Carbohydrate Research, 123 (1983) 267–279
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SOME NEW ASPECTS OF THE MOLECULAR STRUCTURE OF *Acacia senegal* GUM (GUM ARABIC)

SHIRLEY C. CHURMS, EDWIN H. MERRIFIELD*, AND ALISTAIR M. STEPHEN
CSIR Carbohydrate Research Unit, Department of Organic Chemistry, University of Cape Town, Rondebosch 7700 (South Africa)

(Received May 26th, 1983; accepted for publication, June 21st, 1983)

ABSTRACT

The gum polysaccharide of *Acacia senegal*, the main source of gum arabic, has been re-examined by means of two series of sequential Smith degradations, one starting with the whole polysaccharide, the other with a product from which all acid-labile side-chains had been removed by prior partial hydrolysis. Investigation, mainly by methylation analysis and estimation of molecular weight, of the products obtained at each stage of these two series of degradations, both of which ultimately yielded small galactans that appeared to be identical, has afforded evidence for the presence in the polysaccharide chain of uniform blocks of (1→3)-linked D-galactopyranosyl residues; these blocks are comparable in size to those postulated for many arabinogalactans of simpler structure. Some amplification of the structural model proposed for this polysaccharide by earlier workers is possible in the light of these new data.

INTRODUCTION

From studies involving sequential Smith degradations[1], a large body of evidence has been accumulated for the presence of regularly repeating, uniform blocks of (1→3)-linked D-galactopyranosyl residues in the skeletal chains of arabinogalactans from the gum exudates of a number of *Acacia* species[2–8], some *Prosopis* gums[9], and arabinogalactans from other sources, such as larch heartwood[10,11] and rapeseed[12]. The *Acacia* gums examined to date have been those from species included in Series 1 (Phyllodineae)[3,4,6–8] or Series 2 (Botryocephalae)[2,3,5] of the standard classification of the genus by Bentham[13]. In extension of the scope of this investigation, gum polysaccharides from Series 4 (Gummiferae) and 5 (Vulgares) are currently under examination; these have, in general, higher average molecular weight and greater complexity of molecular structure than those from the lower Series[14,15].

*Present address: Department of Clinical Science and Immunology, University of Cape Town, South Africa.

0008-6215/83/$ 03.00

The gum exudate from *Acacia senegal* (Series 5), the major source of the commercially important gum arabic, has been extensively investigated by other workers[16–25], and a model representing a possible structural fragment, based on a study that included a series of seven successive Smith degradations, has been proposed by D. M. W. Anderson *et al.*[20]. This suggested a highly complex molecule, and there was no indication from the data then available of any uniformity in the galactan core or whether the structural model proposed could be regarded as representing a regularly repeating sub-unit in the polysaccharide. However, it was conceded by these authors[20] that it was unlikely that complete reaction had been achieved at every stage in the stepwise Smith degradation scheme, and detailed investigation of this degradation procedure in our laboratory[26,27] has confirmed that, under the conditions used by Anderson *et al.*[20], cleavage of acetal linkages in the reduced, oxidized polysaccharide was, indeed, probably incomplete.

It was therefore decided to re-examine *A. senegal* gum by the modified Smith-degradation procedure, monitored by molecular-weight distribution analysis during the crucial, mild-hydrolysis step, that has been adopted by the present authors[3]. This technique has been applied both to the whole polysaccharide and to a sample from which acid-labile side-chains had been removed by partial hydrolysis with acid, with the objectives of gaining further information about the galactan core and ascertaining the probability of the occurrence of regularly repeating sub-units in the molecule.

EXPERIMENTAL

Purification of gum sample. — The sample used, collected in Kordofan Province, Sudan, was donated by courtesy of Foskor, Ltd, Phalaborwa, Transvaal, South Africa. The gum polysaccharide, isolated by precipitation with ethanol (4 vol.) from a filtered, aqueous solution (5% w/v) of the crude gum (31 g), was purified by dialysis, and the product (23 g) was recovered by freeze-drying.

