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SOME HIGHLY PROTEINACEOUS *ACACIA* GUM EXUDATES OF THE SUBSERIES *JULIFLORAE**

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Abstract—Analytical data for the gum exudates from *Acacia difficilis*, *A. dimidiata*, *A. eriopoda*, *A. maidenii*, *A. stipuligera*, *A. torulosa* and *A. tumida* are presented. Of these, five are highly proteinaceous; they also have high methoxyl contents and very low rhamnose contents. In contrast, *A. dimidiata* shows no unusual analytical parameters, and *A. maidenii* gum has a low arabinose content and a high rhamnose content, thus having a sugar composition of the type first observed in the gum from *A. saligna*. The gum from *A. maidenii* is also of interest as its analytical data are closely similar to those for *A. longifolia*, the only other tetramerous member of the subseries *Juliflorae* to have been studied. The data reported extend even further the unusual ranges of analytical parameters found within the *Juliflorae*, and confirm its great heterogeneity and chemotaxonomic interest.

INTRODUCTION

The *Juliflorae* are regarded [2]as the most highly evolved subseries of the *Acacia* species, both morphologically and chemically. The analytical data published previously [3, 4] for 15 of the *Juliflorae* species showed that they had interesting combinations of chemical properties that are unusual in *Acacia*. It was also tentatively suggested, on the basis of the limited analytical data available, that the groupings within *Juliflorae* suggested by Bentham [5] should be reconsidered.

This paper presents analytical data for a further six *Juliflorae* species. It also presents, for a new specimen of gum from *A. torulosa*, data which show that the nitrogen content of 7% previously reported [4] was not an artefact; indeed, four of the six new species studied also have similarly high nitrogen contents.

RESULTS AND DISCUSSION

The analytical data obtained are shown in Table 1. All the gum specimens were of Australian origin; the data for *A. maidenii* gum are of particular interest as they are closely similar to the values quoted for two samples of *A. longifolia* gum [6] of South African origin. A close similarity in the chemical composition of the gums obtained from Australian and South African specimens of *A. dealbata* [7], *A. saligna* (syn. *A. cyanophylla*) [8] and *A. pycnantha* [8] has been reported. The chemotaxonomic differences [9] between the gums from *A. saligna* and *A. pycnantha* were first pointed out in 1972 [10]. Although there does not appear to have been any botanical reassessment to date of the close relationship attributed to these species by Bentham [5], there are close chemical [6] and morphological similarities between *A. saligna* and *A. longifolia*, to which must now be added *A. maidenii*. *Acacia longifolia* and *A. maidenii* are the only members of the tetramerous *Juliflorae* to be studied to date. It is clearly desirable for more of the gums from this subsection of the genus to be studied promptly.

The gum from *A. dimidiata* has few distinguishing features and can be regarded as a rather unusual member of the *Juliflorae*. Of the species studied to date [3, 4], the data for *A. holosericea* most closely resemble those for *A. dimidiata* and there appears to be justification (Maslin, B. R., personal communication; Pedley, L., personal communication) for adding *A. dimidiata* to Group 2 of the informal groupings proposed earlier [4].

Botanically, *A. stipuligera* is one of the 27 'relictual' Queensland species that are considered (Pedley, L., personal communication) to be taxonomically isolated. Nevertheless, the data in Table 1 indicate that *A. stipuligera* gum has similar features to the gums from *A. torulosa*, *A. tumida* and *A. difficilis* in having a very low rhamnose content, negative rotation, and high values for nitrogen, methoxyl and uronic acid groups. The gum from *A. stipuligera* behaved uniquely for an *Acacia* gum, however, in that it formed a loose gel during dialysis.

The gum from *A. eriopoda* has several features (high nitrogen, methoxyl values; low rhamnose content) in common with the gums from *A. torulosa*, *A. tumida* and *A. difficilis*, but there are major differences in the respect that *A. eriopoda* gum has a positive rotation and an unusually high arabinose content; its very low rhamnose content and high ratio of arabinose to galactose (67/18) is typical of gums from species in the series *Gummiferae* but this ratio is much higher than has been recorded so far, e.g. 59/33 for *A. nubica* gum [11].

The most outstanding correlation, however, is the confirmation of the close relationship that *A. torulosa*, *A. tumida* and *A. difficilis* are considered (Maslin, B. R., personal communication; Pedley, L., personal communication) to show: *A. tumida* and *A. difficilis* can be added to Group 4 of the informal groupings proposed [4]. It is of interest that, for *A. torulosa*, *A. tumida* and *A. difficilis*, the specimens from Northern Territory (samples A, B and B, respectively) are more acidic, more viscous, and have higher nitrogen and methoxyl contents than the speci-

*Part 62 in the series "Studies of Uronic Acid Materials". For Part 61 see ref. [1].

