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SMITH DEGRADATION OF GUM EXUDATES FROM SOME *Prosopis* SPECIES

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ABSTRACT

The gum polysaccharides from two species of *Prosopis*, namely, *P glandulosa* and *P chilensis* growing in different locations in South Africa, have been found to have similar structural features, closely resembling those reported in the literature for the gum of the North American species *P juliflora* (mesquite) On Smith degradation, both of the gums examined in the present study yielded a single polysaccharide product, having molecular weight 6,000, a value also found for the corresponding products from several *Acacia* species Successive, Smith degradations of the polysaccharide produced by a sample of *P glandulosa* have shown that a simple, (1→3)-linked D-galactan is obtained after only two such degradations This suggests the presence of uniform blocks of (1→3)-linked D-galactopyranosyl residues in the skeletal chain of the gum polysaccharide, similar to those postulated for gums from *Acacia* species

INTRODUCTION

Application of the technique of Smith degradation[1] (periodate oxidation followed by borohydride reduction and carefully controlled, mild hydrolysis with acid) to the gum polysaccharides from a number of *Acacia* species (of the series designated Phyllodineae and Botryocephalae in the classification of this genus by Bentham[2]) has afforded a considerable body of evidence for the presence of regularly repeating sub-units in the main, skeletal chains of the polysaccharides constituting these gums[3–7] The molecular weights of the monodisperse products of Smith degradation suggest that, in every case, the basal chain of the gum polysaccharide is composed of blocks of (1→3)-linked D-galactopyranosyl residues, usually about 12, 24, or 36 in number, separated at regular intervals by sugar residues that are attacked by periodate (unless protected by branching[7])

The genus *Prosopis* is a member of the same botanical family (Mimosaceae) and order (Fabales) as *Acacia* The gum of the North American species *P juliflora*, commonly known as mesquite, has been investigated in detail by Aspinall and White-

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head[8,9]: a molecular structure consisting of a galactan core similar to that of the *Acacia* gum polysaccharides, but bearing side chains of far greater complexity, was postulated by these authors. Earlier, Dutton and Unrau[10] had undertaken, for mesquite gum, an extensive study of the course of periodate oxidation and subsequent degradation, from which it was apparent that the average size of the periodate-resistant core-material was no greater than ~5,000, and that little breakdown other than by erosion of arabinosyl and some galactosyl units was caused by subsequent degradation-steps. The present study of gum samples from two other species of *Prosopis* was undertaken in order to ascertain whether these gums, from trees growing in different locations in South Africa, had structural features (*a*) resembling those ascribed to *P. juliflora* gum, and (*b*) including the presence of repeating sub-units in the galactan framework similar to those believed to exist in the molecules of gums from *Acacia*.

EXPERIMENTAL

Origin and purification of gum samples. — The sample of *Prosopis chilensis* gum used in this work was collected in Kimberley, South Africa, in December, 1977. Two specimens of the gum of *P. glandulosa* ("honey mesquite") have been examined, both collected early in 1978: sample I in the Kimberley region, and II at Pofadder, Cape Province. The polysaccharides were isolated and purified in the usual way[4].

General experimental conditions. — Paper chromatography (p.c.) was performed with Whatman No. 1 paper and the following solvent systems (all v/v): *A*, 8:2:1 ethyl acetate–pyridine–water; *B*, 2:1:1 1-butanol–acetic acid–water; *C*, (4:1:5, upper phase) 1-butanol–ethanol–water; and *D*, 18:3:1:4 ethyl acetate–acetic acid–formic acid–water. The *p*-anisidine hydrochloride and ammoniacal silver nitrate spray reagents were used to detect sugars and polyols in p.c. Molecular weights ($\overline{M}_w$) of the gum samples and their Smith-degradation products were estimated by gel-permeation chromatography on agarose (6%) and Bio-Gel P-10, respectively, with M sodium chloride as the eluant[11,12]. Proportions of neutral sugars were determined by g.l.c. of the derived alditol acetates[13] on OV-225 (3%, on Chromosorb W AW DMCS) at 190°, following hydrolysis of the polysaccharides and their degradation products in sealed tubes, under nitrogen, with 2M trifluoroacetic acid for 18 h at 100°. Proportions of uronic acid in the polysaccharides were determined by titration and by comparison of the intensities of the carbonyl infrared absorption at 1720 cm^{-1} given by chloroform solutions of the permethylated derivatives with those of standards of known content of uronic acid.

Methylation analysis. — The mixtures of methylated sugars produced on hydrolysis of the permethylated[14,15] derivatives (under the conditions just given) were analyzed by g.l.c. of the per(trimethylsilyl) ethers of the derived alditols[16] on SE-52 (3%, on Chromosorb W AW DMCS) at 140°.