General experimental conditions. — The solvent systems used in paper chromatography were (v/v): *A*, 8:2:1 ethyl acetate–pyridine–water; *B*, 4:1:5 (upper phase) 1-butanol–ethanol–water; and *C*, 2:1:1 1-butanol–acetic acid–water. Sugars and polyols were detected in p.c. by use of the *p*-anisidine hydrochloride and ammoniacal silver nitrate spray-reagents. The proportions of neutral sugars, and of uronic acid, constituting the gum polysaccharide and products of degradation were determined as previously described[9]. In methylation analysis, permethylation by the Hakomori method[28,29] was followed by hydrolysis with 2M trifluoroacetic acid for 18 h at 100° under nitrogen in a sealed tube, and g.l.c. analysis of the resulting mixture of methylated sugars, as the alditol acetate derivatives[30], on 3% of OV-225 on Chromosorb W-HP (80–100 mesh) at 170°. Base degradation of a sample of the methylated polysaccharide was performed as already desribed[31].

Estimation of molecular weights. — Values of the average molecular weight ($\bar{M}_w$) of the whole polysaccharide, the partially hydrolyzed polysaccharide, and the

TABLE I

SALIENT FEATURES OF SEQUENCES OF SMITH DEGRADATIONS PERFORMED ON *A. senegal* GUM POLYSACCHARIDE AND ON PARTIALLY HYDROLYZED POLYSACCHARIDE

	Starting material					
	Whole polysaccharide				*Hydrolyzed polysaccharide*	
	Smith degradation					
	1	*2*	*3*	*4*	*1*	*2*
Mass of sample (g)	5	0.57	0.10	0.018	1	0.06
Duration of oxidation[a] (h)[b]	117(48)	50(24)	72(48)	72	92(48)	70
Yield of reduced, oxidized polysaccharide (g)	1.1	0.32	0.04	0.011	0.60	0.039
Duration of treatment with acid[c] (h)[d]	96(72)	48(24)	72(48)	48	96(72)	96
Yields of fractions (g)						
Insoluble	0.70[e]	0.134[e]	0.028[f]	0.006[f]	0.20[f]	0.022[f]
Soluble	0.20[e]	0.076[e]	0.005[f]	0.001[f]	0.34[f]	0.006[f]

[a]In 0.12M $NaIO_4$. [b]In parentheses, time after which no further IO_4^- was consumed. [c]M Trifluoroacetic acid at room temperature (22°). [d]In parentheses, time after which no further change in molecular-weight distribution was observed. [e]On fractionation in 1:1 (v/v) methanol–acetone. [f]On fractionation in 1:2 (v/v) methanol–acetone.

products of Smith degradation of each were estimated by steric-exclusion chromatography (in earlier papers, the term gel-permeation chromatography had been used for this procedure, but "steric-exclusion chromatography" is now generally preferred[32]) on Sepharose 4B, Bio-Gel P-300, or Bio-Gel P-10, as appropriate, with M sodium chloride as the eluant, as previously described[33]. Some of these molecular weights were also estimated from the sedimentation and diffusion coefficients of the polysaccharides in 0.2M sodium chloride, determined by courtesy of Dr. A. Polson (Department of Biochemistry, University of Stellenbosch, South Africa) by methods described earlier[34].

Further evidence regarding the molecular weight of the structurally significant fragment obtained after one Smith degradation of the partially hydrolyzed polysaccharide was obtained, by courtesy of Dr. A. Dell (Department of Biochemistry, Imperial College of Science and Technology, London, U.K.), by fast-atom bombardment (f.a.b.)–mass spectrometry of the peracetylated derivative in an instrument fitted with a high-field magnet[35].

Partial hydrolysis of gum polysaccharide with acid. — A sample (10 g) of the purified polysaccharide was heated in 25mM sulfuric acid (100 mL) for 12 h at 100°, the course of hydrolysis being monitored polarimetrically: the specific rotation, initially −26°, became positive after hydrolysis had proceeded for 5 h, and reached its equilibrium value of +38° after 9 h. The acid was neutralized ($BaCO_3$), and the degraded polysaccharide was recovered from the centrifugate by precipitation with ethanol (4 vol.). The precipitate was redissolved in water, and the solution was

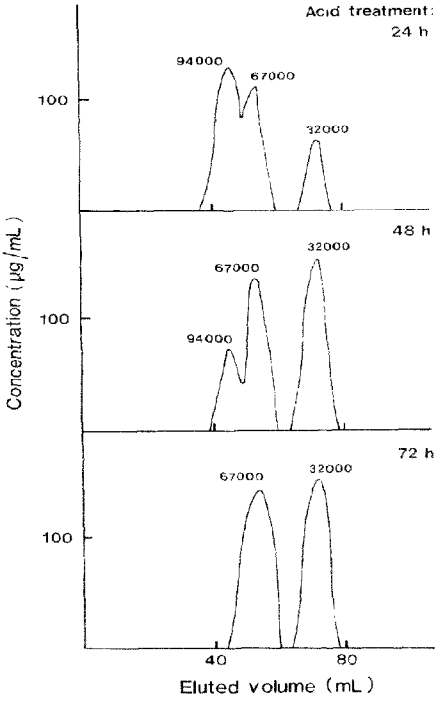


Fig. 1. Chromatography on Bio-Gel P-300 column (90 × 1.5 cm) of samples removed during mild acid hydrolysis in first Smith degradation of whole polysaccharide from *A. senegal* gum.

