STRUCTURAL ASPECTS OF THE GUM OF *Cussonia spicata* THUNB. (*Araliaceae*)

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ABSTRACT

The composition of the gum exuded by *Cussonia spicata* Thunb. has been examined by partial hydrolysis with acid, methylation analysis, and sequential Smith-degradation, with extensive use of gel chromatography in order to determine the molecular-weight distributions of the gum polysaccharide and its degradation products. The evidence thus obtained suggests a molecular core consisting of D-galactose residues, mainly β-D-(1→3)-linked, most of which carry a D-glucuronic acid residue, β-linked, at C-6; the molecular-weight distribution pattern of the degraded polysaccharide obtained on partial hydrolysis with acid indicates a possible repeating unit having a molecular weight of 1,200. L-Arabinose residues (molar percentage 58) occur as short branches, each attached to the galactan framework (at C-4,) with α-L-(1→5)-linkages between consecutive L-arabinose units in some cases; L-rhamnopyranose end-groups are also present. The gum is polymolecular, and variation in composition among the various constituents is indicated by the broad molecular-weight distributions of the methanol-insoluble products of three successive Smith-degradations (in which steps were taken to ensure that acetal cleavage was complete).

INTRODUCTION

The cabbage-wood tree ("*umsenge*", *Cussonia spicata* Thunb., fam. *Araliaceae*), which grows extensively in the Eastern Cape Province, South Africa, occasionally exudes a light-brown gum from its cork-like bark. A study of the composition of the gum was undertaken in order to extend the range of plant gums examined in relation to their botanical sources. Sufficient features of structural interest became apparent to warrant a detailed investigation.

EXPERIMENTAL

General experimental conditions. — The experimental methods involving paper and thin-layer chromatography, paper ionophoresis, and handling of materials have been described[1]. Mixtures of methyl ethers of sugars were analyzed semi-quantitatively by g.l.c. (*i*) as methyl glycosides, on a copper column (3 ft × 0.25 in. o.d.) of 14%

ethylene glycol succinate polyester on Chromosorb W (Johns Manville; 80–100 mesh) at 155°, by use of a Beckman GC-2A chromatograph with helium carrier and a flame-ionization detector (*T*, retention time relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside)[1,2], and (*ii*) as *O*-methylalditol acetates[3], by use of a Beckman GC-4 chromatograph with dual copper columns (12 ft × 0.125 in. o.d.) packed with 3% ECNNS-M on Gas-Chrom Q (Applied Science Laboratories; 100–120 mesh) at 175°, with helium carrier and dual flame-ionization detectors (retention times were relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol)[4,5].

Gel chromatography of 2–8-mg quantities of polysaccharide was conducted on a column (90 × 1.5 cm) of Bio-Gel P-300 (Bio-Rad Laboratories). M Sodium chloride was used for elution[6], each fraction (1 ml) being assayed for carbohydrate by the phenol–sulfuric acid method[7]. As described previously[8–10], prior calibration of the column permitted the assignment of corresponding molecular weights to the peaks in the elution curves, from which $\overline{M}_w$, the weight-average molecular weight, of each polysaccharide sample was calculated.

Purification of the gum. — Samples of the gum exudate, collected during three successive mid-winters from the bark of a specimen of *Cussonia spicata* Thunb. grown in the Alice district, Cape Province, were stirred in water, and the suspension was centrifuged to separate the jelly-like lumps and some contaminating bark from the clear, aqueous solution. Addition of ethanol and acetone to the solution caused precipitation of polysaccharide, which was taken up in water and freeze-dried. The insoluble portion of the gum dissolved rapidly on being stirred in dilute sodium hydroxide solution containing a small proportion of sodium borohydride. The alkali-solubilized material was precipitated with ethanol, dissolved in water, treated in the cold with Amberlite IR-120 (H^+) ion-exchange resin, reprecipitated, and finally dissolved in water and freeze-dried. Apart from a small decrease in molecular weight, the alkali-solubilized gum was not significantly different from the water-soluble fraction: $[\alpha]_D -81°$ (*c* 2.5); equivalent weight 1,600–2,000 (by titration); ash, nil; identical products on acid hydrolysis (paper chromatography); indistinguishable methylation products (see later). Molecular-weight distribution measurements (by gel chromatography) showed several preponderating molecular sizes for each fraction of the gum, the bulk of which had molecular weight above 110,000 (See Fig. 1, A and B, and Fig. 2, A). For one sample, the water-soluble and alkali-solubilized fractions (Fig. 1, A and B) had $\overline{M}_w$ 102,000 and 92,000, respectively.

