Phytochemistry, 1976, Vol. 15, pp. 301–303. Pergamon Press. Printed in England.

PARTIAL STRUCTURAL STUDIES OF FOUR *ACACIA* GUM EXUDATES OF THE SERIES PHYLLODINEAE*

D. M. W. ANDERSON and P. C. BELL
Department of Chemistry, The University, Edinburgh EH9 3JJ, Scotland

(*Received* 3 *July* 1975)

Key Word Index—*Acacia*; Leguminosae; gum exudates; polysaccharides; structural analysis; chemotaxonomy.

Abstract—Methylation and hydrolysis studies have shown that gum specimens from *Acacia difformis*, *A. mabellae*, *A. retinodes* and *A. rubida*, which belong to Bentham's Series I (Phyllodineae), subseries 6F (Uninerves Racemosae), are similar structurally to those from *A. podalyriifolia* and *A. pycnantha*. This is further evidence that *A. cyanophylla*, which was placed next to *A. pycnantha* by Bentham, is atypical of the Series I *Acacias*.

INTRODUCTION

The number of Australian species included in Bentham's Series I (Phyllodineae) of the genus *Acacia* [2] has been increased from the original 277 to 570 in 1969, 625 in 1971, and nearly 700 in 1974 [3]. Analytical data for gum exudates of some 18 species of the Series Phyllodineae have now been published [4,5] but structural studies have been restricted to only three species, viz. *A. cyanophylla*, *A. pycnantha*, *A. podalyriifolia*.

This paper reports hydrolysis and methylation data for the gums from 4 species of Bentham's Series I, subseries 6F (Uninerves Racemosae) viz. *A. difformis*, *A. mabellae*, *A. retinodes* and *A. rubida*; the results are compared with those available from earlier work.

RESULTS AND DISCUSSION

Hydrolysis of each of the gums from *A. difformis*, *A. mabellae*, *A. retinodes* and *A. rubida* with 0·5 M sulphuric acid for 7·5 hr at 100° showed 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose to be the only aldobiuronic acid present. However, small amounts of 4-*O*-methyl-D-glucuronic acid [R_{gal} 1·76 solvent (a); orange–pink spot] were also detectable chromatographically. Although hydrolysis with 1 M sulphuric acid is normally required to break uronosyl linkages, this result is similar to that found for *A. pycnantha* and *A. podalyriifolia* and also for gum exudates from subseries 8 (Juliflorae) [5] of the Phyllodineae. This indicates that the 4-*O*-methyl-D-glucuronic acid residues are more acid-labile than those in gum exudates of *Acacias* in Series IV and V.

Mild acid hydrolysis with 0·25 M sulphuric acid for 1 hr at 100° gave large amounts of 3-*O*-β-D-galactopyranosyl-D-galactose [R_{gal} 0·49, solvent (b)] and much smaller amounts of 6-*O*-β-D-galactopyranosyl-D-galactose [R_{gal} 0·27, solvent (b)] in addition to galactose, arabinose, and trace amounts of rhamnose. Arabinobioses or galacto/arabinobioses (pink spots) were not observed.

Small amounts of each of the four samples were methylated by the classical Haworth and Purdie procedures. Yields, specific rotations and methoxyl contents of the methylated products are shown in Table 1. Methanolysis of portions (*ca* 50 mg) of the methylated polysaccharides, followed by GLC of the mixtures of *O*-methyl sugars, gave the results shown in Table 2. Hydrolysis of the methyl glycosides, followed by chromatography in solvents (c) and (d), indicated the presence of 2-*O*-methyl galactose in addition to those *O*-methyl sugars already characterised by GLC. The relative amounts of each component shown in Table 1 are compared with the *O*-methyl sugars found in *A. cyanophylla*, *A. podalyriifolia* and *A. pycnantha* by Kaplan and Stephen [6].

The GLC profiles of the four samples showed a marked similarity, the main components being 2,3,5-tri-*O*-methyl-L-arabinose, and 2,3,4,6-tetra-, 2,4,6-tri-, and 2,4-di-*O*-methyl-D-galactose. Di-*O*-methyl-L-arabinose was found only in *A. rubida*, which contained more arabinose than the other three samples. Hence in *A. difformis*, *A. mabellae* and *A. retinodes* all the arabinose is present as end-groups, as also occurs with *A. podalyriifolia*. The small amount of 2,3-di-*O*-methyl-L-arabinose found in *A. rubida* suggests that very short arabinose chains, possibly not more than two units long, are involved.