freeze-dried, to yield polysaccharide A, which was found to contain residues of D-galactose and uronic acid only. A portion of the centrifugate was concentrated, and examined by p.c. (solvents *A* and *C*): L-arabinose was the major component, with L-rhamnose and neutral disaccharides present in small proportions, but no free D-galactose or aldobiouronic acids were detected.

Smith degradation of whole polysaccharide and polysaccharide A. — A series of four successive Smith degradations was performed with the whole polysaccharide as starting material, whereas polysaccharide A was submitted to two sequential Smith degradations. The experimental conditions are summarized in Table I. In the first Smith degradation in each case, the consumption of periodate was monitored by the standard, arsenite method[40]; thereafter, this technique was

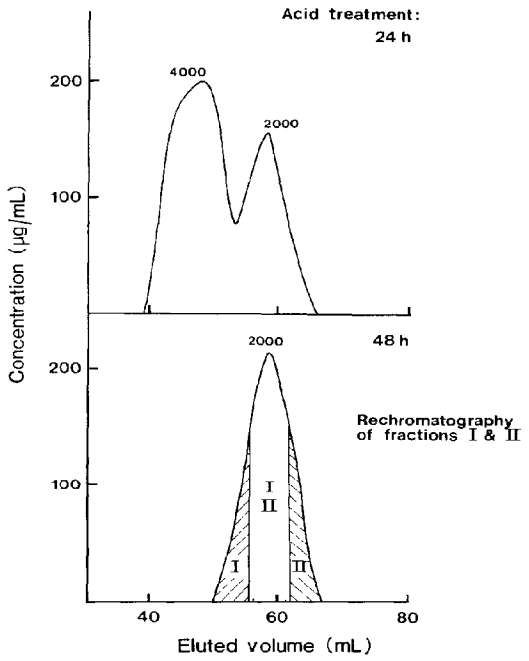


Fig. 2. Chromatography on Bio-Gel P-10 column (52 × 1.5 cm) of samples removed during mild, acid hydrolysis in third Smith degradation of *A. senegal* gum polysaccharide. Lower curve was given by both SD3 and SD1A.

modified by the use of graduated syringes to permit titration of small aliquots (100 μL, or less), as reported previously[9,12]. Reduction of the oxidized, whole polysaccharide with sodium borohydride was terminated by addition of cation-exchange resin (H^+ form), but, in all other instances, this was achieved by acidification of the solution with acetic acid, which was then removed, as fast as possible, by freeze-drying. In most of these Smith degradations, hydrolysis of the reduced, oxidized material with acid at room temperature was monitored by steric-exclusion chromatography of samples removed at intervals (see, for example, Figs. 1 and 2), and the treatment with acid was continued for 24 h after the final distribution of molecular weight had been reached.

After removal of the acid by freeze-drying, fractionation as indicated in Table I gave the products SD1-SD4 (from the whole polysaccharide) and SD1A and SD2A (from polysaccharide A), together with soluble syrups that were found

(p.c., solvents *A* and *B*) to consist mainly of glycerol. Only in the first Smith degradation of the whole polysaccharide was any sugar detected in the soluble fraction; in this experiment, arabinose (~10%, by weight, of the soluble fraction) was found to have been released.

RESULTS AND DISCUSSION

The analytical data for the sample of *A. senegal* gum used (see Table II) are comparable with those published by D. M. W. Anderson and co-workers[15,19,20,23]. The wide variation in the values of $\bar{M}_w$ as determined by the two different methods is indicative of a broad distribution of molecular weights among the polysaccharide components of this gum (suggested also by the behavior of the gum on ultracentrifugation). This polymolecularity had been noted by Anderson *et al.*[19,23], who observed considerable differences in $\bar{M}_w$ among samples of the gum from different sources[2,3]. The value of 560,000 given by steric-exclusion chromatography in the present work is, however, close to that calculated from light-scattering experiments by Anderson *et al.*[21] (580,000), which was considered[23] to be a fairly representative value for this gum.

