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The Water-soluble Polysaccharide of Opium Poppy (*Papaver somniferum* L.)

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Aqueous extraction of dried opium poppy capsules furnished a polysaccharide material in 2.4 % yield containing galactose 10 %, arabinose 6 %, xylose 6 %, rhamnose 4 %, galacturonic acid 60 %, 4-*O*-methylglucuronic acid 4 % and, in addition, trace quantities of fucose, 2-*O*-methylfucose, 2-*O*-methylxylose, and glucuronic acid. Fractional precipitation with sodium acetate resulted in the isolation of an almost pure galacturonan in 0.4 % yield. Chromatography on Amberlite IR-45 in acetate form separated the polysaccharide into two fractions, 87 % and 13 %, respectively, of slightly different composition. The material resisted other methods of fractionation. Periodate oxidation of the polysaccharide followed by reduction with sodium hydridoborate and acid hydrolysis afforded ethylene glycol, glycerol, propylene glycol, and threitol in the approximate molar proportions 1:5:2:1. The composition and the properties of the polysaccharide suggest that it belongs to the group of pectic substances.

The first step in the manufacture of morphine and other opium alkaloids is nowadays mostly performed by aqueous extraction of opium poppy capsules. Such extracts on concentration become slimy and viscous due to the presence of high-molecular substances of carbohydrate nature. Ottestad *et al.*[1] found this material to be a complex polysaccharide yielding, on hydrolysis galactose, glucose, arabinose, xylose, rhamnose, and a uronic acid which was not identified. In view of the pharmaceutical importance of the opium poppy it seemed of interest to study this carbohydrate polymer in some more detail. Furthermore, to the authors' knowledge, no polysaccharide from a plant of the *Papaveraceae* family has been subjected to structural examination so far. The present report describes the characterization of the opium poppy capsular polysaccharide, revealing its close relation to the group of pectic substances.

Pectins are frequently extracted from plant material with hot aqueous ammonium oxalate. However, since this agent as well as hot neutral buffer salt solutions have been found to cause degradation of the polysaccharide,[2,3]

water was used for extraction in the present investigation. The crude polysaccharide had $[\alpha]_D + 180°$; it contained 64 % uronic acid, 5.5 % ester methoxyl, 3.7 % acetyl, and 0.5 % nitrogen. On acid hydrolysis it gave D-galactose, L-arabinose, D-xylose, L-rhamnose, D-galacturonic acid, 4-*O*-methyl-D-glucuronic acid and, in addition, traces of fucose, 2-*O*-methyl-L-fucose, 2-*O*-methyl-D-xylose, and glucuronic acid and its lactone. The proportions of the constituent sugars are listed in Table 1. Auto-hydrolysis of the polysaccharide

Table 1. Per cent composition [a] of some of the polysaccharide fractions obtained.

Component	Original extract	Eluted from the Amberlite IR-45 column		Residual polysaccharide after periodate oxidation
		Fract. I	Fract. II	
Galactose	9.9	9.9	11.4	49
Arabinose	5.9	5.6	8.0	4
Xylose	5.9	5.9	7.1	9
Rhamnose	4.3	3.6	4.5	5
Galacturonic acid	60	65	60	28
4-*O*-Methyl-glucuronic acid	4			0
Ester methoxyl	5.5	5.7	4.1	—
Acetyl	3.7	3.6	3.9	—

[a] In addition to the constituents listed the products contained trace quantities of fucose, 2-*O*-methylfucose, 2-*O*-methylxylose, and glucuronic acid.

in its acid form or heating it 1 h with 0.01 N sulphuric acid at 100° liberated arabinose as the only sugar, suggesting that a part of this component in its furanose form occupies terminal positions.