The small amounts of 2,3,6-tri, 2,6,-di-, and large amounts of 2-*O*-methyl-D-galactose are attributed to undermethylation, and the fact that the results for the four samples show a marked similarity to those found for *A. podalyriifolia* and *A. pycnantha*, with the exception of the much smaller amounts of 2,4-di-*O*-methyl-D-galactose, suggests that the 2-*O*-methyl-D-galactose might arise from undermethylation of 2,4-di-*O*-methyl-D-galactose residues.

The exudates from the four species studied here are very similar to those from *A. podalyriifolia* and *A. pycnantha*, both analytically and structurally. All contain large amounts of end-group galactose and arabinose, smaller amounts of end-group glucuronic acid, and trace amounts of terminal rhamnose. *A. cyanophylla* gum is distinctly different; it has large amounts of end-group

* Part 49 in the Series *Studies of Uronic Acid Materials*. For Part 48, see ref. [1].

Table 1. Methylation data and relative proportions of *O*-methyl sugars present in methylated polysaccharides of the series Phyllodineae

	A. difformis	*A. mabellae*	*A. retinodes*	*A. rubida*	*A. pycnantha*	*A. podalyriifolia*	*A. cyanophylla*
Methylation data							
Weight of polysaccharide used (mg)	296	299	232	304			
Weight of product (mg)	275	272	208	243			
$[\alpha]_D$ of product (degrees)	−50·6	−36·0	−46·7	−44·6	−50	−42	−48
OMe of product (%)	39·2	39·8	39·5	40·0	42·3	41·9	40·6
O-Methyl sugars identified							
2,3,4-tri-*O*-methyl-L-rhamnose	1	tr	2	1	tr	tr	20
2,3,5-tri-*O*-methyl-L-arabinose	26	30	8	21	14	12	3
2,3,4,-tri-*O*-methyl-L-arabinose	tr	—	tr	3	—	—	—
2,3-di-*O*-methyl-L-arabinose	—	—	—	8	1	—	1
3,5-di-*O*-methyl-L-arabinose	—	—	—	—	—	—	1
2,5-di-*O*-methyl-L-arabinose	—	—	—	—	3	—	7
2,3,4,6-tetra-*O*-methyl-D-galactose	23	32	27	29	26	29	7
2,3,6-tri-*O*-methyl-D-galactose	2	2	1	2	1	6	—
2,4,6-tri-*O*-methyl-D-galactose	16	15	14	16	4	2	2
2,3,4-tri-*O*-methyl-D-galactose	6	2	11	5	6	8	7
2,6-di-*O*-methyl-D-galactose	1	—	1	2	2	—	—
2,4,-di-*O*-methyl-D-galactose	20	13	28	9	40	42	28
2,3,4-tri-*O*-methyl-D-galactose	5	6	8	4	2	2	5
2,3-di-*O*-methyl-D-galactose	—	—	—	—	tr	tr	20

rhamnose, very little end-group galactose and arabinose, chains of arabinose, and intra-chain glucuronic acid residues. The equivalent amounts of 2,3-di-*O*-methyl-D-glucuronic acid and 2,3,4-tri-*O*-methyl-L-rhamnose in methylated *A. cyanophylla* gum suggest that rhamnose is linked 1 → 4 to glucuronic acid, which is a feature of Group IV and Group V *Acacia* exudates [7–9].

The gums from Series I *Acacias* (apart from that from *A. cyanophylla*) differ quite markedly from those of Series IV and Series V. Series I gums contain much more uronic acid than rhamnose; only one aldobiuronic acid is detectable (not two as in Series V or four as in Series IV); all uronic acid residues are present as end groups; galacto/arabinobioses were not found during mild acid hydrolysis; arabinose is mostly terminal; and the arabinose chains do not exceed two or three units long.

These studies confirm that Series I *Acacia* gums have a highly-branched galactan-core structure of the type indicated by early work on *A. pycnantha* gum, [10] which has a framework of D-galactopyranose residues with main chains linked 1 → 3 and with side-chains attached by 1 → 6 linkages.