Partial hydrolysis with acid. — The alkali-solubilized fraction (3 g) was kept in sulfuric acid (pH of solution 2.1) for 24 h at 96°, the solution was then made neutral with barium carbonate, and the suspension was filtered. The filtrate was concentrated, and ethanol (8 volumes) was added, yielding a precipitate *A* (1.63 g, dried *in vacuo*), and, after evaporation of solvent, a syrup *B* (1.7 g).

Solid *A* had $[\alpha]_D -24°$ (*c* 2.09), and $\overline{M}_w$ 25,000 (see Fig. 1, C). After reprecipitation, the solid had an equivalent weight of 750 (from the barium content). Further hydrolysis of 1 g in 0.5M sulfuric acid for 3.5 h at 96° caused the release of arabinose, and then increasing amounts of galactose (paper chromatography); from the neu-

tralized (barium carbonate) hydrolyzate there was recovered, by precipitation with ethanol and acetone, a barium salt {0.4 g; $[\alpha]_D$ −4° (*c* 4.0)} that was chromatographically and ionophoretically identical with barium 6-*O*-(β-D-glucopyranosyluronate)-D-galactose. Methylation of a portion of this salt by the methods of Haworth[11] (once) and Purdie and Irvine[12] (three times) gave a product, $[\alpha]_D$ −23° (*c* 2.3, chloroform), identical in t.l.c. with the authentic, fully methylated methyl ester; methanolysis gave methyl glycosides of methyl 2,3,4-tri-*O*-methylglucuronate, 2,3,5-tri-*O*-methylgalactose, and 2,3,4-tri-*O*-methylgalactose (on g.l.c., *T* 2.78m, 3.62s; 5.24w; and 9.3m, respectively). Reduction (lithium aluminium hydride in tetrahydrofuran) of the methylated product gave a syrup which, on methanolysis and g.l.c., gave glycosides of 2,3,4-tri-*O*-methylglucose (*T* 2.78m, 4.05s) and the two tri-*O*-methylgalactoses. Hydrolysis and paper chromatography of both the ester and the reduction product supported this characterization of the component sugar methyl ethers. The syrupy sugars released on hydrolysis of reprecipitated *A* had $[\alpha]_D$ +41°, and their identity as galactose, arabinose, and rhamnose (trace amount) was confirmed by reduction (sodium borohydride), followed by acetylation, and g.l.c. analysis*.

Analyzed by charcoal–Celite chromatography, an aliquot of syrup *B* gave L-arabinose {570 mg; recrystallization from ethanol afforded the β anomer, m.p. and mixed m.p. 160°, $[\alpha]_D$ +149° (7 min) → +114° (60 min) (*c* 2.11)}, α-L-rhamnose {80 mg; from aqueous ethanol as the hydrate, m.p. and mixed m.p. 93°, $[\alpha]_D$ +10° (equil.; *c* 3.01)}, an arabinose disaccharide {50 mg; $[\alpha]_D$ −3° (*c* 3.1)} chromatographically and ionophoretically identical with 5-*O*-α-L-arabinopyranosyl-L-arabinose, and further arabinose-containing oligosaccharides (20 mg).

Quantitative analysis of sugar residues. — Samples of *Cussonia spicata* gum in 0.5M sulfuric acid were heated in sealed tubes for 6 h at 96°, and the neutralized, reduced hydrolyzates were dried and acetylated. G.l.c. analysis showed the acetates of rhamnitol, arabinitol, and galactitol in the molar proportions of 2:15:6.

Galactaric acid, having m.p. and mixed m.p. 217°, i.r. spectrum identical with that of authentic material, was obtained by heating a sample of the gum with nitric acid.