Significant amounts of a uronic acid other than galacturonic acid have not previously been found in a pectin, and the presence of 4-*O*-methylglucuronic acid indicated the existence of at least two different polysaccharides in the extract. However, the material resisted the general methods of fractionation. Fractional precipitation neither with ethanol nor with cetyltrimethylammonium bromide [4] gave fractions of different composition. The latter reagent precipitated all the polysaccharide in solution, thus indicating the absence of neutral components. This was confirmed by electrophoresis in acetate buffer and also by chromatography on diethylaminoethyl (DEAE) cellulose in phosphate and in chloride form. High salt concentrations were required to elute the polysaccharide from the column, and the material appeared essentially in one peak.

On columns of Sephadex G-100 and G-200 the polysaccharide travelled as a broad band, but examination of the head and tail fractions (optical rotation) revealed no significant differences. On Sephadex G-75 the material was eluted with the void volume, on G-100 about half of it was retarded, and it was all clearly retarded on Sephadex G-200. From this the polysaccharide appears to have a broad molecular weight distribution in the range 50 000 to 150 000.

Zitko and Bishop [5] found that pectic acids from a number of sources could be fractionated by precipitation from aqueous solution with sodium acetate. The precipitates obtained at the lowest sodium acetate concentrations used (0.12 – 0.15 M) proved to be pure or almost pure galacturonans. Applying this technique to the opium poppy polysaccharide resulted in the isolation of a product (i), 0.4 % of the original extract, that on hydrolysis furnished galacturonic acid and, in addition, traces only of neutral sugars. The second fraction (ii), obtained in 15.4 % yield, contained galacturonic acid together with a small proportion of neutral sugars and of 4-*O*-methylglucuronic acid. Finally the residual fraction (iii), isolated by precipitation with ethanol, contained all the sugar constituents found in the original extract.

After drying fractions (i) and (ii) proved nearly insoluble in water and also in dilute sodium hydroxide. They could be dissolved, however, by stirring with 8 M urea, but when the solution was dialysed, the material fell out again as the urea was removed. It seems that the decrease in solubility of the uronic acid-rich fractions may be ascribed to hydrogen bonding, since urea in large concentrations is known to break hydrogen bonds within and between macromolecules, thus often promoting the solubility of the polymer.

Chromatography on the weakly basic anion exchange resin Amberlite IR-45 in acetate form resulted in the separation of the polysaccharide into two fractions, the major one, fraction I (87 %), being eluted with water, and the minor one, fraction II (13 %), being eluted with 1 M acetate buffer. Fraction II emerged from the column as a rather broad peak, suggesting the presence of more than one component. But several alterations of the experimental conditions (buffer concentration, pH, gradient instead of stepwise elution) led to no improved fractionation. Fraction II was more strongly retained on the column although it had a lower uronic acid content (60 %) than I, which was eluted with water and contained 65 % uronic acid. Fraction I, however, had a higher degree of esterification than II, 56 % and 43 %, respectively. The proportions of the constituent sugars differed only slightly between the two fractions (Table 1), but paper chromatography of acid hydrolysates showed that II contained a higher proportion of 4-*O*-methylglucuronic acid than I. The 4-*O*-methylglucuronic acid residues were destroyed entirely by periodate oxidation, which means that they occupy end group positions. This fact, coupled with the different degrees of esterification of the two fractions, makes it easier to realize why II is bound more strongly by the resin than I. On paper electrophoresis in acetate buffer fractions I and II travelled at different rates having $M_{\mathrm{Gal\,A}}$ 0.8 and 1.1, respectively (spray reagents d and e). The spots obtained were somewhat elongated, particularly that of II. The indicator spray reagent, d, revealed an additional fast-moving minor component ($M_{\mathrm{Gal\,A}}$ 1.5), thus showing the presence of a fraction devoid of ester groups. The electrophoresis experiments confirm the evidence obtained from the chromatography on the IR-45 column that fraction II is heterogeneous. The chemical homogeneity of fraction I may still be questioned. It probably consists of a mixture of closely related molecules, corresponding to the current concept of polysaccharide chemistry.

