Tetrahedron, 1967, Vol. 23, pp. 193 to 198. Pergamon Press Ltd. Printed in Northern Ireland

APPLICATION OF GAS-LIQUID CHROMATOGRAPHY TO THE STRUCTURAL INVESTIGATION OF POLYSACCHARIDES*—II

THE STUDY OF *ACACIA* GUMS

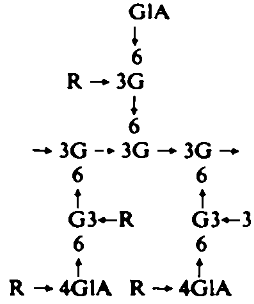
M. KAPLAN† and A. M. STEPHEN
Department of Chemistry, University of Cape Town, South Africa

(*Received* 23 *March* 1966; *accepted for publication* 25 *May* 1966)

Abstract—The structures of a number of *Acacia* polysaccharide gums are compared, using as a basis the analysis by GLC of the cleavage products from the methylated polysaccharides. The four constituent sugars of the gums are linked to one another in a limited number of ways, the proportions of each type of unit varying from species to species.

INTRODUCTION

THE chemistry of "gum arabic" (from *Acacia senegal* and other species), the subject of many investigations, has been adequately reviewed.[1] A recent expression representing the structure of this gum is shown below.[2]



G = D-Galactopyranose GlA = D-Glucopyranuronic acid
R = L-Arabinofuranose..., L-Rhamnopyranose..., D-Gal$_p$ → 3 L-Ara$_f$..., or L-Ara$_p$ → 3 L-Ara$_f$...

Studies of the gums from *A. mearnsii*,‡[3] *A. pycnantha*,[2,4] *A. cyanophylla*,[5] and *A. karroo*,[6] among others, have revealed that the same sugar components are released on acid hydrolysis of the gums, but these sugars are combined in widely differing proportions. A comment on the taxonomic significance of these and similar results on gums of other plant genera has appeared.[7]

---

* Part I—A. M. Stephen, M. Kaplan, G. L. Taylor and E. C. Leisegang, *Tetrahedron, Stephen Memorial issue* p. 233 (1966).

† Present address: Organic Chemical Laboratories, University of Cambridge, England.

‡ Formerly *A. mollissima*.

[1] F. Smith and R. Montgomery, *Chemistry of Plant Gums and Mucilages*, Reinhold, New York (1959).

[2] G. O. Aspinall, E. L. Hirst and A. Nicolson, *J. Chem. Soc.* 1697 (1959).

[3] A. M. Stephen, *J. Chem. Soc.* 646 (1951).

[4] E. L. Hirst and A. S. Perlin, *J. Chem. Soc.* 2622 (1954).

[5] A. J. Charlson, J. R. Nunn and A. M. Stephen, *J. Chem. Soc.* 269 (1955).

[6] A. J. Charlson, J. R. Nunn and A. M. Stephen, *J. Chem. Soc.* 1428 (1955).

[7] E. A. C. L. E. Schelpe and A. M. Stephen, *S. African Ind. Chemist* 12 (1964).

In this paper the examination by GLC of methanolysis products from eight methylated *Acacia* gums is reported, together with a semiquantitative description of the manner in which the different sugars are linked. GLC is shown to be a convenient technique for the rapid analysis of methylated polysaccharides particularly where comparisons within a uniform group (e.g. gums from different species within a genus) are being sought.

EXPERIMENTAL

The *Acacia* gums were collected from trees in Southern Africa between 1963 and 1964. Analysis by GLC of the methanolysis products of the methylated gums was performed using conditions described in Part I; the methylation procedures were standard and were continued until there was no increase in methoxyl content and no further diminution in the size of the peak (IR) in the region of OH absorption. Physical constants and analysis results for the polysaccharides and their fully methylated derivatives are shown in Table 1.