TABLE II

ANALYTICAL DATA FOR WHOLE POLYSACCHARIDE FROM *Acacia senegal* GUM AND FOR POLYSACCHARIDE A

	Whole polysaccharide	*Polysaccharide A*
$[\alpha]_D$ (degrees)	−26	−7
M_w	560,000[a]	260,000[a]
	340,000[b]	200,000[b]
Periodate consumption (mmol.g^{-1})	5.7	6.7
Equivalent weight	1430	840
Hence, uronic acid (mol %)	12	21
Proportions of neutral sugars (mol %)		
Galactose	44	79
Arabinose	35	0[e]
Rhamnose	9	0
Methylation analysis		
Proportions[c] of methylated sugars[d] (mol %)		
2,3,4-Rha	9	—
2,3,5-Ara	17	—
2,5-Ara	18	—
2,3,4,6-Gal	12	9
2,4,6-Gal	trace	17
2,3,4-Gal	—	30
2,4-Gal	20	23
2-Gal	12	—

[a]Sepharose 4B. [b]From sedimentation and diffusion coefficients. [c]Proportions adjusted to allow for uronic acid content. [d]Identities of methyl ethers verified by mass spectrometry

The degraded polysaccharide (A) obtained after partial hydrolysis of the gum with acid was found on analysis (see Table II) to be similar in some respects, although not in others, to the product isolated by Anderson and Stoddart[19,20] after autohydrolysis of *A. senegal* gum at pH 2.8 for 50 h. The most striking difference lies in the values found for the molecular-weight averages: the degraded polysaccharide prepared by Anderson and Stoddart was reported[19] to have $\bar{M}_n$ 4800 (according to end-group analysis by the periodate method), whereas, in the present work, neither steric-exclusion chromatography nor ultracentrifugation indicated the presence, in any appreciable proportion, of fragments as small as this. The two samples are similar in uronic acid content, but the persistence of a small proportion of L-arabinosyl residues in the polysaccharide molecule after autohydrolysis, reported by the earlier workers[19,20], was not observed with polysaccharide A. Except in this respect, the two samples gave similar results on methylation analysis, with 2,4,6- and 2,3,4-tri- and 2,4-di-*O*-methyl-D-galactose constituting major components of the mixture of methylated sugars obtained on hydrolysis of both of the permethylated polysaccharides. Paper chromatography (solvent *C*) of a hydrolyzate of polysaccharide A indicated the presence of the same two aldobiouronic acids, *viz.*, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (major component) and 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose (trace), as were yielded by the autohydrolyzed gum sample[19].

The attachment of the D-glucosyluronic acid groups to O-6 of D-galactosyl residues in *A. senegal* gum was further indicated by degradation of a sample of the permethylated derivative of the whole polysaccharide with sodium methylsulfinylmethanide[37,38]. As reported by Aspinall and Rosell[38], the removal of uronic acid residues by this means results in an increase in the proportion of galactosyl O-6 atoms available for substitution. The appearance of 2,6-di-*O*-methyl-D-galactose in the products of hydrolysis of the remethylated, base-degraded polysaccharide, accompanied by disappearance of 2-*O*-methyl-D-galactose and an increase in the proportion of 2,4,6-tri-*O*-methyl-D-galactose, was noted. The loss of all of the 2,3,4-tri-*O*-methyl-L-rhamnose from the permethylated polysaccharide after base degradation is consistent with the attachment of (terminal) L-rhamnosyl groups to O-4 of D-glucuronic acid in the gum polysaccharide, as shown by Aspinall *et al.*[17,38].

It is only in the molecular size and composition of the products obtained after successive Smith degradations of the gum polysaccharide that the results of the present investigation differ radically from those of earlier workers. After a Smith-degradation procedure in which mild hydrolysis of the reduced, oxidized polysaccharide was allowed to proceed for only 48 h, Anderson and co-workers[20,24] obtained a product having $[\alpha]_D$ −28°, $\bar{M}_n$ ~96,000 (according to steric-exclusion chromatography), and containing some uronic acid (4%) in addition to D-galactosyl (69%) and L-arabinosyl (27%) residues. The analytical data for the SD1 product in the present work (see Table III) show major differences, notably in the positive specific rotation and the much lower value of $\bar{M}_w$, and in the absence of uronic

