Carbohydrate Research, 45 (1975) 291–298

MOLECULAR STRUCTURE OF THE POLYSACCHARIDE EXUDATE FROM *Acacia baileyana* F. Muell.*

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(Received June 16th, 1975; accepted for publication, August 6th, 1975)

ABSTRACT

Study of the molecular-weight distribution of the raw gum exuded from *Acacia baileyana* F. Muell., of partially hydrolysed material, and of products of successive Smith-degradations of the gum suggests the occurrence of sub-units having a molecular weight of ~4000. These sub-units consist of β-(1→3)-linked D-galactopyranose residues, to most of which are attached residues of L-arabinofuranose or D-galactopyranose; the bulk of the pendant groups are removed by a single Smith-degradation to give a monodisperse fragment of molecular weight 2500. Further, successive degradations by the oxidation–reduction–hydrolysis procedure yielded a galactan which contains about ten hexose residues and which is essentially linear.

INTRODUCTION

Previous studies of the gum exudates from *Acacia podalyriaefolia*[1] and *A. elata*[2] have shown that these *Acacia* polysaccharides, which have relatively low molecular weights and are essentially highly branched arabinogalactans, yield, after Smith degradation, galactose-rich polymers that are not monodisperse, but in which components having molecular weights of ~4000 or ~2000 predominate[3]. The present study of *A. baileyana* gum, aspects of which have been referred to in abstract form[4], shows more clearly the uniform nature of the product of a single Smith-degradation of the gum, even though the gum itself is polymolecular.

EXPERIMENTAL

General experimental conditions. — Techniques used in examining the products of acid hydrolysis, and in following the course of successive Smith-degradations, have been described elsewhere[1,3,5]. The solvent systems (v/v) used in paper chromatography were (*A*) ethyl acetate–pyridine–water (8:2:1), (*B*) 1-butanol–ethanol–water

*Dedicated to the memory of Dr. Hewitt G. Fletcher, Jr.

(4:1:5, upper layer), and (*C*) 1-butanol–acetic acid–water (2:1:1). Optical rotations were measured at 18° on a Perkin–Elmer 141 polarimeter, for aqueous solutions in the concentration range 0.5–1% unless otherwise specified.

Purification and properties of the gum. — Small samples of the gum were available from cut stems of the ornamental plant *Acacia baileyana* F. Muell. (Bentham's Series 2, Botryocephalae) grown locally. Small nodules were submitted to molecular-weight distribution analysis by gel-permeation chromatography[1,6–8], while bulked material was dissolved in water and precipitated by slow addition of ethanol to a concentration of ~80%. Experiments were performed upon polysaccharide that had been redissolved in water and freeze-dried. The polysaccharide had $[\alpha]_D$ +6°, equiv. wt. (by titration) 4600, and was homogeneous according to electrophoresis on cellulose acetate strips (pyridine–formate buffer, pH 3.15), though polymolecular (see below) on examination by gel-permeation chromatography; $\overline{M}_w$ was 13,500 for individual nodules of crude gum, and 16,000 for the ethanol-precipitated polysaccharide.

Methylation analysis. — *A. baileyana* polysaccharide, after methylation by the Hakomori[9] and Purdie[10] (thrice) procedures, had $[\alpha]_D$ −40° (chloroform). Gas–liquid chromatography was used to determine the methylated sugars produced on hydrolysis, after conversion into alditol acetates[11]; the results were confirmed by methanolysis and g.l.c. of the methyl glycosides[12,13].

Hydrolysis procedures. — A sample (153 mg, dry weight) of polysaccharide was heated at 100° in 0.01M trifluoroacetic acid (30 ml) for 48 h, aliquots (3 ml) being removed at intervals and examined by gel-permeation chromatography, paper chromatography, measurement of $[\alpha]_D$, and determination of reducing groups by Nelson's method[14]. The acid concentration in the solution remaining (9 ml) was adjusted to 0.1M by addition of M trifluoroacetic acid, heating was continued for 24 h, and samples (2 ml) were removed as before. Finally, the solution (2 ml) was evaporated to dryness *in vacuo*, and the residue was heated in 2M trifluoroacetic acid at 100° for 18 h before being examined as described above. The ratio of neutral sugars in an hydrolysate (2M trifluoroacetic acid, 100°, 8 h) was determined by g.l.c. analysis of the derived alditol acetates[15].