TABLE 1. POLYSACCHARIDE GUMS AND THEIR METHYLATED DERIVATIVES, FROM *Acacia* SPECIES

Polysaccharide		Methylated polysaccharide					
Source	$[\alpha]_D$ (in H_2O)	*Equiv.*	$[\alpha]_D$ (in $CHCl_3$)	OMe%	C%	H%	Ash%
[*] *Acacia mearnsii* de Wild.	−55°	1800	−66°	42·6	52·3	7·7	nil
Acacia karroo Hayne.	+54°	1700	+18°	40·5	51·8	7·7	0·1
Acacia cyanophylla Lindl.	−33°	730	48°	40·6	50·5	7·1	nil
Acacia decurrens Willd.	−46°	3300	−61°	40·5	51·9	8·0	0·9
Acacia pycnantha Benth.	− 2°	9000	50°	42·3	52·0	7·7	nil
Acacia elata A. Cunn.	+ 4°	6100	−39°	43·7	52·0	8·0	nil
Acacia podalyriaefolia A. Cunn.	+ 3°	5000	42°	41·9	53·3	7·9	nil
Acacia giraffae Burch.	+28°	730	−11°	41·0	50·9	7·2	0·5

For OMe contents of some *Acacia* gums, see D. M. W. Anderson, G. M. Cree, M. A. Herbich, K. A. Karamalla and J. F. Stoddart, *Talanta* 1559 (1964).

[*] The same methylated *A. mearnsii* gum sample is being investigated by Dr. G. O. Aspinall *et al.*

Acid hydrolysis of portions of the gums and inspection of the hydrolysates by paper chromatography showed the presence of galactose and arabinose in each, with variable amounts (sometimes traces only) of rhamnose and glucuronic acid (accompanied by its 4-O-methyl derivative). Indications of (6-D-galactose-β-D-glucopyranosid) uronic acid were observed for hydrolysates from all eight *Acacia* gums, and (4-D-galactose-α-D-glucopyranosid)uronic acid as well from *A. karroo* and *A. giraffae*. Paper chromatographic examination of the methylated sugars in hydrolysates of the methylated polysaccharides corroborated the identifications by GLC of methyl glycosides in corresponding methanolysates. These identifications together with relative molar proportions (semiquantitative) of the methylated sugars, as determined by GLC, are given in Table 2. In addition, paper chromatography revealed ca. 1 to 8 molar proportions of monomethyl ethers of galactose in the hydrolysates.

Plots of detector response against time for the methanolysates of methylated *Acacia* gums[*] are reproduced in Figs. 1–8.

DISCUSSION

The quantities listed in Table 2 are subject to the errors pointed out in Part 1 of the current inevstigation by GLC of methanolysates of methylated polysaccharides. It should be noted in particular (i) that the presence of small amounts of partially methylated sugars may be due to de-methylation during methanolysis, (ii) that the correlation of proportions of sugars representing end-groups with those that are

---

[*] M. Kaplan, M.Sc. thesis, University of Cape Town (1965).

TABLE 2. ANALYSIS BY GLC OF METHYLATED *Acacia* GUM METHANOLYSATES

		Acacia species							
Legend	*Sugar*	*A. mearnsii*	*A. karroo*	*A. cyanophylla*	*A. decurrens*	*A. pycnantha*	*A. elata*	*A. podalyriaefolia*	*A. giraffae*
b	2,3,4-Tri-O-methylrhamnose	2	3	20	4	tr	tr	tr	1
c	2,3,5-Tri-O-methylarabinose	32	10	3	39	14	8	11	8
d	2,3,4-Tri-O-methylarabinose	1	2	—	tr	—	—	—	—
e	2,3,Di-O-methylarabinose	2	?	1	1	1	—	—	—
f	3,5-Di-O-methylarabinose	2	11	1	1	—	—	—	2
g	2,5-Di-O-methylarabinose	9	13	7	4	3	—	?	1
h	3,4-Di-O-methylarabinose	—	6	—	—	—	—	tr	—
j	2,3 ,4,6-Tetra-O-methylgalactose	6	2	7	5	26	34	26	6
k	2,3,6-Tri-O-methylgalactose	tr	1	—	1	1	1	5	8
l	2,4,6-Tri-O-methylgalactose	4	3	2	3	4	4	2	6
m	2,3,4-Tri-O-methylgalactose	2	2	7	3	6	5	7	3
o	2,6-Di-O-methylgalactose	4	—	—	3	2	—	—	2
p	2,4-Di-O-methylgalactose	22	36	28	25	40	43	38	36
u	2,3,4-Tri-O-methylglucuronic acid	6	3	5	—	2	—	2	17
v	2,3-Di-O-methylglucuronic acid	5	4	20	3	tr	—	tr	1