Smith degradations. — A sample (1 g) of each of the three *Prosopis* gum polysaccharides was submitted to Smith degradation[4]. Oxidation with 0.12M sodium

metaperiodate was monitored by the standard arsenite titration-procedure[17], the reaction being terminated by addition of barium acetate after ~100 h in all cases. Reduced ($NaBH_4$), oxidized polysaccharide was kept in M trifluoroacetic acid for 48 h at room temperature, in each experiment, no change was observed in the molecular weight of the degraded polysaccharide after this hydrolysis had proceeded for 24 h. Fractionation with 1 : 1 methanol–acetone yielded the degraded polysaccharide (designated SD1) and a methanol-soluble syrup found by p.c. (solvent *C*) to consist mainly of glycerol, together with threitol and some arabinose.

In order to permit a comparison with the results of earlier work[8-10] and with the data available from the investigation of several *Acacia* gum polysaccharides[3-7,18], the Smith-degradation procedure just outlined was repeated, using *P. glandulosa* gum (sample I, 2 g), and the bulk of the product (SD1, yield 538 mg) was used in a series of three further, sequential degradations. Here, the aliquots for determination of periodate consumption were removed by use of a graduated syringe (of capacity 100 μL, or less), and the titration with iodine was performed with similar equipment. In the second, third, and fourth degradations, the quantities of material (mg) oxidized, the contact time (h) of the reduced oxidized products with acid, and the yields (mg) of material insoluble in methanol–acetone were: SD1, 449, 102, and 220; SD2, 185, 70, and 75, and SD3, 55, 48, and 38. The methanol–acetone extracts recovered after the second and third degradations respectively contained glycerol and some arabinose, and arabinose (p.c., solvents *A* and *C*).

TABLE I

ANALYTICAL DATA FOR GUM POLYSACCHARIDES FROM SOUTH AFRICAN *Prosopis* SPECIES

	Gum source		
	P. chilensis	P. glandulosa I	P. glandulosa II
$[\alpha]_D$ (degrees)	−67	−65	−66
$\bar{M}_w$	450,000[a]	400,000[a]	400,000[a]
Equivalent weight	1,430	1,920	2,030
(Hence) uronic acid (mol %)	12[b]	9[b]	9[b]
Proportions of neutral sugars (mol %)			
Galactose	39	34	37
Arabinose	47	55	52
Rhamnose	2	2	2
Periodate consumption (mmol g^{-1})	3.1	2.6	3.5

[a]Single peak in g.p.c. (6% agarose). [b]By titration, values from i.r. absorption at 1720 cm^{-1} for methylated gums were ~13% for *P. chilensis* gum and ~11% for *P. glandulosa* gum (both samples). Acidic di- and tri-saccharides, obtained by partial hydrolysis (0.25M H_2SO_4, 2 h, 100°) and detected by p.c. (solvents *B* and *D*), were identical for *P. chilensis* and *P. glandulosa* I gums, and consistent with those found for *P. juliflora* gum[8].

TABLE II

METHYLATION ANALYSES OF GUM POLYSACCHARIDES FROM SOUTH AFRICAN *Prosopis* SPECIES

Partially methylated sugar[a]	*Molar proportions*		
	P chilensis *gum*[b]	P glandulosa I[b]	P glandulosa II[b]
2 3,4-Rha	2	2	2
2,3,5-Ara	20	19	20
2 5,3,5-Ara	37	32	31
2,3,4,6-Gal	2	6	6
2 3,4-Gal	4	2	2
2,3,6-Gal	5	—	—
2,4-Gal	18	19	20
2,6-Gal	—	4	3
2-Gal	—	7	7

[a]Positions of *O*-methyl groups given by locants [b]$[\alpha]_D$ for chloroform solutions of methylated gums +51° for *P chilensis*, and −45° for *P glandulosa* (both samples)

RESULTS AND DISCUSSION

The analytical data (see Table I) indicate that the two samples of *P glandulosa* gum are identical in chemical composition and $\overline{M}_n$, despite the different locations from which they originated (*cf*, refs. 4, 18, and 19) There are marked similarities between the gums of the two species, namely, *P glandulosa* and *P chilensis*, and the structural units as shown by methylation analysis (see Table II) are likewise in general agreement The observations of Aspinall and Whitehead[8] relating to the structure of *P. juliflora* gum indicated several points of similarity the polysaccharide from *P juliflora* exudate had $[\alpha]_D$ +60°, and contained uronic acid (17%) and rhamnose (a trace), in addition to the main constituents, arabinose and galactose Methylation analysis indicated, *inter alia*, terminal L-arabinofuranosyl (+ + +), D-galactopyranosyl (+), and L-rhamnopyranosyl groups; 2- and 3-linked L-arabinofuranosyl (+ + +) residues in extended chains. 4- and 6-linked D-galactopyranosyl chain-units, and D-galactopyranosyl residues branched 3- and 6- (+ + + +) The highly branched, complex structures of the gums of all three *Prosopis* species are clearly of the type formulated[8,9], after extensive study, for *P juliflora*.