Smith degradation. — Samples of polysaccharide were oxidized by aqueous sodium metaperiodate at room temperature in the dark[3,5], the consumption of oxidant being monitored by the spectrophotometric method[16] and checked by use of buffered sodium arsenite and iodine titration[17]. Work-up of the product by addition of ethylene glycol, concentration, dialysis (Bio-Fiber 50 beaker dialyser, Bio-Rad Laboratories, Richmond, California), reduction with borohydride, dialysis again, and freeze-drying gave the reduced, oxidized polysaccharide. The course of controlled hydrolysis in 0.5M sulphuric acid was followed by periodate oxidation of small aliquots and assay[18] of the formaldehyde liberated[3,5]. Addition of barium carbonate, filtration, and concentration of the filtrate yielded the Smith-degraded products, which were extracted with methanol to give a syrupy, soluble portion and residual polysaccharide (designated *SD1*). The process was repeated three times in modified

form on the insoluble residues to give *SD2*, *SD3*, and *SD4*, together with methanol-soluble extracts; in obtaining these, the dialysis step was omitted entirely, and the periodate-oxidation step was terminated by addition of an excess of sodium borohydride, removal of sodium ions with Amberlite IR-120(H^+) resin, freeze-drying, and removal of borate by repeated distillation in the presence of methanol. The controlled acetal-hydrolysis step was effected, for preference, by cold M trifluoroacetic acid, which was finally removed by freeze-drying. The latter procedure gave up to 82% of the theoretical recovery of insoluble product, whereas considerable losses followed the use of barium carbonate. Analyses of the Smith-degradation products were made, appropriately, using paper chromatography of the products and their hydrolysates, gel-permeation chromatography (Bio-Gel P-10), and measurement of $[\alpha]_D$.

RESULTS AND DISCUSSION

Examination of hydrolysates indicates that the *A. baileyana* polysaccharide exudate contains monosaccharide residues in the relative molar proportions galactose 80, arabinose 12, glucuronic acid 4, and rhamnose 4, and, in this respect, is not unlike the gums of *A. elata*[3], *A. podalyriaefolia*[3], and *A. pycnantha*[19], each of which also has a relatively low molecular weight[3,20,21]. The usual aldobiouronic acid, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, resistant to acid hydrolysis, was detected chromatographically. The main structural units, indicated by analysis of the methylated polysaccharide, consist of (*a*) galactopyranose (30), arabinofuranose (8), and some rhamnopyranose non-reducing end-groups; (*b*) (1→6)-, (1→3)-, and (1→4)-linked galactopyranose chain-units (12); and (*c*) galactopyranose branch-points linked through C-3 and C-4 (some 40 in all). Such a polysaccharide requires, upon oxidation, a periodate consumption of ~5.6 mmol.g^{-1}, and this value was in fact found.

Molecular-weight distributions of the polysaccharide and its acid-hydrolysis products. — The results of examining the polysaccharide by partial hydrolysis with acid are shown in Tables I and II. As reported earlier[4], for three different samples of fresh, whole gum, the molecular weights corresponding to the five peaks observed in the gel-chromatographic elution pattern are 10,500 (50% by weight), 13,400 (24%), 17,800 (20%), 23,500 (4%), and 28,200 (2%). Ethanol-precipitated gum shows a similar elution pattern (Table I), but with lower proportions of the material of lower molecular weight.

The molecular-weight distribution of each hydrolysate, from which the value of $\overline{M}_w$, the weight-average molecular weight, has been calculated in cases where this average may be regarded as meaningful, has been determined from the corresponding gel-chromatogram, the relative proportions of the components being estimated from peak areas as before[1,7]. Typical gel-chromatograms are illustrated in Fig. 1. The values of the hydrolysis rate-constant, k, shown in Tables I and II have been calculated from the values of the degree of scission and the corresponding hydrolysis times, as previously described[1].