A cyanophylla therefore appears to be an anomalous member of the Phyllodineae on the basis of the unusual chemical composition and structure of its gum exudate. Of the *Acacia* spp. studied so far in terms of gum chemistry, the two most extreme species in terms of certain of their analytical parameters are *A. pycnantha* and *A. cyanophylla*. Yet they were placed next to each other in Bentham's classification, and Tindale [3] has advised that "*A. pycnantha* appears to be quite closely related morphologically to *A. cyanophylla*, although a noteworthy difference is that the flowerheads of the former have 50–100 flowers in each capitulum whereas in the latter there are about 40". Independent evidence from the study of heartwood samples is being sought.

Table 2. *O*-methyl sugars identified in methylated *Acacia* gums of the series Phyllodineae

Relative retention time (T) of methyl glycosides*		R_G after hydrolysis‡		
		Solvents (e)		
Column (1)	Column (2)	(C)	(D)	*O*-Methyl sugar identified
0·49	0·48	1·02	1·03	2,3,4-tri-*O*-methyl-L-rhamnose
0·54, 0·69	0·58, 0·73	0·95	1·03	2,3,5-tri-*O*-methyl-L-arabinose
0·85	1·03	0·79	0·78	2,3,4-tri-*O*-methyl-L-arabinose
1·86	1·56	0·79	0·78	2,3-di-*O*-methyl-L-arabinose
1·64	1·72	0·85	0·84	2,3,4,6-tetra-*O*-methyl-D-galactose
2·71, 3·33, (3·91)	(2·96), (3·91), (4·26)	0·71	0·48	2,3,6-tri-*O*-methyl-D-galactose
3·65, (3·91)	(3·91), (4·26)	0·71	0·43	2,4,6-tri-*O*-methyl-D-galactose
6·42	6·58	0·71	0·36	2,3,4-tri-*O*-methyl-D-galactose
9·24	9·28	0·47	0·20	2,6-di-*O*-methyl-D-galactose
14·8, 16·1	14·97, 16·80	0·47	0·12	2,4-di-*O*-methyl-D-galactose
—	—	0·31	0·05	2-*O*-methyl-D-galactose
2·49, 3·00	2·48, (2·96)	—	—	2,3,4-tri-*O*-methyl-D-glucuronic acid†

* Figures in parenthesis indicate T values of components which are not completely resolved. †As methyl ester methyl glycoside. ‡R_G values of *O*-methyl sugars refer to distance moved relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucose.

EXPERIMENTAL

Details of the origin, collection, authentication and subsequent preparation of the gum specimens have been reported previously [4].

PC was carried out on Whatman no. 1 paper in the organic phase of the following systems: (a) HOAc-EtOAc-HCO_2H-H_2O (3:18:1:4); (b) C_6H_6-*n*-BuOH-pyridine-H_2O (1:5:3:3); (c) *n*-BuOH-EtOH-H_2O (4:1:5); (d) Conc. NH_3-MeCOEt-H_2O (1:200:17).

GLC of mixtures of *O*-methyl sugars was carried out at Ar flow rates of *ca* 100 ml min^{-1} on columns of (1) 15% by wt of ethylene glycol adipate polyester on 45–60 mesh Gas-Chrom Z at 176° and (2) 15% by wt of butan-1,4-diol succinate polyester on 80–100 mesh Gas-Chrom P at 176°. Retention times (*T*) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as standard.

Acknowledgements—We acknowledge financial support from Rowntree-Mackintosh Ltd. (York) and Laing-National Ltd. (Manchester).

REFERENCES

1. Anderson, D. M. W. and Bell, P. C. (1975) *Anal. Chim. Acta* **79,** 185.
2. Bentham, G. (1875) *Trans. Linn. Soc. (London)* **30,** 444.
3. Tindale, M. D. Private communications.
4. Anderson, D. M. W., Bell, P. C. and McNab, C. G. A. (1972) *Phytochemistry* **11,** 1751.
5. Anderson, D. M. W. and Gill, M. C. L. (1975) *Phytochemistry* **14,** 739.
6. Kaplan, M. and Stephen, A. M. (1967) *Tetrahedron* 193.
7. Anderson, D. M. W. and Munro, A. C. (1970) *Carbohyd. Res.* **12,** 9.
8. Aspinall, G. O., Charlson, A. J., Hirst, E. L. and Young, R. (1963) *J. Chem. Soc.* 1696.
9. Anderson, D. M. W. and Dea, I. C. M. (1969) *Phytochemistry* **8,** 167.
10. Aspinall, G. O., Hirst, E. L. and Nicolson, A. (1959) *J. Chem. Soc.* 1697.