Carbohydrate Research, 63 (1978) 337–341

Note

A comparative examination of two polysaccharide components from the gum of *Acacia mabellae*

SHIRLEY C. CHURMS, EDWIN H. MERRIFIELD, AND ALISTAIR M. STEPHEN

C.S.I.R. Carbohydrate Research Unit, Department of Organic Chemistry, University of Cape Town (South Africa)

(Received May 30th, 1977; accepted for publication July 13th, 1977)

In continuation of an investigation[1–3] of the structures of gums from *Acacia* species in the *Phyllodineae* and *Botryocephalae* series of the Bentham classification, attention has been focused on the gum of *Acacia mabellae* (Series *Phyllodineae*) for two reasons. Firstly, published analytical data for this gum[4,5] suggest close similarity to gums from other *Acacia* species in this Series that have proved of interest on account of the regular molecular structures indicated by Smith-degradation[6] studies of the gum polysaccharides[2]. Secondly, gel-permeation chromatography of *A. mabellae* gum indicates an unusually simple distribution-pattern of molecular weights, with polysaccharide components, *A* and *B*, of two sizes only ($\bar{M}_w$ 8000 and 16000, respectively). It therefore seemed desirable to fractionate the gum in order to obtain pure samples of each component, and to undertake a comparative structural study to ascertain whether the polysaccharide (*A*) of lower molecular-weight is structurally representative of the gum as a whole, as in the case of the analogous component isolated from the gum of *A. pycnantha*[3].

In fractionating the gum, it was necessary to combine preparative-scale gel-permeation chromatography and fractionation with aqueous ethanol, in order to maximise the yields of the two components. The former procedure was satisfactory for the isolation of the larger polysaccharide (*B*), but the peak-broadening resulting from the adsorptive forces that must inevitably operate on a polysaccharide molecule of this size caused most of the fractions containing the smaller component (*A*) to be contaminated with an appreciable proportion of *B*. The best yield of *A* was obtained from the soluble fraction remaining after precipitation of the bulk of the gum with ethanol from a dilute, aqueous solution. The samples of the two polysaccharides isolated by these methods appeared to be monodisperse; this was verified by gel-permeation chromatography followed by rechromatography of fractions selected from the boundaries of the chromatographic zone in each case.

The properties of each polysaccharide, and data on their acid hydrolysis (both complete and partial), Smith degradation, and methylation analysis, are given in

Tables I and II. It is evident that the two components are virtually identical with respect to composition and structure, and differ only in size. The proportions of constituent sugars (Table I) agree well, in both cases, with those reported by Anderson *et al.*[4] for the whole gum, and are corroborated by the results of the present methylation analyses (Table II), which are somewhat at variance with the methylation data (obtained by different procedures) published by Anderson and Bell[5].

The production of apparently identical, monodisperse, degraded polysaccharides on Smith degradation of *A* and *B* suggests that the only significant difference between *A* and *B* is that the skeletal chain of *B* contains a periodate-vulnerable sugar joining two blocks having the structure of component *A*. In each block, the main chain is evidently composed of periodate–resistant, (1 → 3)-linked galactose residues; this is demonstrated by paper chromatography of partially acid-hydrolysed *A* and *B*, which indicates that the series of oligosaccharides produced (see Table I) consist of (1 → 3)-linked β-D-galactosyl residues, and is confirmed by the methylation analyses (Table II). From the latter data, it may be inferred that each block contains