The constituent sugars not identified previously were characterized after hydrolysis and separation of the neutral and acidic components on a De-

Acidite formate column. The uronic acids were separated on the column by elution with a formic acid gradient and characterized as D-galacturonic acid and 4-*O*-methyl-D-glucuronic acid. The latter constituted 7 % of the total amount of uronic acid present in the polysaccharide. This value cannot be regarded accurate since some of the uronic acid inevitably undergoes degradation during the hydrolysis and, at the same time, a fraction of the uronosyl bonds survives hydrolysis. The presence of minute amounts of fucose, 2-*O*-methylfucose, and 2-*O*-methylxylose appears to be a common feature in pectins.[6] Minor quantities of glucuronic acid have also been found to be integrated in the structure of pectic substances.[7] These constituents were also detected in trace quantities in hydrolysates of the opium poppy polysaccharide; and although only 2-*O*-methylfucose and 2-*O*-methylxylose were fully characterized, fucose and glucuronic acid and its lactone were obviously present, judging from chromatographic and electrophoretic comparison with authentic compounds.

Incubation of the polysaccharide with pectinase produced a limited amount of galacturonic acid in addition to traces of oligosaccharides. Most of the polysaccharide seemed unaffected by the enzyme treatment and was recovered by acetone precipitation after the incubation. Fraction I from the Amberlite IR-45 column proved even less susceptible to enzymolysis, while the galacturonan obtained by precipitation with 0.12 M sodium acetate was largely degraded by the enzyme. In a reference experiment a commercial pectin preparation, containing *ca.* 80 % uronic acid, was extensively degraded by the pectinase, thus indicating satisfactory activity of the enzyme. The very slight effect of pectinase on the major part of the opium poppy polysaccharide may be ascribed to a large degree of branching, preventing the access of the enzyme to the poly-galacturonic acid back-bone.

When the polysaccharide was oxidized by periodate it consumed 0.6 mole of oxidant per anhydrohexose unit with production of 0.12 mole of formic acid. Smith degradation [8] of the polysaccharide furnished a large number of products, emphasizing the complex nature of this carbohydrate polymer. The resulting alcohols were separated in a neutral and an acidic fraction on a Dowex-1 formate column, and from the neutral mixture ethylene glycol, glycerol, propylene glycol, and threitol were isolated in the approximate molar ratio 1:5:2:1. The major compound of the acidic fraction is believed to be D-threonic acid. It seemingly existed as an equilibrium mixture of the lactone and the free acid; it had a negative rotation, corresponding to the D-form of the acid, and gave threitol by reduction. D-Threonic acid is expected to be formed under the conditions used by the presence of 1,4-linked D-galacturonic acid residues.

The yield of the acid amounted to *ca.* 3 molar proportions as compared with the alcohols mentioned above. The expected 4-*O*-methyl-D-glucuronic acid degradation product, 3-*O*-methyl-L-erythronic acid, was not detected. The residual polysaccharide after the Smith degradation, recovered in 2.4 % yield of the starting material, had the relative molecular ratio of neutral sugars: galactose 9.5, arabinose 1, xylose 2, and rhamnose 1. The considerable increase in the galactose content suggests that this sugar is either 1,3-linked or represents branch points in the polymer. It would also be conceivable that

periodate resistant galacturonic acid methyl ester groups in the polysaccharide were reduced by the sodium hydridoborate, thus resulting in the high proportion of galactose. However, when the de-esterified polysaccharide was subjected to Smith degradation under the same conditions, the proportional amounts of galacturonic acid and galactose were essentially the same as obtained for the native, partly esterified material. But a difference was observed in the rhamnose content which was two to three times higher in the residual material from the esterified than from the de-esterified product (judging from chromatograms), indicating that this sugar preferentially carries the acetyl groups present in the polysaccharide. Of the alcohols obtained by the Smith degradation threitol must have originated from galactose linked in its 4-position to a sugar susceptible to periodate oxidation. The propylene glycol obviously has arisen from rhamnose end groups or from 1,2-linked rhamnose residues. Recent studies [9] have shown that at least a part of the rhamnose is linked to galacturonic acid through its 2-position.