Units represent molar proportions; tr — trace
i 2,3,4,6-Tetra-O-methylglucose (internal standard added)
x Identification uncertain

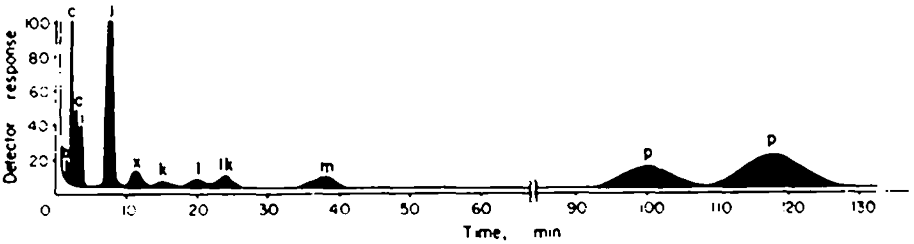


FIG. 1. *A. elata*

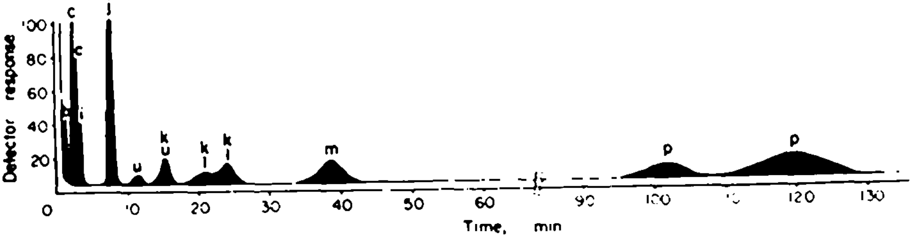


FIG. 2. *A. podalyriaefolia*

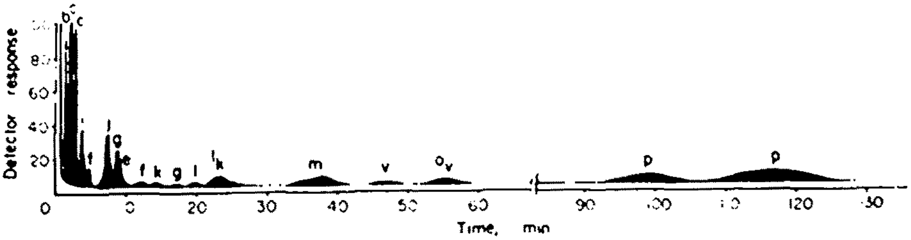


FIG. 3. *A. decurrens*

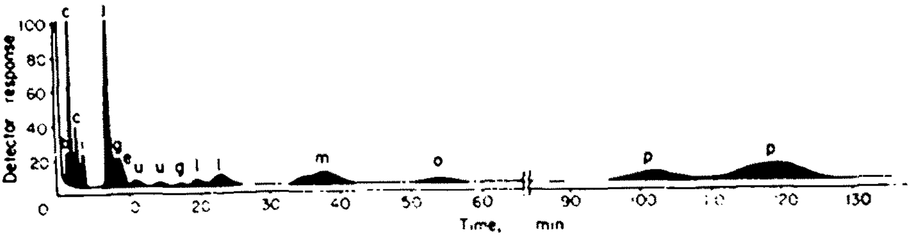


FIG. 4. *A. pycnantha*

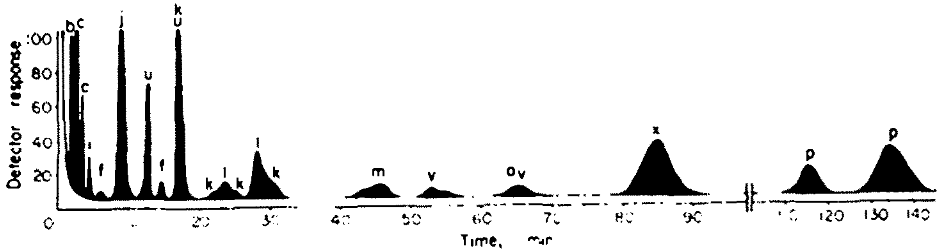


FIG. 5. *A. giraffae*

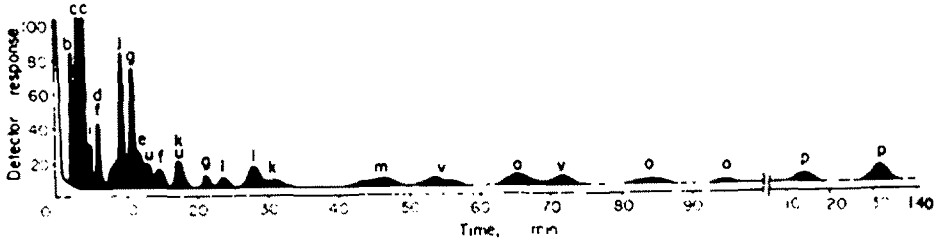


FIG. 6. *A. mearnsii*

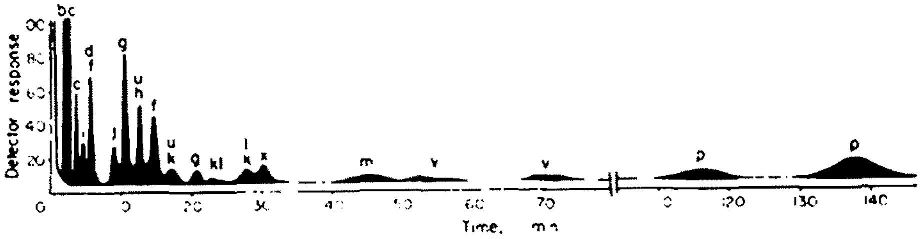


FIG. 7. *A. karroo*