Methylation analysis. — Portions (each 0.6 g) of the water-soluble and alkali-solubilized fractions of *Cussonia spicata* gum were methylated (methods of Haworth, and Purdie and Irvine) until further treatment brought about no change in the i.r. spectrum (Nujol mull) or the behavior in t.l.c.; the two methylated products were not only identical by these criteria, but also had similar $[\alpha]_D$ values of −84° and −87° (*c* 4, chloroform), and indistinguishable n.m.r. spectra (in $CDCl_3$). A sample of each was methanolyzed, and the mixture of methylated sugar glycosides was assayed by g.l.c.; a second sample was hydrolyzed, the mixture reduced and the product acetylated, and the acetates of the alditol methyl ethers were analyzed by g.l.c. Average relative values of the composition, expressed as molecular proportions of methylated sugar residues in a molecule of the methylated gums, were: 2,3,4-tri-*O*-methylrham-

*D-Galactose was isolated by preparative paper-chromatography, which also afforded evidence for xylose and mannose in the hydrolysate of *A*.

nose, 3; 2,3,5-tri-*O*-methylarabinose, 30; 2,3,4-tri-*O*-methylarabinose, 10; 2,3-di-*O*-methylarabinose, 14; 2,4-di-*O*-methylgalactose, 24; methyl 2,3,4-tri-*O*-methylglucuronate, 7; and methyl 2,3-di-*O*-methylglucuronate, 6. Tri-*O*-methylgalactoses (2,3,6-, 2,4,6-, and 2,3,4-) totalled 5 molecular proportions, and there were small proportions of 2,5- and 3,5-di-*O*- and of 3-*O*-methylarabinose. Hydrolysis followed by paper chromatography confirmed the presence of these methylated sugar residues in the methylated gums, and indicated, in addition, the presence of arabinose and 2-*O*-methylgalactose.

Sequential, Smith-degradation experiments. — *First oxidation–reduction–hydrolysis stage.* Sodium metaperiodate (15.8 g) was added to *Cussonia spicata* gum (whole gum, alkali-solubilized; 5 g) in water (500 ml), and the solution was kept in the dark for 6 days at room temperature. By then, the consumption of periodate (0.66 mole/100 g of polysaccharide, by the spectrophotometric method[13,14]. Calc., on the basis of methylation analysis, ~0.64 mole) had approached the maximum. After addition of ethylene glycol, the solution was dialyzed, and concentrated, the contents were reduced with sodium borohydride (5 g, added with stirring during 4 days), and the solution was concentrated to 80 ml. Hydrochloric acid was added to pH 1, and the mixture was kept for 10 h at room temperature, made neutral with sodium hydrogen carbonate, and evaporated. Removal of borate by addition of methanolic hydrogen chloride followed by evaporation gave a product which was treated with mixed ion-exchange resins [Amberlite IR-120 (H^+) and IR-45 (OH^-)]. The resulting, partly degraded, oxidized and then reduced gum was extracted with methanol, giving an insoluble product (1.25 g) that was borate- and chloride-free, but that contained carboxylate groups (equivalent weight, bu titration, 3400; i.r. absorption in potassium chloride at 1740–1720 cm^{-1} for free acid, and at 1640–1610 cm^{-1} for barium salt). The methanol-soluble material was isolated as a syrup (1.67 g, dried *in vacuo*) containing glycerol (identified as the tris-*p*-nitrobenzoate) and ~15% by weight of a mixture of glycosides of arabinose (mainly) and galactose.

A sample of the insoluble product {$[\alpha]_D$ −20° (*c* 3.3), $\overline{M}_w$ 70,000} was kept in 0.5M sulfuric acid at room temperature, and aliquots were treated at intervals with periodate and then assayed for formaldehyde by the chromotropic acid method[15]. A plot of formaldehyde released *vs.* time showed no further increase after 24 h. The bulk of the insoluble product (~1 g) was then hydrolyzed in 0.5M sulfuric acid in the cold for 24 h, the acid neutralized (barium carbonate), the suspension filtered, and the product obtained by evaporation of the filtrate. The residue was extracted with methanol (which removed 0.03 g of soluble material containing glycerol and arabinose), leaving a solid referred to as SDli (0.99 g). This product had $[\alpha]_D$ −20° (*c* 1.02), $\overline{M}_w$ 42,400 (fourteen peaks; see Fig. 2, B) [Found, by g.l.c. analysis of a hydrolyzate, galactose 80, arabinose 19, and rhamnose 1 molecular proportion; glycerol negligible]. No measurable degradation was effected by a further treatment with cold 0.5M sulfuric acid for 48 h.