These studies also have revealed the presence of 1,2-linked xylose, which may explain the formation of ethylene glycol. This alcohol would also be produced from any pentopyranose end group. The glycerol may have originated from different sugar residues. Probably a part of it stems from arabinofuranose end groups. The low yield of formic acid by the periodate oxidation agrees with the evidence that 4-*O*-methylglucuronic acid and arabinofuranose occupy a substantial proportion of the end groups. It is not possible at this stage to propose a structure for the polysaccharide. More information of the structure will require correlation of the present information with methylation analysis and partial hydrolysis studies. The available data, however, have demonstrated the highly complex nature of the water-soluble opium poppy capsular polysaccharide. Despite the presence in the polymer of 4-*O*-methylglucuronic acid, previously not found in pectins, the composition and the properties of the polysaccharide should justify to include it in the group of pectic substances.

EXPERIMENTAL

Paper chromatograms were run on Whatman No. 1 and, for preparative purposes, Whatman No. 3 MM filter paper in the following solvent systems (v/v):

A. Ethyl acetate, pyridine, water, 8:2:1
B. Ethyl acetate, acetic acid, formic acid, water, 18:3:1:4
C. Ethyl acetate, pyridine, acetic acid, water, 5:5:1:3
D. Butanol, acetic acid, water, 12:3:5

Thin layer chromatography was carried out on Kieselgel G in the system (v/v):

E. Chloroform, acetone, 5 M ammonia, 1:8:1.

Zone electrophoresis was performed on Munktell No. 302 filter paper at *ca.* 40 V/cm in 0.05 M sodium tetraborate, pH 9.2, and in pyridine—0.10 M acetic acid, pH 6.0. Caffeine (visible under an ultraviolet lamp) was used as the endosmotic marker. $M_{\text{Gal A}}$ is the rate of migration relative to galacturonic acid. Due to the elongated spots obtained in the case of the polysaccharide fractions the $M_{\text{Gal A}}$ values are given with only one decimal.

Sugars were located on chromatograms and electropherograms with the following reagents:

a. Aniline oxalate, saturated aqueous solution
b. Silver nitrate—sodium hydroxide [10]

c. Periodic acid—benzidine [11]
d. Bromocresol green, 0.1 %, in ethanol
e. Hydroxylamine—ferric chloride [12]
f. 2,3,5-Triphenyltetrazolium chloride, 1 %, in 1 N sodium hydroxide.

Optical rotations were measured at 20° in water.

The elution of polysaccharide from columns was monitored by the phenol-sulphuric acid method.[13] Estimation of the relative proportions of the neutral sugars in the polysaccharide was carried out according to Wilson.[14] Uronic acid was estimated by the carbazole reaction,[15] galacturonic acid being used as the standard. The determinations were corrected for interference by the neutral sugars present in the polysaccharide.

Opium poppy was grown in the Botanical Garden of the University of Oslo. A few days before ripe the capsules were harvested and treated with hot 80 % ethanol. After drying the capsules were ground to a powder. A portion of dried opium poppy capsules, imported from Jugoslavia, was generously provided by Weiders Farmasøytiske A/S, Oslo. Initially the extracts of the two batches were examined separately, but no significant differences could be established, and subsequently no distinction was made between the polysaccharides from the two sources.

Isolation of polysaccharide. Powdered capsules (500 g) were subjected to Soxhlet-extraction with, successively, light petroleum (b.p. 60—80°), benzene, chloroform and methanol to remove lipids, colouring matter *etc.* Without this treatment the following aqueous extraction would result in a very low yield of impure polysaccharide. The pretreated material (300 g) was extracted with water (6 l) at 40—50° under continuous stirring for 6—8 h. The extract was sieved through muslin and clarified by centrifugation. After dialysis against running tap water and subsequent concentration the polysaccharide was isolated by freeze-drying, yield 12 g, $[\alpha]_D + 180°$ (*c* 1.0), uronic acid 64 %, ester methoxyl 5.5 %, acetyl 3.7 %.

Fractionation experiments. Chromatography on DEAE-cellulose [16] was performed on columns (3.2 × 50 cm).

