MOLECULAR-WEIGHT DISTRIBUTION OF HYDROLYSIS PRODUCTS FROM THE GUM OF *Acacia elata* A. CUNN.

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ABSTRACT

The behavior of the polysaccharide gum of *Acacia elata* on acid hydrolysis has been studied by gel chromatography. The changes in the elution pattern and weight-average molecular weight as hydrolysis progresses resemble those found on hydrolysis of the structurally similar polysaccharide of *A. podalyriaefolia* gum. The molecular weights corresponding to several persistent peaks in the elution patterns of the hydrolyzates approximate closely to values predicted for the products obtained on complete hydrolysis of peripheral arabinofuranoside and rhamnopyranoside linkages, and subsequent preferential removal of galactopyranose end-groups, from the various components of the polymolecular gum. The hydrolysis rate-constant, which decreases continuously with increasing degree of depolymerization under given conditions, approaches the value for 6-*O*-β-D-galactopyranosyl-D-galactose in 50mM sulfuric acid when hydrolysis of the gum in this acid is almost complete.

INTRODUCTION

In an earlier study[1], gel chromatography of a hydrolyzate (5mM sulfuric acid for 24 h at 96°) of the polysaccharide gum of *Acacia elata* A. Cunn. gave an elution pattern showing a multiplicity of peaks[1,2]. A detailed investigation by gel chromatography of the course of acid hydrolysis of this polysaccharide was therefore undertaken in an attempt to relate the molecular weights corresponding to these peaks to those of components of the parent polysaccharide, itself polymolecular[1–3], and to compare the behavior on acid hydrolysis of *A. elata* gum with that of other polysaccharides similarly investigated[4,5].

EXPERIMENTAL

Purification of the gum. — The *A. elata* gum was a fresh sample collected in October, 1970, from a tree in Pretoria, South Africa, which was also the source of several gum-samples examined previously[1,3,6,7]. The polysaccharide was purified as described for *A. podalyriaefolia* gum[8], and dried to constant weight *in vacuo* at 20°. The properties of the purified gum were similar to those of the earlier samples.

General experimental conditions. — The experimental methods involving paper and gel chromatography, and the determination of optical rotation and reducing power, were as described elsewhere[4]. The solvent systems (v/v) used in paper chromatography were (*a*) 10:4:3 ethyl acetate–pyridine–water, and (*b*) butyl alcohol–ethanol–water (4:1:5, upper layer).

Partial hydrolysis with 5mM sulfuric acid. — The gum (2.17 g) was heated in 5mM sulfuric acid (100 ml) for 121 h at 96°, the pH being kept constant at 2.00. At intervals, samples (5 ml) of the hydrolyzate solution were removed and immediately cooled, made neutral (barium carbonate), and centrifuged. For each sample, the optical rotation and reducing power of the hydrolyzate were determined, and the degree of scission (x) of the polysaccharide was calculated from the reducing power, as described previously[4]. An aliquot (0.5 ml) of each hydrolyzate solution was concentrated to ~0.1 ml and examined by paper chromatography, and a further aliquot (0.25 ml, containing ~5 mg of carbohydrate) was diluted to 1.0 ml with M sodium chloride and chromatographed on Bio-Gel P-300.

Further hydrolysis with 50mM sulfuric acid. — Sulfuric acid (0.5M; 4.2 ml) was added to the solution (48 ml) remaining after the treatment with 5mM sulfuric acid, and the resulting solution (pH 1.05, rising to 1.07) was heated for a further 22 h at 96°. Samples (5 ml) removed at intervals were examined as already described, except that, in the gel chromatography, 0.1-ml aliquots (containing ~2 mg of carbohydrate), diluted to 1.0 ml with M sodium chloride, were chromatographed on Bio-Gel P-10.

