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STRUCTURAL STUDIES OF SAPOTE (*Sapota achras*) GUM

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ABSTRACT

Sapote gum contains residues of L-arabinose (pyranose and furanose), D-xylose, D-glucuronic acid, and 4-*O*-methyl-D-glucuronic acid in the ratio 1.0:2.8:0.48:0.52. The two uronic acids were conveniently determined by reducing the carboxyl functions with lithium borohydride and measuring the ratio of D-glucose to 4-*O*-methyl-D-glucose. Periodate oxidation of the carboxyl-reduced gum gave *inter alia* 2-*O*-methyl-D-erythritol and 4-*O*-methyl-D-glucose in amounts suggesting that 37% of the 4-*O*-methyl-D-glucuronic acid residues are unsubstituted in the polysaccharide. Acetolysis of the carboxyl-reduced gum gave *O*-α-D-glucopyranosyl-(1→2)-(4-*O*-β-D-xylopyranosyl)$_{0,1,2}$-D-xylose, a hitherto undescribed series of oligosaccharides, together with 2-*O*-(4-*O*-methyl-α-D-glucopyranosyl)-D-xylose. Methylation confirmed that sapote gum has a highly branched structure, and commercial xylanases did not depolymerize the gum. An α-L-arabinofuranosidase liberated a substantial part of the arabinose residues. Sapote gum is a member of the uncommon class of plant gums having a D-xylose backbone and structurally resembles brea gum.

INTRODUCTION

The botanical classification of those plants forming polysaccharide gums has been discussed by Stephen and coworkers[1], and the chemistry of plant gums has been reviewed by Smith and Montgomery[2] and by Aspinall[3]. According to Stephen, gums may be divided into three principal classes depending on whether the backbone is composed of either D-galactose, D-glucuronic acid, or D-xylose residues. The great majority of gums belong to the first two groups, and very few representatives of the third class are known. One such gum containing a D-xylose framework is sapote gum[4] from the South American tree *Sapota achras*. A careful study of sapote gum by White showed the polysaccharide to be highly branched, with a backbone of (1→4)-linked β-D-xylopyranose residues carrying substituents of D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, and L-arabinose[5–7]. On the basis of this work Smith and Montgomery proposed a partial structure[2].

RESULTS AND DISCUSSION

The sapote gum used in this work was part of the same batch used by White[5–7] and was originally obtained from Anderson[4], who first analyzed the constituents of the gum. The gum was purified by precipitation into acidified ethanol and was homogeneous, as judged by elution of the gum as a single band from a column of DEAE-cellulose and by fractional precipitation of the propionate. The propionate was used, as this derivative was to be utilized in subsequent reduction of the uronic acid groups. Analytical data for sapote gum are given in Table I.

TABLE I

ANALYTICAL DATA FOR SAPOTE GUM

Result	*Anderson*[4]	*Present study*[a]	*Degraded gum*
Specific rotation (*c* 1.0, water)	−6°	−8.2°	−8°
Equivalent weight	679	676	850
Nitrogen, %	—	0.5	—
Methoxyl, %	2.80	2.40	—
Uronic anhydride, %	27.5	27.3[b]	
		27.4[c]	
Molecular weight		69,000	14,300

[a]All weights are based on ash-free solids. [b]By g.l.c. [c]Average value determined by decarboxylation of ten nodules chosen at random by Lambert and coworkers[9].

In the present work both the original gum and the polysaccharide recovered after autohydrolysis were methylated, and Table II shows that the results obtained closely parallel those of White[5–7], except that L-arabinose was found in both furanose and pyranose forms. In comparing these results it must be appreciated that White

TABLE II

SUGARS FROM METHYLATED SAPOTE GUM

Compound	*Molar ratios*		
	Whole gum		*Degraded gum*
	Ref. 7	*This study*	
2,3,4-Tri-*O*-methyl-L-arabinose	1	1	
2,3,5-Tri-*O*-methyl-L-arabinose		4	
2,3,4-Tri-*O*-methyl-D-xylose	1	1	2
2,3-Di-*O*-methyl-D-xylose		1.5	1.3
3-*O*-Methyl-D-xylose	3	4	1
D-Xylose		3.5	
2,3,4-Tri-*O*-methyl-D-glucose	1	3	
3,4-Di-*O*-methyl-D-glucose	1	2	

separated the components by fractional distillation, which would also account for the omission of D-xylose from his figures. In our work a small quantity of a partially methylated disaccharide, 4-*O*-(2,3-di-*O*-methyl-β-D-xylopyranosyl)-D-xylose, arising through incomplete hydrolysis, was isolated. This observation corresponds to the isolation of 4-*O*-(2,3-di-*O*-methyl-β-D-xylopyranosyl)-2,3-di-*O*-methyl-D-xylose from other, less highly substituted xylans[8].

Lambert, Dickey, and Thompson[9] partially hydrolyzed the gum and isolated two series of acidic oligosaccharides, one containing D-glucuronic acid and the other 4-*O*-methyl-D-glucuronic acid, thus proving directly that both acids are present in the gum. From the methoxyl and total uronic acid content these authors concluded that the two acids were present in the ratio of 42:58, respectively. We have confirmed the formation, on hydrolysis, of two series of oligosaccharides and have obtained similar percentages (48:52) for the two acids by direct determination on the reduced gum[10].