Second oxidation–reduction–hydrolysis stage. — Oxidized as described in the previous section, the solid product (0.83 g) of the first degradation consumed

0.38 mole of periodate/100 g of polysaccharide. After addition of ethylene glycol, dialysis, reduction, and removal of borate, the residue was dissolved in M hydrochloric acid, and, after 24 h, de-ionized. The solution was evaporated to dryness, and the product extracted with methanol, yielding an insoluble residue referred to as SD2i {0.31 g; $[\alpha]_D$ ~0°, $\overline{M}_w$ 10,100 (sixteen peaks; see Fig. 2, C)}, and a soiuble fraction (0.23 g), consisting largely of glycerol, with traces of arabinose and of glycosidic components. On periodate treatment, a sample of SD2i gave an amount of formaldehyde commensurate with that required for the glycerol end-group; this amount was not increased if SD2i was kept for a further 24 h in 0.5M sulfuric acid. Galactose and a trace of arabinose were the sole products shown by paper chromatography of a hydrolyzate from SD2i; controlled hydrolysis of SD2i with 50 mM sulfuric acid for 1, 3, and 7 h at 96° gave, in addition, three products chromatographically similar to the β-D-(1→3)-linked D-galactose oligomers and one similar to 6-*O*-β-D-galactopyranosyl-D-galactose.

Methylation of SD2i (0.16 g) by the methods of Haworth, Purdie and Irvine, and Kuhn *et al.*[16] (thirteen treatments in all) gave a syrupy product (0.10 g) that had $[\alpha]_D$ −26° (*c* 2.54, chloroform); on methanolysis and g.l.c., the product was found to contain glycosides of 2,3,5-tri-*O*-methylarabinose (trace), 2,3,4,6-tetra-*O*-methylgalactose (++), 2,4,6-tri-*O*-methylgalactose (+++++), 2,3,4-tri-*O*-methylgalactose (trace), and 2,4-di-*O*-methylgalactose (+). In addition to these sugars, 2,6-di-*O*-methylgalactose and 2-*O*-methylgalactose were detected by paper chromatography of the hydrolyzate.

Third oxidation–reduction–hydrolysis stage. — Material SD2i (0.06 g) in water (10 ml) containing sodium metaperiodate (0.15 g) was kept in the dark for 3 days at room temperature (0.32 mole of periodate consumed/100 g of polysaccharide). An excess of barium carbonate was then added, and the mixture was filtered. The material in the filtrate was reduced, and subsequent treatment as already described (including hydrolysis with 0.5M sulfuric acid for 24 h in the cold) yielded methanol-insoluble SD3i (0.022 g), $[\alpha]_D$ +1° (*c* 1.4), $\overline{M}_w$ 7,600 (fourteen peaks; see Fig. 2, D). Treatment with cold acid caused no further degradation. This product absorbed 0.16 mole of periodate/100 g during 3 days.

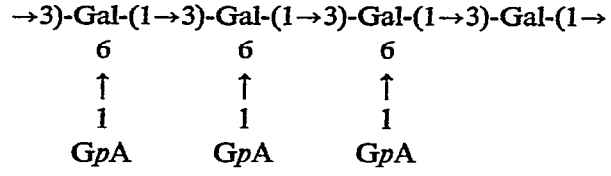
RESULTS AND DISCUSSION

The water-insoluble portion of the gum exudate of *Cussonia spicata* may be solubilized by stirring in cold alkali; the material obtained by reprecipitation has now been shown by a number of tests ($[\alpha]_D$, equivalent weight, component sugars; properties and composition of the methylated derivatives) to be indistinguishable, apart from a slightly lower $\overline{M}_w$, from the soluble portion. Both are polymolecular, although the bulk of the material lies towards the upper limit of the molecular-weight distribution curve. Samples of gum collected from the same tree during three successive winters showed no appreciable differences in properties.

Acid hydrolysis of the gum yields the neutral sugars L-arabinose, L-rhamnose, and D-galactose, and the acidic components D-glucuronic acid and 6-*O*-(β-D-gluco-

pyranosyluronic acid)-D-galactose, all of which are products highly typical of plant gums. The analytical figures obtained by g.l.c. for the proportions of neutral sugars released from the gum by hydrolysis, the equivalent weight of the gum, and the products of methylation show that the molecular proportions of the constituent units of the gum approximate to: L-arabinose 58, L-rhamnose 7, D-galactose 23, and D-glucuronic acid 12. Partial hydrolysis of the gum with acid yields, in addition to the products just mentioned, a number of oligosaccharides which, judged on the basis of chromatographic and electrophoretic mobilities, indicate the presence in the gum of linkages of the types* α-L-Ara*p*-(1→5)-L-Ara*f*, β-D-Gal*p*-(1→3)-D-Gal*p*, and β-D-Gal*p*-(1→6)-D-Gal*p*. Of these, the first has been shown to be present in the gum of *Virgilia oroboides* (fam. *Leguminosae*)[17], and the galactose inter-sugar linkages are characteristic of plant gums built upon a branched galactan framework.