TABLE I

PARTIAL HYDROLYSIS OF *Acacia baileyana* GUM IN 0.01M ACID

Time (*h*)	*Molecular-weight distribution*[a]	$\overline{M}_w$	$[\alpha]_D$[d] (*degrees*)	*Degree of scission*[e]	10^6k (sec^{-1})
0	28,200(5); 23,500(9); 17,800(26); 13,400(33); 10,500(27)[b]	16,000	+6		
					15.3
2	25,400(2); 21,400(8); 16,200(12); 12,000(30); 9,400(37); ⩽1,800(11)[b]	13,000	+15	0.10	
					5.1
4	21,400(4); 16,200(7); 12,000(15); 9,400(22); 7,500(10); 5,800(30); ⩽1,800(12)[b]	9,600	+22	0.14	
					2.3
7	21,400(2); 16,200(7); 12,000(11); 9,400(8); 7,500(20); 5,800(21); 4,000(4); 3,500(4); 3,000(3); ⩽1,800(20)[b]	8,600	+30	0.16	
					1.5
24	16,200(3); 12,000(6); 9,400(9); 7,500(13); 5,800(13); 4,000(15); 3,500(8); 3,000(9); ⩽1,800(24)[b]	6,700	+40	0.23	
					1.4
48	9,400(4); 7,500(6); 5,800(6); 4,000(12); 3,500(9); 3,000(12); ⩽1,800(51)[b]	4,700	+51	0.43	
					1.4
72	4,000(4); 3,500(2); 3,000(2); 2,500(4); 2,000(3); 1,500(4); 1,100(12); 980(6); 800(11); 660(16); 520(23); ⩽300(13)[c]	1,500	+60	0.67	
					1.2
96	1,500(3); 1,100(6); 980(7); 800(13); 660(15); 520(32); ⩽300(24)[c]		+63	0.79	

[a]Molecular weights corresponding to peaks in gel-chromatogram; relative proportions by weight in brackets. [b]Bio-Gel P-300. [c]Bio-Gel P-10. [d]Concentration, 0.51%. [e]Calculated from reducing power as in Ref. 1.

TABLE II

FURTHER HYDROLYSIS OF DEGRADED *Acacia baileyana* GUM IN 0.1M ACID

Time of further hydrolysis (*h*)	*Molecular-weight distribution*	$[\alpha]_D$[c] (*degrees*)	*Degree of scission*	10^6k (sec^{-1})
1	1,500(2); 1,100(2); 980(5); 800(9); 660(9); 520(34); ⩽300(39)[a]	+65	0.90	
				51
3	1,500(1); 1,100(2); 980(3); 800(4); 660(5); 520(30); ⩽300(55)[a]	+69	0.92	
				48
6	800(3); 660(4); 520(16); ⩽300(77)[a]	+70	0.96	
				8
24	360(11); 180(89)[b]	+71	0.98	

[a]Bio-Gel P-10. [b]Bio-Gel P-2. [c]Concentration, 0.46%.