RESULTS AND DISCUSSION

Sugar components of hydrolyzates. — On paper chromatography (solvents *a* and *b*) of the hydrolyzate obtained after treatment of the gum with 5mM sulfuric acid for 2 h ($x = 0.04$; see Table I), the only sugars detected were arabinose (major sugar

TABLE I

PARTIAL HYDROLYSIS OF *A. elata* GUM IN 5mM SULFURIC ACID

Time of hydrolysis (h)	*Degree of scission (x)*	$[\alpha]_D^{20}$ *(degrees)*[a]	$\overline{M}_w$	$10^6 k$ *(sec^{-1})*
0		+4	19,200	
2	0.04	+8	14,900	5.8
5	0.08	+22	10,600	4.2
8	0.11	+28	9.400	2.6
12	0.14	+34	7,200	2.5
24	0.22	+41	6,000	2.3
48	0.33	+46	5,300	1.8
72	0.41	+51	4,200	1.5
96	0.46	+54	3,600	0.90
121	0.49	+56	3,500	0.67

[a]Concentration (c) = 2.17.

component), galactose (trace), and rhamnose (trace). As in the hydrolysis of *A. podalyriaefolia* gum[4], oligosaccharides were detected (in addition to these monosaccharides) only when x reached ~0.08 (after 5 h of treatment with 5mM acid in the present experiment). The proportion of oligosaccharides in the hydrolyzates rose as the time of hydrolysis of the gum in 5mM acid was increased from 5 to 121 h (x increasing to 0.49). The oligosaccharides detected by paper chromatography of these hydrolyzates in solvent *a* were similar to those found in hydrolyzates from *A. podalyriaefolia* gum[4]. In addition to the disaccharides 3- and 6-*O*-β-D-galactopyranosyl-D-galactose, three higher oligosaccharides were resolved; application of the test of Bate-Smith and Westall[9] indicated that these constituted a homologous series (3 to 5 sugar residues per molecule) of β-D-(1→3)-linked D-galactose oligosaccharides. Spots near the origin of the paper chromatograms suggested the presence of higher members of this series, but these could not be resolved.

As further hydrolysis of the degraded gum in 50mM acid progressed, it was found (by paper chromatography, solvent *a*) that these oligosaccharides gradually disappeared, the β-D-(1→6)-linked D-galactobiose persisting to a greater extent than the β-D-(1→3)-linked D-galactose oligosaccharides. After 10 h, only the monosaccharides were detectable; a pink spot appearing at this stage near the origin of the chromatogram was ascribed to the small proportion (3 mole %, by titration[3]) of uronic acid present in the gum.

Molecular-weight distributions. — The change in molecular-weight distribution with increasing degree of hydrolysis of the polysaccharide is demonstrated by the elution patterns obtained on gel chromatography of (*a*) the whole gum (see Fig. 1,A), (*b*) the products of hydrolysis in 5mM sulfuric acid (see Fig. 1, B–J), and (*c*) the products of further hydrolysis in 50mM acid (see Fig. 2, A–D). The corresponding values of the weight-average molecular weight ($\overline{M}_w$), calculated from these elution curves as described previously[4], and of the specific rotation, are shown in Tables I and II.

The elution pattern (see Fig. 1,A) given by the undegraded polysaccharide shows four peaks, as found with all samples of this gum previously examined[1,2].

TABLE II

FURTHER HYDROLYSIS OF DEGRADED GUM IN 50mM SULFURIC ACID

Time of further hydrolysis (h)	*Degree of scission (x)*	$[\alpha]_D^{20}$ *(degrees)*[a]	$\overline{M}_w$[b]	10^6k (sec^{-1})
1	0.75	+65	306	110
3	0.89	+73	244	79
6	0.95	+79	222	
10	1.00	+82	176	
22	1.00	+82	176	

[a]Concentration (c) = 1.96. [b]Relative proportions of monosaccharides were estimated from g.l.c. analysis[3].

