Phytochemistry, Vol. 25, No. 12, pp. 2807–2809, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

ANALYTICAL COMPARISON OF GUMS FROM *ACACIA HEBECLADA* AND OTHER GUMMIFERAE SPECIES

SHIRLEY C. CHURMS, ALISTAIR M. STEPHEN and CHARLENE B. STEYN

Department of Organic Chemistry, University of Cape Town, Rondebosch 7700, Republic of South Africa

(*Received* 6 *March* 1986)

Key Word Index—*Acacia hebeclada*; Leguminosae; gum exudate; chemotaxonomy; arabinogalactan-protein; sugars; hydroxyproline.

Abstract—Analysis of the arabinogalactan-protein isolated from a specimen of *Acacia hebeclada* gum has shown major differences in composition between this and a specimen previously examined in another laboratory. In its much lower proportions of protein and uronic acid, and higher arabinose content, the present specimen of *A. hebeclada* gum resembles more closely gum from the taxonomically related species *A. tortilis*, this similarity extending to the modes of linkage of the constituent sugar and uronic acid residues. The hydroxyproline content of the protein moiety is also close to that in *A. tortilis* gum. The wide variation in composition between different specimens of *A. hebeclada* gum is comparable with that of the gums of *A. karroo* and *A. erioloba*, from the same series (Gummiferae Benth.).

INTRODUCTION

Gum exudates from *Acacia* species of series 4 (Gummiferae) in the Bentham classification [1] have some features of special interest. One is the structure of the arabinogalactan present, in which the galactan core, consisting of chains of β1,6-linked D-galactopyranosyl residues interspersed with small blocks of β1,3-linked D-Gal*p* [2–4], carries side-chains of six or more arabinosyl residues [2]. Another is the wide variation in nitrogen content; this ranges from < 0.4% (protein < 2.5% w/w) for the gums of *A. nilotica*, *A. nubica* and *A. seyal* [5], *A. karroo* [3, 6], *A. arabica* [7] and several other species [8, 9], through 1–3% (protein 6–18%) for gums from *A. drepanolobium* [5], *A. tortilis* [10, 11] and *A. robusta* [4], to 9% (protein 56%) for a glycoprotein exudate from *A. erioloba* (syn. *giraffae*) [12], in which the carbohydrate moiety is markedly different from that in any other *Acacia* gum hitherto described. The present examination of the gum of *A. hebeclada* (series Gummiferae Benth.) was prompted by analytical data published by Anderson and Farquhar [8] for a specimen of this gum, which indicated a protein content comparable with that of the *A. erioloba* exudate.

RESULTS AND DISCUSSION

The analytical data obtained for the present specimen of *A. hebeclada* gum are compared in Table 1 with those previously reported [8], and with analogous data for gum arabinogalactan-proteins (AG-Ps) from other *Acacia* species of the series Gummiferae Benth. [4, 11, 12]. It is evident that this specimen of the *A. hebeclada* gum AG-P differs widely in all respects from the earlier one [8]: the nitrogen content is much lower than 9.4% (the highest recorded for an *Acacia* gum) and the ratio of arabinose to galactose residues in the carbohydrate moiety (*ca* 2:1) higher than those in both the other specimen (*ca* 0.3:1) [8] and the proteinaceous *A. erioloba* exudate (*ca* 1:1) [12]. In these parameters, as in the proportion of 4-hydroxy-L-proline (Hyp), which is generally implicated in carbohydrate–protein linkages in AG-Ps from plant sources [13, 14], the AG-P from the present sample of *A. hebeclada* gum resembles that from the gum of *A. tortilis* [11]. The similarity between these two AG-Ps extends to their specific rotations (both highly positive, a characteristic of many gums from the series Gummiferae [5]) and their high molecular weights. The latter were estimated by chromatography on Sepharose 4B, from which the carbohydrate and protein in the *A. hebeclada* AG-P co-eluted, as observed for *A. robusta* gum [4].

The uronic acid content of this sample of *A. hebeclada* gum is much lower than that previously reported [8], but appreciably higher than the value found for *A. tortilis* gum [11]. However, some variation in the proportion of acid in the latter gum has been noted [10]. Partial acid hydrolysis of a portion of the *A. hebeclada* AG-P released (according to PC) all four of the aldobiouronic acids commonly encountered in gums from the series Gummiferae [2, 15], with a preponderance of 4MeGlcA(α1,4)Gal and 4MeGlcA(β1,6)Gal (*cf.* the sample of *A. erioloba* gum recently examined [12], in which GlcA was absent, all uronic acid occurring as 4MeGlcA).