Table 1. Analytical data for gum polysaccharides from *Acacia* species of the subseries *Juliflorae*

	A. torulosa		*A. tumida*		*A. difficilis*		*A.*	*A.*	*A.*	*A.*
	A	B	A	B	A	B	*eriopoda*	*stipuligera*	*dimidiata*	*maidenii*
Moisture (%)	13.0	9.7	3.3	10.8	4.6	13.1	5.7	5.7	8.8	15.1
Ash (%)*	2.4	3.7	2.2	3.0	2.3	1.2	1.7	4.1	1.3	6.4
Nitrogen (%)*	7.8	7.2	6.7	7.1	7.5	8.5	6.7	6.3	0.26	1.3
Hence protein (%) (N × 6.25)*	49	45	42	44	47	53	42	40	1.6	8.1
Methoxyl (%)†	3.8	1.6	4.2	5.5	2.8	4.2	1.3	4.8	0.23	0.52
$[\alpha]_D$ in water (degrees)†	n.d.	−41	−48	−9	−40	−10	+5	−19	−1	−37
Intrinsic viscosity (ml/g)*	39	69	11	30	18	30	19	21	26	20
Molecular weight, $\bar{M}_w \times 10^5$	1.7	10.0	8.3	1.7	2.9	3.0	3.6	4.8	27	3.1
Equivalent weight	560	957	660	480	930	530	1180	610	2150	650
Hence uronic anhydride (%)‡	32	19	27	37	19	33	15	29	8	27
Sugar composition after hydrolysis										
4-O-methylglucuronic acid§	23	9	25	33	17	25	8	29	1.5	3
Glucuronic acid	9	10	2	4	2	8	7	0	6.5	24
Galactose	43	53	44	49	50	49	18	46	49	51
Arabinose	25	28	29	14	30	21	67	25	39	6
Rhamnose	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	4	17

*Corrected for moisture content.
†Corrected for moisture and protein content.
‡If all acidity arises from uronic acids.
§If all methoxyl groups located in this acid.
n.d. = Not determined.

mens from Queensland and Western Australia. Although geographical factors may be involved, and although the analytical parameters for each *Acacia* gum must be expected to show seasonal and geographical variations that may be quite extensive [1], the differences recorded here for some, but not all, of the analytical parameters for the different specimens of gum from *A. tumida* and *A. difficilis* are possibly a little larger than might have been expected. However, the data for *A. tumida* specimen B are closely similar to those for *A. difficilis* B, both of which originated from Northern Territory. Similarly *A. tumida* A is closely similar to *A. difficilis* specimen A. As *A. difficilis* and *A. tumida* are extremely close morphologically, with *A. difficilis* believed in the field to be a "narrow-leaved variant of *A. tumida*" (Willing, T., personal communication), it appears to be possible that some of the identifications are not strictly correct: scrutiny of the herbarium voucher specimens would be of interest, particularly as the very high nitrogen content of these species of gum make them of outstanding interest.

Unfortunately the *Juliflorae* species do not appear to yield gum copiously. The quantities available so far have precluded studies of the proteinaceous components and their separation from the gum polysaccharide. Evidence was obtained [12] for *A. senegal* gum (gum arabic) that simple fractionation by molecular sieve chromatography leads to a fraction of high MW and high nitrogen content, and low MW fractions of depleted or zero nitrogen content. The availability now of molecular sieves of much higher exclusion limits than were available when these studies were first undertaken [12] makes extensions of these approaches much more practicable. Studies of the amino acid compositions of these highly proteinaceous exudates are also in progress. Notwithstanding the high nitrogen contents shown by the *Juliflorae* species (Table 1), the highest recorded nitrogen content (9.4%) remains that of *A. hebeclada* [13].

Species within the subseries *Juliflorae* continue to provide examples of even wider ranges of values for some of the various analytical parameters for *Acacia* gums that have been reported previously [3, 4]. In particular, the highest methoxyl content is increased greatly from 3.4% (*A. microneura*, *A. resinomarginea* [4]) to 5.5% (*A. tumida*). *Juliflorae* species continue to provide the highest recorded values for MW (*A. holosericea* [3], 3.8×10^6), intrinsic viscosity (*A. torulosa* [4], 69 ml/g) and uronic acid content (*A. microneura* [4], 39.7%). These values, plus the wide variations in rhamnose content from < 1 to 17% (Table 1), uronic acid content from 8 (*A. dimidiata*) to 39.7% (*A. microneura* [4]), methoxyl content from 0.23 to 5.5% (Table 1) and arabinose content from 3 (*A. kempeana* [4]) to 67% (Table 1) stimulate interest in this complex, heterogeneous, highly evolved taxon even more deeply.