TABLE III

ANALYTICAL DATA FOR SMITH-DEGRADATION PRODUCTS SD1–SD4 FROM WHOLE POLYSACCHARIDE

	SD1	*SD2*	*SD3*	*SD4*
$[\alpha]_D$ (degrees)	+4	+16	+28	+30
Molecular-weight distribution[a]	67,000(51) 32,000(49)	30,000(53) 16,000(47)	2000[c]	1500[c]
M_n	50,000[b] 69,000[b]	24,000[b]	2000[b]	1500[b]
Periodate consumption (mmol g^{-1})	4.3	3.6	2.1	
Sugar composition (mol %)				
Galactose	75	97	100	100
Arabinose	25	3		
Methylation analysis[d]				
2,3,5-Ara	16	3	–	–
2,5-Ara	3	–		
2,3,4,6-Gal	13	27	17	22
2,4,6-Gal	32	33	64	67
2,3,4-Gal	–	5		
2,4-Gal	36	32	19	11

[a]Bio-Gel P-300, molecular weights corresponding to peaks are followed, in parentheses, by proportions by weight. [b]Bio-Gel P-10, single, sharp peak. [c]From sedimentation and diffusion coefficients. [d]Molar proportions of methylated sugars, identity verified by mass spectrometry.

acid residues in the degraded polysaccharide. The $\bar{M}_n$ of SD1 was verified by the sedimentation-diffusion method, which gave a value deviating by only 20% from that estimated from steric-exclusion chromatography. It is evident (see Fig. 1) that degradation is not complete when the acid treatment of the reduced, oxidized polysaccharide has proceeded for only 48 h. The final molecular-weight distribution was observed after mild hydrolysis with acid had been continued for 72 h: the gel-chromatogram indicated the presence of two components, one having approximately twice the molecular weight of the other, in equal proportions by weight, so that the molar ratio of the larger to the smaller was 1:2. The pattern obtained on ultracentrifugation confirmed that SD1 was not monodisperse. It must be noted that the release of some arabinose, as such, shows that a few 1-arabinofuranoside linkages were broken in the acid-hydrolysis step to yield SD1, exposing interior sugar residues in the process, with consequences observed at later stages of Smith degradation.

The product, SD2, of the second Smith degradation in this series was also found to contain more than one component: chromatography again indicated the presence of two, each approximately half the size of a component of SD1, the molar ratio remaining at 1:2. The third Smith degradation, in contrast, gave a product (SD3) that appeared to be monodisperse (see Fig. 2) and was of much lower molecular weight, this being consistent with cleavage of several residues in the main skeletal chain.

TABLE IV

ANALYTICAL DATA FOR SMITH-DEGRADATION PRODUCTS SD1A AND SD2A FROM POLYSACCHARIDE A

	SD1A	*SD2A*
$[\alpha]_D$ (degrees)	+26	+30
Molecular weight	2000[a]	1500[a]
	2500[b]	
	2199[c]	
Periodate consumption ($mmol.g^{-1}$)	2.8	
Methylation analysis[d]		
2,3,4,6-Gal	16	22
2,4,6-Gal	66	67
2,4-Gal	18	11

[a]Bio-Gel P-10; single, sharp peak. [b]From sedimentation and diffusion coefficients; sharp peak observed on ultracentrifugation. [c]Calculated from *m/z* value for molecular ion of peracetylated derivative (f.a.b.–m.s.). [d]Molar proportions of methylated sugars; identity verified by mass spectrometry.

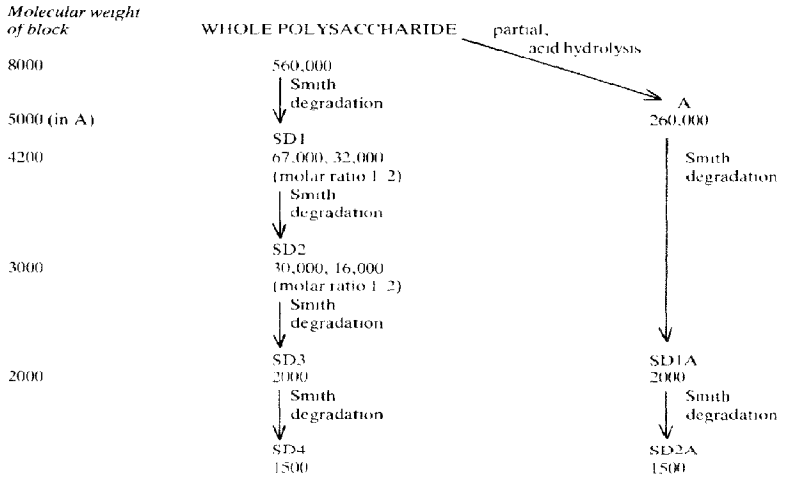
That SD3 may be a fragment representative of a repeating block in the galactan core of the *A. senegal* gum polysaccharide is strongly suggested by the fact that polysaccharide A yields, after only one Smith degradation, a product (SD1A) that is closely similar to SD3 (*cf.*, Tables III and IV). Because, in polysaccharide A, most of the side chains attached to the galactan core in the whole polysaccharide have been removed by partial acid-hydrolysis, and the acid-resistant aldobiouronic acid groups remaining will be destroyed by Smith degradation, it is to be expected that the galactan core will be exposed more easily by this route.