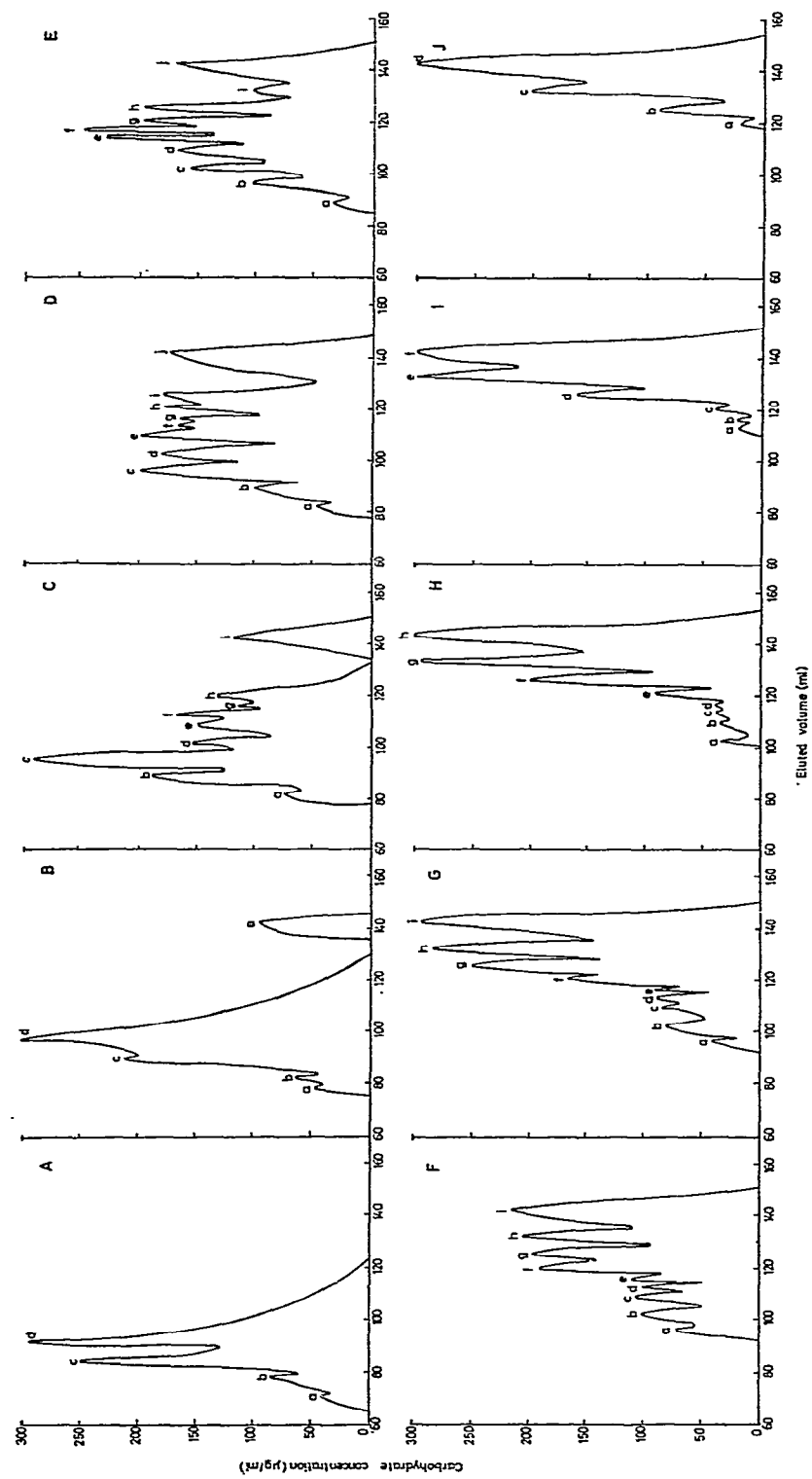

Fig. 1. Elution patterns on a column (90 × 1.5 cm) of Bio-Gel P-300 (M sodium chloride eluant; flow rate 3–4 ml/h) of (A) ethanol-precipitated *Acacia elata* gum, and (B–J) products of hydrolysis of this gum in 5mM sulfuric acid at 96°. The duration of hydrolysis and the molecular weights corresponding to peaks in each case are as follows: A. whole gum; *a* 35,500; *b* 26,500; *c* 21,000; *d* 16,000; B. 2 h; *a* 26,500; *b* 22,400; *c* 17,000; *d* 13,000; *e* ⩽1,800; C. 5 h; *a* 22,400; *b* 17,000; *c* 13,000; *d* 10,200; *e* 7,800; *f* 6,700; *g* 6,000; *h* 5,000; *i* ⩽1,800; D. 8 h; *a–h* as in C; *i* 4,200; *j* ⩽1,800; E. 12 h; *a–h* as *b–i* in D; *i* 3,200; *j* ⩽1,800; F. 24 h; *a–i* as *b–j* in E; G. 48 h; *a–i* as in F; H. 72 h; *a–h* as *b–i* in G; I. 96 h; *a–f* as *c–h* in H; J. 121 h; *a–d* as *c–f* in I.

After hydrolysis of the polysaccharide in 5mM sulfuric acid for 2 h, the elution pattern (see Fig. 1,B) retains the general shape of Fig. 1,A, except for the appearance of a small peak (*e*) caused by the sugars produced, but all four of the peaks corresponding to polysaccharide components shifted to higher elution-volumes; the corresponding decreases in molecular weight amount to ~25% for peak *a*, 15% for *b*, and 19% for both *c* and *d*. The peaks at molecular weights 22,400, 17,000, and 13,000 observed at this stage in the hydrolysis persist in several of the elution patterns obtained subsequently. This persistence applies also to the peaks at molecular weights 10,200, 7,800, 6,700, 6,000, and 5,000 first noted after hydrolysis of the gum in 5mM acid for 5 h (see Fig. 1,C), and to those corresponding to molecular weights of 4,200 and 3,200, which appear after hydrolysis for 8 h (see Fig. 1,D) and 12 h (Fig. 1,E), respectively. As the time of hydrolysis is increased further (see Fig. 1, F–J), the peaks for low molecular weights preponderate to an increasing extent over those corresponding to higher molecular weights, which gradually disappear as the hydrolysis progresses.