Fractional precipitation with cetyltrimethylammonium bromide was carried out as described by Scott.[4] The polysaccharide was subjected to gel chromatography on columns (1.5 × 20 cm) of Sephadex G-75, G-100 and G-200; 0.02 N formic acid was used as eluent.

Fractional precipitation with sodium acetate.[5] The polysaccharide (5 g) was dissolved in water (235 ml) and nitrogen was bubbled through the solution prior to the addition of 1 N sodium hydroxide (15 ml). The solution (pH 11) was kept at room temperature until complete de-esterification (negative hydroxamate test after 1 h) and was then centrifuged. To the clear supernatant was added 6 N hydrochloric acid, dropwise, with vigorous stirring. When precipitation was complete, the material was centrifuged off and washed with 60 % ethanol, ethanol and ether. The air-dried product (2.06 g) was dissolved in water (200 ml) and 1 N sodium hydroxide was added dropwise to pH 6.5. The solution showed no absorption at 235 nm, indicating that degradation by β-elimination had not occurred during the de-esterification by alkali. To the filtered solution was added 2 M sodium acetate slowly and with stirring to give a 0.12 M acetate concentration. The mixture was kept at 5° for 24 h and the precipitate (fraction i) isolated by centrifugation, yield 20 mg. Further addition of 2 M sodium acetate to the supernatant to 0.20 M acetate concentration resulted in a second fraction (ii) 0.77 g. No further precipitates were obtained by the addition of more 2 M sodium acetate. The remaining solution was concentrated, dialysed, and residual polysaccharide was recovered by precipitation with ethanol (fraction iii), yield 1.20 g; $[\alpha]_D + 138°$.

The fractions i and ii proved nearly insoluble, thus preventing accurate measurements of optical rotation. 10 mg portions of fractions i, ii and iii were hydrolysed with 2 N sulphuric acid (1 ml) at 100° for 6 h. Paper chromatography of the neutralized and deionised hydrolysates revealed the presence of galacturonic acid with traces of neutral sugars in fraction i, galacturonic acid with a small proportion of the other constituent sugars in ii and galacturonic acid with a considerable amount of the other sugars in iii.

The de-esterified material that was not precipitated by the addition of hydrochloric acid, was isolated from the solution by precipitation with ethanol (fraction iv), yield 2.7 g. Not this product either showed absorption at 235 nm. Paper chromatography of an acid hydrolysate of iv gave a picture very similar to the hydrolysate of iii.

Chromatography on Amberlite IR-45 resin. The crude polysaccharide (1.2 g), dissolved in water, was applied on a column (3.6×42 cm) of the resin (analytical grade) in acetate form. Elution, first with water, then with 1 M sodium acetate buffer (pH 4.8) yielded fractions I (1.0 g, $[\alpha]_D+184°$) and II (0.15 g, $[\alpha]_D+168°$). A subsequent change to stronger buffer concentration led to no further elution of polysaccharide from the column. Chromatography of the polysaccharide on Amberlite CG-4B (100–200 mesh) resulted in essentially the same elution pattern with the exception that only 50–60 % of the material was recovered from the column.

Characterization of individual sugars. The unfractionated polysaccharide (20 g) was hydrolysed with 2 N sulphuric acid (2 l) at 100° for 6 h. After neutralization of the hydrolysate with barium hydroxide and treatment of the filtrate with Dowex-50 (H^+) the resulting solution was percolated through a column (4.5×62 cm) of De-Acidite FF-IP (SRA 67) in formate form. The column was washed with water until the phenol-sulphuric acid reaction was negative. Of the mixture of neutral sugars thus obtained galactose, arabinose, xylose and rhamnose had been characterized previously.[1] The aqueous eluate was concentrated to a syrup (4.3 g) which was subjected to chromatography on 3 MM filter sheets in solvent A. The fractions with the highest mobility were isolated and rechromatographed in solvent B to give pure samples of 2-*O*-methylxylose and 2-*O*-methylfucose.