The product, SD1A, which, like SD3, gives a single, sharp peak at molecular weight ~2000, has also been examined by other methods of molecular-size estimation. The monodispersity indicated by the superposable peaks obtained on re-chromatography of fractions corresponding to the leading and trailing edges of the gel chromatogram (see Fig. 2) has been verified by ultracentrifugation: use of a synthetic-boundary cell permitted the visibilization of the sedimentation pattern, which showed a sharp peak even after sedimentation (at 58,000 r.p.m.) for 96 min. The value given for the molecular weight of SD1A by calculation from the measured values of its sedimentation and diffusion coefficients is 2500, so that the mean of this and the value from steric-exclusion chromatography is 2250, close to that expected for a molecule containing 13 D-galactopyranosyl residues, with a glycerolyl end-group at the originally reducing end. The presence of such a molecule was also indicated by f.a.b.–mass spectrometry (see Table IV).

The results of methylation analyses of the products SD3 and SD1A showed that both are composed very largely of (1→3)-linked D-galactopyranosyl residues. These data and the amounts of periodate consumed by the two galactans indicated the presence of branches attached to two of these residues, so that further Smith degradation should result in the loss of three end-groups. The decrease in molecular weight is consistent with this concept. Methylation analysis of the products SD4

and SD2A, which appear to be identical (*cf.*, Tables III and IV), suggests that only one of these branches in SD3 and SD1A survives Smith degradation.

Conclusions regarding the molecular sizes of the products of Smith degradation of the gum polysaccharide and its partially hydrolyzed derivative are summarized in Scheme 1. The main conclusions that may be drawn from the results presented here are as follows.



Scheme 1 Chart showing molecular-weight values for polysaccharides and products of degradation, as described in the text.

(*i*) The molecular structure of the *A. senegal* gum polysaccharide, although complex, appears to have a higher degree of regularity than could be concluded from previous work[20]. The molecular weight of the apparently monodisperse products SD3 and SD1A suggests that the galactan core of this polysaccharide consists of regularly repeating blocks, each containing ~13 D-galactopyranosyl residues, a size postulated for such structural units in a number of other arabinogalactans of somewhat simpler structure[2-4,8,10,11,27].

(*ii*) From the molecular weight of SD3 and the proportion of periodate-vulnerable sugars in SD2 (35%, from methylation analysis), the molecular weight of the repeating block in the latter may be estimated at ~3000, and a similar calculation, based on the methylation analysis of SD1 (29% periodate-vulnerable sugars),

predicts a value of ~4200 for the molecular weight of the blocks in that polysaccharide. The repeating sub-unit in the molecular structure of the whole polysaccharide (from which 50% of the constituent residues will be removed by the first Smith degradation) will thus have a molecular weight of ~8000. By way of comparison, the structural fragment suggested[20] as a possibility for *A. senegal* gum would have a molecular weight close to this value.

(*iii*) The observed value of $\bar{M}_w$ for the intact polysaccharide suggests that each molecule contains, on average, 64 of these sub-units; these are separated from one another by sugar residues so linked that they become vulnerable to attack by periodate after removal of protecting branches, either by partial acid-hydrolysis or by Smith degradation. In the former case (polysaccharide A), the blocks (molecular weight ~5000 after removal of acid-labile residues) were completely separated after only one Smith degradation, which removed all periodate-vulnerable sugars (60% of total; see Table II). Complete division of the galactan core into uniform, small fragments required three Smith degradations of the whole polysaccharide, but some cleavage of residues in the main skeletal chain during the first Smith degradation was evident from the tenfold decrease in $\bar{M}_w$ that resulted. The presence in SD1 of two polysaccharide components, having molecular weights of 67,000 (corresponding to 16 of the blocks having molecular weight 4200) and 32,000 (8 blocks), in the molar ratio of 1:2, indicates that periodate-vulnerable residues that are not protected by branching occur at intervals of 16 blocks in two instances, and of 8 blocks in four, in the galactan core of the intact polysaccharide. This is evidently another aspect in which the molecular structure is remarkably regular: a more random distribution of such periodate-vulnerable residues would have resulted in greater polymolecularity in SD1. The larger (L) and smaller (S) periodate-resistant sections of the molecule may be organized in a number of different ways, including 5 that confer symmetry, as indicated next. I, S L S SL S; II, S S L S S L; III, L S S L S S; IV, L S S S S L; V, S S L L S S, where L = a group of 16 sub-units, and S = a group of 8 sub-units. Each sub-unit has a molecular weight of ~8000.