The results of methylation analysis of the arabinogalactan component of the AG-P are shown in Table 2, together with those for the gum arabinogalactans from the other Gummiferae species listed here. The gums of *A. hebeclada* and *A. tortilis* are evidently similar in this respect also, especially in the high proportion of 1,2-linked Ara*f* residues indicated, a feature of several other gum exudates from the series [2, 7]. The two differ only in the presence of terminal Ara*p* and Gal*p* groups in significant proportions in *A. hebeclada* but not *A. tortilis* gum. GC/MS of the acetylated alditols derived from the product of carboxyl-reduction, with lithium aluminium deuteride [16], of the methylated *A. hebeclada* arabinogalactan showed, in addition to the methyl ethers listed in

Table 1. Analytical data for arabinogalactan-proteins from *Acacia hebeclada* gum (two specimens) and other species of the series Gummiferae Benth

	A. hebeclada I*	*A. hebeclada* II [8]	*A. erioloba* [12]	*A. tortilis* [11]	*A. robusta* [4]
N (%)	1.9	9.4	9.0	1.9	2.8
Hence protein (%)†	12	59	56	12	18
$[\alpha]_D$ (degrees)	+70	+28	−43	+75	+36
$\bar{M}_w \times 10^6$	2.4	n.d.	>1.0‡	1.3	0.72
Molar proportions (%) of constituent sugar residues					
Uronic acid	16	34	21	8	9
Galactose	26	44	38	23	40
Arabinose	56	14	36	66	50
Rhamnose	1	8	—	—	1
Mannose	1	—	5	3	—
Proportion of Hyp in protein (% by wt)	10	n.d.	22	8	17

*Present specimen.

†N × 6.25.

‡Value indicated by chromatography on Sepharose 4B (calibrated with dextrans) is uncertain in absence of suitable glycoprotein standards.

n.d. = not determined.

Table 2. Methylation analyses of arabinogalactans from gums of *A. hebeclada* and other Gummiferae species

Methyl ethers*	Methylated arabinogalactan from: *A. hebeclada* I (mol %)†	*A. erioloba* [12] (mol %)†	*A. tortilis* [11] (mol %)†	*A. robusta* [4] (mol %)†
2,3,4-Me_3-Rha	1	—	—	1
2,3,5-Me_3-Ara	10	31	12	11
2,3,4-Me_3-Ara	5	—	tr.	8
3,5-Me_2-Ara	23	5	38	6
2,5-Me_2-Ara	9	—	11	4
2,3-Me_2-Ara / 3,4-Me_2-Ara	1	—	tr.	11
2,3,4,6-Me_4-Man	1	5	1	—
2,3,4,6-Me_4-Gal	8	6	1	7
2,4,6-Me_3-Gal	4	—	6	4
2,3,6-Me_3-Gal	5	14	3	6
2,3,4-Me_3-Gal	1	12	2	2
2,4-Me_2-Gal	14	6	16	21
2-Me-Gal	2	—	2	10

*By GC analysis as acetylated alditols; identities from RR_t (relative to 2,3,4,6-Me_4-Gal) and MS.

†Adjusted to allow for proportions of uronic acid (Table 1).

tr. = trace.

Table 2, the presence in appreciable proportion (*ca* 12 mol%) of 2,3,4-Me_3-Glc, deuterated at C-6 (definitive ions *m/z* 89, 131, 191, 235), and a trace (*ca* 2%) of similarly deuterated 2,3-Me_2-Glc (*m/z* 129, 203, 263). Thus, apart from a small number of 4-linked GlcA residues, the uronic acid in *A. hebeclada* gum is mainly terminal, as in the gum arabinogalactans from *A. tortilis* [11] and *A. erioloba* [12].

