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Note

Structural relationships of whole *Acacia pycnantha* gum and a component of low molecular weight

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There is an increasing body of evidence, mainly from Smith-degradation experiments[1–3], that several gums from *Acacia* species in the *Phyllodineae* and *Botryocephalae* series (Bentham) contain uniform blocks of (1→3)-linked D-galactopyranose residues, separated by periodate-vulnerable sugar units. One such gum, which has been the subject of extensive structural investigation[4,5], is that from *Acacia pycnantha*[3]. We have found that careful addition of ethanol to an aqueous solution of *A. pycnantha* gum permits the separation of a soluble fraction, component *A*, from material of higher molecular-weight that precipitates. On gel-permeation chromatography, *A* shows a single peak with molecular weight 6000, corresponding closely to that calculated for the molecular weight of the postulated repeating-unit in the polysaccharide. In order to ascertain whether the chemistry of *A* was characteristic of the gum as a whole, a comparative structural study of *A* and the precipitated gum polysaccharide was undertaken. The results are given in Tables I and II.

It is evident that, apart from the higher contents of arabinose and uronic acid, there are no significant differences between component *A* and whole *A. pycnantha* gum. The sugar residues, as found by methylation analysis, are similar (Table I), though fewer galactose end-groups appear in *A*, and the i.r. spectra of chloroform solutions of the respective methylated derivatives confirm the higher content of uronic acid in *A*. The monodisperse, Smith-degraded polysaccharides both have molecular weight 3500, this value being in close agreement with that calculated on the basis of removal of end-groups from *A*. The preponderant inter-sugar linkages in the Smith-degraded polysaccharides have been shown by methylation analysis to be (1→3), galactopyranose end-groups also being present.

Partial, acid hydrolysis in 0.01M trifluoroacetic acid at 100° (Table II) proceeds at virtually the same rate for *A* and for the precipitated gum, and the breakdown pattern is similar, in both cases, to that found for other *Acacia* gums of this type[2,6,7].

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TABLE I

PROPERTIES OF *A. pycnantha* GUM AND COMPONENT *A*, AND THEIR SMITH-DEGRADATION PRODUCTS

	A. pycnantha *gum*	*Component* A
Relative molar proportions of neutral sugars		
Galactose	70	66
Arabinose	27	31
Rhamnose	3	3
Equivalent weight[a]	3700	2020
Periodate consumption (mmol g^{-1})	5.7[b]	7.0[c]
Smith-degraded polysaccharide		
$[\alpha]_D$ (degrees)	+28	+24
Galactose–arabinose ratio	95:5	87:13
Molecular weight[d]	3500 (single peak)	3500 (single peak)
Methylated derivative[e]		
$[\alpha]_D$ in $CHCl_3$ (degrees)	−50	−57

[a]By titration. [b]Arsenite method. [c]Spectrophotometric method. [d]Bio-Gel P-10. [e]Sugar residues by g.l.c. analysis (as Me_3Si ethers of derived alditols) as follows (molar proportions for *A. pycnantha* gum and *A* in parentheses): 2,3,4-Rha (2,3); 2,3,5-Ara*f* (18,19); 2,5- and 3,5-Ara (3,4); 2,3,4,6-Gal (20,14); 2,4,6-Gal (3,9); 2,3,4-Gal (7.5); 2,6-Gal (4.7); 2,4-Gal (30,27); mono Gal (6,3).

The molecular weights of the hydrolysis products reflect the usual, rapid removal of arabinose, rhamnose, and terminal galactose residues, followed by gradual breakdown of the galactan core. Paper chromatography (solvent *2*) of the hydrolysates revealed the same pattern of monosaccharides and oligosaccharides as is produced by gums from related *Acacia* species[2,3,6,7].

These results suggest that the other polysaccharide components of *A. pycnantha* gum (molecular weights as shown in Table II) are composed of repeating blocks of sugars which, with minor modifications, have the same structure as component *A*, and whose molecular weights are, in general, simple multiples of that of *A*.

EXPERIMENTAL

Isolation and purification of polysaccharides. — Samples of *Acacia pycnantha* gum were collected near Stellenbosch, Cape Province, South Africa, in November 1974 and October 1975. The bulk of the gum was recovered by precipitation with ethanol (3 volumes) from a clear, 5% aqueous solution. The precipitated polysaccharide was collected by centrifugation, washed with alcohol and ether, and redissolved in water, and the solution was freeze-dried. The centrifugate was concentrated and freeze-dried. From the product, all material soluble in methanol was removed before the water-soluble portion of the remainder was recovered by freeze-drying. The product (208 mg, from 24 g of crude gum) was designated component *A*.