Figure 1 illustrates the breakdown of the *Cussonia* gum after being heated in dilute acid. The $\overline{M}_w$ value (25,000) of the product insoluble in aqueous ethanol is not much lower than the value (37,000) calculated on the basis of removal of peripheral arabinose and rhamnose residues from the four-component starting material (see Fig. 1, B), indicating relatively little cleavage between sugar residues in the molecular core. As has been found in other instances[9,10], partial hydrolysis leaves fragments having favored molecular sizes: the gel chromatogram shows a multiplicity (13) of peaks, pairs of which exhibit the 2:1 molecular-weight relationship noted previously[10]. The molecular weights corresponding to peaks in Fig. 1, C are, to a close approximation, all divisible by 1,200; this suggests the presence in the galactan framework of a repeating unit, containing linkages resistant to acid hydrolysis, of this molecular weight. Such a possible unit is:



which has a molecular weight of 1,176. The (1→3)-linkages between the D-galactose units to which are attached uronic acid residues may, as a result of electronic interaction involving the uronic acid[18], be more resistant to acid hydrolysis. The tendency to hydrolysis of the terminal linkage could be determined by the presence or absence, on the contiguous galactose residue, of a uronic acid residue.

Methylation of gum samples followed by semi-quantitative g.l.c. analysis of their methanolyzates and hydrolyzates established that the main constituent units of the gum are L-Ara*f*→, L-Ara*p*→, D-Gp A→ and L-Rha*p*→ end-groups; →5)-L-Ara*f*→ and →4)-D-GpA→ chain units in much lower proportion; and 3,6- and 3,4,6-linked D-Gal*p* branch-points. As so frequently occurs in the methylation analysis of plant gums, there were also present in the mixture small amounts of methyl sugars of possible structural significance. Should these not arise as a consequence of under-

*Ara = arabinose; Gal = galactose; GpA = glucopyranuronic acid; Rha = rhamnose.

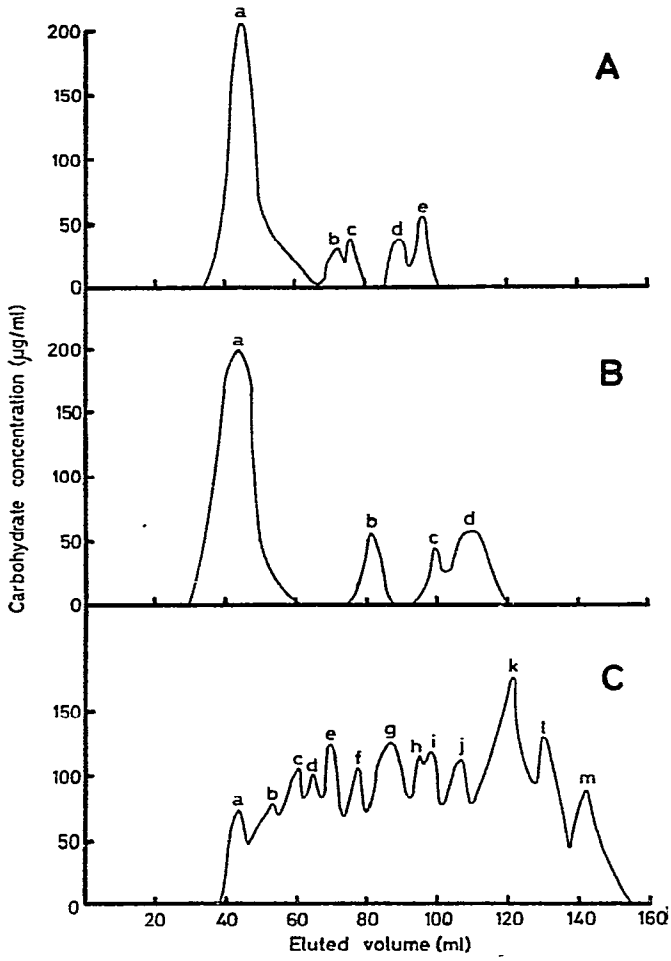


Fig. 1. Elution patterns on Bio-Gel P-300 (flow rate, 3–4 ml/h) of: A. water-soluble portion of *Cussonia spicata* gum, B. alkali-solubilized gum, C. ethanol-insoluble product of partial hydrolysis with acid (pH 2.1, 24 h, 96°) of alkali-solubilized gum. Molecular weights correspond to peaks as follows: A. *a* 125,000; *b* 43,500; *c* 39,000; *d* 23,500; *e* 18,000; B. *a* 125,000; *b* 31,000; *c* 15,500; *d* 11,000; C. (different calibration) *a* 120,000; *b* 70,000; *c* 53,000; *d* 45,000; *e* 37,500; *f* 27,000; *g* 19,000; *h* 14,000; *i* 12,500; *j* 8,700; *k* 4,800; *l* 3,600; *m* $\leqslant$ 1,800.