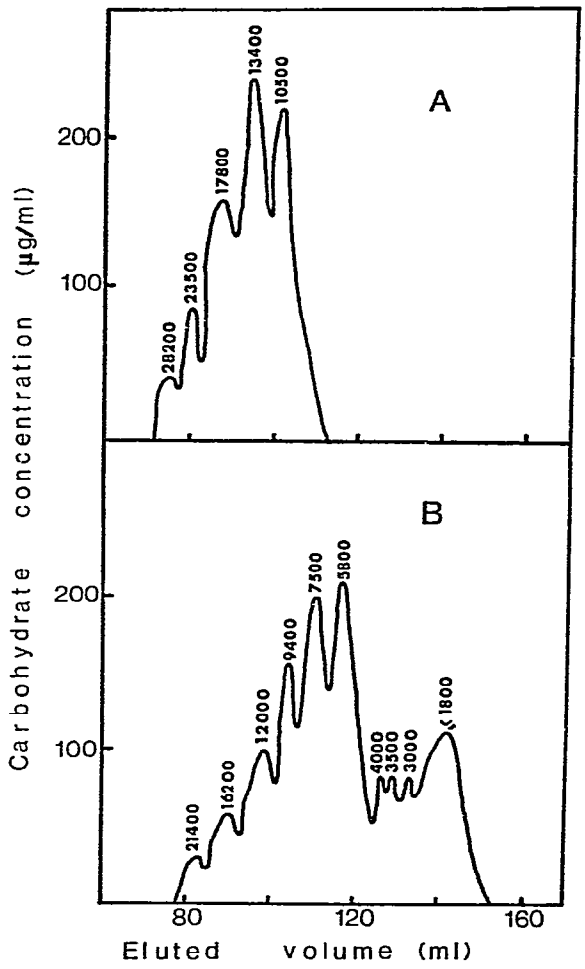

Fig. 1. Bio-Gel P-300 elution patterns of (A) ethanol-precipitated *Acacia baileyana* gum and (B) hydrolysate (0.01M trifluoroacetic acid, 100°, 7 h). Column, 90 × 1.5 cm; M sodium chloride eluent; flow rate, 3 ml.h^{-1}; sample concentration, 5 mg.ml^{-1}; carbohydrate assay by phenol–sulphuric acid method.

The general pattern of the breakdown of this polysaccharide by acid resembles that observed in similar studies of the gums of *Acacia podalyriaefolia*[1] and *A. elata*[2]. It is evident from Table III that the molecular weights corresponding to the peaks observed in the gel-chromatograms of samples removed during the early stages of mild hydrolysis with acid can be rationalised in terms of the usual trends observed on hydrolysis of arabinogalactans of this type. In the hydrolysate obtained after treatment of the gum with 0.01M acid for 2 h (Table I), the degraded polysaccharide components have molecular weights coinciding almost exactly with those predicted (Table III) for the products of complete removal of peripheral arabinose from the

gum components, and the proportion (11%) of carbohydrate of low molecular weight, shown by paper chromatography (solvent *A*) to consist almost entirely of arabinose, approximates closely to the proportion of arabinose in the gum. After mild hydrolysis of the gum with acid for 4 h, galactose and rhamnose were detected (p.c., solvent *A*) in the hydrolysate, and the gel-chromatogram showed additional peaks at molecular weights (7500 and 5800) close to those predicted (Table III) for the degraded polysaccharides formed by removal of terminal residues of those sugars, as well as the arabinose end-groups, from major components of the gum. The hydrolysis products having molecular weights of 4000 and below, which first become evident after treatment of the gum in 0.01M acid for 7 h (Fig. 1B), are probably major fragments formed on cleavage of the skeletal chains of the polysaccharide components; their molecular weights, in general, bear to the higher molecular weights observed the 1:2 relationship noted in several previous studies[1,2,5,22]. The molecular weights of the various degraded polysaccharides formed as hydrolysis of the gum proceeds cannot be expressed as multiples of any repeating unit, possibly because the periodicity that gives rise to such relationships is confined to linkages that are labile to acid only after oxidation and reduction of the periodate-vulnerable chain units involved (see below).

TABLE III

MOLECULAR WEIGHTS PREDICTED FOR PRODUCTS AFTER REMOVAL OF PERIPHERAL SUGAR RESIDUES FROM *Acacia baileyana* GUM COMPONENTS

Mol. wt. of gum component	*Calculated mol. wts. of products*[a]	
	After loss of arabinose end-groups	*After loss of all neutral end-groups*
28,200	25,400	16,000
23,500	21,200	13,300
17,800*	16,000	10,000
13,400*	12,100	7,600
10,500*	9,400	5,900

[a]Molecular weights in this range corresponding to peaks in gel-chromatograms: 25,400; 21,400; 16,200; 12,000*; 9,400*; 7,500*; 5,800*. Asterisks denote major peaks.

In the later stages of hydrolysis of *A. baileyana* gum in 0.01M acid, the hydrolysate becomes rich in oligosaccharides (see Table I). On further hydrolysis of the degraded gum in 0.1M acid, the higher oligosaccharides rapidly disappear (see Table II) until, after 24 h, the elution pattern (Bio-Gel P-2) shows only an aldobiouronic acid in addition to the monosaccharides (p.c., solvents *A* and *C*). The final hydrolysis step, in 2M trifluoroacetic acid, gives a product, $[\alpha]_D$ +76°, containing only monosaccharides (in their usual configuration). These observations from gel-chromatograms of the hydrolysates have been confirmed by paper chromatography (solvents *A* and *C*), which has demonstrated the presence, in addition to aldobiouronic acid, of two neutral disaccharides and a mixture of higher oligosaccharides. The slope of the plot obtained on application of the test of Bate-Smith and Westall[23]

indicates the presence (p.c., solvent *C*) of a series of compounds containing mainly β-(1→3)-linked D-galactopyranose residues, though other linkages must also be present.