The striking persistence of the peaks (corresponding to certain molecular weights) in the elution patterns obtained when the course of hydrolysis of a polysaccharide by acid is followed by gel chromatography has been noted previously[4,5]. In the case of the gum polysaccharide from *Acacia podalyriaefolia*, which is known[1,3,6,7] to be similar in structure to that of *A. elata*, one of the recurrent molecular weights was found[4] to approximate closely to the value predicted for the product obtained on complete removal of the arabinofuranose end-groups from the gum, and another was in accordance with the molecular weight expected after further hydrolysis had also removed all galactopyranose residues present in the gum as end groups or in short branches. *A. elata* gum differs from that of *A. podalyriaefolia* in being polymolecular; in the present case, therefore, each of these hydrolysis steps must be expected to yield several products, which differ in molecular weight, because each originates from a different component of the whole gum. The molecular weights predicted for these products are shown in Table III; these values have been calculated

TABLE III

PREDICTED MOLECULAR WEIGHTS OF PRODUCTS OBTAINED AFTER REMOVAL OF PERIPHERAL SUGAR RESIDUES FROM POLYSACCHARIDE COMPONENTS OF *A. elata* GUM

Component of gum		*Calculated molecular weights of products*[a]	
Molecular weight[b]	*Proportion by weight*[b]	*After removal of Ara and Rha only*	*After removal of Ara, Rha, and Gal end-groups*
35,500	4	29,500	17,700
26,500	8	22,000	13,200
21,000	32	17,400	10,500
16,000	56	13,300	8,000

[a]In this range, the molecular weights found to persist in elution patterns are: 22,400, 17,000, 13,000, 10,200, and 7,800. [b]From Fig. 1, A.

from the molecular proportions, in the whole gum, of galactose end-groups (32%, from methylation analysis[7]), and of arabinose and rhamnose (17 and 3% respectively[3,7]), residues of which occur almost exclusively as end-groups in this gum[3,6,7].