methylation of the gum or of de-*O*-methylation during glycosidic cleavage, their presence suggests the occurrence of arabinose and galactose residues bound in a number of other ways. The proportion of periodate consumed by the gum, as determined spectrophotometrically, agreed favorably with the value calculated on the basis of the sugar units shown by methylation analysis to be present.

A general molecular pattern that is consistent with the evidence is as follows; it is recognized that (*a*) the native gum is not of uniform molecular weight and (*b*) the methylated derivatives are likewise not uniform (according to t.l.c.). The gum is a high polymer of β-D-(1$\rightarrow$3)-linked D-galactose residues to which are bound D-glucuronic acid residues by β-D-linkages to C-6. L-Arabinose comprises about half

of the total carbohydrate present. Most of the L-arabinose occurs as furanose end-group, some as pyranose end-group, and there are α-L-(1→5)-linkages between L-arabinose residues. L-Arabinose residues are attached ultimately to the galactan framework, as short branches, at C-4. L-Rhamnopyranose end-groups, and, possibly, some L-arabinose residues, may be linked 1→4 to certain of the D-glucuronic acid residues.

It has been shown that most of the L-arabinose and L-rhamnose residues are, by mild treatment with acid, removed from the molecular core consisting of D-glucuronic acid linked to D-galactose. In a preliminary attempt to establish the lengths of exterior chains and to remove all but the periodate-resistant, inner galactan framework, a sequence of three Smith-degradation stages was applied to the gum. After the first of these stages, in which the oxidized and then reduced polysaccharide was treated with 0.5M hydrochloric acid for only 8 h in the cold (a recommended procedure[19]), solvent extraction of the product showed that most of the arabinose, rhamnose, and glucuronic acid had been removed, forming glycerol and glycosides of low molecular weight. The insoluble residue, an estimated 35% by weight of the starting material, was shown by gel chromatography to consist of a multiplicity of components of various molecular weights, with $\overline{M}_w$ 86,000. The insoluble products of the second and third such treatments (yields 8 and 3%, respectively, of the original) had $\overline{M}_w$ 40,000 and 26,000. Galactose [(1→3)-linked] was the only sugar detected upon hydrolysis of the third degradation product, which had $[\alpha]_D$ +8°. However, the proportions of glycerol in all three insoluble products were higher than could be accounted for on the basis that glycerol occurs solely as nonreducing end-group; furthermore, there was chromatographic evidence for the presence of glycolaldehyde in the hydrolyzates, certainly in that of the first degradation product. This evidence showed that the acid degradation step in the Smith procedure, applied to *Cussonia spicata* gum, had not fully accomplished its purpose, and that acetal linkages involving glycolaldehyde had survived hydrolysis.

The retarded hydrolysis of acetals wherein an α-hydroxy acid is linked to an aldehyde has been demonstrated before[20], and the problem that this poses in eliminating from acidic polysaccharides those residues of uronic acid that have been cleft by periodate has been recognized[21]. A way of circumventing this property when studying acidic polysaccharides by the Smith degradation is to convert carboxyl groups in the polysaccharide into primary alcohol groups before commencing the Smith degradation, but this procedure results in a measure of breakdown due to cleavage of inter-sugar linkages; for example, after reduction of *Acacia cyanophylla* gum, a diminution in the molecular weight has been found, the proportion having molecular weight above 150,000 decreasing from ~60 to 27% by weight[22]. Alternatively, acid degradation of the oxidized and reduced acidic polysaccharide should be continued, despite possible fission of the more labile glycosidic bonds, until hydrolysis of glycolaldehyde acetal linkages can be shown to be complete. A semi-micro procedure for monitoring the progress of acetal cleavage (in 0.5M sulfuric acid at room temperature) has been devised[23]; this involves the periodate oxidation of samples removed at intervals and the measurement of the formaldehyde released

thereby, which tends to reach a maximum as the acetal cleavage approaches completion. An iodometric procedure such as was employed when the Smith degradation was first developed[24] uses more material[25]. It is clearly imperative to ensure that acetal cleavage is complete if examination of the molecular-weight distributions of the Smith-degradation products is to lead to valid conclusions regarding molecular structure.