The main purpose of our experiments was to determine the nature of the side chains attached to the (1→4)-linked β-D-xylose backbone. Although this objective was not achieved, the results obtained shed some light on the substitution pattern of the uronic acids and demonstrate that the gum is structurally more complex than hitherto realized. These results, a preliminary account of which has been given[11], are now discussed.

Oxidation of an aqueous solution of sapote gum with sodium periodate resulted in an uptake of 0.72 mole IO_4^- per mole of carbohydrate in 70 h, and Smith degradation[12] gave acidic and neutral fractions. From the latter were isolated xylosylglycerol, xylobiosyl-, and xylotriosylglycerols, showing that the branching is random or, alternatively, that there are sequences of different length interrupted by periodate-susceptible units.

Attention was concentrated on the fragments of low molecular-weight obtained by the Smith degradation; a routine check for degraded polysaccharide, recoverable by ethanol precipitation, yielded an insignificant amount of material. In a subsequent experiment it was shown that the amount of ethanol-precipitable material varies from 40% to less than 10%, depending on the conditions used for the hydrolysis of the polyalcohol (0.05M H_2SO_4 for 24 h or 0.5M H_2SO_4 for 3 h) and the method of isolation (see Experimental). These observations do not invalidate the conclusions drawn here, which are only qualitative, but they do serve as a warning not to place too much faith in the quantitative aspects of a Smith degradation unless the conditions are strictly controlled. Since the work here reported was completed we have proposed[13] a method for monitoring the hydrolysis step in a Smith degradation, and have cited other examples of lack of quantitation.

Sapote gum was *O*-propionated[14] and converted into its methyl ester with diazomethane. Reduction with lithium borohydride in tetrahydrofuran[10,15] yielded a neutral polysaccharide, hydrolysis of which gave L-arabinose, D-xylose, D-glucose, and 4-*O*-methyl-D-glucose in the ratio 1.0:2.8:0.48:0.52, assayed as alditol acetates. As pentoses were the only neutral-sugar components of the original sapote gum, the

determination of D-glucose and 4-*O*-methyl-D-glucose after reduction and hydrolysis serves to characterize and assay the uronic acids. This method thus provides a convenient alternative for the determination and identification of uronic acids in certain polysaccharides. The technique is of greatest value when two different uronic acids occur in the same polymer molecule. In agreement with Blake and Richards[16], we have confirmed[10] that the peracetates of galactitol and 4-*O*-methyl-D-glucitol (3-*O*-methyl-L-gulitol) are not separated on a column of ECNSS-M, although they may be resolved on butanediol succinate. Neither column will resolve the peracetates of D-mannitol and 4-*O*-methyl-D-glucitol; such a mixture of uronic acids has not, however, yet been found in a single polysaccharide.

Periodate oxidation of the reduced gum resulted in a consumption of 0.62 moles of IO_4^- per mole of carbohydrate during 96 h. Total hydrolysis of the borohydride-reduced material gave ethylene glycol, glycerol, 2-*O*-methyl-D-erythritol, D-xylose, and 4-*O*-methyl-D-glucose in the molar ratio 1.0:4.0:0.69:2.15:1.85. The presence of 4-*O*-methyl-D-glucose after periodate oxidation indicates that a proportion of the 4-*O*-methyl-D-glucuronic acid residues must be substituted at C-2 in order to make them immune to periodate oxidation. Substitution at C-3 would have given the same result, but the isolation of 3,4-di-*O*-methyl-D-glucose from the reduced, methylated polysaccharide indicates that substitution is at C-2. Conversely, the 2-*O*-methyl-D-erythritol must originate from reduced 4-*O*-methyl-D-glucuronic acid residues that lack such a substituent. The ratio (2.7:1) between 4-*O*-methyl-D-glucose and 2-*O*-methyl-D-erythritol is thus a measure of the proportion of 4-*O*-methyl-D-glucuronic acid residues in the gum that are substituted at C-2 (73%) and those (27%) that are not.

It is now useful to compare three sets of data relating to the uronic acids. Analysis of the reduced gum establishes that D-glucuronic and 4-*O*-methyl-D-glucuronic acids are present in the proportion of 48:52%. The methylation data (Table II) show that 60% of the total uronic acid occupies terminal positions and 40% carries a substituent at C-2. The periodate-oxidation data demonstrate that of the 4-*O*-methyl-D-glucuronic acid 27% is non-substituted (terminal), and the remainder is substituted. Thus, of the total uronic acid, 14% (0.52 × 27%) is represented by terminal residues of 4-*O*-methyl-D-glucuronic acid and it is clear, therefore, that the majority of the terminal acid units are D-glucuronic acid. The absence of both D-glucose and erythritol from the fragments of periodate oxidation indicates that the D-glucuronic acid residues are neither so substituted as to be immune to periodate oxidation nor substituted at C-4; this observation is consistent with the methylation data (Table II).

