AN ANALYTICAL STUDY OF SOME *Acacia* GUM EXUDATES OF THE SERIES *Botryocephalae**

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ABSTRACT

An analytical study has been made of gum specimens from *Acacia deanei*, *A. filicifolia* (three specimens), *A. leucoclada*, *A. parramattensis* (two specimens), *A. parvipinnula*, *A silvestris*, *A. terminalis*, and *A. trachyphloia*, which are species belonging to Series II (*Botryocephalae*) in Bentham's classification of the genus. The three specimens from *A. filicifolia* are all closely similar, but the specimens from *A. parramattensis* differ appreciably in parameters other than their sugar ratios. Several of the analytical values reported increase considerably the range of values established so far for *Acacia* gum exudates. The *Botryocephalae* species give gum exudates of at least 2 chemically distinct types. Group A species (*A. deanei*, *A. parramattensis*, *A. parvipinnula*, and *A. trachyphloia*) have low galactose–arabinose ratios (<2:1) but have strongly negative rotations, high intrinsic viscosities and molecular weights, and relatively high nitrogen, methoxyl, uronic anhydride, and rhamnose contents. Group B species (*A. filicifolia*, *A. leucoclada*, and *A. terminalis*) have high galactose–arabinose ratios (>4/1) but low negative or positive rotations, low intrinsic viscosities and molecular weights, and relatively low nitrogen, methoxyl, uronic anhydride, and rhamnose contents.

INTRODUCTION

A chemotaxonomic review[1] of *Acacia* gum exudates drew attention to the fact that, of the 32 known species in Series 2 (*Botryocephalae*) of Bentham's classification of the genus, only 5 species, *viz. A. dealbata*[2–4], *A. decurrens*[5,6], *A. elata*[6–8], *A. mearnsii*[2,4,6,9], and *A. mollissima*[10], have been studied so far. Unfortunately, there has been considerable confusion, clarified by Brenan and Melville[11], over the botanical nomenclature and identity of *A. mearnsii* and *A. mollissima*, and it appears[6] that gum from the latter species (correctly known as *A. pubescens*) has not yet been studied.

There is therefore comparatively little analytical data on which to base any assessment of the characteristic composition, structure, and properties of exudates from species in the *Botryocephalae* series. To help overcome this deficiency, we now

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report the results of analytical studies of 11 gum specimens, of which all but one (*A. terminalis*) originate from Australian species that have not been studied before.

EXPERIMENTAL

Origins of gum specimens. — Gum from *Acacia deanei* (R. T. Bak.) Welch, Coombs et McGlynn ssp. *paucijuga* (F. Muell. ex N. A. Wakef.) Tindale was collected by Mr. L. Pedley at Brisbane Botanic Gardens, Queensland, in September 1968. Gum specimens from *Acacia filicifolia* Cheel et Welch were collected by Mr. J. Pickard as follows: Specimen I at Ten Mile Hollow, New South Wales, on February 15th, 1970 (Botanical Voucher No. NSW 118410); Specimen II at Putty Creek, New South Wales, on August 3rd, 1969 (No. NSW 104082); Specimen III at Newnes Oil Shale Retorts, New South Wales, on February 7th, 1969 (No. NSW 102996). The specimen from *A. leucoclada* Tindale ssp. *argentifolia* Tindale was collected by Mr. L. Pedley at Brisbane Botanic Gardens in September 1968. Gum specimens from *A. parramattensis* Tindale were collected by Mr. P. Hind at Marayong, near Blacktown, New South Wales, as follows: Specimen I on August 25th, 1968 (No. NSW 102390) and specimen II on July 28th, 1968 (No. NSW 100274). *A. parvipinnula* Tindale was collected by Mr. J. Pickard at Euroka, 4 miles south of Glenbrook, New South Wales, on June 1st, 1969 (No. NSW 103755). *A. silvestris* Tindale was collected by Mr. R. Coveny at Prince's Highway, 12 miles north of Bega, New South Wales, on July 29th, 1970 (No. NSW 119261). *A. trachyphloia* Tindale was collected by Mr. R. Coveny at Prince's Highway, Termeil, New South Wales, on July 29th, 1970 (No. NSW 119119). The gum from *A. terminalis* (Salisb.) Macbride, syn. *A. elata* A. Cunn. ex Benth.) was collected by an officer of the Botanical Research Institute, Pretoria, South Africa, in March 1967*.

Preparation of samples for analysis. — The samples from *A. filicifolia* I, *A. filicifolia* II, *A. filicifolia* III, and *A. leucoclada* were soluble in cold water. After dialysis against tap water for 24 h and then against distilled water for 24 h, the gum solutions were filtered through Whatman No. 42 and No. 1 papers, and then freeze-dried. The yields are shown in Table I.