(*iv*) The two polysaccharide components of SD2 respectively appear to contain 8 and 4 of the blocks that constitute the degraded polysaccharide at this stage. The molar ratio remains 1:2, which suggests that each is derived from a component of SD1 by cleavage of a residue in the middle of the molecule. These particular residues evidently lose their protecting branches during the first Smith degradation: this may indicate that these are shorter than other protecting branches, or it may be a consequence of the cleavage of some L-arabinofuranosidic linkages during the extended period of exposure to acid that was necessary in order to ensure complete hydrolysis of acetal linkages in that degradation process.

The size of the fragment represented by SD3 and SD1A suggests that the galactan core of this repeating sub-unit in the *A. senegal* gum polysaccharide contains more (1→3)-linked D-galactopyranosyl residues than postulated by Anderson *et al.*[20] in their structural model. The present results corroborate the suggested oc-

currence, in the galactan core, of one long branch in each sub-unit, evidence for this being afforded by the methylation analyses of SD4 and SD2A, which indicate the survival of one branch-point even at this stage, when all side-chains attached to the galactan core have been removed. As stated by Anderson *et al.*[20], the low viscosity of solutions of *A. senegal* gum, as compared to those of solutions of equal concentration containing polysaccharides having similar $\bar{M}_w$ but substantially linear structures, is also commensurate with a galactan core branched in this manner. Whether the sub-units are joined to one another linearly, to form a "backbone" to the polysaccharide, or are randomly disposed, remains a matter for conjecture.

What has emerged with some clarity from the present study is the strong evidence for the occurrence of uniform sub-units in the molecular structure of the *A. senegal* gum polysaccharide. The presence of sub-units having a molecular weight of ~8000 has also been postulated for some of the simpler polysaccharides, of lower $\bar{M}_w$, found in the gum exudates from *Acacia* species of the Bentham Series 1, such as *A. mabellae*[4] and *A. implexa*[5]. That the gum polysaccharide of *A. senegal* (Series 5) appears to resemble, in this respect, these polysaccharides from a lower Series serves to emphasize the increasing body of evidence favoring the concept that any arabinogalactan of this general type, irrespective of its molecular complexity, can be regarded as being composed of uniform sub-units.

ACKNOWLEDGMENTS

The financial support of the South African Council for Scientific and Industrial Research and of the University of Cape Town is gratefully acknowledged. The authors thank Mr. E. H. Roux (Foskor, Ltd.) for providing the sample of gum used in these studies, Dr. A. Polson (University of Stellenbosch) for performing the ultracentrifugation and the measurements of diffusion coefficients, Dr. Anne Dell (Imperial College of Science and Technology, U.K.) for valuable information from f.a.b. mass spectrometry, and the Misses F. Dijkstra, A.-L. Wiid, and K. M. Ketelbey for laboratory assistance.

REFERENCES

1 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS AND F. SMITH, *Methods Carbohydr. Chem.*, 5 (1965) 361–370.
2 S. C. CHURMS AND A. M. STEPHEN, *Carbohydr. Res.*, 45 (1975) 291–298.
3 S. C. CHURMS, E. H. MERRIFIELD AND A. M. STEPHEN, *Carbohydr. Res.*, 55 (1977) 3–10.
4 S. C. CHURMS, E. H. MERRIFIELD AND A. M. STEPHEN, *Carbohydr. Res.*, 63 (1978) 337–341.
5 S. C. CHURMS, E. H. MERRIFIELD, A. M. STEPHEN AND E. W. STEPHEN, *S. Afr. J. Chem.*, 31 (1978) 115–116.
6 S. C. CHURMS, E. H. MERRIFIELD, C. E. MILLER AND A. M. STEPHEN, *S. Afr. J. Chem.*, 32 (1979) 103–106.
7 S. C. CHURMS, E. H. MERRIFIELD AND A. M. STEPHEN, *S. Afr. J. Chem.*, 34 (1981) 8–11.
8 S. C. CHURMS, E. H. MERRIFIELD AND A. M. STEPHEN, *S. Afr. J. Chem.*, 34 (1981) 68–71.
9 S. C. CHURMS, E. H. MERRIFIELD AND A. M. STEPHEN, *Carbohydr. Res.*, 90 (1981) 261–267.