*2-O-Methyl-*D*-xylose*, (70 mg) $[\alpha]_D+32°$ (*c* 0.6). M.p. 132–134° after recrystallization from ethanol. It had $R_{Rhamnose}$ 1.44 and 1.35 in solvents A and B, respectively. The sugar gave no stain with 2,3,5-triphenyltetrazolium hydroxide, indicating that its 2-position was substituted.[17] On demethylation[18] it gave xylose.

*2-O-Methyl-*L*-fucose*, (52 mg) $[\alpha]_D-77°$ (*c* 0.4). M.p. 146–149° after recrystallization from water. It had $R_{Rhamnose}$ 1.65 and 1.53 in solvents A and B, respectively. No staining reaction was obtained with 2,3,5-triphenyltetrazolium hydroxide, and the compound gave fucose on demethylation.

The fucose (*ca.* 10 mg) was not obtained in sufficiently pure state for characterization. A part of the sugar was reduced with sodium hydridoborate to the corresponding alcohol, and the two compounds were identified tentatively by their chromatographic and electrophoretic mobilities in comparison with authentic specimens.

After removal of the neutral sugars from the column it was eluted with a formic acid gradient (0–2 N), 500 ml of each; 10 ml fractions were collected. The material appeared in three peaks; there was a slight overlapping, but by rechromatography of the appropriate fractions on 3 MM paper (solvent B) complete separation of the uronic acids was achieved.

D-*Galacturonic acid*, (1.730 g) $[\alpha]_D+55.6°$ (*c* 1.08). M.p. 105–110° (decomp.). Chromatographic and electrophoretic mobility was identical to that of the authentic substance. Oxidation with bromine water gave galactaric acid, m.p. and mixed m.p. 222–224°.

*4-O-Methyl-*D*-glucuronic acid*, (0.153 g) $[\alpha]_D+46°$ (*c* 1.2). It had the same chromatographic and electrophoretic mobility as the reference compound. The syrupy sugar (48 mg) was converted to the methyl ester methyl glycoside by refluxing it with methanol and Dowex-50 (H^+) resin for 24 h. The product (40 mg) was reduced with lithium hydridoaluminate in tetrahydrofuran by gentle reflux for 2 h. The reaction product (29 mg), on hydrolysis, furnished a syrup (20 mg) indistinguishable from 4-*O*-methylglucose in its chromatographic and electrophoretic mobility. The sugar had $[\alpha]_D+57°$ (*c* 0.5) and gave glucose on demethylation.

Digalacturonic acid, (0.358 g) $[\alpha]_D+120°$ (c 0.9). M.p. 110–120° (decomp.). It gave galacturonic acid on acid hydrolysis and had $R_{Gal\ A}$ 0.19 and 0.21 in solvents B and C, respectively, which corresponds to the mobility of 4-*O*-(α-D-galacturonosyl)-D-galacturonic acid obtained by partial hydrolysis of pectins.[19] Conversion of the acid into the methyl ester methyl glycoside followed by reduction with lithium hydridoaluminate in tetrahydrofuran and acid hydrolysis yielded galactose as the only sugar.

Glucuronic acid, found in trace quantities on paper chromatograms of polysaccharide hydrolysates, could not be detected in any of the acid fractions from the De-Acidite column. In its lactone form this uronic acid might have been partly washed out of the column with water and thus escaped detection.

Enzyme digestion of the polysaccharide (200 mg) was carried out with pectinase, Sigma, (20 mg) in 0.05 M acetate buffer, pH 4.5, (100 ml) at 35° for 24 h. For comparison 10 mg of the galacturonan (i) isolated by the sodium acetate precipitation procedure and

a commercial pectin preparation (200 mg) were each digested with 2 mg and 20 mg of pectinase, respectively, under the above conditions. Due to the low solubility of the galacturonan this digest was stirred continuously. At the end of the incubation period residual polysaccharide was precipitated by the addition of acetone (3 vol.). Paper chromatography of the concentrated supernatants showed that the unfractionated polysaccharide had been only slightly affected by the enzyme, while the galacturonan and the commercial pectin sample had been degraded to a much larger extent to galacturonic acid and acidic oligosaccharides.