A portion of the carboxyl-reduced sapote gum was subjected to acetolysis according to the procedure of Ballou[17], and four oligosaccharides containing either D-glucose or 4-*O*-methyl-D-glucose linked to D-xylose were obtained[18]. Disaccharide **2** containing 4-*O*-methyl-D-glucose has been obtained previously by reduction of the corresponding aldobiouronic acid[19] but oligosaccharides **1**, **3**, and **4** represent a series hitherto undescribed. *O*-β-D-Glucopyranosyl-(1→2)-D-xylose is known[20] and has $[\alpha]_D$ 0°; the positive rotation of oligosaccharide **1** indicates an α-D-linkage. Isola-

tion of these oligosaccharides confirms the presence in the gum of the two uronic acids and shows their mode of linkage. Interestingly, although a substantial proportion of the uronic acid residues are non-terminal, no disaccharide could be obtained in which D-glucose or its 4-*O*-methyl derivative was present as the aglycone, nor was any oligosaccharide containing L-arabinose isolated. It was the failure to isolate any such disaccharides by partial hydrolysis or acetolysis that prevented any detailed structural information being obtained.

An attempt was made to fragment the gum by enzymic action. Bishop[21], for example, has shown that a cellulase from the mold *Myrothecium verrucaria* is capable of yielding a series of L-arabinofuranosyl-D-xylose oligosaccharides from wheat-straw xylan. It should, however, be noted that Perlin and Reese[22] have made a detailed study of the spatial requirements of a xylanase that can act on the arabinoxylan from wheat flour. They concluded that the enzyme was capable of cleaving only those glycosidic linkages between unbranched xylose residues. In the case of sapote gum, methylation gives only a small proportion of 2,3-di-*O*-methyl-D-xylose, showing the presence of a very limited number of unsubstituted xylose residues. Recognising that sapote gum is much more highly branched than xylans of the hemicellulose type, the gum was incubated with various enzymes known to metabolize xylans. Only small amounts of L-arabinose and D-xylose were liberated, and no oligosaccharides were detected. The significant difference of sapote gum from other xylans has also been shown by Thompson and Gaillard[36] in their studies on the interaction of iodine with polysaccharides having different degrees of branching. Sapote gum was found not to react with iodine, thus indicating the structure to be highly branched. The highly ramified nature of this gum probably prevents access of the enzyme to the interior linkages. On the other hand, an α-L-arabinofuranosidase[24] liberated the peripheral L-arabinofuranose residues in high yield, thus establishing unequivocally the anomeric nature of this linkage and the presence of part of the L-arabinose in the furanose form.

Sapote gum is thus confirmed as having a (1→4)-linked xylan backbone but which is highly substituted with oligosaccharide chains rather than with short side-chains. Such a structure is rare amongst plant gums, but closely resembles that proposed for brea gum[25] and a related type of polysaccharide isolated from *Watsonia versveldii*[26].

EXPERIMENTAL

General methods. — Descending paper chromatography was performed with Whatman No. 1 and 3 MM papers and the following solvent systems (*v/v*): A, butanone–water azeotrope; B, 8:2:2 ethyl acetate–pyridine–water; C, 18:3:1:4 ethyl acetate–acetic acid–formic acid–water. Sugars were detected with *p*-anisidine trichloroacetate. R_{MG} is movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose. Evaporations were effected *in vacuo* at a bath temperature not exceeding 40°. Melting points are uncorrected, and specific rotations are quoted as equilibrium values at 23° ±2°.

Gas–liquid chromatography was carried out on an F and M 720 dual column instrument fitted with thermal-conductivity detectors and using helium as the carrier gas. Peak areas were measured with an Infotronics model CRS-100 digital integrator. The following columns were employed: A 5% butane-1,4-diol succinate on 80–100 mesh Diatoport S (4 ft. by 1/4 in.); B 3% ECNSS-M on 100–120 mesh Gas Chrom Q (8 ft. by 1/4 in.); C 20% SF 96 on 60–80 mesh Diatoport S (8 ft. by 1/4 in.).

Mass spectra were recorded on an A.E.I. MS-9 mass spectrometer at an ionizing potential of 70 e.V.

The sample of sapote gum was from the same batch as used by Anderson[4] and by White[5–7].

Purification of the gum. — Gum (25.5 g) was dissolved in water (150 ml) and the solution (pH 3.5) was brought to pH 2.5 by the addition of hydrochloric acid. The gum (21.8 g) was recovered by pouring the solution into ethanol and drying the precipitate by solvent exchange. One further treatment gave 19.2 g of gum having the properties shown in Table I.

Fractionation of the gum. — (*a*) *On a column of DEAE-cellulose.* Polysaccharide (60 mg) was dissolved in 5 mM phosphate buffer and poured onto a column (35 cm × 2.5 cm) of washed DEAE-cellulose. The column was eluted with 25 mM sodium dihydrogen phosphate (300 ml) followed by a gradient of sodium hydroxide (0.01–0.5M). The polysaccharide (57 mg), which was eluted[27] as a single band by sodium hydroxide, was recovered by dialysis, passage through cation-exchange resin, and lyophilisation. The gum had $[\alpha]_D$ −6.6° (*c* 1.0, water) and an equivalent weight of 675 by back titration.