General experimental conditions — The solvent systems (v/v) used in paper chromatography were *1*, 1-butanol–ethanol–water (4:1:5, upper layer), *2*, 1-butanol–

TABLE II

PARTIAL HYDROLYSIS (0.01 M ACID) OF *A. pycnantha* GUM AND COMPONENT *A*

Time (h)	*Molecular-weight distribution*[a]	$\overline{M}_w$	$[\alpha]_D$ *(degrees)*	*Degree of scission*[f]	10^5 k (sec^{-1})
A. pycnantha gum					
0	31600(2); 24000(6); 17800(8);[b] 12000(36); 8000(17); 6000(21)	12000	−7[d]		7.7
2	25000(4); 17800(10); 13200(17); 9000(40); 6000(10); 4500(14); ≤ 1800(5)	10000	0	0.05	5.6
5	25000(1); 17800(6); 13200(15); 9000(40); 6000(8); 4500(18); ≤ 1800(12)	9300	+10	0.11	2.8
24	15800(2); 13200(4); 11000(7); 9000(8); 7000(10); 6000(6); 4800(10); 4500(5); 3400(14); ≤ 1800(34)	7100	+38	0.26	1.2
48	13200(2); 11000(4); 9000(7); 7000(8); 6000(8); 4800(13); 4500(9); 3400(14); ≤ 1800(35)	5900	+46	0.33	1.1
96	9000(3); 7000(3); 6000(6); 4800(3); 4500(9); 3400(12); 2900(11); ≤ 1800(53)	4600	+55	0.45	
Component A					
0	6000 (single peak)[c]	6000	−7[e]		7.9
3	5500(45); 4500(22); 3500(15); ≤ 300(18)	4900	+14	0.08	6.5
6	4500(40); 3500(28); ≤ 300(32)	4100	+26	0.16	1.8
24	4500(3); 3500(6); 1700(2); 1500(2); 1100(6); 980(5); 800(8); 660(6); 520(9); ≤ 300(53)	3800	+39	0.25	1.5
48	1300(1); 1100(2); 980(2); 800(5); 660(7); 520(13); ≤ 300(70)	[g]	+47	0.34	1.0
96	660(7); 520(18); ≤ 300(75)	[g]	+55	0.44	

[a]Molecular weights corresponding to peaks in the gel chromatogram; relative proportions by weight in brackets. [b]Bio-Gel P-300. [c]Bio-Gel P-10. [d]Concentration, 0.58%. [e]Concentration, 0.44%. [f]Calculated from reducing power as in Ref. 6. [g]M_w not meaningful; oligosaccharide mixture.

acetic acid–water (2:1:1), and *3*, ethyl acetate–pyridine–water (8:2:1). Molecular-weight distributions were estimated by gel-permeation chromatography on Bio-Gel P-300 or P-10, with M sodium chloride as eluent, as described previously[6]. Molar proportions of neutral sugars in hydrolysates of the polysaccharides were determined by g.l.c. of the derived alditol acetates[8].

Methylation analysis. — The polysaccharides were methylated by the Hakomori[9] and Purdie[10] (three times) procedures to give fully methylated derivatives, which

were hydrolysed (2M trifluoroacetic acid, 100°, 18 h, under nitrogen), and analysed by g l c of the trimethylsilyl ethers of the derived alditols[11]

Partial, acid hydrolysis — Samples of the polysaccharides were hydrolysed in 0.01M trifluoroacetic acid at 100° for 96 h, aliquots being removed at intervals and examined by various methods, including gel-permeation and paper chromatography, as described previously[2,6,7] The values of the hydrolysis rate-constants in Table II were calculated from the values of the degree of scission and the corresponding hydrolysis times[6]

Smith degradation — The polysaccharides were oxidised with aqueous sodium metaperiodate, and the products reduced with sodium borohydride as described elsewhere[2,3] The reduced-oxidised polysaccharides were kept in M trifluoroacetic acid solution at room temperature for periods ranging from 48 h (for component *A*) to 120 h (for the bulk gum). This mild-hydrolysis step was monitored in each case by gel-permeation chromatography of samples of the products removed at intervals[3] When the molecular weight of the degraded polysaccharide was no longer affected by further exposure to M trifluoroacetic acid at room temperature, the hydrolysis was terminated by removal of the acid by freeze-drying Each degraded polysaccharide was then recovered by removal of solubles by methanol extraction as before[2,3]

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