EXPERIMENTAL

Origin of gum specimens. Gum from *A. torulosa* F. Muell: Sample A was collected near Elliot, Northern Territory, Australia, by P. K. Latz on 28 May 1975 (Latz 5790); sample B was collected 42 km WNW of Lakeland Downs, Laura, Queensland on 9 September 1975 (Coveney and Hind, NSW107876). Gum from *A. tumida* F. Muell. ex Benth.: Sample A was collected by T. Willing near Broom, Western Australia, in December 1981 (T. Willing 41); sample B was collected by P. K. Latz on 30 May 1975, 32 miles south of Elliot, N. T. (Latz 5999). Gum from *A. difficilis* Maiden: Sample A was collected by T. Willing near Broom, Western Australia, in December 1981 (T. Willing 20); sample B was collected by Dr. M. Tindale (M.T. 6088) 40 km south of Pine Creek, Northern Territory, on 10 July 1979 (NSW 108566). Gum from *A. eriopoda* Maiden and Blakely was collected by T. Willing at Broom, Western Australia, from fire-damaged trees in December 1981 (T. Willing 42). All specimens collected by T. Willing were verified by B. Maslin, Western Australia Herbarium; the voucher specimens are deposited at Perth. Gum from *A. stipuligera* F. Muell. was collected by P. K. Latz, 300 km north of Alice Springs, on 28 May 1975. Gum from *A. dimidiata* Benth. was collected by Dr. M. Tindale (MT 6064) 41 km south of Katherine at King River Crossing, Northern Territory, on 9 July 1979 (NSW 108562). Gum from *A. maidenii* F. Muell. was collected by L. Pedley from a tree in his garden in Brisbane, March–May 1979.

Preparation of samples for analysis. As *Juliflorae* species yield gum only sparingly, the amounts of crude gum available for purification and analysis were small (1–10 g). The amounts of gum from *A. maidenii* and *A. difficilis* sample B were particularly small; analyses were carried out on the powdered, crude gum. Previous difficulties [3, 4] in dissolving gums from *Juliflorae* species were again encountered; dissolution of all of the remaining specimens listed in Table 1 was achieved by adding 1% $NaBH_4$. Further evidence that this does not lead [14] to significant degradation is given by the high values of intrinsic viscosity, MW and uronic acid content shown in Table 1. All of the solns obtained were dark in colour, except for *A. tumida* sample A. After the dissolution (1% $NaBH_4$), filtration (muslin, then paper), dialysis (2 days vs tap H_2O; 1 day vs distilled H_2O) and recovery by freeze-drying, the recoveries of the gums from *A. tumida* sample A, *A. eriopoda* and *A. difficilis* were 64, 59 and 61%, respectively.

Analytical methods. The standard analytical methods used have been described [10].

Presence of 4-O-*methylglucuronic acid.* In all previous structural studies of *Acacia* gums (e.g. [11]), acidic hydrolyses followed by chromatography in a range of solvents have not revealed the occurrence of naturally methylated neutral sugars and all methoxyl content is customarily attributed to the presence of 4-*O*-methylglucuronic acid. The unusually high methoxyl contents reported in Table 1 led to a check for the presence of major amounts of this substituted uronic acid. Examination of hydrolysates (0.5 M H_2SO_4, 8 hr at 100°) by PC in solvents (a) (EtOAc–HOAc–HCO_2H–H_2O, 18:3:1:4); (b) (C_6H_6–*n*-BuOH–pyridine–H_2O, 1:5:3:3); and (c) (EtOH–H_3PO_4 (0.1 N)–*n*-BuOH, 10:5:1) showed the presence of 6-*O*-(β-D-glucopyranosyluronic acid-D-galactose [R_{gal} 0.18 (solvent (a)], 0.19 [solvent (b)], 4-*O*-(-4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose [R_{gal} 0.55 (solvent (a)], 0.43 [solvent (b)], and 4-*O*-methylglucuronic acid [R_{gal} 2.49 (solvent (a), 1.44 (solvent (c)]. The occurrence of this methylated uronic acid in 0.5 M acid hydrolysates of *Acacia* gums of the *Vulgares* and *Gummiferae* series is unusual, but has been observed by several workers (Bell, P. C., Farquhar, J. G. K. and Gill, M. C. L., unpublished work) in studies of gums from the *Juliflorae*.

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