(*b*) *Fractional precipitation of propionate.*[14] A solution of finely powdered, purified gum (5 g) in formamide (60 ml) was treated with pyridine (45 ml) and redistilled propionic anhydride (30 ml). After 2 days at room temperature, the solution was poured into ice-cold 2M hydrochloric acid (1 liter). The recovered propionate (6.2 g) was retreated for 3 days in the absence of formamide to give fully esterified material (6.8 g). The propionate (1.70 g) was dissolved in chloroform (50 ml) and petroleum ether (30–60°) was added with stirring. Three fractions were obtained [% petroleum ether added; % propionate precipitated; $[\alpha]_D$ (*c* 1.0, in chloroform) of precipitate]: fraction 1 (37; 80; −41.2°), fraction 2 (41; 12; −42.4°) and fraction 3 (47; 5; −40.3°).

Molecular weight determination. — This was carried out by the method of Unrau and Smith[28]. The difference in production of formaldehyde from original and borohydride-reduced gum was 0.2 mg for a sample of 457 mg. On the assumption that each xylitol end-group yields one mole of formaldehyde, this corresponds to a molecular weight of 69,000.

Hydrolysis of the gum. — (*a*) *With 5 mM sulfuric acid.* Separate samples were heated with 5 mM sulfuric acid for different periods of time with the following results (time in h, $[\alpha]_D$): 0, −6.0°; 2, +23.7°; 4, +41.0°; 6, +44.7°.

(*b*) *With 0.5M sulfuric acid.* Purified gum (2 g) dissolved in 0.5M sulfuric acid (40 ml) was heated for 8 h on a steam bath. The solution was neutralized with barium

carbonate and was divided into acidic and neutral fractions by successive passage through cation (Amberlite IR-120) and anion (Duolite A-4) exchange resins. The acidic components (802 mg) were eluted by 10% formic acid and isolated by freeze-drying. They were separated into four fractions (R_{Xylose} 1.16, 0.97, 0.75, and 0.62) on Whatman 3 MM paper with solvent C.

Fraction 1 (31 mg, R_{Xylose} 1.16) had $[\alpha]_D$ +83° (*c* 1.2, water) and was chromatographically identical to 4-*O*-methyl-D-glucuronic acid. Fraction 2 [113.2 mg, R_{Xylose} 0.97, $[\alpha]_D$ +105° (*c* 1.0, water)] was chromatographically identical to 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. Fraction 3 [26.7 mg, R_{Xylose} 0.75, $[\alpha]_D$ +35° (*c* 1.0, water)] was chromatographically indistinguishable from D-glucuronic acid. Fraction 4 [107.0 mg, R_{Xylose} 0.62, $[\alpha]_D$ +87° (*c* 1.0, water)] was chromatographically identical to 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose.

For the t.l.c. separation of the aldobiouronic acids, silica gel (35 g) and calcium sulfate (2.5 g) were slurried in 0.3M disodium hydrogen phosphate and the plates were developed in butyl alcohol–ethanol–0.1M hydrochloric acid (1:10:5 v/v). The time of development was 2 h and the foregoing fractions 2 and 4 had R_F 0.58 and 0.15, respectively.

Examination of the neutral fraction by paper chromatography in solvent B showed only L-arabinose and D-xylose. This was confirmed by examination of the *O*-trimethylsilyl derivatives[29] on column C operated isothermally for 3 min at 190° and then programmed at 3°/min to 230°.

Methylation of sapote gum. — Purified sapote gum (1.24 g) was passed through a 200-mesh sieve, dried for 6 h *in vacuo* at 60°, and methylated by the method of Hakomori[30]. The reaction mixture was diluted with water and dialyzed overnight against running water. Methylated sapote gum (1.38 g, OMe 39.3%) was recovered by continuous chloroform extraction and had $[\alpha]_D$ −48.2° (*c* 6.2, chloroform). A portion (1.02 g) of the methylated gum was hydrolyzed[31] to give a syrup (996 mg) that was separated by ion-exchange resins into neutral (617 mg) and acidic (355 mg) portions. A small portion (96 mg) of the neutral fraction was separated by chromatography on Whatman No. 1 paper with solvent A. The remainder (500 mg) of the neutral sugars was separated[32] on a cellulose–hydrocellulose column (40 × 3 cm) with solvent A. Fractions were collected at 15-min intervals for 115 tubes and then at 30-min intervals.

Paper-chromatographic separation. — *Components 1 and 2.* The syrup (37.2 mg) had $[\alpha]_D^{23}$ −27.6° (*c* 1.5, methanol) indicating 2,3,5-tri-*O*-methyl-L-arabinose (lit.[33] $[\alpha]_D$ −38.5°), and 2,3,4-tri-*O*-methyl-D-xylose (lit.[33] $[\alpha]_D$ +18.5°) in the ratio 4.1:3. G.l.c. of the alditol acetates[34,35] (column B kept isothermally for 3 min at 160° and then programmed at 2°/min to 180°) gave a ratio of 4.5:1.

Components 3 and 4. The mixture (15 mg) showed two spots having R_F values 0.65 and 0.57, chromatographically identical with 2,3,4-tri-*O*-methyl-L-arabinose and 2,3-di-*O*-methyl-D-xylose. G.l.c. of the alditol acetates under the same conditions as for components 1 and 2 confirmed these assignments.