All the other samples were incompletely soluble in cold water, but dissolved in 1% aqueous sodium borohydride[12] within 24 h. After dialysis against tap water for 96 h and against distilled water for 24 h, the solutions were filtered as described above and then freeze-dried. The yields are shown in Table I.

Analytical methods. — The standard analytical methods been have described[13,14], with the following exceptions. Equivalent weights were found by potentiometric titration (to pH 7) of weighed amounts of gum that had been exhaustively electrodialysed (ash content <0.1%). The ratios of galactose–arabinose–rhamnose were determined with a Technicon Carbohydrate Analyser (by courtesy of

**Footnote added in proof.* For this species we use the nomenclature quoted by the Botanical Research Institute, Pretoria. The name *A. elata* is, however, preferred by Dr. M. D. Tindale since the holotype has never been found and Salisbury's very brief description is poor.

TABLE I

ANALYTICAL DATA FOR PURIFIED GUM POLYSACCHARIDES FROM *Acacia* SPECIES OF THE *Botryocephalae* SERIES

	A. deanei *ssp.* paucijuga	A. filicifolia *I*	A. filicifolia *II*	A. filicifolia *III*	A. leucoclada *ssp.* argentifolia	A. parramattensis *I*	A. parramattensis *II*	A. parvipinnula	A. silvestris	A. terminalis	A. trachyphloia
Recovery from crude gum, %	53	72	87	84	79	59	69	60	55	63	50
Moisture, %	11.5	11.3	10.4	10.4	11.3	13.0	12.6	11.6	9.1	11.4	8.9
Ash, %[a]	2.4	0.9	0.7	1.1	1.2	2.7	3.8	2.5	2.3	1.1	2.2
Nitrogen, %[a]	1.25	0.27	0.29	0.21	0.04	1.17	1.55	1.01	0.21	0.55	1.03
Hence protein, % (N × 6.25)[a]	7.8	1.7	1.8	1.3	0.3	7.3	9.7	6.3	1.3	3.4	6.4
Methoxyl, %[b]	0.75	0.47	0.43	0.50	0.65	0.84	1.23	0.84	1.34	0.44	1.02
$[\alpha]_D$, degrees[b]	−66	+4.3	−9.6	−2.3	−4.4	−49	−77	−54	−8.4	+5.4	−57
Intrinsic viscosity $[\eta]$, ml.g^{-1}[a]	12.5	4.2	6.3	6.4	4.4	15.1	23.0	12.0	6.1	6.4	16.6
Molecular weight, $\bar{M}_w \times 10^4$[a]	36	5.8	7.5	5.6	13	37	140	34	20	4.7	97
Equivalent weight[b]	1350	4260	4070	3610	3020	1255	1115	1200	1255	3850	1415
Hence uronic anhydride, %[b,c]	13.0	4.2	4.3	4.9	5.8	14.0	15.8	14.7	14.0	4.6	12.4
Sugar composition[b] *after hydrolysis*											
4-*O*-Methylglucuronic acid[d]	4.5	2.8	2.6	3.1	4.0	5.0	7.4	5.0	8.0	2.6	6.1
Glucuronic acid	8.5	1.4	1.7	1.8	1.8	9.0	8.4	9.7	6.0	2.0	6.3
Galactose	38	80	76	77	80	38	39	40	71	82	56
Arabinose	43	15	18	16	12	42	41	40	12	12	27
Rhamnose	6	1	2	2	2	6	4	5	3	1	5

[a]Corrected for moisture content. [b]Corrected for moisture and protein content. [c]If all acidity arises from uronic acids. [d]If all methoxyl groups are located in this acid.

Dr. J. M. McNab). In the calculation of $\bar{M}_w$ from light-scattering data, the mean value of *dn/dc* (0.146) established[15] in a survey of *Acacia* species was used.

RESULTS AND DISCUSSION

The analytical data obtained are given in Table I. In agreement with earlier reports[12,13,16], the use of 1% aqueous sodium borohydride does not appear to cause any degradation during the dissolution process. Indeed, the water-soluble species (*A. filicifolia* I, II, III and *A. leucoclada*) have lower intrinsic viscosity values and much smaller molecular weights than the species solubilised with borohydride, with the exception of *A. terminalis*, which (under the synonym *A. elata*) was reported[8] to have an even lower molecular weight (ultracentrifuge, $\bar{M}_w = 29{,}500$; molecular-sieve chromatography, $\bar{M}_w = 22{,}000$) than our value (47,000) for borohydride-solubilised material. The other analytical data available[7,8] for *A. elata* (equivalent weight = 6,100 and hence uronic anhydride = 3%; $[\alpha]_D$ +4°; galactose–arabinose–rhamnose, 80:17:3) are very close to our corresponding values for *A. terminalis*.