In view of this complexity of structure, the production of a single polysaccharide, having molecular weight 6,000, on Smith degradation of the gums of *P chilensis* and *P. glandulosa* (see Table III) is remarkable, even bearing in mind the recorded[10] formation of a component of average d p 30 on degradation of *P juliflora* gum The degraded polysaccharide has the same size as that of (*a*) the single fragment obtained on Smith degradation of the gum of *Acacia mearnsii*[6], and (*b*) constituents of the mixtures obtained similarly from the gums of *A saligna*[7] and *A longifolia*[18] Whereas the degraded polysaccharides from the two samples of *P glandulosa* gum

TABLE III

PROPERTIES OF METHANOL-INSOLUBLE PRODUCTS OF SMITH DEGRADATION OF SOUTH AFRICAN *Prosopis* GUMS

	SDI product from		
	P chilensis *gum*	P glandulosa I	P glandulosa II
$[\alpha]_D$ (degrees)	−36	+14	+14
Molecular weight	6,000[a]	6 000[a]	6,000[a]
Sugar composition (mol %)			
Galactose	70	90	87
Arabinose	30	10	13
Methylation analysis[b]			
2,3,5-Ara	10	6	6
2,3,4-Ara	—	5	3
2,5,3,5-Ara	13	5	3
2,3,3,4-Ara	—	1	2
2,3,4,6-Gal	15	8	10
2,4,6-Gal	14	9	13
2,3,4-Gal	19	22	22
2 4-Gal	24	33	31
2-Gal	5	11	10

[a]Single peak in g p c (Bio-Gel P-10) [b]Molar proportions of methylated sugars

TABLE IV

PROPERTIES OF SMITH-DEGRADED POLYSACCHARIDES SD1–SD4 FROM *P glandulosa* GUM (SAMPLE 1)

	Product			
	SD1	*SD2*	*SD3*	*SD4*
$[\alpha]_D$ (degrees)	+14	+25	+26	+26
Molecular weight	6,000[a]	4,400[a]	4,100[a]	3,900[a]
Sugar composition (mol %)				
Galactose	90	97	100	100
Arabinose	10	3	—	—
Periodate consumption (mmol g^{-1})	2 4	1 8	1 6	

[a]Single peak in g p c (Bio-Gel P-10)

may be regarded as identical, there are minor differences (see Table III) between these and the corresponding product from *P chilensis* gum In comparison with SD1 products from the *Acacia* gums, those from *Prosopis* gums contain a higher proportion of L-arabinosyl units and a lower proportion of unbranched, (1→3)-linked, D-galacto-pyranosyl residues

Results obtained when a larger sample of *P glandulosa* gum was submitted to a series of four successive, Smith degradations are shown in Table IV The decrease

in molecular weight from 6,000 to 4,400 accompanying the second degradation may be ascribed to removal of end groups, or chains of sugars which, in the intact, gum polysaccharide, were protected by sugar units (removed during the first degradation), reference to the molecular model proposed[8 9] for *P juliflora* gum shows how this can be so The methanol-insoluble product of the third Smith degradation (SD3) contains D-galactopyranosyl units as the only remaining sugar residues, and the decreases in molecular weight resulting from this and the fourth degradation indicate removal of no more than two end-groups from what appears to be essentially a (1→3)-linked D-galactan. Aspinall and Whitehead[8] noted the formation of such a product after two successive, Smith degradations of carboxyl-reduced, partly hydrolyzed, *P. juliflora* gum

The results of Smith-degradation experiments afford evidence both for a general similarity in the molecular structures of gums from two different species of *Prosopis* and for the presence, in their main skeletal chains, of uniform blocks (very approximately 30 in number) of (1→3)-linked, D-galactopyranosyl residues which are heavily substituted by branching involving D-galactose and the other constituent sugars of the gums. The existence of repeating sub-units of structure in the polysaccharide gum-exudates of both *Acacia* and *Prosopis* species (Mimosaceae, order Fabales) indicates that such uniformity of structure may be more widely found amongst gums from other families and orders of plants

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