The sequence of three Smith-degradation stages was, therefore, repeated on the *Cussonia spicata* gum, but on a larger scale and under hydrolysis conditions, described in the Experimental section, which ensured that acetal hydrolysis was complete, even though there was evidence of the release of traces of arabinose at the first and second stages. The first degradation product, after having been given the conventional treatment with acid, was shown by titration and i.r. spectroscopy to contain carboxyl groups, the number of which agreed with the number of acetal linkages subsequently cleaved by further treatment with 0.5M sulfuric acid for 24 h at room temperature. Glycerol, mostly free, constituted the bulk of the soluble material produced at each stage; in addition, the soluble products included small proportions of glycosides of arabinose and galactose.

The three insoluble degradation-products, namely, SD1i, SD2i, and SD3i, represented ~20, 7, and 3% (by weight) of the *Cussonia* gum used, and were polymolecular, with $\overline{M}_w$ 42,400, 10,100, and 7,600, respectively (see Fig. 2, B, C, D). Both the yields and the $\overline{M}_w$ values are generally lower than those of the corresponding products in the preliminary series of Smith degradations (in which acetal hydrolysis was certainly incomplete during the first stage).

As before, most of the D-galactose in the gum was found to be accumulated in SD1i; galactose was the only sugar component other than a trace of arabinose in SD2i, the product of the second stage of degradation. Methylation analysis of SD2i showed multiple branching (~24% of nonreducing end-groups), the preponderant chain units being (1→3)-linked, and the branch points 3- and 6-linked. The periodate uptake of SD2i was as expected for such a galactan structure, and indicated an average of 15 unprotected end-groups or (1→6)-linked chain units, and 48 periodate-resistant sugar residues per molecule. The insoluble product SD3i, on the basis of its $\overline{M}_w$ and consumption of periodate, appears to be similarly constituted, but with less branching (~12% of nonreducing end-groups; an average of 5–6 end-groups and 40 inner residues per molecule, although Fig. 2, D shows that the degree of polymerization covers the range of ~25 to 170 galactose units). The $[\alpha]_D$ values indicate β-linkages between the D-galactose units.

The small decrease (2,500) in $\overline{M}_w$ accompanying the third stage of degradation suggests that the periodate-vulnerable residues in SD2i are all end-groups. However, the value found for $\overline{M}_w$ of SD2i is about one-third of the value (30,600) predicted from the $\overline{M}_w$ of SD1i on the assumption that only end groups are removed during the second Smith-degradation; this suggests an average of two periodate-vulnerable, (1→6)-linked units per molecule in the galactan framework of SD1i. The range of molecular weights covered by the peaks in Fig. 2,C indicates that the lengths of the