It is evident from Table III that the molecular weights (22,400, 17,000, 13,000, 10,200, and 7,800) corresponding to five of the persistent peaks found in the elution patterns shown in Fig. 1 approximate closely to calculated values. Furthermore, the consistently low yield of the product having molecular weight 22,400 (see Fig. 1, B–D) is in accordance with the small proportion of the parent polysaccharide (molecular weight 26,500) in the whole gum; the high proportions of the products having molecular weights 17,000 and 13,000 observed in the early stages of hydrolysis (see Fig. 1,B,C) could be due to the fact that all four of the polysaccharide components of the gum may be expected to yield products having molecular weights of these magnitudes at one or other of the two stages represented in Table III. The occurrence and distribution, in hydrolyzates of *A. elata* gum, of the products having molecular weights 22,400, 17,000, 13,000, 10,200, and 7,800 is thus consistent with the usual, rapid cleavage, from the various polysaccharide components of the gum, of the acid-labile arabinofuranoside[10] and rhamnopyranoside[11] linkages, followed by preferential removal of terminal galactopyranose residues[12].

The other persistent peaks in Fig. 1 occur at molecular weights that are approximately half of the values corresponding to peaks at lower elution volumes (*e.g.*, the series 13,000, 6,700, and 3,200). This 2:1 molecular-weight relationship among peaks in the elution patterns of hydrolyzates from polysaccharides has been noted in several instances[4,5,13], and is discussed elsewhere[4].

On further hydrolysis, in 50mM acid, of the mixture represented by Fig. 1,J, all of the polysaccharide products rapidly disappear; after only 1 hour, the hydrolyzate consists exclusively of mono- and oligo-saccharides (1–6 sugar residues per molecule; see Fig. 2,A). As the time of hydrolysis is increased, progressive degradation of the oligosaccharides is observed (Fig. 2,B,C), until, after 10 hours, the elution curve

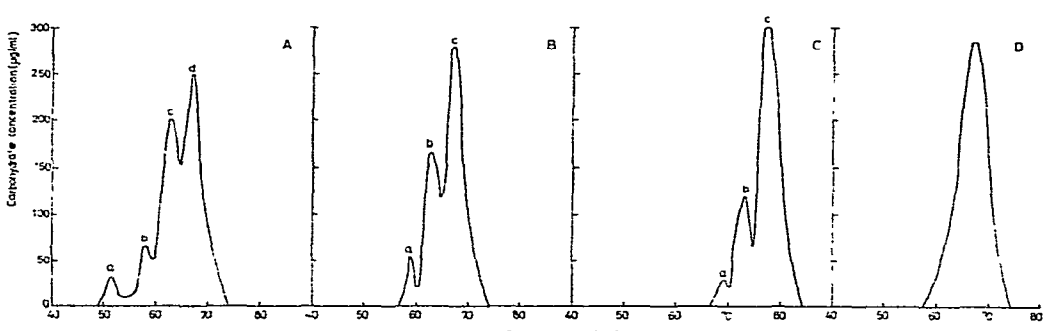

Fig. 2. Elution patterns on a column (55 × 1.2 cm) of Bio-Gel P-10 (M sodium chloride eluant; flow rate 10 ml/h) of products of further hydrolysis of partially hydrolyzed gum in 50mM sulfuric acid at 96°. A. 1 h; *a* 940; *b* 530; *c* 350; *d* ⩽250; B. 3 h; *a* 480; *b* 350; *c* ⩽250; C. 6 h *a–c* as in B; D. 10 h; ⩽250.