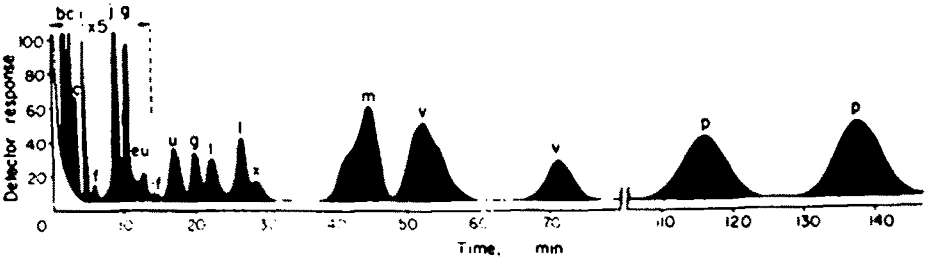


FIG. 8. *A. cyanophylla*

branch-points is not entirely satisfactory in some instances, (iii) that the identities of peaks in the chromatograms have not as yet all been confirmed by collection (from the effluent gas stream) and characterization of the responsible substances and (iv) that small quantities of methylated sugars (e.g. 2,3-di-O-methylarabinose) may not have been observed because of peak overlap with other sugars. On the whole the total proportions of the sugars found by the GLC technique agree well with published results.