The small group of bipinnate wattles studied here are all fairly close botanically. On the basis of the flavonoid and condensed-tannin contents of the heartwood and bark, Tindale and Roux[17] found that all the species studied in this paper, except *A. deanei* and *A. parvipinnula*, could be assigned to a trihydroxyflavonoid (mollisacacidin)-containing group; *A. parvipinnula* was not studied, and *A. deanei* was assigned to a group containing both mollisacacidin and a dihydroxyflavonoid (guibourtacacidin), although only a trace of the latter was detected. It is therefore of interest that the range of analytical parameters for the gum exudates should be as extensive as is shown in Table I. The ranges of values, *e.g.* for specific rotations, intrinsic viscosity, nitrogen, molecular weight, and ratio of galactose to arabinose, extend considerably the extreme values for these parameters established previously for *Acacia* exudates.

The species formerly giving the most strongly negative rotations were the putative *A. mollissima*[10] (−49°) and *A. mearnsii* (−55°, Ref. 6; −61°, Ref. 9). *A. deanei* (−66°) and one specimen of *A. parramattensis* (−77°) exceed these: the range established for *Acacia* exudates is now −77° to +108° (*A. nilotica*[18]).

An *Albizia* species[19] has been found to have an intrinsic viscosity of 4.7 ml·g^{-1}, but values lower than 9–10 (*A. nubica*[3], *A. nilotica*[18], and *A. arabica*[20]) have not been found for *Acacias*, except for a proportion of nodules of *A. seyal* gum, which is a very variable species[21]. The values of $[\eta]$ found for *A. filicifolia*, *A. leucoclada*, *A. silvestris*, and *A. terminalis* gums are therefore much lower than previously known. Although they also have low molecular weights, it will be of interest to investigate whether their molecules are even more highly branched than that of *A. arabica*[20] gum, the present model for gum molecules of high molecular weight but low viscosity. The present group of *Botryocephalae* species show a remarkable range of intrinsic viscosity ($[\eta] = 4.2$–23) and molecular weight (47,000–1,400,000); *A. parramattensis* I and *A. trachyphloia* are as viscous as good specimens of gum arabic (*A. senegal syn. verek*[22]) and *A. parramattensis* II equals the most viscous *Acacia* known (*A. laeta*[23]).

The *Botryocephalae* species all have significant methoxyl contents; the value for *A. silvestris* (1.34%) is exceeded only by that reported[4] for *A. giraffae*.

The nitrogen content of plant gums is an important analytical parameter[24]; values for the present species vary widely. *A. deanei* has a nitrogen content that equals the highest previously recorded (*A. drepanolobium*[12]), but this is considerably exceeded now by *A. parramattensis* II (1.55%). Only one species (*A. nilotica*[18]) has a nitrogen content (0.02%) lower than that of *A. leucoclada*.

A rhamnose content of 1–6% is not now regarded as an unusual feature in *Acacia* gums[25]. The *Botryocephalae* and *Gummiferae* (Series 4) species do not show the molar correspondence between rhamnose and uronic acid that is characteristic of Series 5 (*Vulgares*).

The species with unusually high contents of galactose (*A. filicifolia*, *A. leucoclada*, and *A. silvestris*) now join the species (*A. elata*, and *A. podalyriifolia*) that are regarded[7,8] essentially as arabinogalactans. Although *A. cyanophylla*[26] remains as the species having the lowest arabinose content, the ratio of galactose–arabinose differs markedly in the species studied here. Two chemically distinct sub-groups are apparent. The first (group A), comprising *A. deanei*, *A. parramattensis*, *A. parvipinnula*, and *A. trachyphloia* have low galactose–arabinose ratios (<2:1) and high values of the other parameters, *e.g.* nitrogen >1%; $[\eta]$ >12 ml·g^{-1}; $\bar{M}_w$ >340,000; uronic anhydride >12%; $[\alpha]_D$ more negative than −49°; methoxyl >0.75%; and rhamnose > 4%. In contrast, group B (*A. filicifolia*, *A. leucoclada*, and *A. terminalis*) have high galactose–arabinose ratios (>4:1) and low values of the other parameters, *e.g.* nitrogen <0.55%; $[\eta]$ <6.4 ml·g^{-1}; $\bar{M}_w$ <130,000; uronic anhydride <6%; $[\alpha]_D$ less negative than −10°; methoxyl <0.65%; and rhamnose <3%. *A. silvestris* has the properties of the group B species, but its high methoxyl and uronic anhydride values fall within the limits for group A, and it has an intermediate value of $\bar{M}_w$. These chemically distinct groups indicate the possibility that species of the *Botryocephalae* may be characterised by the existence of at least two different structural types of gum molecule. This possibility will be investigated.

The three different specimens of *A. filicifolia* gum, collected in different years at different places in New South Wales, are all closely similar. The two specimens from *A. parramattensis*, which originated from the same district, are similar in terms of sugar composition, but their other parameters are more divergent; since their high nitrogen content, low viscosity, and strongly negative rotation are unusual features, further specimens will be sought so that the inter-nodule variation can be studied.

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