The arabinogalactan moiety in the present sample of *A. hebeclada* gum resembles not only that from *A. tortilis* [11] but also those from several other *Acacia* species of the series Gummiferae, notably *A. nubica* [5], *A. arabica* [6] and *A. sieberana* var. *sieberana* [9]; these other gums, however, had low nitrogen content (< 0.4%). The general similarity between this specimen of the AG-P from *A. hebeclada* gum and that from *A. tortilis* does not extend to the other two AG-Ps isolated from gums of *Acacia* species of the series Gummiferae, *viz. A. robusta* [4] and *A. erioloba* [12]. However, gums from this series have been found to be more than usually variable in chemical composition [6, 9]: for example, analysis of 15 different specimens of *A. karroo* gum [6] has shown $[\alpha]_D$ to vary

from +38 to +67°, uronic acid content from 10 to 18%, and the ratio of galactose to arabinose residues from *ca* 1:1 to *ca* 3:1, with $\bar{M}_w$ also widely variable ($1.5–48 \times 10^5$). In this case the nitrogen content of the gum samples was uniformly low (0.09–0.24%), but specimens of the gum of *A. erioloba* examined previously [15, 17] differed from that recently investigated [12] in having low nitrogen content, as well as positive specific rotation and a much lower arabinose–galactose ratio (*ca* 0.2:1). Other significant differences include the production of all four aldobiouronic acids on partial acid hydrolysis [15], and of 2,3,5-Me_3-Ara and 2,4-Me_2-Gal in molar ratio *ca* 1:4 (*cf.* Table 2) on hydrolysis of the methylated arabinogalactan from an earlier sample of *A. erioloba* gum [17].

In the light of these observations, the major differences between the specimen of *A. hebeclada* gum described here and that previously examined [8] are less surprising. It is possible that the mechanism governing the biosynthesis of arabinogalactan and AG-P in these gums may be particularly susceptible to variations in external conditions, such as drought, attack by insects, etc.

EXPERIMENTAL

Origin of gum specimens. Gum from *Acacia hebeclada* DC. ssp. *hebeclada* was collected by Mrs. Susan Brown (S.W.A. Herbarium, Windhoek, S.W.A.) during September, 1984, from trees growing in the Windhoek municipal area. The sources of the gums from *A. robusta* Burch. var. *clavigera*, *A. tortilis* (Forsk.) Hayne ssp. *heteracantha* (Burch.) Brenan, and *A. erioloba* E. Mey. are given elsewhere [4, 11, 12].

Isolation and purification of AG-P. The gum dissolved (with sonication) in H_2O to form a clear soln (concentration *ca* 6% w/v) which set to a gel on standing. A portion of the gel was dissolved in H_2O and the soln was freeze-dried to yield sample *A*; a second sample, *B*, of the AG-P was isolated from the aq. soln by precipitation with EtOH (4 vols), followed by centrifugation and freeze-drying of an aq. soln of the ethanol-insoluble fraction. *A* and *B* were identical with respect to $[\alpha]_D$, M_w and nitrogen content, which indicated that fractionation with EtOH caused no significant change in the composition of the AG-P. Sample *B* was used in the analyses reported here.

Analytical methods. The conditions used in PC, GC/MS and gel chromatography have been described [4, 11]. The uronic acid content of the AG-P was determined by the specific colorimetric method of Blumenkrantz and Asboe-Hansen [18] and the proportion of hydroxyproline by that of Leach [19]. For the latter assay a sample was hydrolysed under nitrogen, in a sealed tube, with 6 M HCl for 24 hr at 110°. For the determination of the proportions of neutral sugars hydrolysis, also under nitrogen in a sealed tube, was carried out with 2 M trifluoroacetic acid for 18 hr at 100°, corrections being applied to the analytical results to allow for degradation of sugars, especially arabinose, under these conditions [4]. The sugars were analysed by GC, both as the alditol acetates [20, 21] and as the peracetylated aldononitriles [22, 23], molar response factors that were experimentally determined immediately prior to and after these analyses being used for quantitation. In methylation analyses the effective carbon response factors of Sweet *et al.* [24] were used to quantitate GC of the partially methylated alditol acetates derived from the hydrolysates (2 M TFA, 18 hr, 100°) of the methylated [25] arabinogalactan and its carboxyl-reduced derivative [16].

Acknowledgements—We thank the Council for Scientific and Industrial Research and the University of Cape Town for financial support, Miss Bridget Williamson (Mass Spectrometry Unit, University of Cape Town) for MS analyses, and Mrs. Susan Brown (S.W.A. Herbarium, Windhoek) for collection and identification of the gum specimen.

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