α] —36° (*c* 2, in water) was the β-anomer. From the latter was obtained 2-O-(β-D-mannopyranosyl)-D-erythritol hepta-*p*-nitrobenzoate m.p. 235–238°. Anal. calc. for $C_{59}H_{41}O_{30}N_7$: N, 7.93%. Found: N, 7.98%.

Fraction 6 (122 mg) on complete hydrolysis of a portion (12 mg) gave mannose and erythritol (1:1) with traces of glycerol and lyxose. Conversion of the remainder of the fraction to the trimethylsilyl derivatives and examination by v.p.c. gave the

results shown in Fig. 2. Each component was desilylated by refluxing with 80% ethanol for 16 h and analyzed. Component 6a (10 mg) gave on hydrolysis of a part (5 mg) mannose and erythritol (1:1). Periodate oxidation of the remainder (5 mg) gave formaldehyde (0.52 mg) corresponding to a mol.wt. of 290 (theory 284). Hydrolysis of the reduced material gave only glycerol. This material was hence judged to be 2-O-(α-D-mannopyranosyl)-D-erythritol. Component (6b) (25 mg) had [α] —35° (*c* 2, in water) and analysis gave the same results as (6a). It was therefore 2-O-(β-D-mannopyranosyl)-D-erythritol. Component (6c) (8 mg) gave on hydrolysis mannose and erythritol (1:1). Component (6d) was the major fraction (58 mg) and had [α] —28° (*c* 2, in water). Hydrolysis of a portion (8 mg) gave mannose and erythritol (1:1). The formaldehyde (2.55 mg) produced on periodate oxidation of a part (25 mg) indicated a mol. wt. of about 285 and hydrolysis of the reduced material gave ethylene glycol and glycerol (1:1). The remainder was converted to 1-O-(β-D-mannopyranosyl)-erythritol hepta-*p*-nitrobenzoate m.p. 239–243°. Anal. calc. for $C_{56}H_{41}O_{30}N_7$:N, 7.93%. Found: N, 8.00%.

When part (8 mg) of fraction 7 was hydrolyzed there were obtained mannose, lyxose, erythritol and glycerol in a ratio of 3:1:1:1 or a sugar:alcohol ratio of 2:1. Analysis by v.p.c. as before only gave overlapping peaks.

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SUMMARY

Methods are given for the separation and analysis of (i) the methylated sugars obtained from the methylated polysaccharide, and (ii) the glycosides obtained by a SMITH degradation of the mannan. Analysis of a mixture of isomeric methylated sugars was facilitated by successive borohydride reduction, periodate oxidation and borohydride reduction. In this way isomeric C_6 sugars were selectively degraded into C_6, C_5, C_4, C_3 and C_2 alditols which were readily separated by vapour-phase chromatography (v.p.c.). Details are given for the separation of tri-, di-, and mono-O-methyl-D-mannoses.

SMITH degradation of the mannan gave a series of glycosides of which 1-O-(α- and β-D-mannopyranosyl)-glycerol, 1-O-(α- and β-D-lyxofuranosyl)-erythritol and 2-O-(D-lyxofuranosyl)-D-erythritol were separable by paper chromatography. Other glycosides separated by v.p.c. of their trimethylsilyl derivatives were 2-O-(α-D-mannopyranosyl)-D-erythritol, the β-anomer (*p*-nitrobenzoate, m.p. 235–238°); 1-O-(β-D-mannopyranosyl)-erythritol (*p*-nitrobenzoate, m.p. 239–243°) and the α-anomer. The mannan was shown to contain D-mannofuranose and D-mannopyranose residues, to be highly branched and to contain mainly (1 → 4) and (1 → 6) linkages.

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