Component 5. This material (10 mg) had $[\alpha]_D^{23}$ −25.3° (*c* 1, water) and R_F 0.40

in solvent A. Assignment as 4-*O*-(2,3-di-*O*-methyl-β-D-xylopyranosyl)-D-xylose was consistent with the following: (*a*) Acid hydrolysis of 3 mg followed by paper chromatography showed D-xylose and 2,3-di-*O*-methyl-D-xylose. (*b*) Borohydride reduction of 3 mg before hydrolysis gave xylitol and 2,3-di-*O*-methyl-D-xylose. (*c*) The remainder (4 mg) was methylated by the method of Hakomori and the product refluxed with methanolic hydrogen chloride for 6 h. The solution was neutralized with silver carbonate, concentrated, and injected onto column A kept isothermally for 5 min at 120° and then programmed at 2°/min to 170°. Peaks corresponding to the methyl glycosides of 2,3,4-tri- and 2,3-di-*O*-methyl-D-xylose were observed in approximately equal ratio.

Component 6. This material (8.5 mg) was trimethylsilylated[29] and injected in cyclohexane onto a column of SE-52 (8 ft × 0.25 in.) kept for 3 min at 110° and then programmed at 3°/min to 140°. The chromatogram was identical with that from authentic 3-*O*-methyl-D-xylose similarly treated[36–39].

Component 7. This component (20.1 mg) was chromatographically identical with D-xylose.

Cellulose-column separation. — Fraction 1. The syrup (152 mg) was passed a second time through the column[32], and fractions were collected every 15 min. Tubes 20–25 contained component 1, and component 2 was found in tubes 32–36. *Component 1* (75 mg) had $[\alpha]_D$ −38° (*c* 1.0, methanol) and was identified chromatographically as 2,3,5-tri-*O*-methyl-L-arabinose. *Component 2* (25 mg) had $[\alpha]_D$ +16° (*c* 1.5, methanol) and had the same mobility as 2,3,4-tri-*O*-methyl-D-xylose. A part (10 mg) was converted into the methyl glycosides and separated by g.l.c. with column A at 120°. Two peaks were observed and a sample of each glycoside was collected. One crystallized spontaneously and was identical with methyl 2,3,4-tri-*O*-methyl-β-D-xylopyranoside[40], m.p. and mixed m.p. 49–50°.

Fraction 2. This corresponded to tubes 31–50 and was separated on paper to yield *component 3* as a syrup (29 mg) having $[\alpha]_D$ +123° (*c* 1.0, water) (lit.[40] +120°, 127°) and the same chromatographic properties as 2,3,4-tri-*O*-methyl-L-arabinose. Demethylation with 48% hydrobromic acid and examination in solvents A and B gave arabinose and partially demethylated compounds.

Fraction 3. The syrup from tubes 51–70 was separated by double development on paper in solvent A to yield *component 4* (18 mg), identified as 2,3-di-*O*-methyl-D-xylose by paper chromatography and by examination of its methyl glycosides on column A. *Component 5* (23 mg) had $[\alpha]_D$ +25.3° (*c* 1.0, water) and was not further examined as it was chromatographically identical with component 5 from the separation on paper.

Component 6 was isolated as a syrup (31 mg) having $[\alpha]_D$ +24.8° (*c* 1.5, methanol). On long standing the syrup crystallized and had m.p. 94–96°, undepressed by authentic 3-*O*-methyl-D-xylose[40].

Component 7 (97 mg) appeared to be D-xylose, as judged by paper chromatography and by g.l.c. of the trimethylsilyl derivative on column C, temperature-programmed between 190° and 220° at 3°/min.

Acidic sugars. — These (340 mg) were eluted from the resin with 10% formic acid, were refluxed for 6 h with 3% methanolic hydrogen chloride (30 ml), and the product was reduced with lithium aluminum hydride (1 g) by refluxing for 4 h in tetrahydrofuran (30 ml). The neutral material (327 mg) was hydrolyzed with 0.5M sulfuric acid (20 ml, 8 h, 100°) and paper-chromatographic examination of the syrup (308 mg) in solvent A showed three components chromatographically identical to 3-*O*-methyl-D-xylose, 3,4-di-*O*-methyl-D-glucose, and 2,3,4-tri-*O*-methyl-D-glucose. Part of the material (107 mg) was separated on paper by using solvent A. The two slowest-migrating components overlapped, but the fastest-migrating component (R_{MG} 0.64) was obtained pure as a syrup (42 mg) having $[\alpha]_D$ +75.3° (*c* 1.1, water). This material was identified as 2,3,4-tri-*O*-methyl-D-glucose from the melting point (93–94°) of the methyl β-glycoside (lit.[41] 92–95°) obtained by g.l.c. separation on column A. Confirmation was provided by periodate oxidation and borohydride reduction of the derived tri-*O*-methylglucitol. The product was identified as 2,3,4-tri-*O*-methyl-L-xylose by paper-chromatographic comparison with the D-enantiomorph[40] and by optical rotation, $[\alpha]_D$ −17.5° (*c* 1.0, water).