Periodate oxidation of the polysaccharide (81 mg) was carried out with 1 mmole of sodium metaperiodate in 0.2 M acetate buffer, pH 3.6 [20] (50 ml). The oxidation was allowed to proceed at 10° in the dark; at intervals 5 ml aliquots were removed and the reduction of periodate was measured iodometrically.[21] Results from a typical experiment given as moles of periodate reduced per anhydrohexose unit: 0.34 (1 h), 0.46 (4 h), 0.60 (19 h), 0.61 (48 h).

For the estimation of formic acid liberated a corresponding oxidation was performed in unbuffered solution. Aliquots of 5 ml were titrated with 0.01 N sodium hydroxide after reduction of the periodate with ethylene glycol. Results given as moles of formic acid formed per anhydrohexose unit: 0.054 (1 h), 0.082 (4 h), 0.116 (19 h), 0.150 (48 h).

In a Smith degradation experiment the polysaccharide (4.86 g, *i.e.* 0.03 moles of anhydrohexose units) was oxidized by sodium metaperiodate (0.06 moles) in water (1000 ml) at 10° in the dark. The reaction was terminated after 48 h by adding ethylene glycol, and the mixture was dialysed against running water followed by reduction of the polyaldehyde with sodium hydridoborate (2 g). After 20 h the excess of sodium hydridoborate was destroyed by the addition of dilute acid, and the polyalcohol was isolated by precipitation with ethanol, yield 3.6 g, $[\alpha]_D + 50°$ (*c* 1.0). Hydrolysis of the polyalcohol was effected with 1 N sulphuric acid for 5 h at 22° followed by neutralization with barium hydroxide and treatment of the filtrate with Dowex 50 (H^+) resin. Residual polysaccharide was recovered by precipitation with ethanol, yield 112 mg. The composition of this material is given in Table 1. The supernatant from the ethanol precipitation was concentrated *in vacuo* and the mixture of alcohols was isolated as a syrup (2.1 g); it was shown by chromatography to contain a number of both neutral and acidic components. A portion of the syrup (1.2 g) was passed through a column (3.4 × 25 cm) of Dowex-1 resin in formate form. Elution with water afforded the neutral fraction (0.62 g); subsequent elution with 1 N formic acid gave the acid fraction (0.51 g). The mixture of neutral alcohols was subjected to chromatography on 0.5 mm layers of Kieselgel G in solvent E. Sections of the plates were sprayed with reagent c, and the appropriate zones were scraped off and extracted with ethanol. After rechromatography in the same solvent the following yields were obtained: ethylene glycol (4 mg), glycerol (39 mg), propylene glycol (12 mg), and threitol (10 mg). The compounds behaved as the corresponding authentic specimens on chromatography and electrophoresis. Glycerol, propylene glycol, and threitol were characterized as their *p*-nitrobenzoates; glycerol tri-*p*-nitrobenzoate, m.p. and mixed m.p. 195–196°; propylene glycol di-*p*-nitrobenzoate, m.p. and mixed m.p. 124–126°; threitol tetra-*p*-nitrobenzoate, m.p. and mixed m.p. 213–215°. Attempted preparation of the corresponding ethylene glycol derivative was unsuccessful.

The acidic fraction from the Dowex-1 formate column was subjected to chromatography on 3 MM filter paper in solvent B, and the major component isolated as a syrup (30 mg), $[\alpha]_D - 22°$ (*c* 1.9). On paper chromatography in acidic solvents it usually gave rise to two spots (reagents b and c) of mutually varying intensity. In solvent B the predominant spot had $R_{Glucose}$ 2.80, while in solvent C the main spot had $R_{Glucose}$ 0.76. Esterification of the material (15 mg) with diazomethane, followed by reduction with lithium hydridoaluminate yielded a syrupy product (5 mg), indistinguishable from threitol in its chromatographic and electrophoretic mobility.

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