TABLE I

PROPERTIES OF *Acacia mabellae* GUM POLYSACCHARIDES AND THEIR DEGRADATION PRODUCTS

	Polysaccharide A	*Polysaccharide* B
Molecular weight	8000	16000
$[\alpha]_D$ (degrees)	+1.5	+6.5
Equivalent weight[a]	3300	2800
Hence, uronic acid (mol %)[b]	5	6
Proportions of neutral sugars (mol %):		
Galactose	77	78
Arabinose	15	13
Rhamnose	3	3
Molecular-weight distribution of partial hydrolysate[c] (proportions by weight in parenthesis)	1600(2); 1300(2); 1100(2.5); 950(3); 800(6); 660(8); 520(27.5); ⩽300(49)	13000(3); 7200 (4); 3500(4.5); 1900(2.5); 1600(2); 1300(2.5); 1100(4); 950(6.5); 800(7); 660(8); 520(17); ⩽300(39)
Periodate consumption[d] ($mmol.g^{-1}$)	6.0	6.0
Smith-degraded polysaccharide		
$[\alpha]_D$ (degrees)	+30	+33
Galactose–arabinose ratio	96:4	97:3
Molecular weight	3500	3500

[a]By potentiometric titration with 0.01M NaOH. [b]Corroborated by infrared spectroscopy (KCl disc); absorbances at 1720–1700 (un-ionised acid) and 1640–1620 cm^{-1} (ionised form) in accordance with relative acid contents from titrations. [c]0.01M Trifluoroacetic acid, 100°, 48 h. [d]Arsenite method.

TABLE II

METHYLATION ANALYSES OF COMPONENTS *A* AND *B* FROM *A. mabellae* GUM

	Polysaccharide A	*Polysaccharide* B
$[\alpha]_D$ for methylated product[a] (degrees)	−48	−46
Molar proportions[b] of *O*-methyl sugar residues[c,d]		
2,3,5-Ara	17	15
2,5-Ara 3,5-Ara 2,3,4-Rha	3	3
2,3-Ara 3,4-Ara	1	1
2,3,4,6-Gal	23	23
2,4,6-Gal	6	5
2,3,6-Gal	4	3
2,3,4-Gal	3	3
2,4-Gal	34	37
2-Gal	4	4

[a]In $CHCl_3$. [b]Means, in each case, of two assays of the same methylated product and also duplicate methylations. [c]Positions of *O*-methyl groups given by locants. [d]Assayed as Me_3Si ethers of the derived alditols.

~22 such residues, most of which carry a branch, terminated usually by galactose or arabinose. End-groups will be removed on Smith degradation of the polysaccharide, and the molecular weight of the resulting degraded polysaccharide is predicted from these considerations to be ~4000, which is close to the value found.

The nature of the linkages joining the two blocks in component *B* is uncertain, but glycosidic linkage is considered more probable than an ionic bond. Aggregation resulting from ionic binding forces involving the uronic acid groups and divalent cations, such as Ca^{2+}, has been demonstrated by Greenwood *et al.*[7] to occur in acidic polysaccharides of the type present in plant gums; in the present case, however, the fact that virtually complete deionisation (verified by atomic absorption and X-ray fluorescence spectroscopy) produced no detectable change in the molecular weight of *B* indicates that such association is not responsible for the existence of this large component. Furthermore, *B* was unaffected by sodium *iodate* and work-up.

The Smith-degraded polysaccharide produced by the two components of *A. mabellae* gum is strikingly similar, in all respects, to those yielded by the gums from two other *Acacia* species of the Series *Phyllodineae*, namely *A. pycnantha*[2,3] and *A. difformis*[2], and the methylation and other data also indicate the close similarity of these three gums from related *Acacia* species. *A. mabellae* gum has afforded the most clear-cut illustration to date of the highly uniform molecular structure that is exhibited by several gums from this Series and the *Botryocephalae*[1,2].