A portion (90 mg) of the neutral mixture was converted into the methyl glycosides and separated by g.l.c. on column A kept for 5 min at 120° and then programmed at 2°/min to 170°. On the basis of retention times, peak enchancement with standards, and mass spectrometry, the presence of the following compounds was indicated: methyl 2,3,4-tri-*O*-methyl-D-glucosides [13.0 *min* (w), 17.5 *min* (s)], methyl 3,4-di-*O*-methyl-D-glucosides [22.5 min (s), 25.0 min (w)] and methyl 3-*O*-methyl-D-xylosides [11.0 min (w), 14.0 min (s)]. The xylosides were collected and hydrolyzed. A portion was examined by paper chromatography and the remainder was trimethylsilylated and examined by g.l.c. on column B kept for 3 min at 110° and then programmed at 3°/min to 140°. In each case the behavior was consistent with authentic 3-*O*-methyl-D-xylose similarly treated.

Autohydrolysis. — Purified sapote gum (20.0 g) in water (300 ml) was boiled for 60 h under reflux. The filtered solution was dialyzed against several changes of distilled water and finally against running water for 24 h. The combined dialyzates were concentrated to a syrup (10.7 g) and the non-dialyzable, degraded gum (8.2 g) was recovered by freeze drying. A portion of the diffusate was separated on ion-exchange resins into neutral and acidic fractions each of which was examined by paper chromatography. The same results were obtained as from the hydrolysis of sapote gum with M sulfuric acid described previously.

Methylation of the degraded gum. — Degraded gum (2.05 g) was methylated as before to yield 2.21 g of product showing no hydroxyl absorption in the infrared. A part (100 mg) of this material was hydrolyzed and the neutral fraction was shown by paper chromatography to contain 2,3,4-, 2,3-, and 3-*O*-methyl-D-xyloses. This result was confirmed by examination of the alditol acetates on column B kept for 3 min at 145° and then programmed at 2°/min to 180°.

Reduction of the methylated degraded gum. — A second portion (600 mg) of the methylated degraded gum was dissolved in tetrahydrofuran (50 ml) and added to

a solution of lithium aluminum hydride (1.05 g) in ether (250 ml), which was then refluxed for 18 h. The reduced material (580 mg), recovered by chloroform extraction, showed no absorption in the region of 1700 cm^{-1}. Examination in solvent A of a hydrolyzate was inconclusive as 2,3-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose have similar R_F values. However, g.l.c. of the derived alditol acetates on column B showed clearly the presence of 2,3,4-tri-, 2,3-di-, and 3-*O*-methyl-D-xylose, together with 2,3,4-tri-*O*-methyl-D-glucose (retention times 16, 25, 38, and 34 min respectively).

Smith degradation of sapote gum. — Purified sapote gum (5.04 g) was dissolved in water (125 ml) and 0.5M sodium metaperiodate solution (125 ml) was added. The reaction was allowed to proceed in the dark for 92 h at 25° when 0.72 moles of periodate had been consumed. The polyalcohol (4.63 g) was obtained as a thick syrup after addition of ethylene glycol (10 ml), reduction with sodium borohydride (2.5 g), dialysis, and concentration. The polyalcohol (2.05 g) was hydrolyzed with 0.5M sulfuric acid (100 ml) for 3 h at room temperature. The solution was neutralized with barium carbonate, centrifuged, and passed through cation- and anion-exchange resins to yield the neutral components as a thick syrup (1.48 g), part of which was resolved on Whatman 3MM paper with solvent B. A second portion (156 mg) was separated on a column (26 cm × 2 cm) of Dowex-1 X2 (OH^-) resin by elution with water[42]. The flow rate was adjusted to 40 ml per h and fractions of approximately 5 ml were collected.

Component 1 was identified as ethylene glycol by formation of the bis(*p*-nitrobenzoate), m.p. and mixed m.p. 139–141°.

Component 2 was identified as glycerol by formation of the tris(*p*-nitrobenzoate), m.p. and mixed m.p. 187–190°.

Component 3 had $[\alpha]_D$ −34.0° (*c* 1.0, water), R_{Xylose} 0.85 in solvent B and behaved as 2-*O*-β-D-xylopyranosylglycerol. Thus, hydrolysis gave D-xylose and glycerol in the ratio 1.0:0.90 as determined by phenol–sulfuric acid and chromotropic acid or 0.90:1.0 as determined by analysis of the trimethylsilyl derivatives[29,43] on column C kept for 3 min at 110° and then programmed at 3°/min to 230°. The xyloside (9 mg) was oxidized by periodic acid (0.01M, 10 ml), and the consumption determined spectrophotometrically[44] was 2.10 moles (theory 2.00 moles). No formaldehyde was detected. Reduction of the dialdehyde and hydrolysis gave glycerol and ethylene glycol (paper chromatography) in the ratio of 0.95:1.0 as determined from the trimethylsilyl ethers on column C kept for 3 min at 90° and then programmed at 3°/min to 200°. A portion of the xyloside (3 mg) was methylated according to Perila and Bishop[45] and, after hydrolysis, the presence of 2,3,4-tri-*O*-methyl-D-xylose was demonstrated on paper in solvent A or as the methyl α- and β-glycosides on column A at 130°.