Comparison of the hydrolysis rate-constants given in Table I with those obtained for the gums of *Acacia podalyriaefolia*[1] and *A. elata*[2] suggests that *A. baileyana* gum is more labile to mild hydrolysis with acid than are the other two; this is indicated also by the more rapid rise in $[\alpha]_D$ and fall in $\overline{M}_w$, and reflects a higher proportion of acid-labile (1→3)-linkages in the *A. baileyana* gum polysaccharide.

Products of Smith degradations, and their significance. — The first Smith-degradation sequence, which was performed in triplicate, gave the methanol-insoluble *SD1* (53% of total product). Apart from 1% by weight of a component of molecular weight 550, *SD1* consisted entirely of material, $[\alpha]_D$ +15°, having a molecular weight of 2500. Further treatment of a sample with acid at room temperature produced no detectable change in molecular weight and negligible release of glycolaldehyde (diphenylamine assay[24]), showing that acetal cleavage was complete. Complete hydrolysis of a sample gave galactose and small proportions of arabinose and possibly glycerol and threitol, and methylation analysis indicated the main linkages to be (1→3). The methanol-soluble material contained glycerol (27% by weight), glycolaldehyde that had escaped volatilisation, and a trace of what appeared on paper chromatography to be threitol. It is clear, therefore, that whatever the degree of polymerisation of the components of the native gum, the material of molecular weight 2500 comprises a block of periodate-resistant sugar residues which becomes detached in the degradation procedure. Allowing for one polyol end-group, the data give a value of 2400 for the molecular weight of the carbohydrate portion of *SD1*, and, from the number of end-groups removed by the degradation procedure, the corresponding unit in the whole gum has a molecular weight of 4600. It will be noted that the molecular weights predominating in the gum itself are, to a close approximation, multiples (2, 3, 4, 5, and 6) of this quantity, which also coincides with the value found for the equivalent weight of the gum.

Periodate oxidation of *SD1* (oxidant consumed in 48 h, 2.6 mmol.g^{-1}), followed by reduction and hydrolysis, yielded methanol-insoluble *SD2*, $[\alpha]_D$ +32°, which gave a single peak on gel-permeation chromatography corresponding to molecular weight 2100, and contained galactose residues. The methanol-soluble extract consisted mainly of glycerol, with traces of glycosides of galactose. The fall in molecular weight of the insoluble portion corresponds to the loss of two end-groups from *SD1*; the oxidant consumption, however, indicates three.

Similar treatment of *SD2* (periodate consumed, 2.6 mmol.g^{-1}) yielded *SD3*, which had $[\alpha]_D$ +34°, a molecular weight of 1800 (single peak), and contained residues of galactose as the only detectable sugar together with the expected low proportion of polyol. The periodate consumed (1.4 mmol.g^{-1}) by *SD3* was just over that expected for the removal of non-reducing end-group only, as was the drop in molecular weight (to 1600, single peak) on Smith degradation to *SD4*, which had $[\alpha]_D$ +36°.

This shows that, after the first Smith-degradation had cleaved the skeletal structure of the polysaccharide at regular intervals and removed most of the peripheral sugars, two further Smith-degradations were adequate to remove the short chains remaining. The resulting, linear β-(1→3)-linked D-galactopyranose chains, terminated by polyol, contain ~10 residues.

These results suggest a molecular structure for the *A. baileyana* gum polysaccharide in which the main chain, to which are attached numerous branches each comprising a single residue in most cases, consists of blocks of approximately 12 periodate-resistant galactose residues [(1→3)-linked], separated by periodate-vulnerable galactose residues. This structure possesses a remarkably high degree of uniformity, in comparison even with the other *Acacia* gums of low molecular-weight which have shown some indication of being built-up of relatively simple sub-units[3].

ACKNOWLEDGMENTS

The assistance of Mr. E. H. Merrifield and Mrs. J. C. Timm, and financial support from the South African Council for Scientific and Industrial Research and the University of Cape Town (Staff Research Fund) are gratefully acknowledged. Mrs. J. McCarthy provided the plant specimens, which were classified by Dr. A. V. Hall (Bolus Herbarium, University of Cape Town).

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