The general pattern of structure of a number of *Acacia* gums has been described many times, and examination of the methylation/methanolysis results for the eight species reported here shows immediately a general resemblance from one example to the next.* All are in conformity with the established structural pattern.

The simplest structures are those of *A. elata*, *A. podalyriaefolia* and *A. pycnantha* gums in which the uronic acid and rhamnose contents are very low, and (to judge by the similarity in proportions of galactose and arabinose linked in each specific manner) in which there appears to be great similarity in detailed structure. That for a different sample of *A. pycnantha* gum has been the subject of detailed study[2] which established the main chain as composed of $\beta 1 \rightarrow 3$-linked D-galactopyranose units, with multiple short branches attached at $C_{(6)}$. Arabinose is present in the three gums to a small extent, practically exclusively as furanoside endgroup.

A. decurrens and *A. mearnsii* gums contain uronic acid, as endgroup or linked through $C_{(4)}$. Here as is usual the rhamnose content corresponds to the amount of 4-linked uronic acid, and in some examples this mode of inter-sugar linkage has been rigidly proved.[9] L-Arabinofuranose end-groups are predominant rather than D-galactopyranose. The similarities between the gums of these species is of interest because of a close botanical connection.

A. cyanophylla is characterized by high rhamnose and uronic acid contents, the former constituting (as pyranoside) the bulk of the nonreducing end-groups.

---

* All eight contain the same seven constituent units $Rh_p\rightarrow$, $A_f\rightarrow$, $G_p\rightarrow$, $\rightarrow 3G_p\rightarrow$, $\rightarrow 6G_p\rightarrow$, $\underset{\underset{\uparrow}{6}}{\rightarrow 3G_p\rightarrow}$ and $\overset{\downarrow}{\underset{\underset{\uparrow}{6}}{\rightarrow 3G_p\rightarrow}}$. Five or six contain in addition $Gl_pA\rightarrow$, $\rightarrow 3$ & $\rightarrow 2A_f\rightarrow$ and $\rightarrow 4Gl_pA\rightarrow$. $A_p\rightarrow$, $\rightarrow 2A_p\rightarrow$, $\rightarrow 5A\rightarrow$ and $\underset{\underset{\uparrow}{4}}{\rightarrow 3G_p\rightarrow}$ feature rarely.

[9] G. O. Aspinall, A. J. Charlson, E. L. Hirst and R. Young, *J. Chem. Soc.* 1696 (1963); G. O. Aspinall and R. Young, *J. Chem. Soc.* 3003 (1965); A. J. Charlson, private communication.

In the examples given thus far evidence is to hand for a β-linkage between the uronic acid and $C_{(6)}$ of a galactopyranose unit. *A. karroo* and *A. giraffae* gums contain, in addition, part of the uronic acid linked to $C_{(4)}$ of galactopyranose. Of these two gums the latter contains much the higher proportion of total acid but less arabinose; both contain negligible rhamnose. In *A. karroo* some of the arabinopyranose units are end-groups but others are attached through $C_{(3)}$ to some other sugar; possibly to arabinofuranose, which is detached on autohydrolysis (so enabling 3-*O*-β-L-arabinopyranosyl-L-arabinose to be isolated).

In view of possible heterogeneity in such complex substances as the *Acacia* gums, the present study must be regarded as a description of the nature of all the intersugar linkages present in the *Acacia* exudates without reference to their possible distribution in molecules of different types.

Acknowledgments—The authors are indebted to the Council of the University of Cape Town (Staff Research Fund) and to the South African Council for Scientific and Industrial Research (post-B.Sc.Hons. grant). Professor H. Shaw and Dr. A. V. Hall kindly assisted in the obtaining and identifying of gum specimens, and Dr. A. J. Charlson and Miss D. C. Vogt in the compilation of the manuscript.