A portion (4 mg) of *component 3* was trimethylsilylated[29] and injected onto column C which was kept isothermally for 3 min at 150° and then programmed at 2°/min to 239°. Per-*O*-(trimethylsilyl)-2-*O*-β-D-xylopyranosyl-glycerol was eluted in 52 min, and under these conditions per-*O*-trimethylsilyl-D-xylopyranose was eluted at 28 and 32 min. The mass spectrum of per-*O*-(trimethylsilyl)-2-*O*-β-D-xylopyranosyl-glycerol showed strong peaks for *m/e* 204, 349, 389, 569 (M-15).

Component 4 (29 mg) had $[\alpha]_D$ −48.2° (*c* 1.0, water), R_{Xylose} 0.60 and was identified as 2-*O*-β-D-xylobiosylglycerol by methods similar to those used for component 3. The ratio of D-xylose to glycerol, determined colorimetrically, was 1.9:1.0 and 1.8:1 by g.l.c. The methylated xyloside (5 mg) gave equal quantities of the acetates of 2,3,4-tri- and 2,3-di-*O*-methyl-D-xylitol when examined on column B kept for 3 min at 140° and programmed to 180° at 3°/min. Identification was by peak enhancement with standards. Periodate oxidation (3.15 moles consumed) of the xyloside (8 mg) followed by reduction and hydrolysis gave ethylene glycol and glycerol in the ratio of 1.0:2.0, as determined by g.l.c. of their trimethylsilyl ethers.

Component 5 (15 mg) had $[\alpha]_D$ −57° (*c* 1.0, water) and R_{Xylose} 0.32. Hydrolysis gave xylose and glycerol in the ratio of 2.8:1.0, and periodate oxidation (4.10 moles consumed) gave ethylene glycol and glycerol in the ratio of 1.0:2.8. These data show the compound to be 2-*O*-β-D-xylotriosylglycerol.

Xylobiose (5 mg) was characterized by the fact that hydrolysis gave D-xylose only (paper chromatography).

Periodate oxidation. — (Supplementary experiments by Dr. P. E. Reid.) Two samples of sapote gum polyalcohol (each 800 mg) were hydrolyzed as follows. Sample A was hydrolyzed for 24 h at room temperature with 0.05M sulfuric acid, neutralized with barium carbonate, filtered, passed through Amberlite IR-120 resin, concentrated, and poured into ethanol. The yield of degraded polysaccharide was 338 mg. Sample B was hydrolyzed for 3 h at room temperature with 0.5M sulfuric acid and decationized as for sample A up to and including passage through the cation-exchange resin. The eluate from the resin was divided in half and one part (B1) was concentrated and poured into ethanol: yield of degraded polysaccharide 87 mg. The other half (B2) was passed through an anion-exchange resin (Duolite A-4) before concentration and precipitation into ethanol; yield of degraded material 25 mg.

Carboxyl-reduced gum[10,15]. — Propionated sapote gum (500 mg) was dissolved in tetrahydrofuran (15 ml) and the solution added to a cold (−73°) solution of diazomethane in ether (50 ml). The solution was stirred for 1 h at −73° and the methyl ester (550 mg) was recovered by precipitation into petroleum ether (b.p. 30–60°). The esterified material (450 mg) was dissolved in tetrahydrofuran (50 ml) and to the refluxing solution lithium borohydride (1 g) in tetrahydrofuran (25 ml) was added dropwise during 90 min. The reaction mixture was refluxed for 18 h, during which time a gelatinous precipitate formed. The flask was then cooled in ice and water (25 ml) added during 2 h. The reduced product (225 mg), which was isolated by dialysis and lyophilisation, gave a negative test with carbazole[46]. A hydrolysate examined by paper chromatography in solvent B showed L-arabinose, D-xylose, D-glucose, and 4-*O*-methyl-D-glucose. Analysis of this mixture as alditol acetates on column A kept for 25 min at 210° and then programmed at 10°/min to 225 gave a ratio of 1.0:2.8:0.48:0.52. The retention time of xylitol pentaacetate was about 21 min and the relative times for the four compounds are 0.71, 1.00, 1.73, and 1.53 respectively.

Periodate oxidation of carboxyl-reduced gum. — A sample (203 mg) was

dissolved in sodium metaperiodate solution (0.1M, 25 ml) and the oxidation was allowed to proceed for 96 h, whereupon 0.66 moles of periodate had been consumed. The reaction was stopped by the addition of ethylene glycol (1 ml) and the solution was dialyzed. Sodium borohydride (100 mg) was added, and after 24 h the solution was again dialyzed. Residual polysaccharide was recovered as a syrup (182 mg), part of which was hydrolyzed, deionized and examined on paper in solvent B. A portion of the hydrolyzate was trimethylsilylated and separated on column C kept for 5 min at 80° and then programmed at 3°/min to 240°. The presence of ethylene glycol (14 min), glycerol (32 min), 2-*O*-methyl-D-erythritol (40 min), 4-*O*-methyl-D-glucose (55,60 min), and D-xylose (51,54 min) was observed.