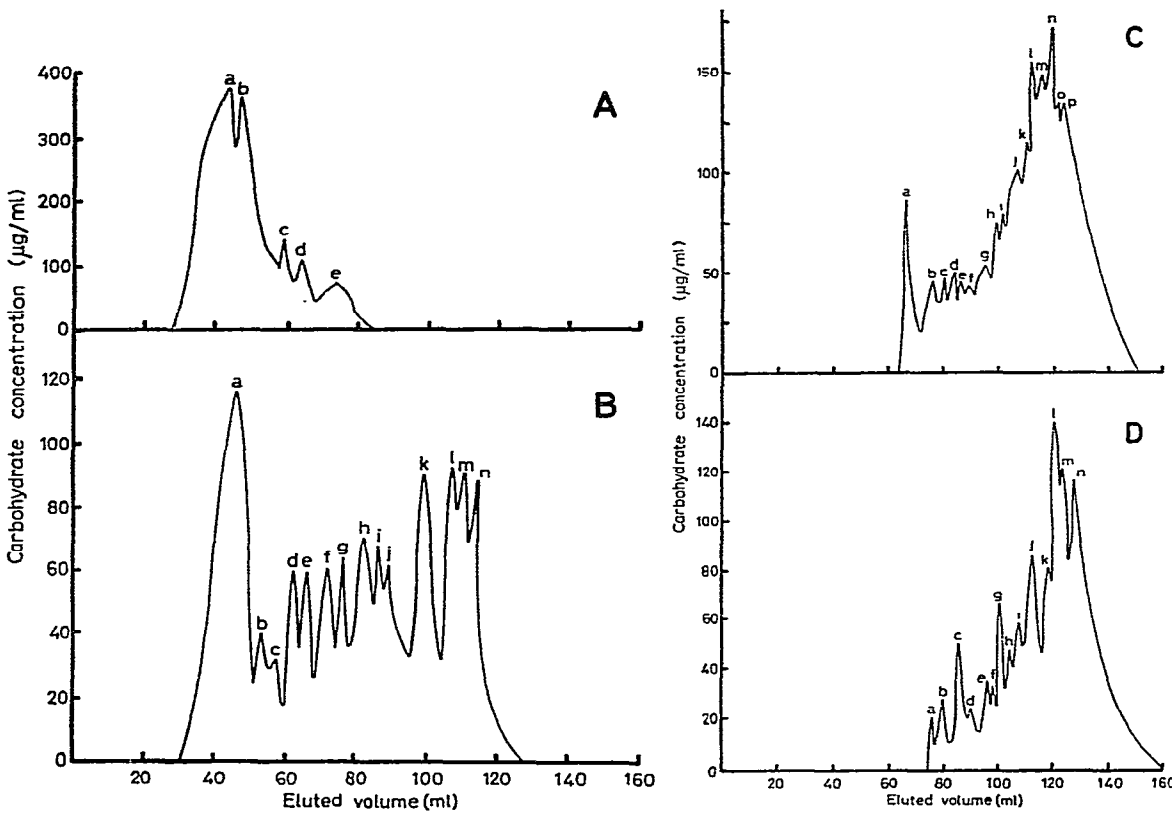


Fig. 2. Bio-Gel P-300 elution patterns of (A), alkali-solubilized, *Cussonia spicata* gum (second sample), and (B–D), the methanol-insoluble products of successive Smith-degradations of this gum (B, SDli; C, SD2i; D, SD3i). Molecular weights correspond to peaks as follows: A. *a* 112,000; *b* 89,000; *c* 54,000; *d* 44,500; *e* 29,500; B. *a* 95,500; *b* 70,000; *c* 59,500; *d* 48,000; *e* 41,500; *f* 33,000; *g* 28,000; *h* 22,500; *i* 19,000; *j* 17,000; *k* 11,500; *l* 8,400; *m* 7,500; *n* 6,500; C. *a* 40,000; *b* 28,000; *c* 24,000; *d* 21,000; *e* 19,500; *f* 17,000; *g* 13,500; *h* 11,500; *i* 10,500; *j* 8,400; *k* 7,500; *l* 6,900; *m* 6,000; *n* 5,300; *o* 4,900; *p* 4,500; D. *a* 28,000; *b* 24,000; *c* 20,000; *d* 16,000; *e* 13,000; *f* 12,000; *g* 11,000; *h* 9,500; *i* 8,400; *j* 6,900; *k* 5,500; *l* 5,000; *m* 4,500; *n* 4,000.

galactose chains separated by (1→6)-linkages in SD1i, and which therefore appear in SD2i, vary from ~28 to ~250 galactose units.

This scatter of molecular sizes in SD2i and SD3i is clearly evident from the gel-chromatograms (Fig. 2,C and D), because of the high degree of resolution made possible by the use of optimal conditions[26,27] in this process of chromatography. Although the overall profiles of Figs. 2,C and 2,D are regular, the multiplicity of peaks due to the accumulation of different fragments of favored molecular weights is obvious.

Calculation, from the molecular weights of the five components of the whole gum (Fig. 2,A) and the known proportions of arabinose, rhamnose, and glucuronic acid removed, of the molecular weights expected for the products of the first Smith-degradation predicts for SD1i an elution curve having five peaks, at molecular weights

39,000, 31,000, 18,500, 15,500, and 10,000, with $\overline{M}_w$ 32,000. The elution pattern actually observed on gel chromatography of SD1i (see Fig. 2,B) shows 14 peaks, at molecular weights ranging from 95,000 to 6,5000, with $\overline{M}_w$ 42,400. This result suggests that the proportions of periodate-vulnerable residues in the components of high molecular weight are smaller than those in the molecules of low molecular weight. It appears, therefore, that *Cussonia spicata* gum consists of a mixture of polysaccharides that differ not only in molecular weight but also in composition. Separation of the various components on a preparative scale is clearly a prerequisite to any further elucidation of the structure of the constituents of this gum.

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