EXPERIMENTAL

Fractionation of gum. — The specimen of *A. mabellae* gum used, which was collected in New South Wales, Australia, in January, 1970, was made available by courtesy of Dr. D. M. W. Anderson (Edinburgh). Gel-permeation chromatography (Bio-Gel P-300 and P-10, M sodium chloride eluent[8]) of a small sample of the gum, as received, indicated the presence of polysaccharide components with molecular weights 8000 (~40% by weight) and 16000 only; $\overline{M}_w$ was 13000. A monodisperse sample of the smaller component (*A*) was isolated by fractionation of a 3% aqueous solution of the gum with ethanol (2 vol.); after removal of the precipitated material by centrifugation, freeze-drying of the soluble fraction yielded *A*. It was necessary to perform this fractionation twice to obtain *A* in sufficient quantity (720 mg, from 4 g of gum) for examination. Addition of an equal volume of ethanol to a 5% aqueous solution of the polysaccharide mixture gave a precipitate that consisted of the larger polysaccharide (*B*) only, but the yield was very low (~100 mg, from 3 g of mixture). A higher yield of *B* was obtained by chromatography of a sample (1.4 g) of the mixture on a large column (70 × 4 cm) of Bio-Gel P-10, with water as the eluent; the sample was applied in a volume of 25 ml and eluted at a flow rate of 50 ml.h^{-1}; fractions (50 ml) were screened for carbohydrate[9], and subsequently freeze-dried to yield polysaccharides which were then examined by analytical-scale gel-permeation chromatography. An initial fractionation, followed by rechromatography of the material recovered from the first fractions, resulted in the isolation of monodisperse *B* (500 mg).

Examination of polysaccharides. — The various techniques used in the examination of the two polysaccharides and their degradation products have been described previously[1-3]. Removal of cations [by treatment with Amberlite IR-120(H^+) resin] from samples to be used in potentiometric titrations and methylation analyses was monitored by atomic absorption and X-ray fluorescence spectroscopy. In the Smith degradations, the course of the mild hydrolysis step with acid was followed, as described previously[2,3], by gel-permeation chromatography of samples of the products removed at intervals, and also by polarimetry and by determination of the glycerol[10] and glycolaldehyde[11] produced. In each case, the hydrolysis was terminated when the molecular-weight distribution of the degraded polysaccharide (considered the most sensitive criterion[2]) was no longer affected by exposure to M trifluoroacetic acid at room temperature for a further 24 h; this required contact with the acid for a total of 48 h for *A*, but 72 h for *B*. The monodispersity of the resulting Smith-degraded polysaccharides (yield, ~100 mg from 400 mg of polysaccharide in each case) was verified by gel-permeation chromatography, with rechromatography of selected fractions as described.

ACKNOWLEDGMENTS

We thank the South African Council for Scientific and Industrial Research

and the University of Cape Town (Staff Research Fund) for financial support, Dr. D. M. W. Anderson (University of Edinburgh) for providing the gum specimen, and Mr. J. P. Willis and Mrs. E. Abramowitz for performing analyses by X-ray fluorescence and atomic absorption spectroscopy.

REFERENCES

1 S. C. Churms and A. M. Stephen, *Carbohydr. Res.*, 45 (1975) 291–298.
2 S. C. Churms, E. H. Merrifield, and A. M. Stephen, *Carbohydr. Res.*, 55 (1977) 3–10.
3 J. W. H. Adam, S. C. Churms, A. M. Stephen, D. G. Streefkerk, and E. H. Williams, *Carbohydr. Res.*, 54 (1977) 304–307.
4 D. M. W. Anderson, P. C. Bell, and C. G. A. McNab, *Phytochemistry*, 11 (1972) 1751–1754.
5 D. M. W. Anderson and P. C. Bell, *Phytochemistry*, 15 (1976) 301–303.
6 F. Smith and D. R. Spriestersbach, *Abstracts Papers Am. Chem. Soc. Meeting*, 128 (1955) 15D; I. J. Goldstein, G. W. Hay, B. A. Lewis, and F. Smith, *Methods Carbohydr. Chem.*, 5 (1965) 361–370.
7 C. T. Greenwood and N. K. Matheson, *Chem. Ind. (London)*, (1956) 988.
8 S. C. Churms and A. M. Stephen, *Carbohydr. Res.*, 15 (1970) 11–19.
9 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
10 G. W. Hay, B. A. Lewis, and F. Smith, *Methods Carbohydr. Chem.*, 5 (1956) 377–380.
11 Z. Dische and E. Borenfreund, *J. Biol. Chem.*, 180 (1949) 1297–1300.