(Fig. 2,D) shows only one peak, at the maximum elution volume for the column, which corresponds to $\overline{M}_w \leq 250$. The slight dissymmetry of the peak may be due to the presence of an unresolved component of somewhat higher molecular weight, possibly an aldobiouronic acid (not yet isolated), in addition to the monosaccharides identified by paper chromatography.

As in the case of *A. podalyriaefolia* gum[4], the decrease in $\overline{M}_w$ with increasing degree of hydrolysis of *A. elata* gum is generally much smaller than that predicted, from the equation of Montroll and Simha[14], on the assumption of random hydrolysis of the galactopyranoside linkages in the gum. For $\overline{M}_w$, the experimentally determined values agree with those predicted on this basis at high x ($\sim$0.75) only, at which stage the remaining hydrolyzable carbohydrates are oligosaccharides (see Fig. 2) that are mainly β-D-(1→3)-linked (according to paper chromatography), so that hydrolysis may be expected to be largely random. The factors responsible for non-random degradation at higher degrees of polymerization, which have been discussed in connection with the hydrolysis of *A. podalyriaefolia* gum[4], probably affect hydrolysis of the structurally similar gum of *A. elata* to a comparable extent.

Kinetics of hydrolysis. — The mean values of the hydrolysis rate-constant, k, over the time intervals between consecutive samples are given in Tables I and II; these values of k were calculated from the corresponding values of x as described previously[4]. The decrease in k with increasing x (under given conditions of hydrolysis) that is observed here has also been noted with *A. podalyriaefolia*[4] and *Brabeium stellatifolium*[5] gums. On hydrolysis of *A. elata* gum in 5mM acid, the relative decrease in k during 96 h is of the same order of magnitude as that found for *A. podalyriaefolia* gum[4]. For these and other arabinogalactans[15], this decrease is considerably less than that expected from the ratio ^{12,16}(17:1) of the hydrolysis constants, measured under comparable conditions, for the glycosides methyl α-L-arabinofuranoside and methyl β-D-galactopyranoside, which have linkages similar to those cleft during partial hydrolysis of these polysaccharides in 5mM acid.

The values of k reported here are higher than those found on hydrolysis of *A. podalyriaefolia* gum[4] under comparable conditions. However, the average value (5.8×10^{-6} sec^{-1}, from Table I) found initially on treatment of the gum with 5mM sulfuric acid remains considerably lower than the published[16] value (260×10^{-6} sec^{-1}) of k for the hydrolysis of methyl α-L-arabinofuranoside in 5mM acid at 100°. On the other hand, the values of k found on further hydrolysis of the partially degraded polysaccharide in 50mM acid (see Table II) are of the same order of magnitude as those calculated from the half-hydrolysis times of the D-galactobiose under comparable conditions[17] [$\sim 200 \times 10^{-6}$ and $\sim 80 \times 10^{-6}$ sec^{-1} for the β-D-(1→3)- and β-D-(1→6)-linked isomers respectively]. This observation is consistent with the high proportion of disaccharides in the hydrolyzable material remaining at this stage (see Fig. 2). As hydrolysis of the more labile β-D-(1→3)-linkages between D-galactose residues reaches completion, k approaches the value for 6-*O*-β-D-galactopyranosyl-D-galactose, which is probably (on the basis of the evidence from paper chromatography) the last of the neutral oligosaccharides to disappear.

The discrepancy between the k values presented here and those obtained on similar hydrolysis of *A. podalyriaefolia* gum[4] is believed to be due mainly to some difference in the conditions of hydrolysis. In the present work, the pH was carefully controlled during hydrolysis, so that the rise noted in the earlier study[4] was prevented. The lower rate of hydrolysis and incomplete degradation of *A. podalyriaefolia* gum can probably be ascribed to this increase in pH, rather than to any real difference in behavior between the two gums, a possibility belied by the similar trends in $\overline{M}_w$ and in the elution patterns obtained on gel chromatography, during the course of acid hydrolysis.

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