Acetolysis of carboxyl-reduced gum. — Reduced polysaccharide (1.10 g) was swollen in formamide (15 ml) over a period of 1 h at 60° at which time pyridine (15 ml) was added[14]. Acetic anhydride (15 ml) was added dropwise during 1 h and the reaction kept at 60° for a further 5 h, after which time the solution was added dropwise to ice–water (1 liter). The precipitate was washed with water and air dried to give 1.43 g of acetate. The acetate (712 mg) was dissolved in acetic anhydride (24 ml), with subsequent addition in order of acetic acid (24 ml) and concentrated sulfuric acid (1 ml). The reaction[17] was kept at 40°, monitored by t.l.c., and quenched after 2.5 h by the addition of pyridine (50 ml). Acetolyzed material was recovered by evaporation of volatile materials *in vacuo* at 50°, and the residue, dissolved in methanol at 5°, was treated with sodium methoxide (1 g) and kept for 16 h at 5°. The solution was poured into water (500 ml), passed through Amberlite IR-120 resin, and evaporated to a syrup (475 mg).

The resultant oligosaccharides (380 mg) were dissolved in water (10 ml) and added to a column (10 cm diameter by 4 cm height) of charcoal–Celite (1:1, 30 g) formed in a Büchner funnel[18]. The column was eluted stepwise with water (2 liters), 5% ethanol (2 liters), 10% ethanol (2 liters), and 15% ethanol (2 liters). Each fraction was concentrated and examined on paper in solvent B, which revealed the presence of four oligosaccharides in addition to monosaccharides.

Aqueous eluate. This fraction (175 mg) contained only L-arabinose and D-xylose.

5% Ethanol. This fraction (65 mg) contained 3 components which were separated by double development for a total of 40 h in solvent B. The two fastest-moving components (R_{Xyl} values 1.0 and 0.75) were chromatographically identical to D-xylose and L-arabinose, and were unchanged on acid hydrolytic treatment. *Oligosaccharide* **1** (8 mg) had $[\alpha]_D$ +102.5° (*c* 1.0, water) and acid hydrolysis gave D-xylose and D-glucose in a ratio of 1:1 (paper chromatography, or g.l.c. of the trimethylsilyl derivatives on column C). Methylation of 2 mg by the method of Perila and Bishop[45] followed by methanolysis and g.l.c. gave methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose (column A, isothermal 120°, 5 min and then to 170° at 3°/min, T_R 1.00, 1.33 and 1.14, 1.33). This is consistent with oligosaccharide **1** being 2-*O*-(α-D-glucopyranosyl)-D-xylose.

10% Ethanol. This fraction (25 mg), had $[\alpha]_D$ +109° (*c* 1.0, water) and on paper chromatography showed one component only, having R_{Xyl} 0.75. Hydrolysis of this

oligosaccharide **2** and qualitative examination on paper followed by g.l.c. of the alditol acetates on column A kept isothermally for 20 min at 210° and programmed at 10°/min to 225° indicated the presence of D-xylose and 4-*O*-methyl-D-glucose in the ratio of 1:1. Methylation gave the same sugars as for oligosaccharide **1**, indicating that oligosaccharide **2** is 2-*O*-(4-*O*-methyl-α-D-glucopyranosyl)-D-xylose[19].

15% Ethanol. This fraction (18 mg) showed the presence of two components on double development for a total of 48 h in solvent B. The faster migrating, *oligosaccharide* **3**, (8 mg) had $[\alpha]_D$ +50° (*c* 0.5, water). Hydrolysis and analysis of the trimethylsilyl derivatives showed D-xylose and D-glucose in the ratio of 2:1. Methylation (of 3 mg) and analysis showed 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3- (T_R 1.22, 1.41) and 3,4-di-*O*-methyl-D-xyloses in equimolecular amounts. The slower-moving fraction (7 mg), *oligosaccharide* **4**, had $[\alpha]_D$ +25° (*c* 0.5, water) and was similarly shown to be composed of D-xylose and D-glucose in the ratio of 3:1. The same methylated sugars were obtained as for oligosaccharide *3* but in the ratio of 1:2:1 respectively. Thus oligosaccharides **3** and **4** are polymer homologs of oligosaccharide *1*.

Additionally, a sample (2 mg) of each oligosaccharide was reduced with sodium borohydride and hydrolyzed. In all cases xylitol was obtained.

Action of enzymes on sapote gum. — The following enzyme preparations were used: (*a*) pectinase (Nutritional Biochemicals Corp.), (*b*) hemicellulase (NBC), (*c*) cellulase (NBC), and (*d*) α-L-arabinofuranosidase[24]. Sapote gum (0.5 g) was dissolved in a buffer solution (25 ml) of potassium acid phthalate (pH 4.0) containing 0.2% of enzyme. The solution was placed in a dialysis bag for enzymes *a* and *b* or in a beaker for *c* and *d*. The solutions were maintained for 96 h at 30° and in cases *a* and *b* the water surrounding the dialysis bag was changed every 24 h. Blanks were conducted similarly. Enzyme and polysaccharide were precipitated with ethanol, the mixtures were centrifuged, and the supernatants concentrated. Paper chromatography showed the presence of D-xylose in cases *b* (+) and *c* (+), L-arabinose (+) and D-xylose (+); while *d* gave a strong response for L-arabinose (+ + + +). Enzymes *a*, *b* and *c* were highly active on a preparation of birch xylan.

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