10 S. C. CHURMS, E. H. MERRIFIELD, AND A. M. STEPHEN, *Carbohydr. Res.*, 64 (1978) c1–c2.
11 A. M. STEPHEN, S. C. CHURMS, E. H. MERRIFIELD, D. R. WALWYN, AND A. J. A MULLER, *AGP News (Melbourne)*, 3 (1980) 5–8
12 S. C. CHURMS, A. M. STEPHEN, AND I. R. SIDDIQUI, *Carbohydr. Res.*, 94 (1981) 119–121.
13 G. BENTHAM, *Trans. Linn Soc. London*, 30 (1875) 335–664.
14 D. M W. ANDERSON AND I. C. M. DEA *Phytochemistry*, 8 (1969) 167–176.
15 D. M W. ANDERSON, *Kew Bull.*, 32 (1978) 529–536.
16 F. SMITH AND R. MONTGOMERY, *The Chemistry of Plant Gums and Mucilages*, Reinhold, New York, 1959, pp. 241–255.
17 G. O ASPINALL, A. J. CHARLSON, E. L. HIRST, AND R. YOUNG, *J. Chem. Soc.*, (1963) 1696–1702.
18 G O ASPINALL AND R. YOUNG, *J. Chem. Soc.*, (1965) 3003–3004.
19 D M. W ANDERSON AND J. F. STODDART, *Carbohydr. Res.*, 2 (1966) 104–114.
20 D. M. W ANDERSON, SIR EDMUND HIRST, AND J. F. STODDART, *J. Chem. Soc., C*, (1966) 1959–1966.
21 D. M. W ANDERSON, SIR EDMUND HIRST, S. RAHMAN, AND G. STAINSBY, *Carbohydr. Res* , 3 (1967) 308–317.
22 D. M. W. ANDERSON AND S. RAHMAN, *Carbohydr. Res.*, 4 (1967) 298–304.
23 D. M. W. ANDERSON, I. C M DEA, K. A. KARAMALLA AND J F. SMITH, *Carbohydr. Res.*, 6 (1968) 97–103
24 D M W. ANDERSON AND I. C M DEA, *Carbohydr. Res.*, 6 (1968) 104–110.
25 D. M W ANDERSON AND I. C. M. DEA, *J Soc. Cosmet. Chem.*, 22 (1971) 61–76.
26 S. C. CHURMS AND A M. STEPHEN, *Carbohydr Res.*, 19 (1971) 211–221.
27 P. I. BEKKER, S. C. CHURMS, A. M. STEPHEN, AND G R. WOOLARD, *J S. Afr. Chem. Inst.*, 25 (1972) 115–130.
28 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208
29 P. A. SANDFORD AND H. E. CONRAD, *Biochemistry*, 5 (1966) 1508–1517.
30 H. BJORNDAL, B. LINDBERG, AND S. SVENSSON, *Acta Chem. Scand.*, 21 (1967) 1801–1804.
31 S. C. CHURMS, F H MERRIFIELD, AND A. M STEPHEN, *S. Afr. J Chem.*, 33 (1980) 39–40.
32 H. G. BARTH, *J. Chromatogr. Sci.*, 18 (1980) 409–429.
33 S. C. CHURMS AND A. M. STEPHEN, *Carbohydr. Res.*, 15 (1970) 11–19.
34 S. C CHURMS, A. M. STEPHEN, AND A. POLSON, *S. Afr. J. Chem* , 72 (1976) 151–152.
35 H. R. MORRIS, A. DELL, AND R. A. MCDOWELL, *Biomed. Mass. Spectrom.*, 8 (1981) 463–473.
36 P. F. FLEURY AND J. LANGE, *J. Pharm. Chim.*, 17 (1933) 107–113.
37 B. LINDBERG, J. LONNGREN, AND J. L. THOMPSON, *Carbohydr. Res.*, 28 (1973) 351–357.
38 G. O. ASPINALL AND K.-G. ROSELL, *Carbohydr. Res.*, 57 (1977) c23–c26.